The population dynamics of a hybrid zone in the Alpine grasshopper *Podisma pedestris*: an ecological and genetic investigation

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Katherine Sarah Jackson

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ABSTRACT

This thesis describes ecological and genetic investigations of a hybrid zone between incompatible genotypes in the alpine grasshopper *Podisma pedestris*. The two races are distinguished by a Robertsonian fusion between the X chromosome and an autosome. The cline between them is usually between 400 and 800m wide, and is thought to be maintained by a balance between selection against heterozygotes and dispersal.

The *Podisma* hybrid zone provides an interesting system in which to investigate fitness differences in nature. Measurements of fitness components have been made in the field across the hybrid zone. Counts through the season, in matched vegetation types, show (surprisingly) a substantial difference between the two races in the number of young nymphs: this difference is consistent across years and across transects. Hybrid populations are less dense than the average of the pure populations, but are not significantly different from the sparser of the parental races. Differences in density across the zone decrease through the season, suggesting density-dependent mortality. This is supported by cage and transplant experiments in the field, and by simulation experiments.

Less direct ways of measuring fitness components are also explored. Theoretical relations derived by Barton (1983) allow one to infer parameters such as selection pressures from the observed cline shape. Here, computer simulations show that these estimates are robust.

Where the cline coincides with a physical barrier, the pattern of chromosome frequencies combine with measures of dispersal to show that selection is acting on many genes, causing an additional barrier to gene flow between the divergent populations.

An assessment of the density of *Podisma* over a wide area allows the expected position of the cline to be estimated. Computer simulations show that the observed position of the cline is consistent with that expected from both direct density estimates, and densities inferred from a vegetation survey.
Chapter 1

Introduction: an evolutionary perspective

In this thesis I will be describing ecological and genetic investigations of a hybrid zone between incompatible genotypes in the alpine grasshopper *Podisma pedestris*. In this chapter I will discuss the relation between population genetics and ecology (Section 1.1); in Section 1.2 I will discuss how hybrid zones can provide insights into important questions in both ecology and evolution. In Section 1.3 I will discuss models of speciation; the controversies that have dominated this area of research over the last two decades, and the important contribution made by studies on natural hybrid zones and their interaction with theoretical population genetics. Finally, in Section 1.4 I will provide an overview of the questions addressed in this thesis.

1.1 Ecological genetics

Evolutionary biologists have long been interested in the role of population structure in evolution. The factors that determine spatial patterns - how these interact and how and under what conditions they might cause local differentiation and speciation - have been central to these studies. For example, does gene flow bind species into single units, thus preventing adaptation to local conditions? Does environmental heterogeneity maintain genetic variation? Can a single population split into two morphologically distinct and/or reproductively isolated groups? Spatial patterns of gene frequency in natural populations are determined by the combination of evolutionary forces such as genetic drift, natural selection, mutation and gene flow; ecological factors will be very important in determining the effects, magnitude and relative importance of these forces.

Natural selection is perhaps the evolutionary force most closely linked to ecology:
both changes in population numbers and allele frequency depend on fitness. Absolute fitness determines population size: in a continuous population, it is measured by the Malthusian parameter, \( r \), which gives the rate of increase in size of the population: \( \frac{dN}{dt} = rN \). In a population with discrete generations, \( N^* \), the population size in generation \( t+1 \) is equal to fitness, \( W \) multiplied by the population size in generation \( t \): \( N^* = WN \). Thus absolute fitness determines the absolute rate of increase in a population. Relative fitness is the rate of transmission of a genotypes genes relative to that of another genotype. Thus a healthy fertile genotype might have a relative fitness of 0.5 instead of 1 only because another genotype is reproducing twice as fast.

There is an interesting analogy between genetic drift and "demographic" drift - both being caused by random differences in fitness between individuals. The importance of random genetic drift in small local demes has been emphasised in Wright's (1931) shifting balance theory. Because different combinations of genes can give the same phenotype, a population can respond to selection in different ways and can move towards alternative stable equilibria. Wright illustrated this with his "adaptive landscape": a plot of mean fitness against allele frequencies or character means. Ignoring frequency-dependence or linkage disequilibria, mean fitness increases under selection and so a population will move uphill towards an adaptive peak. Wright argued that a single panmictic population will reach the nearest peak - but not necessarily the global optimum - whereas population subdivision would allow evolutionary advance: migration from demes which had drifted to high peaks could shift neighbouring demes to that peak; demes at superior peaks might send out more emigrants and might be less likely to go extinct.

Another important connection between ecology and population genetics is through population regulation. Populations do not often occur in large panmictic units; they are usually sub-divided to some extent. The distinction between "hard" and "soft" selection is whether population size is regulated at the level of local deme or the whole population. Perhaps the most direct way to connect ecology and population genetics is through models of density-dependent selection, in which one set of equations determines population regulation, another set determines gene frequency change: the equations are interdependent (Roughgarden, 1971).

Some of the most intractable puzzles in biology have been the mechanisms responsible for the origin of new species. There is an interesting relation between species diversity and genetic theories of speciation. Are species numbers limited by
niches or by the rate of reproductive isolation? What limits a species range? Clearly, ecology and demography in combination with population genetics theory are vital in assessing the plausibility of models in evolution.

1.2 Hybrid zones

Hybrid zones provide excellent natural systems for investigating many of these questions. These are the narrow regions where two closely related but genetically distinct forms meet and interbreed. There has been some confusion over the definition of a hybrid zone (see Barton and Hewitt, 1985). In this thesis I use it to refer to a cline or set of clines - that is gradients or a set of gradients in gene frequency or morphology, at one or more loci.

Clines can be formed in several ways. In the absence of selection, mixing of two previously separated genotypes would result in a transient cline, and eventual polymorphism. A wave of advance of an advantageous gene would similarly result in a transient cline (Fisher, 1937). The two genotypes could meet along an ecotone, where each genotype would be most suited to its own environment (Haldane, 1948). In this case the hybrids would not be at an advantage in either environment, though they might be at an advantage in the transitional habitat (Moore, 1977). In these cases, hybrids would remain in a stable hybrid zone where the two forms met. A stable hybrid zone will also form if there is selection against recombinant or heterozygous genotypes: “hybrid inferiority”. Where the two genotypes meet they produce relatively unfit hybrids which leave fewer offspring. An animal migrating into foreign territory is most likely to mate with the resident genotype, producing hybrid progeny which will be selected against. This is likely to produce a stable zone, the shape of the cline will typically follow a tanh (or logistic) curve, and it will have width determined by the relative strengths of the selection against hybrids and gene flow into the zone. Bazykin (1969) suggested the simplest model: if heterozygotes have fitness \(1 - s\) and homozygotes fitness 1 and if \(p\) is the allele frequency, then the change in allele frequency is (for small \(s\)):

\[
\frac{\partial p}{\partial t} = \frac{\sigma^2}{2} \frac{\partial^2 p}{\partial x^2} + spq(p - q)
\]  

(1.1)

This equation has the solution:

\[
p = \frac{1}{1 + exp[-(x - x_0)/l]}
\]  

(1.2)
where $l = \sqrt{\sigma^2/2s}$ is the characteristic distance over which selection changes allele frequencies and the cline width is $4l$.

The concept of a tension zone is central to researches into the dynamics of hybrid zones maintained by a balance between dispersal and selection. Tension zones are so called because they tend to move so as to minimize their length (Key, 1968). The theory of clines and tension zones will be discussed in more detail in chapter 5. Here it will be useful to summarise the distinctive features and behaviour of tension zones as they will be central to the discussions to follow.

### 1.2.1 The dynamics of tension zones

The theoretical behaviour of tension zones has been investigated in detail by Bazykin (1969) and Barton (1979c). The important feature of tension zones is that they are maintained by internal genetic factors rather than by external selection differentials. This means that they are mobile and can be perturbed from their initial position by a variety of forces. Barton (1979c) has shown that an asymmetry in selection (where one parental race is fitter than the other), for example, will tend to move the tension zone in the direction of the less fit race - in a homogeneous environment the less fit race would eventually be swamped. Higher population density or an increased dispersal rate on one side of the zone relative to the other will similarly result in advance - simply by weight of numbers. One would expect such differences in natural tension zones to be quite common: it is unlikely that two populations will be equally fit, and it is quite likely that there will be at least some asymmetry in the size or dispersal rate of the populations at different "adaptive peaks". The different genotypes might be better suited to different environments, in which case one would expect the tension zone to move either until one type is eliminated or until it reaches a point on an environmental gradient where the selective forces balance. And indeed, tension zones often are at ecological boundaries (for example, the zone between *Mus musculus* and *Mus domesticus* is associated with the climatic gradient in Denmark (Hunt and Selander, 1973) and in the Australian grasshopper, *Caledia captiva*, the Moreton and Torresian races meet in a hybrid zone running close to the 30\% contour of the coefficient of variation of rainfall (Shaw and Wilkinson, 1980); the zone between *Bombina bombina* and *Bombina variegata* is broadly correlated with the transition from lowland to mountainous habitat in central Europe (Szymura and Barton, 1986, 1991)). Barton (1979c) has shown that population structure will also be very important in determining the position and movement of tension zones.
Since a larger population size or dispersal will tend to push the zone forward, tension zones will be trapped by local barriers to dispersal, and by areas of low density: thus variations in population structure can override a selective imbalance and immobilise the zone.

1.2.2 Hybrid zones and speciation

For some time hybrid zones have been viewed as giving insights into speciation: for example it was thought that selection against hybrids would provide a strong selective force for the development of pre-mating isolating mechanisms, to prevent parental types from wasting gametes and effort in hybrid matings (Dobzhansky, 1940; Wilson, 1965). However with increasing research, it appears that hybrid zones can be stable for thousands of years without the development of pre-mating isolating mechanisms (Moore and Buchanan, 1985). More recent reviews (Barton and Hewitt, 1981b; Endler, 1977) conclude that it is difficult to make inferences from present hybrid zones about the historical origins of species. Parameters such as cline shape, they argue, are more indicative of current fitness relations than they are of historical process. This has led to a shift in emphasis in hybrid zone studies, (though relating no less to speciation) to concentrate on what insights hybrid zones can provide into the nature and effects of differences between incipient species; the interactions between diverging populations and the ecological and demographic factors which influence these; and the role of coadaptation in the spread of new gene combinations (Barton and Hewitt, 1989).

Investigations into the causes of zone position have revealed some interesting patterns. In the alpine grasshopper *Podisma pedestris* for example, two chromosomal races meet along a mountain ridge and form a long narrow cline (usually ≈ 800m wide) which, for the most part, follows local regions of low population density or physical barriers. Apparent anomalies in zone position can be accounted for by local density (Nichols and Hewitt, 1986; see Chapter 2). In contrast, the hybridizing crickets *Gryllus firmus* and *G. pennsylvanicus* are distributed in a mosaic across the eastern U.S. in direct response to soil type (Harrison, 1989).

In the next sections I will discuss the major models of speciation and the controversies that have dominated this area of research over the last two decades. I will discuss the important contribution made by studies on natural hybrid zones and their interaction with theoretical population genetics.
1.3 Speciation

Because clustering of organisms into similar phenotypes can arise in several ways (see Maynard Smith, 1989, 1990; Templeton, 1989), a single all-inclusive definition of species does not (and perhaps cannot) exist. However, perhaps the most widely accepted definition of species is the “biological species” definition suggested by Dobzhansky (1937) and Mayr (1942) in which species are defined as “groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups” (Mayr, 1942). The definition applies only to sexually reproducing organisms (for other definitions, see Templeton, 1989). There may be practical difficulties in defining species based on Mayr’s definition, and we do need to be aware that morphological and ecological species do not necessarily correspond to biological species. Nonetheless, Mayr’s definition provides an unambiguous - and therefore useful - basis for investigating the mechanisms responsible for speciation in sexually reproducing organisms.

But is the key process in evolution of new phenotypes and adaptations among sexual organisms reproductive isolation? Although by definition biological species cannot exchange genes under natural conditions, it is not at all clear that inhibition of gene flow is necessary to maintain distinct types. Hybrid zones suggest not, since distinct types are maintained despite gene flow.

Mayr (1963) proposed a number of mechanistically different modes of speciation. The multiplication of species by what he called “true speciation” might occur either instantaneously (by polyploidy for example) or through gradual divergence of populations. He divided gradual speciation into three geographic modes: sympatric, semigeographic (clinal divergence) and geographic. This outline was used to guide the study of speciation for nearly a decade. Mayr was struck by the phenotypic uniformity across the main range of a species, relative to the divergence found in peripheral isolates. For this reason he favoured the peripatric model, where gene flow and genetic homeostasis confer “genetic cohesion” on large central populations. Populations at the edges of a species distribution occasionally become isolated. If sufficiently small, these populations may experience considerable genetic drift, and a reduction in heterozygosity. Mayr argues that this loss of variation will displace a population from its adaptive peak (Wright, 1931). Selection may drive a population towards a new adaptive peak, but in doing so will break up many “coadapted complexes”. He called this process a genetic revolution.
Mayr (1963) forcefully opposed the idea that speciation could occur in a sexually reproducing species without effective geographic isolation (sympatric speciation) and challenged biologists to devise realistic models of such a process. Maynard Smith (1966) took up the challenge. He considered genetic changes at two loci and modelled a heterogeneous environment divided into two niches. He showed that a stable polymorphism can exist between two alleles each conferring a selective advantage in one of the niches (disruptive selection), even if adults form a single random mating population. The conditions were that population size be regulated separately in the two niches, and that the selective advantages be large.

How likely is such a polymorphism? Can a single gene difference produce selective coefficients large enough to satisfy the necessary conditions? The conditions for sympatric speciation demanded by Maynard Smith's model are quite severe (e.g. see later work by Maynard Smith 1970; Maynard Smith and Hoekstra 1980; Hoekstra et al 1985) and the assumption of simple genetic control of niche separation is probably not met by many species. The model has been of great value, however, in that it clarified the components of speciation and provided stimulus and a theoretical framework for the development of subsequent models. Wilson and Turelli (1986) point out that a new niche will be underexploited, and so gives high fitness to new rare forms - thus making underdominant polymorphisms more plausible.

Since Maynard Smith's model, the equations of population genetics theory have been used increasingly both to devise new models and to evaluate existing models of speciation. Advances in molecular biology - in particular the advent of electrophoretic analysis - and the flourishing of experimental population genetics in the late 1960's have also been very important to ideas on the modes and mechanisms of speciation. But what have they told us?

1.3.1 Mechanisms of speciation

Studies of speciation are inherently difficult: because of the long time scale over which speciation occurs it is clearly not possible to witness the processes that might cause speciation. There is also a major difficulty in explaining how changes that might lead to reproductive isolation are established in the first place. There are two related issues here: first, genotypes that are partially reproductively isolated from the rest of their species would be at a disadvantage, leave fewer offspring and so would be eliminated; and the second issue is that gene flow might swamp changes that have evolved in limited regions. Evolutionary biologists have proposed
several possible mechanisms of speciation. I will consider the first issue above and will discuss ways in which reproductive isolation might evolve, overcoming selection against rare types. I will then consider the second issue, the effects of gene flow and geography. In a recent survey, Barton (1988) classified the mechanisms of speciation into four different groups: random drift; changes in selection; "quasi-neutral models"; and the accumulation of incompatible mutations.

Drift

The main argument given in support of drift as a mechanism comes from the observed diversity found between small isolates (Mayr, 1942), though the pattern is supported only by anecdotal evidence. Chromosomal evolution is often quoted as an example of this mechanism (Wright, 1941): since a chromosomal rearrangement may cause meiotic non-disjunction in heterozygotes it is hard to see how else such rearrangements could become established. However, there has been much argument over the importance of drift to speciation (Provine, 1986). An important example is Barton and Charlesworth (1984) criticism of Carson’s (1968) "founder flush" a model similar to the "genetic revolution" proposed by Mayr (1963). Carson set out to explain speciation in Hawaiian Drosophila. He proposed that new populations are founded by a very small number individuals or even one fertilized female. On this interpretation, genetic drift associated with the founding event changes the genetic composition of the new population, and causes reproductive isolation from the progenitors. Several models have been based on such founder events, differing only in the exact mechanism by which the rapid genetic change occurs (e.g. see Mayr, 1963; Carson, 1968; Templeton, 1980). Barton and Charlesworth (1984) have evaluated the likelihood of speciation by calculating the probability of peak shifts across a Wrightian fitness surface. Their model indicated that the probability of speciation occurring in a short time in small founder populations was very low. Reproductive isolation is more likely to arise by the gradual accumulation of many small selective barriers than by the erection of one large barrier during a founder event (Walsh, 1982).

The importance of non-disjunction in the evolution of chromosomal rearrangements is also not clear. In some cases, the meiosis in heterozygotes for such rearrangements has been more regular than would be expected (Capanna et al, 1977; Barton, 1980a; Shaw et al 1985). Non-disjunction may also be less important relative to other effects of the chromosomal differences on (for example) spermato-
genesis, DNA content, meiotic drive, and linkage relations (e.g. Charlesworth and Charlesworth, 1980; Moran, 1981; Westerman et al, 1987). These arguments suggest no clear evidence for the importance of drift and founder events as causes of speciation. However comparisons across large numbers of species have found a striking correlation between the rate of speciation and the rate of chromosomal evolution (Wilson et al, 1975; Bush et al, 1977). White (1978a) argued that chromosome rearrangements cause speciation. However most of these rearrangements cause little reproductive isolation. The correlation is most easily explained by genetic drift, which can establish underdominant rearrangements in small populations (Wright, 1941; Lande, 1979) and can also promote speciation.

Changes in selection

A change in selective conditions could result in the evolution of new genotypes or morphs. An example of this occurs in the snail *Partula suturalis*. There are two races which differ in the direction of shell coiling and which meet in a narrow zone of polymorphism (approximately 1km). Here mechanical incompatibility results in assortative fertilization and reduced fitness of crossed matings and leads to partial reproductive isolation (Murray and Clark, 1980; Johnson, 1982). It is hard to see how one form could have arisen from the other, because the rare form would be at a disadvantage. However, another form, sinistral *Partula mooreana* parallels the range of the dextral *P. suturalis* morph and mates with it, but produces inviable offspring. When more than a third of *P. suturalis* matings are with *P. mooreana* the dextral *P. suturalis* morph is at an advantage even when rare (Barton, 1988): the disadvantage of partial incompatibility with its own species is outweighed by the advantage of partial isolation from matings with *P. mooreana* (Johnson, 1982; Johnson et al, 1990).

Another example is suggested by Turner (1971). Here, shifts away from a warning pattern in Mullerian mimics are usually selected against - because predators will not recognize the new morph as being distasteful. However a change in predator abundance or in the mimic’s distastefulness could allow the evolution of new morphs, provided that the mimic is sufficiently abundant.

Quasi-neutral models

Barton (1988) has distinguished two classes of 'quasi-neutral' models. Stabilising selection could produce an optimal phenotype by a variety of different gene combina-
tions, such that although transitions between neighbouring combinations will only be opposed by weak selection, substantial incompatibility may be revealed when different combinations recombine - an example of this is provided by ring species such as are found in *Partula* (Murray and Clarke, 1980). In the second class, a similar phenomenon could result from sexual selection. Here if female preference and male character coevolve by Fishers "runaway process", populations could drift to any one of several possible equilibria, and substantial pre-mating isolation could be produced between populations which happen to reach different equilibria (Lande, 1981). However, Barton and Charlesworth (1984) have argued that if there is some gene exchange and the ancestral form has not been lost, the 'missing links' through which the populations evolved will eventually be restored, leading to a breakdown of isolation.

**Accumulation of incompatible mutations**

The classic view of allopatric speciation was that substantial isolation could result if the populations evolved independently and so accumulated different, incompatible alleles (Wright, 1940; Muller, 1942). The chromosomal races of the shrew *Sorex araneus*, for example, evolved from an ancestral acrocentric karyotype and have accumulated different metacentric combinations of chromosome arms by centric fusions (Searle, 1986). In crosses between metacentric combinations there are several cytotic problems and substantial sterility. However in the hybrid zones the ancestral acrocentric chromosomes are common reducing the level of non-disjunction (Hatfield et al, in press). Such breakdown of isolation might be expected in simple cases, but becomes increasingly unlikely as more intermediate genotypes must be recovered.

**1.3.2 The geography of speciation**

Speciation is inseparable from a geographic perspective. Many of the events and forces involved in speciation are influenced by the degree to which movement between populations is interrupted (by physical barriers for example). Thus there may be considerable differences in the mechanisms comprising each mode of speciation.

The three main modes currently recognised by biologists are *allopatric*, *parapatric* and *sympatric*. (for more detailed classifications see White, 1978a). The allopatric mode is generally agreed to be the principal one in most plants and animals (but see Tauber and Tauber, 1989). In this model, an ancestral species becomes subdivided by a physical barrier that prevents gene flow: the subdivided populations then di-
In contrast, sympatric speciation involves the evolution of new species entirely within the geographical range of the parental form. Disruptive, frequency-dependent or density-dependent natural selection are usually invoked here, to maintain polymorphism between partly incompatible genotypes.

In the parapatric mode of speciation, divergence occurs in populations that are spatially separated but share a common border. Here, a small proportion of individuals in each population encounters the other - as would occur in hybrid zones. Smooth or stepped clinal variation in morphological, behavioural, physiological and life-history traits are common in geographically widespread species. The steepness and structure of the clines will influence the flow of genes. Differences in selection pressures along the clines and restrictions on gene flow (caused, for example, by linkage) will contribute to parapatric diversification (Endler, 1977, Barton and Hewitt, 1981 - and for a review of the theory, see chapter 5). Reproductive isolation could evolve as an indirect result of genetic diversification through natural or sexual selection; or as a direct result of natural selection for premating reproductive isolating mechanisms (Dobzhansky, 1940). Its evolution could be accelerated by strong disruptive selection in adjacent areas, reduction in gene flow along the cline, strong natural or sexual selection on premating traits in the zones of overlap (but see Butlin, 1989), interaction among genes (Endler, 1977; Barton and Hewitt, 1981; Lande, 1982; Slatkin, 1982).

In a recent review, Barton and Coyne (1988) have expressed disappointment in the slow progress made in understanding speciation since the classic reviews of Dobzhansky (1937) and Mayr (1942). They suggest that too much effort has been spent on investigating controversial modes of speciation such as sympatric, chromosomal or genetic drift (founder effect) which, they say, are hard to distinguish from "better established alternatives".

We may still not understand speciation. However, there have been some important theoretical, conceptual and experimental advances which have brought us closer to understanding the conditions that might promote divergence, the processes that might bring it about, and the number of genetic differences that separate two species. In this section I will review some of these. In particular, I will focus on the important contributions made by studies of hybrid zones. In addition to providing useful systems in which to investigate the interplay of evolutionary and ecological forces, they have inspired some interesting theoretical investigations.
1.3.3 Species as alternative stable equilibria and Wright’s shifting balance

An important conceptual advance which has been used to provide new and valuable insights is that a species can be seen as being at a distinct equilibrium, stable to new mutants and to the introgression of foreign genes (see Carson, 1985; Rouhani and Barton, 1987a; Barton, 1988; 1989). The speciation process then involves the movement of a population from one stable genetic equilibrium to another (see Provine, 1986). This is analogous to Wright’s (1931; 1980) “shifting balance” theory, where transitions between “adaptive peaks” are involved in adaptation within a species.

Theoretical investigations aimed at understanding population divergence have recently been based on an 'adaptive topography’ or 'fitness surface’ such as that envisaged by Wright. For example, Barton and Charlesworth’s (1984) criticism of Carson and Templeton’s (1984) "founder flush" model was based on such a model (see above). Rouhani and Barton (1987a,b); Barton and Rouhani (1987, 1991) have also used this approach to investigate the probability of shifts between alternative peaks and the effect of tension zones on the establishment and spread of coadapted gene combinations. Their investigations have provided some interesting results, which will be discussed below.

1.3.4 The polygenic nature of species differences

How many genes are involved in reproductive isolation? Reproductive isolation can result from simple genetic differences, for example hybrid parthenogenesis (in which hybridisation between two sexual species produces a parthenogenetic species) and infectious incompatibility, which causes some degree of isolation in Drosophila simulans (Turelli and Hoffmann, 1991). Another example occurs when D. melanogaster females are inseminated by D. simulans only sterile female offspring are produced, but if the male D. simulans has the mutation “Lethal hybrid rescue” (Lhr), sterile male offspring are produced as well (Hutter et al, 1990).

However, evolutionary biologists have recognised for some time that subspecies and congeneric species usually differ by many genes with small phenotypic effects. This was revealed in hybridisation experiments (Dobzhansky, 1951; Mayr, 1963; Wright, 1978) and by the polygenic nature of quantitative variation in natural populations (Wright, 1968). Which genes and how many genes are responsible for differences between species? Population genetics theory, in combination with ex-
Experimental and molecular methods have provided some interesting insights. For example, Lande (1981) (extending earlier work by Castle (1921) and Wright (1968)) compared the variance of F1 and F2 progeny. He showed that the number of genes, \( n \), will be \( \frac{(\Delta \text{means})^2}{8(\text{Var}(F_2) - \text{Var}(F_1))} \) (though this will give an underestimate of the number of genes, since genes segregate in blocks (see Lande, 1981)).

A more powerful technique is to use molecular methods to actually map the genes responsible for species differences. For example, Zouros (1981) crossed *Drosophila mojavensis* and *Drosophila arizonensis*, since there are no recombinants in male *Drosophila*, chromosomes from F1 males remained intact. He then backcrossed F1 males with *Drosophila mojavensis* females and examined autosomes 2, 3, 4 and 5 and the insemination rate of females by *Drosophila arizonensis* males. *Drosophila arizonensis* chromosomal types inseminated poorly in comparison with *Drosophila mojavensis*; furthermore, the insemination rate increased with similarity to *Drosophila arizonensis* which suggested that the genes responsible for insemination rate were present on all four chromosomes. In principal, one could use markers to pin down the exact position of the genes responsible. For example, Coyne and Orr’s (1989) investigated hybrid sterility and inviability in which hybridization between a pair of species results in one fertile and one sterile sex. A species carrying recessive mutations on all of its chromosomes is crossed to a species with no genetic markers. Homogametic F1 hybrids are then backcrossed to the marked parental species. The cross produces a number of genotypic classes identified by the marker alleles, and the fertility and viability of these classes indicate which chromosomes or chromosome arms carry the genes causing postzygotic isolation. The advent of DNA markers will allow more species and more genomic segments to be analysed in this way.

Another powerful method for estimating the number of genes contributing to differences between incipient species is provided by population genetics analysis of hybrid zones (e.g. Szymura and Barton, 1991). Hybrid zones have given a clear indication that even subspecific differences are usually polygenic. For example, from 21 detailed surveys on hybrid zones, an average of 20% of enzyme loci are fixed for different alleles or show substantial frequency differences (Barton and Hewitt, 1985). These differences might not all contribute to reproductive isolation, but the number that do has been estimated to be large (for example, in the grasshopper, *Podisma pedestris*, Barton and Hewitt (1981c) have estimated that it will be \( \approx 150 \), and the number of genes responsible for reproductive isolation between *Bombina bombina* and *Bombina variegata* is \( \approx 55 \) (Szymura and Barton, 1991; see Chapter 5).
1.3.5 Populations are not homogeneous

Almost all species are subdivided, in that not all pairs of individuals are equally likely to mate. Such a population need not be strictly divided into distinct local demes, but may be spread out over a continuous range. Density and dispersal may vary from place to place. This makes the distinction between peripatric and parapatric divergence arbitrary (Barton, 1988). This is an important conceptual advance, because traditionally theoretical models of speciation have been based on the assumption that populations are subdivided into discrete demes - and even though this was largely for mathematical convenience, it reinforced the belief that strict isolation was necessary for speciation (Mayr, 1942; Carson and Templeton, 1984; Key, 1982; Futuyma and Mayer, 1980). Even in Lande's model (Lande, 1979) neighbouring demes could only exchange $\approx 1$ individual per generation.

1.3.6 Tension zones and the "shifting balance"

One can think of hybrid zones as steep clines which have formed between races fixed at different equilibria. Such clines can form when the organism is distributed in demes (Karlin and McGregor, 1972) or when it is distributed continuously (Bazykin, 1969; Rouhani and Barton, 1987a). As discussed above, if the cline is maintained by internal genetic factors it is called a "tension zone". Barton and Hewitt (1989) have pointed out that since tension zones connect groups of populations at different stable genetic equilibria, or roughly "adaptive peaks", they form an important component of Wright's "shifting balance". In nature more or less continuous tension zones are common (Barton and Hewitt 1985, Hewitt 1988); the shifting balance under these conditions has been modelled by Rouhani and Barton (1987a).

In their investigation, Rouhani and Barton show that stochastic divergence can occur in an essentially continuous population (i.e. in parapatry). A local group of demes (if the population is distributed in demes) or neighbourhoods (if it is continuously distributed) are linked by migration and drift to new gene frequencies. Then, when a critical unstable equilibrium has been passed, natural selection pushes the local demes or neighbourhoods to a new equilibrium. Once a critical number of demes (or neighbourhoods) have reached the new equilibrium (or adaptive peak), it will be 'protected' from the old form by a set of clines making up a stable tension zone. The tension zone can move out to spread the new equilibrium, or collapse inwards in which case the new form is swamped. Whether it will spread out or not
depends on its fitness, as well as on variation in population density and dispersal (Barton 1979c; Rouhani and Barton 1987a). In order to have any chance of establishment, the new form must be at a selective advantage, so that it can overcome swamping by surrounding populations.

In most models which have invoked sampling drift as the mechanism, divergence has originated either in single small isolates (Mayr, 1942; Carson and Templeton, 1984) or in a network of demes connected by very low migration rates (White, 1968; Lande, 1979). However, Rouhani and Barton (1987b) have shown that strongly selected incompatibilities can become established relatively easily in regions where neighbourhood size is sufficiently low; and their calculations show that rapid cline movement will preserve and spread the new equilibrium (adaptive peak) through an essentially continuous habitat (in previous models the new equilibrium was spread either through expansion of the single founding population or by movement of secondary hybrid zones (White, 1978b; Key, 1968) or by extinction and recolonisation of discrete demes (Lande, 1979, 1985).

Rouhani and Barton also point out that selection across continuous tension zones (as well as interdemic selection) makes Wright's "shifting balance" more likely than might previously have been thought. The spread of higher adaptive peaks is analogous to the way individual selection allows the spread of rare advantageous mutations within a population (Wright, 1980).

1.4 Overview

In the above discussions, we have seen how studies of hybrid zones can contribute to our understanding of species formation. We have seen that local environment and variations in population structure such as population density and dispersal rate can perturb tension zones and are therefore important factors in an investigation of hybrid zones as they relate to diverging species. Whether or not a tension zone can move from where it first formed and the relative importance of population structure and differential adaptation in determining their position will be important. Studies on the position, maintenance and movement of tension zones in combination with the conflict between gene flow and selection towards alternative stable equilibria gives valuable information on both the nature and effects of differences between incipient species and the role of coadaptation in the spread of new gene combinations (Barton and Hewitt, 1989).
In hybrid zones natural selection tests the fitness of hundreds of crosses between coadapted populations at different equilibria each generation. The results of these selective tests can be measured by studying the populations within these zones. Thus hybrid zones provide useful systems in which to study fitness relations in nature. The relative fitness of genotypes will be of fundamental importance to the dynamics of the hybrid zone itself, for example, in determining whether it is a tension zone or not - and if it is a tension zone, an understanding of the fitness relations will be essential if we are to make any predictions as to whether it is likely to move out to spread to a new equilibrium (Barton, 1979; Rouhani and Barton, 1987a,b).

Since both ecological factors and a reduction in the fitness of hybrids can maintain a tension zone, we need detailed field investigations into the habitat through which the zone runs as well as field measurements of fitness to assess their relative importance. The relative fitness of pure and mixed populations could be estimated directly under laboratory conditions, where crosses could be set up and the survival of F2 progeny compared. However there are the usual problems of rearing in captivity and extrapolation to the field. Thus, while these measures are useful in demonstrating viability differences between genotypes, they cannot give quantitative measures of fitness in the field.

Apart from the valuable insights which field fitness measures would provide to understanding the nature of hybrid zones, such measures are of general importance to both ecology and evolution. Absolute fitness is of fundamental importance in regulating species abundance, and it is the relative fitnesses of different genotypes that respond to natural selection within populations. Similarly, while field measures of density are essential to an interpretation of the pattern of gene frequencies, population density is also of more general interest to studies in both ecology and evolution. For example, the extent to which populations are regulated by density-dependent factors is an area of much current debate (see Chapter 4). Furthermore, the effect of gene flow can only be assessed if we know the density of the population into which the genes are flowing. This has important implications in assessing the factors which limit a species range, for example.

In this thesis the hybrid zone between the two chromosomal races of Podisma pedestris will be used to investigate such factors and hopefully to gain both general insights as well as a better understanding of the factors responsible for the position and maintenance of the hybrid zone. I will also review the theoretical relations derived by Barton (1983), which allow one to infer parameters such as the
selection pressures and number of genes involved from the observed pattern of gene frequencies. I will describe an indirect way of measuring fitness in the field and will describe simulation experiments used to test the theoretical predictions when some of the assumptions are violated, as they must be in nature (chapters 5 and 6).

The hybrid zone between the two chromosomal races of the alpine grasshopper, *Podisma pedestris* provides an excellent system for field investigations into many of the questions discussed above. The zone has been investigated extensively, and detailed surveys of its position and population structure have yielded some interesting patterns. In the next chapter I will introduce *Podisma pedestris* and will describe the hybrid zone between the two races. The detailed studies by Hewitt (1975, 1985), Hewitt and Barton (1980), Barton and Hewitt (1981a,c, 1982), Barton (1979a), Nichols (1984), Nichols and Hewitt (1986, 1988) have provided good background for the investigations described in this thesis. I will review Barton’s investigation in Section 2.3.1 and Nichols’ investigation in Section 2.3.2. The work described in the chapters to follow were to a large extent, motivated by their results.
Chapter 2

The hybrid zone between two races of the alpine grasshopper

*Podisma pedestris*

2.1 *Podisma pedestris*

*Podisma pedestris* is a flightless grasshopper distributed throughout Siberia, the Scandinavian peninsula and the Alps, Appenines and Pyrenees. In the Southern Alps, *Podisma* is found mostly between altitudes of 1500 and 2500 metres; below $\approx 1500\text{m}$ summer temperatures are too high and above $\approx 2500\text{m}$ vegetation is too sparse. *Podisma* is found consistently and abundantly in areas containing a mixture of alpine herbs; in particular, it seems to prefer sites with substantial cover of whortleberry (*Vaccinium* spp.) and *Rhododendron* shrubs, which provide shelter and which presumably cover sites suitable for laying pods (Marty 1969; Dreux, 1962; Nichols, 1984; pers. obs.).

*Podisma* overwinters as eggs, laid in soil. The eggs hatch in June or July and the nymphs go through four or five instars before reaching adulthood. The nymphs are a cryptic mottled brown-black (see Figure 2.1), though slight variations in colour are common. The adults are more brightly coloured, particularly the males, which have bright red markings on their tibia (see Fig 2.2). The adults are wingless, and in the field, survive until August/September. *Podisma* have a dispersal rate of about 20m per generation. This estimate is based on several field investigations which have used both genetic and physical marks for individuals (Barton and Hewitt 1982, Nichols 1984, Currie, 1992, Mason, 1988). It is not known what factors make the grasshoppers move. Dispersal could depend on habitat suitability and density, for example, and stage of development could be important. There is some evidence that dispersal rates are higher through unsuitable habitat (see Nichols, 1984), and
that adults disperse more than nymphs (Currie, 1992; but see Mason, 1988).

Over most of its range, *Podisma* has the standard acridid karyotype of 22 acrocentric autosomes and an XO/XX sex determining system. However in the Maritime Alps, a neo XY sex chromosome system (Figure 2.3) replaces the standard karyotype and the two races (referred to as XY and XO) meet in a hybrid zone which follows the central ridge of the Alpes Maritimes (see Fig 2.4). The XY race was probably formed by a centric fusion between the X chromosome and a large autosome (a Robertsonian translocation) (John and Hewitt 1970). The fusion segregates as an X-linked Mendelian allele. In most places the cline separating the two races is approximately 800 metres wide. Where the cline coincides with a physical barrier, or regions of inhospitable habitat (and consequently low population density) the width is reduced sometimes to only a few metres (Nichols and Hewitt, 1986; Currie, 1992; see Chapters 6 and 7).

The historical evidence for the origin and spread of the XY race and the present distribution of the two races has been discussed in some detail by Nichols and Hewitt (1986). Reconstruction of *Podisma*’s past distribution is fundamental to
Figure 2.2: Adult male *Podisma pedestris* on *Vaccinium* (photograph by R.A. Nichols).
Figure 2.3: (a) First meiotic metaphase in an “XY” male. This photograph shows 8 pairs of autosomes and the fused chromosome (top left). (b) First meiotic metaphase in an “XO” male, 7 pairs of autosomes are visible; the unfused chromosome can be seen on the bottom left of the photograph.
Figure 2.4: The hybrid zone separating the two chromosomal races of *Podisma pedestris* near the main ridge of the Alpes Maritimes. Triangles represent peaks. Open circles, samples of males fixed for the ancestral "XO" karyotype; filled circles, "XY" samples of about 20 males fixed for the Robertsonian fusion between the X and an autosome. The solid line shows the 2500m contour (except in (a) where it shows the 1900m contour). a-f Detailed transects: (a) Seyne, (b) Lac d'Allos, (c) Col de la Lombarde, (d) Col de Fenestre, (e) Vallée des Merveilles (lower right) and Lac Autier (upper left), (f) Vallon de Fontanalbe (near Tende). All insets are to the same scale.
any interpretation of the nature of the hybrid zone (see Barton and Hewitt, 1985, Nichols and Hewitt, 1986). *Podisma* probably established in central Europe in the late tertiary and extended southwards to the Alps with the palearctic ice ages. *Podisma*’s range would have been fragmented and rejoined as the climate warmed and cooled in regular fluctuations and the interglacial and glacial periods. The chromosomal fusion probably became fixed in a small population isolated during these changes, somewhere to the south west of the Alpes-Maritimes, and spread as the population expanded with further climatic change (Nichols and Hewitt, 1986). As the ice retreated, the ridge of the Alpes Maritimes would have been the last strip to be recolonised. Here, the two races met forming a hybrid zone. The zone is likely to have formed several times, most recently after the last ice age; since then the zone has not moved far.

The present day geographic pattern of frequencies of the two karyotypes can contribute to our understanding of the original spread of the two races and the possible extent of genetic differences other than the chromosomal fusion between them. If this leads to reduced hybrid fitness and a "tension zone", it may give a clue as to the likely extent and direction of any movement of the tension zone. Bazykin (1969) and Barton (1979a,b,c, 1983) have developed a substantial theory of the dynamics of tension zones. The theory of clines and of tension zones will be discussed more fully in chapter 5 where we will see how the width and shape of a cline can be used to infer parameters such as the number of genes involved and the selection pressure maintaining the cline. In the last chapter I discussed factors which will influence the position and movement of tension zones; for example, theoretical arguments have shown that variation in density and dispersal can greatly distort the pattern of gene frequencies (Barton, 1979c). Clearly a knowledge of population structure is necessary to interpret the patterns of gene frequencies.

Detailed field investigations are essential to obtain accurate estimates of cline position and width as well as estimates of the rate of dispersal and local population densities and information on local habitat. These parameters can be used to determine whether the cline is at an ecotone; whether it is likely to be a wave of advance, or (if it is a tension zone), the relative importance of reduced hybrid fitness, local environmental features and population structure, in determining its position.

The results of two extensive field surveys - one by Barton (1979a), (and see Barton and Hewitt, 1981a) at the eastern end of the zone, near Tende and the other by Nichols (1984) (and see Nichols and Hewitt, 1986, 1988) at the far western end,
near Seyne les Alpes - have formed the motivation and foundation of much of the work in this thesis.

Barton's analysis of the population structure and shape of the cline near Tende, in the light of theoretical arguments suggests that the zone is maintained by selection against incompatible genotypes. This view is supported by laboratory investigations. However, some paradoxes remain (see below). In an attempt to resolve these and to determine whether similar properties were revealed elsewhere along the zone, Nichols investigated a section at the far western end of the zone. The area near Seyne was chosen because it is most distant from Tende and because it runs through an isolated block of mountains which has probably not been connected to the rest of the range for 8000 years (Nichols and Hewitt, 1986).

In this chapter I will describe the overall position of the zone (Section 2.2); review the results of the two detailed surveys at Tende and Seyne (Sections 2.3.1 and 2.3.2) and discuss the aims of my research in the light of these (Section 2.5). In Section 2.4 I will introduce the section of the cline at Col de la Lombarde where most of my research has taken place.

2.2 The position of the zone

Figure 2.4 shows the distribution of the two chromosomal races. Several investigators have contributed to the data used to map the position of the zone. John and Hewitt (1970) Hewitt and John (1972) and Hewitt (1975) established the approximate position of the zone. They collected scattered samples over a wide area and found four mixed populations. Since then, Halliday et al (1984), Barton and Hewitt (1981a), Nichols and Hewitt (1986), Jones (unpublished data) and Humpage (unpublished data) have mapped the zone in several areas.

Barton (1979a) surveyed a region near Col de Tende (region “a” in Figure 2.4) at the eastern end of the zone in detail. He collected over 3,000 grasshoppers, and so the position and width of the zone in this area are known accurately (Fig. 3.6). At the western end, near Seyne (region “b” in Fig. 2.4) a detailed survey by Nichols has also yielded an accurate map of the zone position and width (Fig. 3.4). The region at Col de la Lombarde (region “c” in Fig. 2.4) described in this thesis has been surveyed extensively over the last 4 years - see below.
2.3 The nature and maintenance of the hybrid zone

2.3.1 Barton’s investigation near Tende

Population structure and position and shape of the cline

Barton (1979a) surveyed a 3km long region near Tende. The karyotype data are shown in figure 3.6. He divided the area into four transects and fitted one-dimensional tanh curves by maximum likelihood to estimate cline width and position. He compared the fitted curve with the actual sample frequencies. Some populations differed substantially and significantly from expectation in all 4 areas.

The shape of the cline can only be interpreted if the rate of dispersal is known. Mark-release-recapture experiments were used to obtain an estimate of the dispersal rate, and it was found to be \( \approx 21 \text{m}. \text{gen}^{-1/2} \). This estimate has since been confirmed by several other investigations elsewhere along the zone (Nichols, 1984; Currie, 1992; Cox, 1989; and see Chapter 6).

Because the cline is wide, relative to the rate of dispersal, it must be maintained by weak selection (Barton and Hewitt, 1981c). The selective force needed to explain the consistently narrow width of the zone need not be strong. Barton (1979a) estimated it at \( \approx 0.55 \% \) (maximum likelihood limits 0.35% - 1.1%). One would expect some meiotic non-disjunction due to the Robertsonian translocation, which would make female heterozygotes sterile. However, Barton (1980a) found no detectable increase in nondisjunction related to the fusion (< 1%). He found no apparent change in ecology from one side of the zone to the other. He therefore concluded that it is unlikely that the maintenance and position of the zone relies on forms being suited to different habitats.

It is difficult to tell just how the selective force operates. For example, the width of a cline maintained by selection, \( s \) against heterozygotes will be approximately the same as the narrow cline which would form if one race had a selective advantage, \( s \) over the other. If one karyotype had a selective advantage of (\( \approx 0.55\% \)) over the other, this would be enough to maintain the cline: it would move forward at only 2m per generation (Barton, 1979a). Since the position of the cline has only been determined to within 40m at Tende, such a rate would only be detectable after 20 years. Furthermore, the small selective difference needed to maintain the cline would be impossible to detect in laboratory crosses, so that if no difference between the
pure races were detected, this would not necessarily mean that they are of equal fitness.

Barton (1979a) found that at Tende, the cline position is generally determined by natural barriers and it does tend to run through inhospitable low density areas; but in the short section where the zone is free to move, the XY race bulges forward - as one might expect if the XY karyotype had a selective advantage over the XO type.

Since variation in abundance will be important in determining the pattern of gene frequencies, Barton estimated densities over a wide area at Tende, and used these estimates to calculate the expected position of the cline. He concluded that the XY race bulges forward more than would be expected from the pattern of densities (see Chapter 7).

The pattern Barton observed in the field is equally consistent with selection against heterozygotes or with a selective advantage of one karyotype over the other. This uncertainty is important: Barton has shown that gene flow past a moving cline will be much faster than past a static cline. Laboratory investigations can be used to help elucidate the nature of the zone.

Laboratory investigations

Barton (1980a) and Barton and Hewitt (1981c) investigated the selection acting on hybrids in a series of laboratory experiments. They found that in crosses of pure XY populations with pure XO populations, and in intra-population crosses, a greater proportion of hybrid eggs failed to hatch successfully than non-hybrid eggs. Hybrid viability was reduced by 50%, indicating a very much greater incompatibility between the races than that required to maintain the chromosomal zone. Crosses from a mixed population near the centre of the zone showed no significant association between karyotype and performance. Barton and Hewitt (1981c) concluded that the reduced fitness in hybrids in the first experiment must result from genetic differences other than the chromosomal arrangement. They found no significant difference in viability between XO and XY races. However, in similar experiments, Nichols (1984) found that the offspring from pure XY populations were significantly more viable than those from XO populations.
Arguments for weak selection at many loci

The chromosomal cline is much wider than *Podisma*'s estimated dispersal range (800m vs 20m). This implies that it is maintained by weak selection (≈ 0.5% - Barton and Hewitt, 1981c). However, laboratory crosses show that both grasshoppers from the centre of the zone and F1 hybrids have reduced viability. The region over which viability is reduced (estimated from these crosses) is narrower than the chromosomal cline (350m rather than the 800m). This is still much wider than the dispersal range, and implies that the inviability results from weak selection against incompatibilities at many loci rather than from strong selection at a single locus (about 3.5% per locus; Barton, 1980a; Barton and Hewitt, 1981c). It shows also that other viability clines coincide with the chromosomal cline. The fitness of hybrids from the centre of the zone is lower than that of F1 heterozygotes; from this Barton and Hewitt (1981c) estimated that 87% of the inviability was due to the breakup of coadapted gene complexes, rather than selection against heterozygotes.

Differences other than the chromosomal fusion

The races are indistinguishable in terms of allozyme variation at 21 loci (Halliday et al, 1983, 1984; Currie, 1992). However the enzymes investigated may not be representative of the whole genome. Sites for HindI11 and PvuII in the intergenic spacer sequence of X-linked ribosomal DNA repeats do show racial divergence, closely associated with the fusion (Dallas et al 1988). The C-value (DNA content) of males with the unfused chromosome (the XO race) is 5% higher than that of the neo-XY race. A comparison of variation in DNA content across the zone found greater differences in the centre of the zone suggesting that this is not merely a pleiotropic effect of the fusion (Westermann et al, 1987). Barton et al. (1983) found a region of increased electrophoretic variation coinciding with the chromosomal cline, possibly due to intragenic recombination or elevated mutation rates. Barton (1979a) measured ten body characters reflecting gross body shape, and found a consistent difference in shape due to the chromosomal difference. The fusion is also associated with differential fertilization (Hewitt et al., 1987).

In summary, Barton's investigation demonstrated the importance of population structure and how detailed field investigations are necessary for an understanding of the nature and dynamics of a tension zone. In particular, the relationship between the position of the zone and patterns of density and local habitat were found to
be crucial. In Nichols' investigation at Seyne, the emphasis was on the ecological genetics of *Podisma*: he examined the relation between density and habitat type and how this affects the position of the zone, and he measured viability difference in the field.

### 2.3.2 Nichols investigation near Seyne

**Population structure and the shape of the cline**

To examine the relationship between the position of the zone and population density as well as density and vegetation type, Nichols (1984) mapped the pattern of gene frequencies and estimated densities in a 1560m by 780m grid, sampled every 60m. Density estimates were made by recording numbers seen in walks at 60m/minute. Habitat was classified according to altitude, aspect and "vegetation type". Altitude and aspect were read off IGN maps of the area, and vegetation type was determined from aerial photographs. Five types were distinguished: *Vaccinium*; patchy *Vaccinium*; broken ground; bare rock/scree and grass or crops. He used this relationship to predict *Podisma* density over the entire study area (Nichols and Hewitt, 1986).

Nichols (1984) observed that, as at Tende, over most of its length in this region, the cline runs along an area of low population density. However, in one area it unexpectedly runs through a very high density area. In an attempt to understand this, Nichols used a computer simulation to calculate the expected position of the cline. For the observed pattern of gene frequencies and estimated densities from the grid, he found that after 1000 generations the position of the zone had not moved substantially from its observed position. Furthermore, when he changed the observed densities to lower values in the simulation, the XY race pushed forwards. Thus the observed position of the zone in a high density area can be explained by details of the population structure: the position of the zone is determined by the effects of drift, via the absolute density, and not by the relative density alone. Nichols concluded that these results agree well with the expected position of a cline maintained by a balance between dispersal and selection against hybrids.

A consideration of the climatic history of the area along with the present position of the zone at Seyne suggests that the zone is not a wave of advance comes from Nichols and Hewitt (1986) argue that as the climate warmed after the last ice-age, the habitable area near Seyne would have become isolated from the rest of the range, because temperatures in the surrounding valleys would have increased to unbearable
levels. This would probably have happened about 8000 b.p. (Nichols and Hewitt, 1986). For a zone of the observed width to be a wave of advance, it would have to be moving at over 1km per 1000 years (Barton, 1979a) - at this rate, they argue, the fitter race would have driven the less fit race off the block of mountains near Seyne over 4000 years ago.

**Ecological survey and measures of viability in the field**

I have described how the relative fitnesses of pure and mixed races can be estimated from laboratory crosses. Such experiments are useful in demonstrating viability differences but they cannot be applied quantitatively to the field. Field measures are clearly highly desirable. Another way of measuring relative fitness is from population counts. Nichols followed the rate of development and density changes in the field, and investigated the relation between *Podisma* density and habitat type.

Nichols and Hewitt (1988) established 120 1m$^2$ quadrats each spaced 8m apart at Seyne. The quadrats were divided into four blocks: two hybrid, one pure XO and one pure XY. The sites were chosen to be in areas of similar altitude and aspect since these variables will effect *Podisma*'s distribution. Another variable of obvious importance to the distribution of these grasshoppers is the type of vegetation. *Podisma* will be directly affected by plants for food, shelter and suitable laying sites. The species present in an area are likely to be correlated with each other as well as with other environmental factors such as soil moisture and acidity, (e.g. Southwood, 1978; Gauch, 1982) and so the vegetation in sites can be classified into “types” which would be expected to have varying effects on *Podisma*’s density, rate of development and possibly dispersal rate.

Nichols identified some 62 species in the area and was able to classify the habitat into 11 groups, using TWINSPLAN (Hill, 1979; this method will be described in more detail in chapter 3). These groups included quadrats from both inside and outside the zone. Counts of grasshoppers were made throughout the season. To compare density and viability measures from quadrats within the zone with those on either side, he was able to take into account the differences in habitat type between the quadrats.

Nichols found populations from the hybrid zone to be retarded by over a week on the final day of scoring; the difference was highly significant even when the effect of habitat type was taken into account. He found nymph density inside the zone to be less than half that of the density in pure populations. Although adult
densities were slightly lower in the zone the difference in adult densities was not significant, suggesting some form of density-dependent population regulation. The retarded development and lower nymph density suggest reduced viability in the hybrid population - of the same order as that found in the laboratory (Nichols and Hewitt, 1988).

In this thesis I will present investigations aimed at exploring some of the implications from the studies near Tende and Seyne. Most of the investigations have been at a new site near Col de la Lombarde, ≈ 100Km east of Seyne.

2.4 The zone at Col de la Lombarde

Col de la Lombarde is situated at 2423m immediately on the Italian side of the French-Italian border in the Alps (see Fig. 2.5).

Some very important contributions to our knowledge of the zone in this area have been made by students at University College, London, in their final year projects. The position of the hybrid zone was mapped roughly by E. Brunet (1986). In 1987, D. Hewitt (1988) and T. Wickham (1988) mapped the position of the zone more extensively. Wickham collected many samples over a wide area, and Hewitt collected 42 samples each consisting of approximately 20 individuals over a finer scale. Several extra samples have been collected and karyotyped to fill in gaps and give more detail in specific areas (by N.H. Barton, A. Leibowitz, D. Currie, F. Cox, J.S. Jones, K. Jackson).

Fig. 2.5 shows the approximate distribution of karyotype frequencies in the area. Theodolite surveys of the region in 1988, 1989 and 1990 were used to construct a more accurate map of the area. Appendix A.1 lists frequencies of the fused chromosome on the coordinate system from this survey. Detailed karyotype maps corresponding to these data are provided in Chapter 3. In most areas the cline approximates to a tanh curve and is about 600m wide. The zone follows the stream to the valley floor, and then bulges out again on the other side of the valley. At the top, it seems likely that the extensive and inhospitable scree slopes and cliffs hold it in position. However, these areas were too steep for detailed investigation. The position of the zone will be addressed further in chapter 7, where computer simulations will be used to help elucidate this question.

In addition to mapping a large area at the site near Col de la Lombarde, the third year projects provided some very valuable insights for some of the investigations
Figure 2.5: Map of the study area at Col de la Lombarde (1:25000). The overlay shows karyotype frequencies. Filled circles: pure XY samples; open circles: pure XO samples.
described in this thesis. D. Hewitt explored the relationship between habitat type and *Podisma* density in 1987: his results are particularly useful for comparing ways of measuring densities in this area. The effect of the stream, a local barrier to gene flow, on the width and shape of the cline was explored by F. Cox (1989). Further data and analyses of this area will be described in Chapter 6.

### 2.5 Implications of the studies at Tende and Seyne

By investigating a new area, we can determine how general the patterns at Tende and Seyne are. In this thesis I am particularly interested in measuring the relative fitness of the different genotypes in the field.

#### 2.5.1 Field measures of fitness

Despite its importance to both ecology and evolution (see Chapter 1) fitness is hard to measure in natural populations. However, Nichols (1984) showed that hybrid populations contained fewer hatchlings and were retarded in development relative to pure populations living in the same vegetation type. This suggests reduced viability in the hybrid populations; an effect of the same order as that found in the laboratory (Nichols and Hewitt, 1988).

This result was exciting: field observations on relative fitness of populations spanning the tension zone could lead to a more accurate knowledge of hybrid zone dynamics such as the width and depth of the “hybrid sink” (this term refers to the effect of lower densities in the centre of the zone where individuals are less fit - see Barton, 1980b). This in turn could give an estimate of the selection on and number of loci involved in reproductive isolation (Barton and Hewitt, 1982; Barton, 1986), based on field measures rather than laboratory measures. Further, although F1 hybrids have reduced viability in the laboratory, one cannot be sure that the cline in inviability is really coincident with the chromosomal cline. In principle this could be resolved by field measures of fitness from populations spanning the zone. One of the aims of my research was therefore to investigate the relative fitness, under natural conditions, of populations spanning the tension zone in *Podisma pedestris*.

In chapter 3 I will describe measurements of fitness components which were made in the field at Nichols original site near Seyne, and at Col de la Lombarde.

The field measures of density at Col de la Lombarde contrast with those of Nichols and Hewitt (1986): the hybrids do not show a clear reduction in density,
which although interesting in itself means that we cannot estimate the width over which fitness components change across the zone, as originally hoped. In chapter 5 I will review a less direct way of measuring fitness components, which is based on Barton’s theory of multilocus clines (Barton, 1983). The quantitative results of his investigation have led to some very useful relations, which allow one to infer parameters such as selection pressures from cline shape. I will describe simulation experiments aimed at testing these relations when some of the assumptions are violated. The results of Cox’s (1989) investigation suggest an interesting way to measure fitness and the number of genes. Across either side of the valley at Col de la Lombarde, the cline is wide ($\approx 600m$). In between, however, chromosome frequencies change abruptly across a small stream: here the zone is only 1 metre wide. The pattern across this barrier can be combined with measures of dispersal to show that although selection on the chromosome fusion itself is weak, there is strong overall selection against hybrids; selection is acting on many genes, causing an additional barrier to gene flow between the divergent populations. In chapter 6 Cox’s investigation will be described in more detail, and experiments aimed at expanding her investigation will be presented. The results of this investigation raise an interesting question relating to the joint effects of a physical barrier and one resulting from linkage effects.

### 2.5.2 Density-dependent population regulation?

Nichols and Hewitt (1988) showed that at Seyne, although hybrid populations contained fewer hatchlings, the densities of adults were similar, suggesting some form of density regulation. How general is this pattern? Is it due to movement into low density areas from the outside or is it due to increased mortality in dense populations? These questions will be addressed in chapter 4. I will describe population counts through the season in quadrats, on two different scales. More direct tests in which densities of first and second instars are manipulated in field cages and by transplanting young nymphs between isolated patches of vegetation will also be described.

### 2.5.3 Parasite load

Parasite load is of relevance to several aspects of this thesis. Disease (in the broad sense - i.e. pathogens or parasites) are one of the most likely mechanisms of density-dependent regulation (see Hassel, 1978; Holmes 1982; May, 1982). Recent theory
has emphasised the ecological importance of parasites (Anderson and May, 1979), however most of the literature deals with induced infections and very little is known about how parasites affect natural populations. Resistance to pathogens seems to be genetically based in several insects (Anderson and May 1981; May, 1982). Hybrid zones provide useful systems for exploring ecological and evolutionary questions relating to parasite-host dynamics. For example: is there a negative relation between individual fitness and parasite load? Is there heritable variation in parasite resistance? Do preferred mates have fewer parasites? Parasite load will be investigated in chapter 3.

2.5.4 The maintenance, position and movement of the zone

The position of the hybrid zone, the shape of the cline, and how these relate to ecological differences and to population structure were explored by Barton (1979a) and Nichols (1984).

The evidence from Tende and Seyne strongly suggests that the zone is maintained by reduced hybrid fitness. In particular the reduction in early survival seen in the laboratory and the stability of the hybrid zone suggest this. The data presented in chapter 3 is therefore surprising: extensive surveys on the pattern of densities across two transects at Col de la Lombarde (600 different 2m² quadrats in all; and replicated across years) as well as across transects at three new sites (Col de Fenestre; Vallée des Merveilles and Jas du Plan Tendasque) will be described. These show, unexpectedly, that the XY race is consistently more numerous; and the XO quadrats have densities which are on average similar to those of hybrids.

Is the XY race pushing forward, as is suggested by the observed densities? At both Seyne and Tende the zone runs through low density areas over most of its length (as one would expect of a zone maintained by reduced hybrid fitness). However, over a short distance at Tende the XY race seemed to bulge out more than was expected; and at Seyne the zone ran through a high density area over part of its length. Both Barton and Nichols used simulations to test whether the observed karyotype distribution is consistent with the pattern of densities. Calculating the expected position of the cline is an ingenious way of investigating the nature of the zone. This topic will be explored and discussed in more detail in chapter 7. Data from Col de la Lombarde will be used to determine whether the observed karyotype distribution is consistent with the density in this region.

Nichols investigated the ecology of the area at Seyne in detail, and was able to
predict densities and thus the position of the zone from habitat type using aerial photographs. This possibility is exciting: if it works, we should be able to predict the position over a large area simply by inferring densities from habitat type. Vegetation has been mapped extensively across the zone at Col de la Lombarde and can be used to infer densities. This will be described in Chapter 7. Local densities have also been measured directly in the same area; we therefore have a comparison of the two methods.
Chapter 3
Measuring fitness in the field

3.1 Introduction

Darwin’s theory of evolution by natural selection is based on three premises. The first is that all species produce more offspring than can survive and reproduce. The second is that organisms vary in their ability to survive and reproduce and the third is that this variation is to some degree inherited. Certain genotypes are better adapted to their environment than others, and this is reflected in their relatively greater fitness (i.e. ability to survive and reproduce in that environment). Genotypes with greater fitness leave, on average, more offspring than do less fit genotypes and so favourable alleles that promote higher fitness will increase in frequency.

Measuring fitness in natural populations is difficult and as a consequence there has been much controversy over how tightly population density is regulated and over the heritability of fitness within populations. There are several stages during an organism's development at which selection could act. It could act by differential viabilities of genotypes at any stage during growth and development from zygote to adult (viability selection includes development rate and age of reproduction); or it could occur among mature adults by differential mating success (sexual selection). After mating, there could be differential production of gametes (e.g. meiotic drive) or differential success of gametes in accomplishing fertilization, (i.e. gametic selection), or different matings could produce different numbers of gametes, zygotes or offspring (i.e. fecundity selection).

Measuring fitness is difficult for several reasons. An allelic substitution could have effects at any of the stages discussed above, and the different fitness components will have different effects on the dynamics and equilibrium properties of allele frequencies. So the first problem is to isolate the components of fitness - and therefore to identify the relevant life stages for measuring them. Another problem is the
genetic complexity of fitness. The probability of surviving and the chance of successfully attracting mates are likely to involve genetically complex traits, and many genes, and so the question is whether the marginal effects of one or a few identified genes are important. Another difficulty is a statistical one. Selection coefficients as small as, say, a 1% difference in viability or fecundity can have a major effect on the fate of a gene. If sample sizes are inadequate such weak selection will not be detected.

There are several approaches to estimating the components of fitness. Broadly, these fall into two classes: laboratory manipulations, and field sampling of different age classes (Manly, 1985). In laboratory experiments, the relative fitnesses of genotypes can be measured directly, for example, by setting up crosses and comparing birth or hatch rate, offspring survival and F2 progeny. However, to obtain useful estimates, large numbers of crosses must be set up; and in addition, there are often difficulties in rearing in captivity, and always difficulties in extrapolation to the field. Thus although such measures are useful in demonstrating viability differences between genotypes, they cannot be applied quantitatively to the field.

Several methods have been used to attempt to detect natural selection in the field (for a review see Endler, 1985). One of the most direct methods for detecting natural selection is cohort analysis in which an attempt is made to obtain detailed information on the complete demography of the population with respect to the relevant traits or phenotypes. By gathering detailed data on individuals, information on components such as survival, fertility, fecundity, mating ability etc. can be obtained. However, very few attempts at life-time fitness measurements have been made. Examples are tits Parus major (McGregor et al, 1981) and red deer Cervus elaphus (Clutton-Brock et al, 1982) - though both are concerned with non-heritable variation. Other examples are summarised in Clutton-Brock (1983).

It is often impractical to keep track of individuals. In an alternative approach known as fitness component analysis, intensive sampling of as many age classes or life-history stages in a population as possible also allows one to separate the effects of natural selection into components. If the investigation includes data from parents and their offspring, then one can also obtain information on heritability. Good examples are found in the fish Zoarces (Christiansen, 1977, 1980; Christiansen et al., 1977) and barley Hordeum (Clegg et al, 1978).

Unfortunately, there are several practical problems to fitness component analysis: intensive sampling is very time consuming; it is often difficult to distinguish
between ecological and genetic effects; population response to un-detected natural perturbations and population cycles could complicate such investigations and the results could be misleading if the sampling scale is inappropriate. As with all methods, it is difficult to detect weak selection which might nonetheless have a profound effect on the fate of a gene.

### 3.1.1 Demonstrating natural selection in hybrid zones

The *Podisma* hybrid zone should provide a useful system in which to investigate fitness differences in nature. Several sources of evidence suggest that the zone is maintained by reduced hybrid fitness (see Chapter 2) and so components of fitness can be investigated under natural conditions in populations spanning the zone. Indeed, Nichols and Hewitt (1988) found that in natural populations of *Podisma pedestris*, hybrid populations contained fewer hatchlings and were retarded in development relative to pure populations living in the same vegetation type. This suggests reduced viability in hybrid populations, an effect of the same order as that found in the laboratory (Nichols and Hewitt, 1988; Chapter 2). Such field estimates of fitness across a hybrid zone are rare: apart from the investigation by Nichols and Hewitt, I am aware of only one other - that of Moore and Buchanan (1985) (and see Moore and Koenig, 1986) (and this was based on very small samples).

In Section 3.2, I will describe investigations into the relative fitness, under natural conditions, of populations from inside and outside the tension zone in *Podisma pedestris*. Viability (including rate of development) and fecundity components of fitness were investigated by sampling quadrats along transects spanning the tension zone several times within a generation and by sampling the same localities over three generations. Other components of selection such as sexual and gametic selection are not investigated here.

One of the greatest obstacles to investigating fitness in natural populations is the complexity of the environment in which they are found (including the presence of other species, parasites and predators) and its variation in both space and time. It is essential to distinguish between 'ecological' and 'genetic' effects on fitness. The choice of sites is therefore very important and an ecological investigation is necessary. Although there is no evidence for a consistent difference in environment across the *Podisma* hybrid zone, any area is likely to have some environmental gradients running in various directions. Thus an arbitrarily chosen transect across the zone is quite likely to show some significant correlation by chance alone (Clarke,
1975, 1978). It is therefore important to sample more than one locality.

It has often been suggested that parasites are important in maintaining genetic diversity and that they might play a role in reproductive isolation, race formation and speciation. To assess the plausibility of such theories, it is essential to have a better understanding of the genetics of host resistance and the interaction between host susceptibility and parasite virulence in natural populations. Sage et al (1986) found evidence to suggest that populations from a hybrid zone between *Mus domesticus* and *Mus musculus* have significantly more nematodes and cestodes than populations from the two parental races. One explanation is that hybrids have recombinant genotypes which fail to confer resistance (Sage et al, 1986). This study also suggests that hybrid zones might provide interesting systems in which to investigate the interaction between host susceptibility and parasite virulence, in nature. Although there is a great deal of literature on induced infections, relatively little is known about the genetics of host resistance in natural insect populations. In section 3.3 I will describe observations on parasite load across the hybrid zone in *Podisma pedestris*.

In addition to providing information on components of fitness, repetitive sampling within a generation gives detailed information on *Podisma*’s density distribution in space and time. This is of considerable interest to the nature, position and stability of the hybrid zone and should also provide insights into the spread and establishment of coadapted genomes. I will return to this in Chapters 7 and 8.

### 3.2 Population structure and measures of fitness in the field

#### 3.2.1 Introduction

Previous investigations

In their investigation at Seyne (see figure 2.4), Nichols and Hewitt (1988) (see Chapter 2) found that hybrid populations contained fewer hatchlings, and were more retarded in development, relative to pure populations for a given habitat. Densities of adults were similar, however, suggesting some sort of density-induced regulation (Nichols and Hewitt, 1988). They followed density and development in 120 $1m^2$ quadrats spaced 8m apart. The quadrats were divided into four blocks - two hybrid, one pure XO and one pure XY (see figure 3.5) and were matched as closely as possible for altitude and aspect (both of which could affect mortality and develop-
Habitat differences between quadrats were scored and the numbers and stage of development were recorded at intervals from 28 July to 14 August, 1983. Analysis of deviance was used to assess the extent to which differences in density and rate of development between populations resulted from differences in habitat type. Habitat type explained 35% of the variance in second instar nymph density between different quadrats. After accounting for habitat, density in the zone was significantly lower than in pure populations (at the 1% level). When the effects of habitat were not taken into account, the average density was 1.940 (s.e. 0.142) in the zone and 3.295 (s.e. 0.324) in pure populations (4.290 in the pure XY region and 2.30 in the pure XO region) (Nichols, 1984). Differences in density within each block suggested that there were significantly fewer young nymphs in the zone than would be expected from the adult density ($P < 0.05$). Nichols and Hewitt (1988) also found that peak nymph density has only a small effect on adult density, suggesting either density-induced mortality or migration (the regression of adult on peak nymph density can be described by: \[ \text{adult density} = \text{peak density} \times 0.103 \text{ (s.e. 0.015)} + 0.233 \text{ (s.e. 0.079)} \].

Could the differences observed by Nichols and Hewitt be due to random variation between the separate locations? The investigation into fecundity was based on a single year's observation, which could be misleading. Also, since the area over which the quadrats were chosen represents only a single transect, the trends may simply be due to chance.

Here, I will describe further investigations aimed at estimating relative viabilities and rates of development of the genotypes, as well as density trends, from population counts along transects spanning the tension zone.

The methods in this investigation are complicated. Several different transects have been scored, each necessarily in a slightly different range of habitats. Some of the transects were sampled at regular intervals throughout the season (five times) over three consecutive years, but time was limited and so others were sampled only once or twice over two consecutive years or over just one year. It will therefore be helpful to provide a rough description of the transects, methods and observed density trends, before discussing how sites were chosen, the methods of density and instar estimation and the results in more detail. Since it is important to distinguish between "ecological" and "genetic" effects on fitness (see Section 3.1.1) it was necessary to investigate habitat differences across the transects. In Section 3.2.2 I will discuss habitat surveys in detail and in Section 3.2.4 and 3.2.5 I will describe sta-
tistical analyses aimed at disentangling ecological differences in density from those due to intrinsic fitness differences. In Section 3.2.6 we will then be in a position to compare density patterns before and after accounting for ecological differences. Finally in Section 3.2.7 I will discuss what the density patterns tell us about the relative fitnesses of the races.

Further investigations: a summary

To find out whether Nichols' results were repeatable and to check our methods against his, a pilot investigation was carried out at his original site, near Seyne in 1987. A new site was surveyed in the same year at Col de la Lombarde to repeat the experiment in a different range of habitats. Both surveys consisted of counts of individuals in each of approximately 100 quadrats by two scorers (usually Dr. N.H. Barton and myself). At Seyne the transects were extended across the full width of the zone, with the aim of mapping the region of reduced fitness. 104 quadrats were laid out in five blocks spanning the cline (XO, ZO, Z, ZY, and XY in figure 3.5). The quadrats were \((2m)^2\) in area to increase the sample size (Nichols used \((1m)^2\) quadrats). The site at Col de la Lombarde had not been mapped before and a large survey of karyotype frequencies was performed (see figure 3.1; Appendix A.1). One hundred and five quadrats were established in habitats matched by eye as being suitable to *Podisma*. However, the zone had not yet been mapped thoroughly, and the quadrats did not quite span the chromosomal cline. The comparison in 1987 is therefore only between XY and mixed sites.

Density and stage of development (instar) were followed throughout the life-cycle. *Podisma* goes through five instars before reaching adulthood. These can be distinguished by size (there are no other obvious features distinguishing instars). This is a somewhat subjective measure and so, to ensure consistency in scoring, nymph sizes were compared against a standard from each instar, until the scorers' abilities to judge the stage of development became consistent.

In 1988, the investigation was concentrated at Col de la Lombarde. The transect established the previous year (*transect 1*) was re-scored, to investigate repeatability in time and to allow a parent-offspring density comparison. Extra quadrats were chosen to extend the transect so as to include the pure XO region (see Figure 3.2). Thus in 1988, *transect 1* comprised 120 quadrats, 40 of which had been scored in 1987 (20 from the XY region and 20 from the mixed region). Additional XY and mixed sites were chosen to include a broader region than that scored in 1987 (20 of
Figure 3.1: (a) Frequency of the chromosomal fusion near Col de la Lombarde. Distances are in metres. Transect 1 and Transect 2 used for the quadrats in 1987-1989 are indicated (for details see Figs. 3.2 and 3.3). The solid line shows the stream which the hybrid zone follows to the valley floor, and the river in the valley. The coordinate system shown here was constructed from a theodolite survey of the region. Pies show data from Appendix A.1. (b) Vallone d’Orgials. The overlay indicates Transect 1 and Transect 2, the river, the Col and the approximate position of the stream.
each) and 40 XO quadrats were chosen.

A new transect, transect 2 (Fig. 3.3), was established on the other side of the valley from transect 1 (Figure 3.1) in 1988, to check repeatability in space. This transect comprised 100 quadrats which, as for transect 1, were chosen by eye to be as similar in physical features and vegetation types as possible; this time vegetation types could be chosen on the basis of the results from a multivariate analysis on the vegetation from the previous year (see below).

In 1989 both transects at Col de la Lombarde were re-scored three times during the season to estimate hatchling density; peak nymph density and adult density. An extra three transects in geographically distinct areas: Col de Fenestre (see Fig. 3.8 and 3.9), Vallée des Merveilles (see Fig. 3.10 and 3.11), and Tende (see Fig. 3.6 and 3.7) (and see Fig. 2.4) were also established in 1989, to determine whether the density trends observed at Seyne and at Col de la Lombarde are general or whether they are simply due to local effects. These transects were scored only once, near peak density (peak nymph density occurs, on average, at mean instar ≈ 3.0; see below).

Table 3.1 summarises mean density and mean instar for the transect at Seyne, and compares trends with those found by Nichols (1984). Tables 3.1 to 3.4 summarise mean density and mean instar for the transects at Col de la Lombarde, Vallée des Merveilles, Tende and Col de Fenestre. Figure 3.12 shows density trends over three consecutive years for those quadrats for which we have the comparison (Transect 1, Col de la Lombarde). Note that in these tables it is the trends in density and instar across the transects (i.e. difference between genotypes in any given transect) that are of interest. Clearly, mean density will depend partly on when the transects were scored and on Podisma's stage of development at the time of scoring. Since transects were not all scored at the same times and since the stage of development varies from site to site even at a given time, it is not meaningful to make comparisons between transects. The point of sampling different transects was to determine whether the trends across the zone can be replicated. The methods used to obtain these estimates and the observed differences in mean instar and density across the transects will be discussed in detail below. Direct comparisons of densities within a transect should be made with caution because densities will depend on stage of development and on habitat. In Section 3.2.5 I will discuss possible sources of variation in density and will compare density trends before and after accounting for these. Similarly, trends in mean instar will be compared before and after accounting
Figure 3.2: Transect 1 at Col de la Lombarde. Numbers refer to quadrats. Dashed line, road; solid line, stream; shaded area, scree. The overlay shows karyotype frequencies. Filled circles, pure XY samples; open circles, pure XO samples. Quadrats 1-105 were scored in 1987. In 1988 the transect was extended.
Figure 3.3: *Transect 2* at Col de la Lombarde. Numbers refer to quadrats. Dashed line, path; shaded area, cliffs. *Lb 103-112* are border beacons between France and Italy. The overlay shows karyotype frequencies. Filled circles, pure XY samples; open circles, pure XO samples.
Figure 3.4: Map of the study area near Seyne (1:25000).
Figure 3.5: The Seyne sites. Detail from Figure 3.4 to show quadrat positions. The 1700m and 1900m contours are shown. The overlay shows the frequency of the chromosomal fusion. Filled circles: pure XY samples, open circles: pure XO samples (data from Nichols and Hewitt (1986), Halliday et al (1984)). Crosses show the location of the five blocks of quadrats sampled in 1987 (from left to right, these are denoted O, ZO, Z, ZY, Y). Squares show the locations of Nichols and Hewitt's (1988) sites, where these are not the same (Site O = Nichols and Hewitt’s O2; Site Z = H3 and H4; ZO, ZY and Y were not sampled by Nichols and Hewitt).
Figure 3.6: Map of the study area near Tende (1:25000). The overlay shows karyotype frequencies. Filled circles: pure XY samples; open circles: pure XO samples.
Figure 3.7: The sites near Tende. Detail from Figure 3.6 to show quadrat positions. The 2050m contour is shown. Quadrats were positioned along the transects as indicated. Numbers give the range of quadrats making up the transects.
Figure 3.8: Map of the study area near Col de Fenestre (IGN 1:25000). The overlay shows karyotype frequencies. Filled circles: pure XY samples; open circles: pure XO samples.
Figure 3.9: The sites at Col de Fenestre: detail from Figure 3.8 to show quadrat positions. Solid lines, paths; dashed line, stream; shaded areas, lakes. Quadrats were established in clusters, each represented by a square in this figure. Numbers give the range of quadrats making up the clusters.
Figure 3.10: Map of the study area around the Vallée des Merveilles (IGN 1:25000). The overlay shows karyotype frequencies. Filled circles: pure XY samples; open circles: pure XO samples.
Figure 3.11: The sites at Vallée des Merveilles: detail from Figure 3.10 to show quadrat positions. Solid lines, paths; dashed line, stream; shaded areas, lakes. Quadrats were established in clusters, each represented by a square in this figure. Numbers give the range of quadrats making up the clusters.
for other possible sources of variation. I will also investigate whether differences in mean instar indicate differences in rates of development between genotypes.

### 3.2.2 Choosing the quadrats

Although there is no obvious transition in habitat type across the zone, there will always be small scale differences. Ideally for density counts across the zone to be comparable, differences due to habitat type should be accounted for so that the resulting differences can be attributed to genotype. At Col de la Lombarde, quadrats were chosen to have similar ecological features, but otherwise were distributed randomly across the zone (usually no closer than 10 metres apart to minimise autocorrelation). A detailed survey of habitat in each quadrat along the Seyne transect and along transects 1 and 2 at Col de la Lombarde were used to determine any subtle differences objectively and to classify the range of habitats sampled. A 'point quadrat' method was used to score plant species abundances. This involved throwing a sharp knife at 18 random points in each quadrat and recording plant species found at its tip. A list of the species found in both areas, is provided in Appendix B.1. For a more general description of quadrats the percentage cover of the obvious features such as soil, rock, rhododendron, *Vaccinium* and grass were recorded. In 1988 the
<table>
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<tbody>
<tr>
<td></td>
<td>Nichols, 1984</td>
<td>Data from 1987</td>
</tr>
<tr>
<td>race</td>
<td>site</td>
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<tr>
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<tr>
<td></td>
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<tr>
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<tr>
<td></td>
<td>O2</td>
<td>2.31</td>
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</table>

Table 3.1: Variation in mean density and mean instar across the hybrid zone at Seyne. Figures have not been corrected for the effects of habitat. (site O2 = site O, site H3,H4 = site Z: for site locations see 3.5)

<table>
<thead>
<tr>
<th></th>
<th>Col de la Lombarde</th>
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<tr>
<td></td>
<td>1987 transect 1, 1988</td>
<td>transect 1, 1989</td>
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<tr>
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<td>mean density</td>
<td>mean instar</td>
</tr>
<tr>
<td>XY</td>
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<td>3.21</td>
</tr>
<tr>
<td>H</td>
<td>3.46</td>
<td>3.92</td>
</tr>
<tr>
<td>XO</td>
<td>1.571</td>
<td>3.72</td>
</tr>
</tbody>
</table>

Table 3.2: Variation in mean density and mean instar across the hybrid zone at Col de la Lombarde. Figures have not been corrected for the effects of habitat.

<table>
<thead>
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<th>Col de la Lombarde</th>
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</thead>
<tbody>
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<td>transect 2, 1989</td>
</tr>
<tr>
<td>race</td>
<td>mean density</td>
<td>mean instar</td>
</tr>
<tr>
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<td>3.5</td>
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<tr>
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</tr>
<tr>
<td>XO</td>
<td>4.15</td>
<td>4.13</td>
</tr>
</tbody>
</table>

Table 3.3: Variation in mean density across the hybrid zone at Col de la Lombarde: transect2. Figures have not been corrected for the effects of habitat.
Table 3.4: Variation in mean density and mean instar across the hybrid zone at Tende, Col de Fenestre and Vallée des Merveilles. Figures have not been corrected for the effects of habitat or stage of development.

variables were extended to include ground cover for each quadrat, mean vegetation height (of species recorded at each of the 18 sample points) and vegetation height diversity (variance in height) (see MacArthur, 1975). It became apparent that the choice of XY sites on transect 2 had been inappropriate: instead of the usual spread of ground-cover types found in other areas, these quadrats were dominated by thick lichen. The ground cover may be of direct importance to the grasshoppers, or may reflect large-scale ecological differences. Since the lichen ground cover occurred almost exclusively in this area any comparison between karyotypes would be confounded. It was necessary to choose 20 new XY quadrats with ground-cover similar to the XO and mixed quadrats of this transect. Unfortunately this meant that early scorings were missed for these extra sites.

### 3.2.3 Estimating densities

The 'fixed-time' (sensu Southwood, 1978) method for estimating population density was used. This method is most often useful for estimates within a given habitat and is especially appropriate for an extensive survey which compares population densities in both space and time. A similar method was used by Nichols and Hewitt (1988). Some compromise had to be reached between numbers of quadrats searched and time allowed for each search. An initial calibration in 1987 indicated that \( \approx 80\% \) of third instar nymphs were caught by two scorers, in the first three minutes of a 15 minute search (but note that this is only \( \approx 50\% \) of the total present- see below). A \((2m)^2\) wooden quadrat was dropped on the pre-located quadrats (see Fig. 3.13) and the number and stages of grasshoppers caught in three minutes by two scorers were recorded. This was repeated throughout the season. The data are provided in Appendix G.
In 1987 the Seyne and Col de la Lombarde quadrats were scored five times throughout the season. In 1988, as a compromise between numbers of quadrats to be scored and availability of time in which to score them, Transect 1 was divided into two subsets, each consisting of 20 quadrats from each karyotype. One subset was scored nine times at \( \approx 8 \) day intervals, and the other, three times at 14 day intervals. Transect 2 was scored three times during the season at 14 day intervals. In 1989 the transects at Col de la Lombarde were scored only three times- adequate to obtain estimates of hatchling densities, peak nymph densities and adult densities.

**Calibration**

The efficiency of searching will depend on the skill of observers, and could also depend on habitat type, density and conspicuousness of the grasshoppers, weather and time of day. Such variation in efficiency could limit the value of relative estimates even for comparative purposes, and so it was essential to check the density estimates.

Mark-release-recapture experiments were used to estimate density in the calibration quadrats, and to find the proportion of the total present that are caught in the first three minutes of searching, by two scorers over a nine minute search.
period. The calibrations were to determine the extent of migration into and out of the sites and the consistency of density estimates within a short time interval. Twelve \((2m)^2\) calibration sites were established at Col de la Lombarde in the same range of habitat types as those of the density survey. Some vegetation types are easier to search than others. For example, *Juniperus nana* is spiky and usually dense (see Fig. 3.14) and so is difficult to search thoroughly; observability could be different in tall versus low vegetation. There could also be a difference in the proportion (of the total number present) caught in a three minute search between high density and low density sites. Thus six of the calibration quadrats were chosen in low relatively easily searched vegetation, and six in tall, dense vegetation (see Fig. 3.15). Three of the *low* quadrats were in high density regions and three in low density regions; and similarly three of the *tall* quadrats were chosen in habitats that support low numbers, and three in those that support high numbers. Estimates of density and dispersal were obtained from mark-release-recapture experiments. Individuals were counted into a tin cushioned with vegetation and dusted lightly with reddish-brown fluorescent dust. Numbers were recorded every three minutes for 9 minutes. At the end of the scoring period, the grasshoppers were released into the centre of the quadrats. Three hours later the site was re-scored for 3 minutes, and in addition, four surrounding areas (each \((2m)^2\) in area) were searched for 3 minutes each (Figure 3.16). The results are summarised in Table 3.5 and Table 3.6.

Before estimating the fraction caught in three minutes of scoring, there are a number of points to note. Dispersal from the calibration sites during the experiments was negligible: only eight of the 122 marked individuals were recaptured in surrounding areas. Overall, the number of individuals recaptured from the calibration quadrats is 57- roughly half of the total number marked (122). However, note that the number on the second recapture is slightly lower than the number seen in the first three minutes of the initial capture (90 versus 114). This could be due to disturbance: grasshoppers tend to drop into the vegetation if disturbed too much. The absolute population size could be estimated in two ways. Moran’s (1951) method, modified by Zippin (1956, 1958) is based on maximum-likelihood. It could be used to estimate population size from the rate at which removing individuals reduces the sizes of successive samples at the three minute intervals. However, this method assumes that the probability of capture does not fall off with time. Table 3.5 shows that ninety-three per cent of the grasshoppers caught during the nine-minute search were caught in the first three minutes and yet, we only re-captured \(\approx 50\%\) of
Table 3.5: Calibration data. In column 2, Type refers to the habitat: $L$ is low, easy to search vegetation; $T$ is tall vegetation which is more difficult to search and in which grasshoppers are less conspicuous (see text). The initial numbers seen in the first, second and third three-minute searching intervals are provided. The numbers released are not always the same as the total numbers seen: some of those seen jumped out of the calibration quadrat and escaped.

Table 3.6: Calibration data. The numbers of marked (M) and unmarked (U) individuals caught in four $(2m)^2$ areas surrounding each calibration site (see Figure 3.16. Each surrounding area was searched for three minutes.
the marked individuals. This suggests that the probability of recapture does fall off with time. Thus the Lincoln index method for estimating population size has been used: if \( r \) individuals are marked and released in a given area and if, on a subsequent scoring, \( n \) individuals are caught of which \( m \) are marked and \( n-m \) are unmarked, then the chance of catching a grasshopper is estimated as \( m/r \). The estimated population size is then \( \hat{N} = \frac{n}{m/r} \). This method assumes no loss by dispersal or mortality, which seems reasonable here. Pooling all data, \( r=122, m=57 \). Therefore the chance of catching a marked grasshopper is 0.467, and \( \hat{N} = 192.72 \). The fraction caught in the first three minutes of scoring is 0.59.

Is the fraction of individuals observed in the tall quadrats the same as that observed in the low quadrats? Is there any relation with density? The results are summarised in Tables 3.7 and 3.8 and suggest no difference in percentage captured between habitat types, and between high and low density sites. They suggest that on average approximately 59% of the total numbers present are caught in the first three minutes of scoring.
Figure 3.15: Typical calibration sites. (a) "low" vegetation and (b) "tall" vegetation.
Figure 3.16: Sketch to show the plan of the calibration site and four surrounding areas that were searched on the re-capture (see text).

<table>
<thead>
<tr>
<th>Tall, difficult vegetation</th>
<th>Proportion seen</th>
<th>Low, easy vegetation</th>
<th>Proportion seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.40</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>0.57</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>0.37</td>
<td>7</td>
<td>0.56</td>
</tr>
<tr>
<td>11</td>
<td>0.57</td>
<td>8</td>
<td>0.57</td>
</tr>
<tr>
<td>12</td>
<td>0.45</td>
<td>9</td>
<td>0.40</td>
</tr>
</tbody>
</table>

\[ \text{mean: } 0.43 \quad \text{mean: } 0.49 \]

No difference between proportion seen : \( G = 0.0185 \text{ - ns} \)

Table 3.7: The proportion seen in the first three minutes of search in tall, hard-to-search versus low, easy-to-search vegetation (see text). Figures in the first and third columns give the calibration quadrat numbers. \( G = 2 \sum \text{obs} \cdot \ln(\text{obs}/\text{exp}); \text{ns=not significant.} \)

<table>
<thead>
<tr>
<th>Low density sites</th>
<th>Proportion seen</th>
<th>High density sites</th>
<th>Proportion seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.00</td>
<td>11</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>1</td>
<td>0.40</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>4</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>0.57</td>
<td>7</td>
<td>0.56</td>
</tr>
<tr>
<td>6</td>
<td>0.57</td>
<td>9</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>0.37</td>
<td>12</td>
<td>0.45</td>
</tr>
</tbody>
</table>

\[ \text{mean: } 0.460 \quad \text{mean: } 0.463 \]

No difference between proportion seen : \( G = 7.22 * 10^{-5} ; \text{ ns} \)

Table 3.8: The proportion seen in the first three minutes of search in low density versus high density sites. Figures in the first and third columns give the calibration quadrat numbers. \( G = 2 \sum \text{obs} \cdot \ln(\text{obs}/\text{exp}); \text{ns=not significant} \)
3.2.4 Distinguishing habitats

In this investigation it is essential to distinguish between “ecological” and “genetic” effects on fitness. We saw in Section 3.2.1 that there is a consistent trend in density across the zone, but this is before accounting for any ecological differences. Could the difference in density be due to habitat? The quadrats along each transect were chosen to be as similar as possible (see Section 3.2.2). In addition, a detailed survey of habitat in each quadrat along the same transect (1987) and along transect 1 (1987,1988) and transect 2 (1987,1988) at Col de la Lombarde allowed a classification of the range of habitats sampled and was used to determine any subtle differences objectively. Abundance scores (0-18) of 82 (those listed in Appendix B.1) different plant species were recorded for each quadrat and the percentage cover of major physical and ecological features were also recorded. In 1989 time was limited and so a detailed habitat survey was not possible for the transects at Tende, Vallée des Merveilles and Col de Fenestre. However the choice of quadrats along these were to some extent based on the results of the multivariate analysis described in this section.

There are two approaches to disentangling ecological differences in density from those due to intrinsic fitness differences. The most obvious is regression analysis, in which grasshopper density would be regressed on, say, abundance or percentage cover of plant species and other ecological features. This approach requires multiple regression on a large number of variables (in this case, 82 species abundance scores), some of which may be of negligible importance. One could choose a sub-set of the variables, but then one has to decide which of these are important to *Podisma*. This is not immediately obvious. The second approach is to reduce the number of variables using multivariate-statistical methods. A quadrat-by-species data matrix has a large degree of redundancy: many quadrats are likely to be similar in their species composition and so knowledge of the presence or abundance of one species allows the estimation of another.

There are several multivariate methods available for analysing such data. These fall broadly into three classes: ordinations, classifications and gradient analysis. Ordinations attempt to represent sample and species relations as closely as possible in a low-dimensional space, usually resulting in a two-dimensional graph in which similar samples or species are near each other and dissimilar ones far apart. The simplest ordination method is Principal Component Analysis (PCA) which projects
Figure 3.17: Plate # 1. From left to right: *Aster alpinus*, *Nigritella cornellia*, *Dianthus gratianopolitanus*. Illustration by Nuala Gregory.
the data swarm onto a differently orientated species-space (without any differential weighting of the species) in such a way that the pattern of the data swarm is as simple as possible relative to the new axes. In other ordination methods, a score is assigned to each quadrat by some objective species weighting method. The scores are then ranked giving an 'ordination' of the data. Classification methods combine similar samples in a step-wise fashion resulting in an hierarchical dendrogram. Gradient analysis is appropriately used when at least some of the quadrats can be assigned to a position along some environmental gradient. Species associated with the extremes of the gradient can then be identified.

One can wade through volumes of arguments for various combinations of 'best methods'. However, too much adjusting and checking is often counter productive (see Gauch, 1982; Pielou, 1984). Keeping in mind that the aim of the project is to investigate differences in fitness across the zone, and that the multivariate methods are merely an attempt at reducing 'environmental noise', I have avoided comparing methods on the data. Instead, the vegetation has been analysed using TWINSPLAN only (Hill, 1979), the same method as that used by Nichols (1984) and the method deemed most appropriate: TWINSPLAN yields an ordination and classification simultaneously and is designed to ignore rare species and to work with fairly coarse measures of species abundance. Further details and the results of TWINSPLAN analysis on the vegetation surveys from the Seyne and Col de la Lombarde transects are provided in Appendix B.2. The TWINSPLAN groupings used in the models described below are summarised in Table 3.9 (the groups into which quadrats fall are listed in Appendix G).

3.2.5 Disentangling the causes of density differences

In the last section I described the statistical analysis used to group quadrats into different habitat types. In this section I will describe a way of estimating the effects of these habitat types and other variables on density. Clearly the density in a quadrat will depend to some extent on the stage of development, and since this differs between karyotypes (see Section 3.2.1) it was necessary to correct densities for stage of development before estimating the effects of habitat types and other ecological variables. The expected percentage change in density between the observed instar and the mean for the transect was estimated for each quadrat (from Figure 3.20) and was used to correct the densities for stage of development.
Figure 3.18: Plate # 2. From left to right: *Vaccinium myrtillus*, *Viola biflora*, *Viola nummularifolia*. Illustration by Nuala Gregory.
### a) VEGETATION

Based on TWINSPAN 'indicator' & 'preferential' species

<table>
<thead>
<tr>
<th>Category</th>
<th>Year</th>
<th>Characteristic Vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1987</td>
<td>Vaccinium myrtilus, grasses</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>Vaccinium myrtilus, rock</td>
</tr>
<tr>
<td>2</td>
<td>1987</td>
<td>Vaccinium myrtilus, earth</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>Vaccinium myrtilus, grasses</td>
</tr>
<tr>
<td>3</td>
<td>1987</td>
<td>Vaccinium uliginosum, Vaccinium myrtilus absent</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>Vaccinium uliginosum, rock, earth</td>
</tr>
<tr>
<td>4</td>
<td>1987</td>
<td>Vaccinium uliginosum, Vaccinium myrtilus</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>Vaccinium uliginosum, herbs</td>
</tr>
</tbody>
</table>

### b) GROUND COVER

<table>
<thead>
<tr>
<th>Transect &amp; Year</th>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>transect 2, 1988</td>
<td>1</td>
<td>lichen, no grass, little litter</td>
</tr>
<tr>
<td>transect 1, 1988</td>
<td>1</td>
<td>litter, earth/grass, little rock</td>
</tr>
<tr>
<td>transect 2, 1988</td>
<td>2</td>
<td>litter, earth/grass, little rock</td>
</tr>
<tr>
<td>transect 1, 1988</td>
<td>2</td>
<td>rock, earth, no litter, no lichen</td>
</tr>
<tr>
<td>transect 2, 1988</td>
<td>3</td>
<td>rock, earth, no litter, no lichen</td>
</tr>
</tbody>
</table>

### c) TOPOGRAPHY

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flat (slopes up to 13°)</td>
</tr>
<tr>
<td>2</td>
<td>South Facing, gentle slope (14° to 30°)</td>
</tr>
<tr>
<td>3</td>
<td>North Facing, gentle slope</td>
</tr>
<tr>
<td>4</td>
<td>South Facing, steep slope (greater than 30°)</td>
</tr>
<tr>
<td>5</td>
<td>North Facing, steep slope</td>
</tr>
</tbody>
</table>

### d) VEGETATION HEIGHT

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean Height (ht)</th>
<th>Category</th>
<th>Variance of Height (hvar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ht&lt; 4cm</td>
<td>1</td>
<td>hvar&lt; 3cm</td>
</tr>
<tr>
<td>2</td>
<td>4cm&lt;ht&lt;7cm</td>
<td>2</td>
<td>3cm&lt;hvar&lt;5cm</td>
</tr>
<tr>
<td>3</td>
<td>7cm&lt;ht&lt;11cm</td>
<td>3</td>
<td>5cm&lt;hvar&lt;7cm</td>
</tr>
<tr>
<td>4</td>
<td>11cm&lt;ht&lt;14cm</td>
<td>4</td>
<td>7cm&lt;hvar&lt;10cm</td>
</tr>
<tr>
<td>5</td>
<td>ht&gt;14cm</td>
<td>5</td>
<td>hvar&gt;10cm</td>
</tr>
</tbody>
</table>

Table 3.9: Definitions of categorical variables used in the General Linear Models analysis of data from transects near Col de la Lombarde. Note that vegetation was scored twice (1987 and 1988) and the TWINSPAN analysis did not give identical results. This is because different quadrats were scored in 1987 and 1988 (see text).
Figure 3.19: Plate # 3. Top: *Rhododendron ferrugineum*, bottom: *Gentiana kochiana*. Illustration by Nuala Gregory.
Figure 3.20: Graph of (a) mean density and (b) log mean density against mean instar, for transect 1, averaged over three successive years (1987, 1988, 1989). Peak nymph density occurs when the grasshoppers are between first and third instars, and will partly depend on the extent to which hatching is staggered. To interpolate or extrapolate densities, the percentage change in density for a given change in instar can be estimated from this figure.
Regression analysis

Simple regressions of density on percentage cover of the major vegetation types and the major physical features (see Section 3.2.2) were performed. Multiple regressions were performed on each transect first for all karyotypes and then for karyotypes individually. An examination of the models and their errors indicated that most of the differences in density were not accounted for by habitat. However, regressions of density on ecological features for XY, XO and mixed races separately reduced the noise, and suggested differences in density between karyotypes.

There are several possible sources of variation which could account for the remaining noise. The variation could result from deterministic "chaos" or truly random fluctuations or it could be due to variables not included in the regression analysis such as variation in topography, soil-type, interaction with other species, predators, parasites etc. Since at least some of these variables might be accounted for indirectly in the more thorough habitat survey, the other approach was to use general linear models to estimate the effects of the habitat type as defined by TWINSPAN and other variables such as topography and ground cover.

General Linear Models (GLM)

The relative effects of variables on density can be estimated using GLM (see Appendix B.3). These estimates can then be used to adjust densities and so to reduce environmental noise. A comparison of adjusted densities allows estimates of density differences between XO, XY and hybrid populations. The SAS (1985a,b) general linear model procedure was used to produce estimates of effects of variables on density. The effects of vegetation type (as classified by TWINSPAN), topography, ground cover, mean vegetation height, variation in vegetation height, and karyotype (whether pure XO, pure XY or hybrid) were investigated in the models. In the SAS GLM procedure, estimates can be based on class variables but there is an option in the model-building procedure for reverting to linear regression analysis for continuous numeric variables. This allowed a comparison of the relative importance of discrete and continous variables on density.

Initially, the classifications used in the models were based on narrow ecological categories. Categories were then refined according to the magnitude of differences in the effects of each (based on a comparison of F-values and degrees of freedom for the models). The refined variables used in the models and their definitions are
summarised in Table 3.9.

Histograms of the observed frequency distribution of quadrat densities suggested that these were approximately normally distributed and so analysis of variance was used to compare means. The observed variation in nymph densities was partitioned into portions associated with vegetation differences, ground cover, topography, karyotype and time (\(day, day^2\)). In analysis of variance, partitioning is in terms of sums of squares\(^1\) (SS) with corresponding partitioning of the associated degrees of freedom (df). F ratios were then used to compare the amount of variability associated with each source of variation. The results are presented in Table 3.10.

Mean height of the vegetation and vegetation height diversity had negligible effects on density. The range of slopes and aspects used in this investigation showed no trend if included as separate variables, but were usually significant in combination. Genotype, date of scoring, vegetation type and ground cover had significant effects on density for at least one of the transects. However, effects were not consistent between transects. This is not surprising, because variable distributions in each transect can be quite different (see Figures 3.21, 3.22 and 3.23) (note also that not all categories were recorded and that categories were not always defined in the same way (e.g see Table 3.9(a),(b)) therefore a comparison of sources of variation between transects should be made with caution. For all transects \(\sqrt{density}\) as the dependent variable gave a better fit than density, and fitting a quadratic with respect to \(day\) (i.e. \('day', \('day^2'\)) gave the best fit (including \('day^3'\) did not improve the fit significantly). Interaction effects did not improve the fit significantly. The models described below were the best fit models for a series of tests.

**Interpretation of GLM models**

The estimates of relative effects of each variable resulting from GLM were used to adjust densities. Since corrections were always made to the type most commonly found in the transect, these corrections produce the densities expected in a representative or typical quadrat for the transect. Appendix B.3 provides a summary of corrections.

Although SAS GLM for analysis of variance can cope with unequal representation of habitat types across the zone, it is important to know the distribution of these types for a meaningful comparison of karyotypes and transects. Habitat types may

\(^1\)The SAS GLM "Type IV" SS for analysis of variance on unbalanced data was used, see Appendix B.3 and Freund et al (1986).
Table 3.10: Sources of variation in nymph densities: Partitioning of variation was by analysis of variance, using SAS TYPE IV SS (see text). Class variables are defined in Table 3.9. DAY and DAY² are continuous numeric variables in the model. In all cases, the square-root of density as the dependent variable gave the best fit. Pr>F is the significance probability associated with the F-value. (d) includes the inappropriate XY sites.
occur exclusively in one or other karyotype region and in these cases it is hard to
distinguish a habitat related difference in density from one due to inherent genetic
effects.

Figures 3.21, 3.22 and 3.23 illustrate the distribution of class variables between
genotypes for each transect. Definitions of the class variables are provided in Ta-
ble 3.9. Within-transect comparisons are essential for a valid interpretation of the
density patterns. Comparisons between different transects however, should be made
with caution: the transects were on different sides of the valley and so the class
variables defined in table 3.9 were not distributed evenly between the transects (see
Figure 3.21, Figure 3.22 and 3.23). This is not a problem, since we are interested
in differences between karyotypes: the point of sampling more than one transect is
to replicate the experiment.

Since the sources of variation (Table 3.10) differ according to the characteristics
of each transect, the results should be considered separately. Consider the Col de
la Lombarde 1987 transects. Karyotype clearly accounts for most of the variability.
However, as Figures 3.21, 3.22 and 3.23 indicate, the XY quadrats are predomi-
nantly north-facing and gently-sloping, whereas this topography constitutes only a
small proportion of mixed quadrats. This will clearly bias the distribution of effects
in the models and the corresponding F ratios. It is interesting to compare this with
the results from transect 1 in 1988. Some of the quadrats chosen for this transect
were identical to those of the 1987 transect. However, recall that some new quadrats
were established (see Section 3.2.2). As a consequence transect 1 1988 had a more
similar range of topographies and here karyotype accounts for a higher proportion
of effects (see Table 3.10). Nonetheless there is still confounding of variables: vege-
tation type 2 occurs predominantly in mixed and XO karyotype regions.

In the field, I suspected that the lichen-dominated ground-cover of the original
transect 2 XY sites were inhospitable to Podisma and so chose extra XY sites with
ground-cover similar to that of the XO and mixed sites. The major contribution
of ground-cover in Table 3.10 d relative to that in other transects suggests that the
lichen dominated cover in transect 2 (or some correlated variable) is of importance
to the grasshoppers. This argument is supported in that this ground cover was not
represented at all in XO and mixed karyotypes in transect 2 and in the extra XY
sites (Fig. 3.22 and 3.23) and in these regions densities were consistently higher.
Table 3.10(d) shows F values for transect 2 when the XY sites with lichen-dominated
ground cover were included, and Table 3.10(e) gives values when these sites were
Figure 3.21: The distribution of categorical variables across the zone: $T$=topography; $V$=vegetation; $G$=ground cover. The bars show the numbers of quadrats falling into the given categories (categories are coded 1 to 6) as a percentage of the total number of quadrats. For definitions of categories 1 to 6, see Table 3.9.
Figure 3.22: The distribution of categorical variables across the zone: 
T=topography; V=vegetation; G=ground cover. The bars show the numbers of 
quadrats falling into the given categories (categories are coded 1 to 6) as a percentage 
of the total number of quadrats. For definitions of categories 1 to 6, see 
Table 3.9.
Figure 3.23: The distribution of categorical variables across the zone: 
T=topography; V=vegetation; G=ground cover. The bars show the numbers of 
quadrats falling into the given categories (categories are coded 1 to 6) as a per­
centage of the total number of quadrats. For definitions of categories 1 to 6, see 
Table 3.9.
In Section 3.2.1 we saw that there is a consistent trend in density across the zone. Tables 3.1 to 3.4 give mean uncorrected densities across each transect and Figure 3.24 and Table 3.12 show trends in mean peak density across transects.

In 1987 and 1988 detailed habitat surveys were done and so it was possible to compare density trends across the zone before and after correcting for differences due to vegetation type, other ecological features and stage of development. Table 3.11

---

Figure 3.24: Average density trends across the zone for six different transects (see text). T1, T2 refer to Transect 1 and Transect 2 at Col de la Lombarde, respectively. In general, confounding of variables complicates the interpretation of the data and may be difficult to overcome because of large-scale geographical differences across the karyotype regions (see Chapters 7 and 8 for further discussion).
Table 3.11: Variation in mean density across the hybrid zone at Col de la Lombarde. Figures corrected for the effects of instar and habitat.

<table>
<thead>
<tr>
<th></th>
<th>transect 1, 1987</th>
<th>transect 1, 1988</th>
<th>transect 2, 1988</th>
</tr>
</thead>
<tbody>
<tr>
<td>race</td>
<td>mean density</td>
<td>mean density</td>
<td>mean density</td>
</tr>
<tr>
<td>XY</td>
<td>8.36</td>
<td>5.09</td>
<td>7.17</td>
</tr>
<tr>
<td>H</td>
<td>4.54</td>
<td>1.88</td>
<td>5.54</td>
</tr>
<tr>
<td>XO</td>
<td>1.27</td>
<td>5.98</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.12: A comparison of mean peak nymph densities and mean adult densities across the zone (1988 XY figures are extrapolated - see text).

<table>
<thead>
<tr>
<th></th>
<th>transect 1, 1988</th>
<th>transect 1, 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>race</td>
<td>peak stderr</td>
<td>adult stderr</td>
</tr>
<tr>
<td>XY</td>
<td>14.35 1.4</td>
<td>3.09 0.36</td>
</tr>
<tr>
<td>H</td>
<td>3.2 0.78</td>
<td>0.59 0.16</td>
</tr>
<tr>
<td>XO</td>
<td>2.90 0.6</td>
<td>3.7 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>transect 2, 1988</th>
<th>transect 2, 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>race</td>
<td>peak stderr</td>
<td>adult stderr</td>
</tr>
<tr>
<td>XY</td>
<td>10.81 1.32</td>
<td>4.12 0.42</td>
</tr>
<tr>
<td>H</td>
<td>7.81 0.79</td>
<td>2.90 0.39</td>
</tr>
<tr>
<td>XO</td>
<td>7.58 0.81</td>
<td>3.12 0.44</td>
</tr>
</tbody>
</table>

In an analysis of variance, karyotype accounts for most of the variability in density and this is consistent between years (see Table 3.10). Table 3.2 gives the means of uncorrected densities and Table 3.11 gives the means of densities corrected for the effects of habitat and instar, for those transects for which a detailed habitat survey was done.

The SAS General Linear Model (GLM) procedure for analysis of variance on unbalanced data was used to test the level of significance in the difference between means. This analysis assumes that densities are normally distributed, a valid assumption here. In 1987 and 1988 detailed habitat surveys were done. Accounting
Table 3.13: The values from Table 3.12 expressed relative to the XY densities. In most cases the ratio is smaller for adults than for nymphs.

for the effects of ecological variables and stage of development has little effect on the trend in density across transects. Comparisons for the 1989 transect are necessarily for uncorrected densities.

**The pure XY race has consistently higher density than the mixed and pure XO races.** The pure XY race is significantly more dense than both the XO and mixed races at Seyne (P>0.001 and P>0.003 respectively). At Col de la Lombarde, XY is significantly more dense than mixed for *transect 1* (P>0.001) in 1987 (recall, XOs were not sampled that year) and in 1988 XY is significantly more dense than both mixed and XO (P>0.0001). In 1989 the XY race is significantly more dense than both Mixed and XO (P>0.0001).

*Transect 2* in 1988 shows a similar pattern. Recall that the initial sampling of XY race was done in inappropriate habitat. However, it was possible to extrapolate densities based on values from the more appropriate quadrats sampled later in the season, using Figure 3.20. Based on these values, the XY race is again significantly more dense than the XO and mixed races (P>0.0001); and the same pattern is observed in 1989 (P>0.0001).

On average, there is no significant difference in density between pure XO and mixed races. There was no significant difference between XO and mixed densities at Seyne in 1987, nor were there differences between these races across *transect 1* at Col de la Lombarde for all three years (1987, 1988, 1989). However across *transect 2* the XO race was significantly more dense in both 1988 and 1989 (P>0.0041 and P>0.0001 respectively).

A comparison of Tables 3.1 to 3.11 shows remarkably similar trends for the transects at Seyne and Col de la Lombarde. At most the corrections account for 30% of the variation in density between quadrats, and in some transects, for none.

These observations could have several causes: It may be that in choosing similar quadrats, most of the variation in density due to vegetation or topography had already been accounted for. There could be large-scale geographical differences across the transects, which might affect density distributions. For example, the
Table 3.14: Variation in mean density across the hybrid zone at Tende, Col de Fenestre and Vallée des Merveilles. Figures have been corrected for the effects of stage of development - see text.

vegetation and topography *surrounding* the quadrats could be important to *Podisma* densities: these would be missed by choosing to score only those patches of habitat that were ecologically similar. It could also be that interactions with other species, predators and parasites result in patchiness.

The observed trends in density could be due to undetected chance differences across transects: there might always be hidden differences which could affect density. No amount of classification of habitat could exclude this possibility. Therefore an extra three independent transects were scored in 1989 to give a robust test of whether there is a consistent trend in density across the zone. The three transects were in geographically distinct areas: one near Col de Fenestre, one near Vallée des Merveilles and one near Tende (see Figure 2.4 and Figures 3.6 through 3.11). Time was limited, and it was not possible to do a detailed habitat survey along these transects. However we have seen that after matching quadrats by eye, any further attempts at correcting for ecological effects have little effect on the trends in density across transects at Col de la Lombarde. Care was taken to match quadrats, and the choice was based on the results of the TWINSPAN and GLM results from the analyses described above (i.e. *indicator* and *preferential* species as well as other features found to characterise similar quadrats were used as a guide).

Tables 3.4 and 3.14 show the difference in mean density and mean instar across the transects. The trends in density across the zone are the similar to those observed across other transects (see Figure 3.24). Since no habitat survey was done along these transects, I was not able to correct for ecological effects. However, it was possible to account for stage of development. Analysis of variance was used to test the level of significance of differences between means.

On average the XY race has significantly higher densities than XO
or mixed. For uncorrected data, the XY race has significantly higher densities than XO and mixed near Tende (P>0.005 and P>0.05 respectively). At Vallée des Merveilles, the XY race has significantly higher densities than the XO race (P>0.005) but not than mixed (P>0.1). At Col de Fenestre, mean densities were not significantly different.

When densities are corrected for instar, the XY race is significantly more dense than XO and mixed at both Tende and Col de Fenestre (P> 0.05; P>0.05 respectively) and at Vallée des Merveilles the XY race is significantly more dense than the mixed race, though still not significantly different from the XO race.

On average the difference between mean densities of XO and mixed populations are not significantly different. In all comparisons, there was no significant difference in density between XO and mixed races. Correcting for instar did not alter this pattern.

In summary, after choosing matched quadrats, little of the variation in density could be accounted for by regional differences in vegetation. This contrasts with Nichols and Hewitt (1988), who found that after allowing for the effects of vegetation, there is no significant variation between XO and XY regions. At Col de la Lombarde, this may be because we have already eliminated most vegetation effects by using matched quadrats. At Seyne, it is quite possible that some confounding of variables or some hidden local effect could account for the discrepancy. (Figure 3.5 shows that the areas sampled by Nichols are not identical to those sampled in 1987 and some of his sites are well away from mine). However, note that the before correcting for ecological variables, his density trends are similar to mine (see Table 3.1). There could also be some time effect since we sampled the area three years later (but note that at Col de la Lombarde, replicability in time is remarkably good).

The investigations described here have revealed a consistent pattern is found across years and across transects: at the beginning of the season, the XY race is always more numerous, and the XO quadrats have densities which are, on average, similar to those of hybrids. This is a surprising finding in view of the reduction in early survival seen in the laboratory and the stability of the hybrid zone (see chapter 2).

Do the density trends suggest that the hybrid zone is moving? Tension zones tend to move down gradients of population density (Hewitt, 1975; Barton, 1979 and see chapter 5). An asymmetry in density is likely to result in asymmetric gene flux and since the allele from the less dense population will be less frequent in the zone it
will occur predominantly in heterozygotes. Since heterozygotes have reduced fitness, the rare allele will have a selective disadvantage and will eventually be eliminated. Thus one would expect the zone to move towards the sparse population. These questions will be addressed in Chapters 7 and 8.

The other interesting pattern to emerge from this survey is the pattern of change in density with time. Table 3.13 compares mean peak density and mean adult density. Despite large differences in nymph densities, adult densities were similar, suggesting some density dependent process. This will be investigated further in the next chapter.

### 3.2.7 Measuring relative fitneses

The original aim of the extensive surveys described above was to investigate relative fitness, under natural conditions, of populations from inside and outside the tension zone (for a discussion of absolute and relative fitness, see Chapter 1). In the last section I compared density trends before and after accounting for ecological variables. We saw that there is a consistent density pattern across transects and across years and that the pattern is the same whether or not densities are corrected for ecological variables and for instar. What do the density patterns tell us about the relative fitneses of the races?

**Survival**

There are several problems in interpreting the data. Consider the viability component of fitness. Within-generation changes in density should give information on survival. We assume that the populations are at equilibrium, so their absolute fitneses must be 1 \((N^* = WN)\) and at equilibrium \(N^* = N\) so \(W = 1\). Thus if a greater proportion of hybrid eggs fail to hatch successfully than non-hybrid eggs (as observed in the laboratory, see Chapter 2), then one might expect the hybrids to have higher nymph to adult viability to compensate for this.

The observed density-dependent reduction in population size complicates the interpretation of survival differences further. If there is density-dependent mortality (as the investigation in Chapter 4 suggests), one might expect the less dense hybrid and XO sites to have higher nymph to adult viability. However the density-dependent reduction in population size within a generation complicates the interpretation of survival differences.

**Fecundity**

I have used the ratio of average adult density per quadrat in 1988 to average hatchling density per quadrat in 1989, as a measure of fecundity. A similar comparison between numbers of adults in 1987 and numbers of hatchlings in 1988 is rather crude because sampling in 1988 started when most individuals were second instars, and so any differential early nymph mortality would give misleading results.

*Adult* density is defined as the average density when the mean instar is 5.5 (estimated by regression) (note that when all individuals are adult (instar=6.0), the density of potential breeders is likely to be underestimated due to adult mortality). *Hatchling* density in 1989 was calculated from the numbers of first and second instar nymphs seen on the first and second scorings (8 days apart). Since the races were at slightly different stages of development, it was necessary to correct for mean stage of development. Table 3.15 gives ratios of adult densities in 1988 to hatchling densities in 1989 for transect 1 at Col de la Lombarde. Again the interpretation of fecundity differences is not straightforward: fecundity may also be density-dependent (see Table 3.15).

**Rate of development**

Differences in rate of development between the races can be made by comparing the change in mean instar through time. The stage of development of all individuals caught in the density survey were recorded. General linear models were used to investigate the relative importance of variables such as topography and vegetation (where these had been recorded, i.e. the 1987 and 1988 transects) on stage of development, and to disentangle these from inherent differences between races.
Figure 3.25: Graph of average density against average instar through time (thick lines) for XY and Mixed (hybrid) sites (1987 data). Thin lines join days of scoring. At Col de la Lombarde the stage of development at XY sites is consistently lagged with respect to that of Mixed sites. At Seyne the pattern is reversed. This figure suggests that the observed differences in mean instar between XY and Mixed populations reflect differences in date of hatching rather than rate of development.

These variables were found to be of negligible importance to stage of development. The analyses do not indicate a difference in rate of development; however, they do show a difference in mean stage. At Col de la Lombarde, mixed sites are significantly more advanced than XY sites in 1987 (P>0.0001). In 1988 the mixed sites were more advanced than either XY or XO sites (P>0.001 and P>0.008 respectively), but pure populations were not significantly different. This pattern contrasts with that observed at Seyne: here mixed populations were at a significantly earlier stage of development than either pure XY or pure XO populations (P>0.0001 and P>0.0001 respectively), the same pattern as that observed by Nichols in this area. However, Figure 3.25 suggests that the differences in stage reflect a difference in date of hatching rather than in rate of development, at both Seyne and Col de la Lombarde.
Population size

The surveys show that the XY race is consistently more numerous, and that the XO sites have densities which are on average similar to those of hybrids. This is a surprising result: in a homogeneous environment, we would expect a consistently larger XY population size to push the zone forward and eventually eliminate the less dense XO race. This is because populations at the centre of the zone would receive more immigrants from one side than from the other, so that the frequency of introgressing alleles would rise (Hewitt, 1975; Barton, 1979c; and Chapters 1 and 5). We would therefore expect to find tension zones trapped by regions of low density, flanked on either side by denser populations. Indeed, over most of its length at Tende (Barton and Hewitt 1981a) and Seyne (Nichols and Hewitt, 1986) the zone is found to follow such low density areas.

In Chapter 7 I will investigate whether the position of the zone at Col de la Lombarde is consistent with the asymmetric pattern of densities: would one expect the cline to move to a new position? Should the XY race swamp the XO race?

3.3 Parasite load in *Podisma pedestris*

3.3.1 Introduction

One of the clearest examples of natural selection is the rapid evolutionary change that can occur in the 'red queen' race between parasite virulence genes and host resistance genes (e.g. Flor, 1971 in Begon et al, 1990). For example, Sage et al (1986) found evidence to suggest that populations from a hybrid zone between *Mus domesticus* and *Mus musculus* have significantly more nematodes and cestodes than populations from the two parental races. One explanation is that hybrids have recombinant genotypes which fail to confer resistance (Sage et al, 1986). Whitham (1989) found a similar pattern in the distribution of aphids across the hybrid zone between the cottonwoods *Populus fremantii* and *Populus angustifolia*. Both observations on natural populations and transplant experiments showed that significantly more aphids survive on trees within the hybrid zone than on those from pure populations. Is there any evidence to suggest a difference in parasite load inside and outside the *Podisma* hybrid zone?

Studies on variation in insect susceptibility to parasites lag behind those in vertebrates (see Gupta, 1986). However, recent research in pest management focusing on parasites as potential biological control agents for insects has stimulated an interest
in variation in host response to parasite infections (see Burger, 1981; Pedigo, 1989). The nature of immunity in insects is different from that in vertebrates. Most vertebrates possess an acquired humoral immune response characterised by host antibody formation whenever 'non-self' particles are recognised by host defences. Invertebrate resistance includes encapsulation and humoral responses (Lackie, 1980) but lacks the immune response system of vertebrates. In invertebrates, populations of phagocytic cells are responsible for most of a host's response to invaders: they engulf and digest small foreign bodies and encapsulate and isolate larger ones. The recognition of 'self' from 'non-self', presumably depends on the surface properties of the invader, although the process is still largely obscure (Begon et al., 1990). However, resistance to pathogens does seem to be genetically based in several insects (Anderson and May, 1981; May, 1982). The response of invertebrates gives poorer protection to the individual than that of vertebrates, and recovery of a population after infection depends more on high reproductive potential of the survivors than on the recovery of those that have been infected (Tripp, 1974). Because insects do not have the plastic, facultative response exhibited by organisms with more complex immune systems, resistance to many parasites is likely to depend on whether individuals are susceptible or not to start off, rather than on their ability to respond (May, 1982).

Most of the literature deals with induced infections and very little is known about the genetics of host resistance to parasitism in natural populations. However, there is some evidence for variation in susceptibility (Salt and Van den Bosch, 1967; Pedigo, 1989).

The hybrid zone in *Podisma pedestris* should provide an interesting system in which to investigate whether there is heritable variation in resistance to parasites in this insect. Is there any evidence for increased parasitism in the hybrid zone?

### 3.3.2 A parasite survey

#### The parasites

There have been no previous investigations on *Podisma* parasites. However, Barton and Nichols (pers. comm.) have noticed individuals parasitised by mermithid nematodes, dipteran larvae and mites, and Nichols (pers. comm.) observed gregarines in the gut of some individuals.

In 1988, several different populations from the hybrid zone and from populations pure for the XY and for the XO karyotypes were sampled at Col de la Lombarde (see
below). Time was limited in the field, and facilities rudimentary, so that it was necessary to prepare slides of the appropriate tissues for a thorough examination back in the laboratory. In this investigation I made appropriate tissue preparations to search for parasites that had previously been found in *Podisma* as well as some other widely distributed Orthopteran parasites that would be likely to occur in *Podisma*. Mites (Acari of the family Podapolipodidae); mermithid nematodes - probably *Mermis hexameremis* (Hominick, pers. comm.); dipterans; gregarines (Sporozoa) had all been observed. *Maloemeba locusta* (Amoeba), and *Nosema sp.* (Microsporidia) are likely to occur (Canning, pers. comm.).

**Mermithid nematodes**

The infective stage in *Mermis hexameremis* penetrates first or second instar larvae. The nematode develops in the hemocoel until the grasshopper has reached fifth instar or adulthood. When they emerge, the mermithids burrow into the soil where they complete their development. Mermithid nematodes have a profound effect on their hosts: they can affect the morphology, behaviour, sexual development, and physiological balance, and when they emerge, the host is destroyed (Poinar, 1975). Observations on natural populations of *Podisma* suggest that the mermithid nematodes are fatal: they destroy the grasshopper when they emerge, if not before. In 1987 about 5% of the individuals dissected for karyotyping were found to be infected by mermithid nematodes, and all these individuals had little or no spermatheca. There is no known response in Orthoptera to Mermithid nematodes, though one cannot rule out differences in susceptibility or behavioural responses (Hominick, pers. comm.).

**Protozoa**

*Nosema* spores are ingested with food and pass across the gut wall to the fat bodies (Canning, 1962a). Canning (1962b) found that locusts infected with *Nosema locustae* suffered greater hypertrophy of the fat body, and 92% of nymphs infected with the parasite died before adulthood. In the laboratory, microsporidians can cause chronic disease that results in lowered fecundity and sterility (Burger, 1981). Gregarines are common invertebrate gut parasites. They develop from spores which can be ingested by the host at any stage of its life-cycle (Hyman, 1940; Kudo, 1954). The preliminary phase is in the cecae, where gregarines attach to the cell walls. They then pass into the host fore- and midgut, where they pair up and attach to
the epithelial wall. Gregarines feed on material present in the gut lumen (Kudo, 1954). They can damage the host if large numbers block the passage of food across the gut or when the epithelium is eroded—thus allowing the passage of bacteria and viruses into the haemocoel (Bucher, 1959).

Malomeba locustae are commonly found in Orthopteran malpighian tubules (Canning, pers com). Investigations into the effect of protozoan parasites in nature are rare. Protozoans have been shown to have a negligible effect on mortality in natural populations of the British grasshopper, Chorthippus parallelus and C. brunneus (Richards and Waloff, 1954; Dempster, 1963; Greathead, 1963; Monk, 1980). On the other hand, Zuk (1987a) has found that while gregarines are not by themselves a major source of mortality in field crickets, they could induce vulnerability to other pathogens, particularly under adverse conditions, such as food shortage. She has also found that spermatophore numbers are negatively correlated with natural levels of gregarines, in male field crickets (Zuk, 1987b) and that paired males were significantly less parasitised by gregarines than unpaired males (Zuk, 1988). Malomeba locustae have been known to damage orthopteran populations in nature, for example this species prevented an outbreak of the brown locust Locusta parolalina in one area of South Africa (Venter, 1966).

The survey

In 1988 several different populations from the hybrid zone and from populations pure for the XY and for the XO karyotype were sampled at Col de la Lombarde. Sites were chosen in a similar range of habitats in pure and mixed areas. Between ten and fifteen individuals (on average fourth instar nymphs) were collected from each site, and the number of mites (if any) on each individual were recorded. Individuals were dissected in 0.7% saline solution the same evening. Mermithid nematodes are large and easily visible (see Figure 3.26). Observations on other Orthoptera suggest that gregarines would be found in their preliminary phase in the ceca or, in a later phase, paired-up close to the gut wall where they should be visible with a dissecting microscope (pers obs on locusts). The amoeba should be visible as oval cysts approximately 10 microns in diameter. These are usually abundant when present, and so should be easily recognised (Canning, pers com). Similarly, microsporidian spores, although small (∼ 5 microns) are usually abundant and unmistakable when present. Twenty individuals from the hybrid zone sites and five from sites within each of the pure areas were dissected in the field. Fat bodies,
caeca, mid-gut, hind-gut and malpighian tubules were dissected out with two pairs of fine forceps. No gregarines, microsporidia, or amoeba were found in any of these individuals. However, since time was limited, slides of the relevant tissue smears were prepared for a more thorough search back in the laboratory. Individual malpighian tubules were squashed in a drop of saline on slides, to search for amoeba. To search for the preliminary gregarine phase, three caeca from each individual were pulled off and smeared in a drop of saline on a slide. To search for the microsporidia, a fat body from each individual was dissected out, and smeared over a slide. The slides were allowed to dry and then were fixed in methanol for thirty seconds. The bodies were frozen for further examination. In the laboratory, slides were stained in 10% Giemsa for half an hour, and those with malpighian tubules were mounted in Euparal vert. So far, I have examined tissue preparations from 3 individuals from each of the 33 populations sampled at Col de la Lombarde and have not found any of these parasites (surprising given that they are such widespread orthopteran parasites). However about ten percent of all the grasshoppers were infected by mermithid nematodes.

Is there any difference in nematode abundance between pure and mixed populations? Table 3.16 summarise the data from Col de la Lombarde. Overall, pop-
Figure 3.27: The frequency of individuals parasitised by mermithid nematodes across Parasite Transect 1 at Col de la Lombarde. 20% and 80% contours of XY frequency show the approximate limits of the hybrid zone.

...ulations from the hybrid zone do have a significantly larger number of parasitised individuals ($G = 11.0142; P < 0.001$) than populations from the pure sites. Comparing the transects separately: data from Parasite transect 1 suggest that mixed populations have significantly more parasitised individuals than pure populations (XY and XO combined; $G=13.897; P<0.001$; and see Figure 3.27). However Parasite Transect 2 does not show this pattern, indeed only two individuals out of 104 from Parasite Transect 2 were parasitised. The pattern across Parasite Transect 1 (Figure 3.27) could suggest a patch of parasites that happened to coincide with the hybrid zone. Clearly to rigorously determine whether there is a difference in susceptibility between pure and mixed populations one would need to sample several different transects across the hybrid zone.
<table>
<thead>
<tr>
<th>Parasite transect 1</th>
<th>Col de la Lombarde</th>
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<tr>
<td></td>
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<td>13</td>
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<tr>
<th>Parasite transect 2</th>
<th>Col de la Lombarde</th>
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<td>Mixed</td>
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Table 3.16: A comparison of the numbers of grasshoppers parasitised by mermithid nematodes from parental populations and from mixed (hybrid) populations at Col de la Lombarde. a) P transect 1 b) P transect 2

Discussion

To determine whether hybrid populations are more susceptible to parasitism than pure populations, it is clearly important that there is some host response to the parasite. If *Podisma* cannot respond to *Mermis hexamemis*, it is an inappropriate parasite to test the hypothesis of reduced hybrid resistance. Fungi of the genus Entomophthora might be worth investigating in *Podisma*: *E. grylli* infects grasshoppers, and the infected individuals typically climb to the tops of plants and die with their legs wrapped tightly around the plant (Evans, 1984). During the density survey in 1989 some *Podisma* individuals were found in this attitude.

As with the other field investigations into fitness differences between pure and mixed populations, investigating possible variation in resistance to parasites in the field is complicated by several ecological variables. Parasite population dynamics as well as the interaction between host and parasite populations will further complicate an interpretation of any observed pattern in parasite load.

To start with, the distribution of parasites within populations of hosts need not be random. For example, patches of disease might develop from centres of infection and some populations would have no parasites while others could be heavily infected. In general, when dispersal is low, very dense, very local aggregates of parasites are common. The aggregates could arise because hosts vary in their susceptibility to infection, but susceptibility could be affected by environmental, or
behavioural factors as well as by genetic ones. For example, Henry (1972) found that *Nosema locustae* infections in populations of grasshoppers in Southern Idaho, varied markedly between sampling sites, but were most common at sites characterised by mixed vegetation that supported consistent and moderate densities of several species of grasshoppers. A lag-type density relationship is typical of many host-parasite interactions: host and parasite population sizes typically fluctuate out of phase so that the time of sampling is important: in a widely distributed but sub-divided host population such as *Podisma*, some parasite populations could be in the low-density phase and others in the high density phase.

Different host densities in space and time are also likely to affect parasite load: for example, Hassel (1978) reviewed several studies of parasitoids and predator-searching behaviour, and concluded that over at least part of the range of host densities, enemies spend more time searching where prey are abundant, and increase their residency time.

The patchy environment at Col de la Lombarde and differences in population structure are likely to affect parasite load. A further complication is that a parasite with a free-living stage, such as the mermithid nematodes discussed here, will only be found in the appropriate environment.

The investigation by Sage et al (1986) on parasite load across a hybrid zone between *Mus musculus* and *Mus domesticus* was slightly different from the one described here. Their 'wormy' mice were defined as those individuals containing more than 500 nematodes; 'normal' mice had fewer than 250: the important difference between their investigation and the one described here is that almost all of the mice they looked at had at least some nematodes. A difference in load between hybrid and pure populations might be more likely to reflect a difference in resistance (response) to parasites. However, in light of the investigations described in this section, it would be more convincing if they had looked at more than a single transect. Another problem with their investigation is that it gives no evidence of greater hybrid mortality or reduced hybrid fitness in the *Mus* populations.

### 3.4 Discussion

Natural selection is not easy to detect in the field. In this chapter we have seen some of the difficulties that can arise in attempting to compare components of fitness between races in natural populations: apparent density-dependent effects
complicate the interpretation of measures of fitness in the field and because hybrid populations do not show a clear reduction in density, we cannot estimate the width over which fitness components change across the zone. However, in chapter 6, I will describe another approach which gives estimates of strong selection on many loci from field investigations.
Chapter 4

Density-dependent population regulation

4.1 Introduction

In the last chapter, extensive density surveys revealed a consistent pattern across the zone: at the beginning of the season, the XY race is always more numerous than the XO race and hybrids. Unexpectedly, the XO quadrats have densities that are on average not significantly different from those of the hybrid zone. This density pattern is surprising in view of the reduction in embryo and young nymph survival seen in the laboratory, and the stability of the hybrid zone. I will discuss the implications of this trend on the position and nature of the zone further in chapter 7. Another interesting pattern revealed in the density surveys was that despite large and significant differences in nymph densities, adult densities usually differed by a smaller ratio (Table 3.12). This supports observations by Nichols (1984) and suggests some density dependent process. In view of the unexpected density trend, it would be interesting to investigate this further: in laboratory experiments (see chapter 2), survival of hybrid embryos and young nymphs was lower than that of pure individuals. However, if there is density-dependent population regulation, this reduced embryo and young nymph survival need not lead to reduced numbers of later instar nymphs or adults. For example, in a series of experiments on blowfly populations, Nicholson (1958) found that reducing larval survival could increase the numbers of adults. In this chapter I will analyse the density-dependent pattern observed along transect 1 and transect 2 in more detail. Is the observed density-dependent decrease in numbers between hatchlings and adults due to mortality or due to increased dispersal from crowded areas? Since the $(2m)^2$ quadrats used for the survey described in the last chapter are smaller than the distance moved by
nymphs, one could argue that the observed density-dependent pattern is due to increased dispersal from crowded areas, rather than to increased mortality. To test this possibility, the survey was repeated on a larger scale. In Section 4.4 simulation experiments aimed at testing the reliability of inferences from the field observations will be described. In sections 4.6 and 4.5, I will describe manipulation experiments aimed at investigating the density-dependent trends further, and at disentangling the effects of hybridity from density-dependent regulation in population change.

Apart from being of interest to the hybrid zone, the differences in density across the zone provide a useful system in which to investigate population dynamics and density dependence, so notoriously difficult to detect in natural populations.

### 4.2 Density dependence

Ecologists recognise several different relations between population density and percentage mortality. Under the simplest model of population dynamics, the growth rate will vary randomly from one year to the next due to changes in climate and other environmental factors, but will be unaffected by population size. For example, if \( x_t \) is the log of population size in year \( t \) the logarithmic rate of increase between years \( t \) and \( t+1 \) is \( x_{t+1} - x_t \); under this model, \( x_{t+1} - x_t = e_t \), where \( e_t \) is an independent random variable, usually assumed to be normally distributed. There will be no deterministic trend in population size, and no tendency for it to return to an equilibrium value. The population is said to be density-independent or unregulated. However, many populations appear to fluctuate about an equilibrium value rather than to wander randomly in size and early authors such as Howard and Fiske (1911), Nicholson and Bailey (1935) and Smith (1935) and others emphasised that density dependent factors must be responsible for stabilising animal populations. To stabilise a population the growth rate needs to decrease with population size, for example:

\[
(x_{t+1} - x_t) = -\alpha (x_t - \mu) + e_t \tag{4.1}
\]

or

\[
(x_{t+1} - \mu) = \beta (x_t - \mu) + e_t \tag{4.2}
\]

where \( \beta = 1 - \alpha \) (\( 0 < \alpha, \beta < 1 \)). If \( x_t \) is larger than the equilibrium value, \( \mu \), the growth rate is on average negative, while if \( x_t \) is less than \( \mu \), it is positive and so the population will tend to be restored to its equilibrium value. Such a population is said to be 'regulated'.
Contemporary ecology arose from a debate over population dynamics that concerned the extent to which population sizes result from density-dependent processes or from less predictable stochastic processes (Nicholson, 1958; Andrewartha and Birch, 1954). Entomologists had seen little evidence of explicit density-dependence in the field. But theoreticians argued that without regulation populations would inevitably attain very high densities or alternatively, premature extinction: persistent populations must be regulated. Their arguments were reinforced by abundant evidence of explicit density dependence in simple laboratory microcosms (see Varley and Gradwell, 1970).

There is now widespread agreement that population size varies as a result of both density-dependent and density-independent processes. However, disentangling these in natural populations has proved to be very difficult (see Hassel 1986, Strong, 1986, Varley & Gradwell, 1970). Most of the literature on density-dependent population dynamics concentrates on long term population fluctuations: traditional methods of analysis have been life-table analysis on population data collected over several generations. However, despite large numbers of field studies (particularly on insects and birds), not many have been able to detect important density-dependent processes, and hardly any have both demonstrated a specific mechanism of density-dependence and the central importance of that mechanism to population regulation (Varley & Gradwell, 1970; Dempster, 1975; Klomp, 1966; Krebs, 1970). Recent reviews of published life-tables have concluded that density-dependent regulation is infrequent in most populations (Stiling, 1988). This has prompted a debate on whether traditional methods for the detection of density-dependent regulation are adequate.

Hassel (1986, 1987) and May (1986, 1989), for example, have criticized traditional approaches for not paying attention to population structure: they have tended to assume homogeneous panmictic populations, and ignore finer scale structuring. Bulmer (1975) has pointed out that temporal trends in time series data can mask the existence of density-dependence and Turchin (1990) has shown that traditional analyses will not, in general, detect density-dependent regulation in populations that are characterized by time lagged regulation or complex dynamic behaviour. Because of the difficulty of explicit mathematical analysis, simulation has been used to test the reliability of traditional methods of analysis (e.g. Hassel, 1987; Strong, 1986; Gaston and Lawton, 1987; Mountford, 1988).

The conventional methods of analysis are aimed at detecting underlying popu-
lalation regulation from the obscuring noise which might result from environmental and demographic parameters. However, one of the criticisms of conventional methods is that they are not designed to detect the kind of density-dependent process that acts differentially between segments of a population within a generation: traditional methods tend to assume that birth and death rates are a function of average population sizes from generation to generation. However, there may be differential survivorship between 'patches' of different local population density (DeJong, 1979; Atkinson and Shorrocks, 1981) or fitness might vary between individuals due to their behaviour, timing or phenotype (Lomnicki, 1980).

Hassel (1987) has modelled a single panmictic population with a known spatial scale over which clustering occurs, thus segmenting it. His simulations demonstrate that density-dependence that acts within a generation can be important to population regulation. He has shown that although density dependence acting between segments of a population within each generation will be detected by analysis based on average population sizes per generation, if sufficient random variation in parameters affecting the spatial distribution of the insects, and in their probabilities of survival is introduced, density-dependence will not be detected by this method of analysis.

Hassel et al (1987) have applied these insights to about 20 years of data on the abundance of whitefly sub-populations on leaves of viburnum bushes and have detected density-dependent effects that were not revealed by conventional analysis of the overall (averaged) whitefly population in successive generations. Mountford (1988), on the other hand, found that for a given mean size of a population at equilibrium, stochastic heterogeneity is neutral in its effect on the detection of density-dependence by traditional methods and that spatial heterogeneity (due to ecological effects) actually enhances it.

May (1989) points out however, that the difference between Hassel and Mountford's simulations arise from a difference in the behaviour of individuals in their models. Hassel assumes "scramble" competition amongst larvae (when resources are sparse, all individuals suffer) which has potential for highly nonlinear or chaotic dynamics. Mountford on the other hand, assumes "contest" competition in which resources are distributed such that a few individuals do well even in hard times. It seems that while patchiness and stochastic effects by themselves are insufficient to upset traditional methods of data analysis, if there are severe nonlinearities in density dependent effects, conventional analysis may not detect underlying density-
dependence.

Most of the literature on population dynamics concerns single panmictic populations, and most of the methods for detecting density dependence use comparisons across time. The investigation presented here uses a different approach from the traditional time-series studies: *Podisma* is distributed more or less continuously, and I attempt to infer density-dependence by making comparisons between sites within this distribution (i.e. comparisons across space rather than time), for a continuously distributed organism rather than a single panmictic population. An inherent difficulty with this approach is that variation in initial density could reflect subtle, undetected environmental differences, and that differences in mortality could be caused by these environmental effects, rather than by initial density itself. However, this problem also applies to the more conventional comparisons of patterns across time yet is usually ignored. The problem can be minimised by sampling areas of as uniform a habitat as possible.

Since there are no natural sampling units, the unit size must be arbitrarily defined. If quadrats are scattered randomly, they cannot give direct information on the spatial structure of the population. Measures of aggregation (or clumping) based on quadrat data will typically depend on the size of the quadrat. If *Podisma* is randomly distributed within a given habitat type, then, the expected distribution of the number of nymphs per quadrat is Poisson, with parameter $\lambda$ - the mean number of nymphs per quadrat. Changing the quadrat size simply alters the magnitude of $\lambda$ ($\lambda$ being proportional to quadrat size) and the distribution remains Poisson for all quadrat sizes. However, if *Podisma* has a clumped distribution for a given habitat, (i.e. if some patches have high expected density and others low), the observed distribution across quadrats will be influenced by quadrat size (see Pielou, 1977; Kareiva, 1986). For example, a large quadrat might contain the whole of a densely occupied patch or several of these patches, whereas a small quadrat may contain only part of a dense patch. As a result, the observed (clumping) pattern will depend on the size of the quadrat.

Theoretical investigations can give an indication of the conditions under which we would expect to see density dependence occurring in a continuously distributed insect. They can also be used to investigate whether we would expect randomly scattered quadrats to be an appropriate sampling method for detecting density-dependence. Barton (unpublished) has used models extended from population genetics (Slatkin and Barton, 1990) to address these questions. He assumed a uniform environment.
and density dependence of the same strength everywhere, and investigated the spa-
tial distribution of small variations in density caused purely by demographic fluctu-
ations. The results suggest that unless neighbourhood size is extremely small, such
demographic fluctuations will be negligible, and that comparison between quadrats
would not give significant evidence of density dependence. Models in which the
underlying dynamics are chaotic, or where the environment itself varies, can give
much larger fluctuations. For example, if density-independent fecundity varies, and
one looks at density-dependent viability, one should see a density dependent pat-
tern. However, one would expect to detect density-dependence only if the size of
the sampling unit matches the scale of the density-dependent process.

4.3 Observations on natural populations

Density surveys repeated throughout the life-cycle, have been described in detail
in the last chapter. In these surveys, two transects were established, spanning the
hybrid zone, at Col de la Lombarde, and \( (2m)^2 \) quadrats matched as far as possible
for vegetation, slope and aspect were searched by two scorers for 3 minutes each.
Grasshoppers were collected into a tin packed with vegetation (for protection), and
released into the middle of the quadrat after the searching period. Calibration
indicated that 59% of the grasshoppers are caught by two scorers in a three minute
searching period and that there is no significant difference in the proportion observed
from high and low density quadrats.

Since the \( (2m)^2 \) quadrats may be smaller than the nymph dispersal radius (see
Mason, 1988), one could argue that any observed density-dependent pattern may be
due to increased dispersal from crowded areas, rather than to increased mortality.
To exclude this possibility the survey was repeated on a larger scale. Four replicate
40m by 40m grids each consisting of sixteen adjacent \( (10m)^2 \) quadrats, were set up in
1989, thus establishing 64 replicate quadrats of size larger than the nymph dispersal
radius. The grids were established in a relatively uniform area in the XY karyotype
region (see Figures 4.1 and 4.2), covering a range of habitats similar to that of the
transect 1 XY sites described in chapter 3. The grids were scored when nymphs were
at their peak density (the average instar corresponding to peak density was estimated
from the surveys described in chapter 3: peak density occurs when the population is
on average at third instar) and again when \( 3/4 \) of the individuals were adult (adult
density is defined as the density when the average instar is 5.5: when all individuals
are adult (i.e. average instar: 6.0), densities would be underestimated because of adult mortality (see chapter 3)). Two scorers searched each (10m)$^2$ quadrat for ten minutes. Calibration indicated that roughly 40% of the grasshoppers are caught in a ten minute search period and there was no significant difference in the proportion caught in high and low density sites. The calibration data, methods and results are described in detail in Appendix C.1.

For this investigation, the quadrats along transect 1 and transect 2 were classified by locality and karyotype frequency. Density-dependent effects were investigated

<table>
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<th>D1</th>
<th>E2</th>
<th>E3</th>
</tr>
</thead>
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<td>C2</td>
<td>C3</td>
</tr>
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<tr>
<td>A0</td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
</tr>
</tbody>
</table>

Figure 4.2: Diagram showing the quadrat layout within a grid. Quadrats are labelled A0 to E3 and each measured 10m by 10m.
both within and across karyotype regions. Clearly data sets with very low numbers or with a very narrow range of peak densities would not be appropriate for an investigation into density-dependence, and so only data sets with a sufficient range of peak nymph densities were used in the analyses described below. They include sites of mixed karyotype for transect 1 in 1988 and 1989 (referred to below as M188 and M189 respectively); the pure XY sites from transect 1 in 1988 and 1989 (XY188 and XY189 respectively) and the pure XY sites from transect 2 in 1989 (XY289); pooled data sets: M188+XY188, and M189+XY189 provide two further data sets for a comparison across karyotype regions; and the four grids described above (Grid 1, Grid 2, Grid 3, Grid 4) (the data are provided in Appendix G).

There are several ways of establishing whether density-dependence is occurring. The reliability of these will depend on how realistic the underlying assumptions are. I will discuss five different methods: the first three (a comparison of variances; polynomial regression of adult density on peak density; unweighted least-squares regression of mortality on peak nymph density) assume that measurement errors are normally distributed; the fourth (weighted least-squares regression) assumes that adult densities vary around some expected value (which is a function of nymph densities) with Poisson error; and the last method (based on maximum likelihood) assumes that Podisma has a clumped distribution and that further variance is introduced by Poisson sampling error.

F-tests

The simplest approach is to compare the variance of peak density with the variance of adult density. Because adult densities are lower than nymph densities, we would expect them to have a lower variance even with no density-dependence and so in order to establish whether there is density-dependence, a measure of variances relative to mean densities is needed. F-tests based on the variance-to-mean ratio for peak and adult densities are presented in table 4.1. In most cases the variance was significantly greater for nymph densities, strongly suggesting density-dependence. However, F-tests are sensitive to deviations from normality. The theoretical distribution that would best fit the observed densities is unclear here because of small sample sizes.
<table>
<thead>
<tr>
<th>Data set</th>
<th>N</th>
<th>Mean (peak)</th>
<th>Variance (peak)</th>
<th>V/M (peak)</th>
<th>Mean (adult)</th>
<th>Variance (adult)</th>
<th>V/M (adult)</th>
<th>F ratio</th>
</tr>
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<td>16</td>
<td>73.94</td>
<td>408.99</td>
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<td>21.69</td>
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<td>0.78</td>
<td>7.09**</td>
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<td>50.14</td>
<td>266.74</td>
<td>5.32</td>
<td>24.93</td>
<td>36.38</td>
<td>1.46</td>
<td>3.64**</td>
</tr>
<tr>
<td>Grid 3</td>
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<td>22.00</td>
<td>68.13</td>
<td>3.10</td>
<td>9.06</td>
<td>4.99</td>
<td>0.55</td>
<td>5.64**</td>
</tr>
<tr>
<td>Grid 4</td>
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<td>23.33</td>
<td>49.95</td>
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<td>14.21</td>
<td>19.41</td>
<td>1.36</td>
<td>1.57ns</td>
</tr>
<tr>
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<td>32</td>
<td>3.20</td>
<td>16.90</td>
<td>5.28</td>
<td>0.59</td>
<td>0.87</td>
<td>1.47</td>
<td>3.59**</td>
</tr>
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<td>35.92</td>
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<td>3.09</td>
<td>2.81</td>
<td>0.91</td>
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</tr>
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<td>8.66</td>
<td>2.75</td>
<td>1.73</td>
<td>1.65</td>
<td>0.95</td>
<td>2.89**</td>
</tr>
<tr>
<td>XY189</td>
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<td>9.45</td>
<td>19.68</td>
<td>2.08</td>
<td>2.67</td>
<td>2.91</td>
<td>1.10</td>
<td>1.89**</td>
</tr>
<tr>
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<td>102.47</td>
<td>6.63</td>
<td>3.00</td>
<td>3.68</td>
<td>1.23</td>
<td>5.39**</td>
</tr>
</tbody>
</table>

Table 4.1: A comparison of variances relative to mean densities, for each of the eleven field data sets (first column - see text). The second column gives the number of quadrats in each data set. The mean and variance of peak (nymph) densities and adult densities are provided. Column 5 gives the variance:mean ratio of peak (nymph) densities and column 6, the variance-to-mean ratio of adult densities. F-tests (last column) are used to determine the level of significance in the difference between these ratios (** = significant at the 1%; * = significant at the 5% level; ns = not significant).

Quadratic regression analysis

Simple plots of adult density \((F)\) against peak nymph density \((P)\) suggest density-dependence of the form: \(F = (1 - a)P - bP^2\), which gives \(1 - F/P = a + bP\) as the mortality, \(a\) as the density independent mortality and \(bP\) as the density-dependent contribution to mortality. We expect values of \((1 - a)\) and \(b\) such that \(P < (1 - a)/2b\) \((P,\) the position of the maximum of the function. Values of \(a\) and \(b\) were such that \((1 - a)/2b > P\) in all cases). To test whether the model is reasonable, polynomial regression analysis was performed on the field data sets, using SAS. Appendix C.2 summarises the results: in most cases a no-intercept quadratic regression gave a better fit than a linear regression, and higher order terms did not improve the fit significantly.

Regression of mortality on peak nymph density

Plots of proportional drop in numbers \((1 - F/P)\) on peak nymph density are provided in Figures 4.3 and 4.4. They suggest a strong density-dependent effect. This is particularly marked for the \((10m)^2\) quadrats. Note in Figure 4.4 that the effect is not as strong for Grid 1 as it is for Grids 2, 3 and 4. Grid 1 has higher peak densities than the others and so these may have been underestimated (though the calibration suggests there is no difference between the proportion caught in high density and
low density quadrats) or it could be that the carrying capacity is higher in Grid 1 for obscure reasons.

Note that when the peak density is low, detecting density dependence by regression of mortality on peak density breaks down because sample sizes are small. In some of the very high density sites peak densities may have been underestimated. Unweighted least-squares regression was used to estimate strength of density-dependence (given by the slope of the regression of mortality on peak density). In most cases this slope was positive (see Figures 4.3, 4.4 and Table 4.2).

Clearly a problem with all the methods described so far is that they assume that measurement errors are normally distributed. In a more realistic model, patches would vary in carrying capacity around some expected nymph density, $E(P)$. As a rough approximation assume that the true nymph density is approximately equal to the observed value (here the sampling error contributed by nymph densities is ignored because peak densities are much larger than adult densities). Then \(1 - F/P\) is distributed around some expected value, \(\hat{F} = aP - bP^2\), with, in the simplest case, \(F\) varying because of Poisson sampling error only. A weighted regression analysis of mortality on peak density assuming a Poisson error can then be used to analyse the data.

\[
\text{var}(1 - F/P) \approx \frac{\text{var}(F)}{P^2} \quad (4.3)
\]

Since \(F\) is assumed to have Poisson error

\[
\text{var}(1 - F/P) \approx \frac{\text{var}(F)}{P^2} \approx \frac{E(F)}{P^2} \approx \frac{(aP - bP^2)}{P^2} \approx (a/P - b) \quad (4.4)
\]

The weighting factor is therefore: \(1/\text{var}(F)\) i.e. \(\frac{1}{(a/P - b)}\) and using a least squares method, \(\hat{b}\) is given by:

\[
\hat{b} = \frac{\sum \frac{(P - \hat{P})(m - \hat{m})}{a/P - b}}{\sum \frac{P - \hat{P}}{a/P - b}} \quad (4.5)
\]

Thus \(b\) (a measure of the density-dependent mortality) is estimated iteratively, starting with an initial estimate derived from simple least-squares regression. This method assumes that all error is in \(F\) not \(P\), a fair assumption because \(P >> F\).
Figure 4.3: Mortality ($M$) (i.e. proportional drop in numbers: $1 - F/P$) against initial peak nymph density ($P$) at Col de la Lombarde for five sets of $(2m)^2$ quadrats. The line gives the best fitting linear relation, fitted by maximum likelihood (see Table 4.2). a: Hybrid sites, Transect 1, 1989. b: XY sites, Transect 1, 1988. c: Hybrid sites, Transect 1, 1988. d: XY sites, Transect 1, 1989. e: XY sites, Transect 2, 1989. Datasets M188, XY188, M189, XY189, XY289 are defined in the text. $F$=adult density; $P$=peak nymph density. Note that for the model $F = aP - bP^2$, a plot of $1 - F/P$ versus $P$ will be a straight line with intercept $(1 - a)$ and slope $b$. 
Figure 4.4: Mortality ($M$) (i.e. proportional drop in numbers: $1 - F/P$) against initial peak nymph density ($P$) at Col de la Lombarde for four sets of $(10m)^2$ quadrats. The line gives the best fitting linear relation, fitted by maximum likelihood (see Table 4.2).
Estimates using maximum likelihood

Perhaps the most appropriate method for determining whether \( b \) is significantly different from zero or not is maximum likelihood. Maximum likelihood is useful in finding the plausible range of parameters (here, \( b \)) from a limited set of experimental data. One judges the plausibility by asking: what is the chance of obtaining the data given \( b \)? (i.e. what is the likelihood of \( b \)). Because the sample sizes are small, it would be useful to be able to combine results from several data sets. Maximum likelihood enables one to do this: a log transformation of the likelihood curves allows one to add up the results of different experiments (i.e. to multiply the probabilities of the 'b's' resulting from different independent experiments).

To estimate \( b \) by maximum likelihood, the distribution of \( F \) must be specified. The simplest assumption is that \( F \) is Poisson distributed with expectation \( aP - bP^2 \). However, this neglects underlying variation in density between quadrats. Contagious or clumped distributions are common in insect populations and can result from several different mechanisms (see Karieva, 1986, and Pielou, 1977). If we assume that Podisma is clumped, then the number of individuals per quadrat will have a distribution with a large variance. Thus we can assume that the actual (or expected) densities have an underlying Gamma distribution. Indeed, whatever the true distribution of densities, it is likely that some Gamma distribution can be found to approximate it closely:

\[
\psi(x) = x^{\beta-1}e^{-ax} \frac{\alpha^\beta}{\Gamma(\beta)} dx
\]  

(4.6)

where \( x \) is the expected adult density. Further variance is likely to be introduced by Poisson sampling error: the probability of sampling \( j \) grasshoppers will be:

\[
P_j = \frac{e^{-x}x^j}{j!}
\]

(4.7)

where \( x \) is the expected adult density. Thus the overall probability \( P_j \), of sampling \( j \) grasshoppers from a quadrat will be:

\[
P_j = \int_0^\infty (x^{\beta-1}e^{ax} \frac{\alpha^\beta}{\Gamma(\beta)}) (e^{-x}x^j/j!) dx
\]

(4.8)

which reduces to the negative binomial distribution:

\[
P_j = \frac{\alpha^\beta}{\Gamma(\beta)j!} \int_0^\infty x^{j+\beta-1}e^{-(a+1)x} dx
\]

(4.9)

\[
= \frac{\alpha^\beta \Gamma(j + \beta)}{\Gamma(\beta)j!(\alpha + 1)^{j+\beta}}
\]
The mean of this distribution is: \( \beta/\alpha \) and the variance is: \((\beta/\alpha) + (\beta/\alpha^2)\). Thus the variance is greater than a simple Poisson distribution by: \((\beta/\alpha^2)\). Let the variance to mean ratio \( v/m = k \). Then \( \beta = m/k - 1 \), and since \( \beta/\alpha = m \), \( \alpha = 1/k - 1 \). In terms of David and Moore's index of clumping (i.e. \( I = (v/m) - 1 \)), \( \beta = m/I \), and so low values of \( \beta \) indicate pronounced clumping and high values, only slight clumping.

Comparisons of parameter estimates using unweighted and weighted regression and maximum-likelihood are provided in Table 4.2. Log-likelihood support curves for the field datasets are provided in Figures 4.5, 4.6 and 4.7. All maximum-likelihood estimates were obtained assuming a variance-to-mean ratio of \( k = 2 \), since Table 4.1 suggests that \( k \) will be \( \approx 2 \) (results are similar for \( k = 1 \)).

The results from these various approaches, although noisy, do all suggest density-dependence. The larger scale quadrats show a convincing density-dependent pattern, arguing that density-dependent mortality rather than dispersal is the cause.

In the next section I will describe simulation experiments which aim to show whether it is reasonable to infer density-dependence from the relations observed in natural populations, or whether these are a statistical artefact of the sampling procedure. They will also be used to assess which method of analysis gives the most reliable estimates.

### 4.4 The simulation experiments

#### 4.4.1 Methods

In the model, \( F = aP - bP^2 \). Naive argument would suggest that if \( b \) is significantly different from zero, then there is evidence of density-dependent mortality. However, it could be that sampling error or clumping create the spurious impression of density-dependent mortality.

As discussed above, it is likely that the actual (or expected) densities approximate to an underlying Gamma distribution, and that further variance will be introduced by Poisson sampling error. Thus, the overall distribution of nymph densities can be described by a negative binomial, as before (see equation 4.10). The expected adult density is a function of the peak nymph density: \( E(F) = aP - bP^2 \) (where \( P \) is the true 'peak' or nymph density). Thus, the expected adult densities were calculated for different strengths of density-independent and density-dependent mortality, by varying the parameters \( a \) and \( b \) in the model. Observed adult densi-
<table>
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<tr>
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<th>PARAMETER</th>
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<th>WEIGHTED REGRESSION</th>
<th>MAXIMUM LIKELIHOOD</th>
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<td></td>
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Table 4.2: A comparison of estimates from field data sets of the parameters “a” and “b” in the model $F = aP - bP^2$, where $F$ is the adult density and $P$ is the peak nymph density (see text). For dataset code definitions see text.
Figure 4.5: 1988 Transect 1: Log-likelihood support curves from maximum-likelihood analysis of field data sets. Horizontal axis: the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value.
Figure 4.6: 1989 Transect 1 and Transect 2: Log-likelihood support curves from maximum-likelihood analysis of field data sets. Horizontal axis: the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value.
Figure 4.7: Combined datasets, 1988, 1989: Log-likelihood support curves from maximum-likelihood analysis of field data sets. Horizontal axis: the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value.
ties were generated from these expected values by Poisson sampling alone, or with Gamma error followed by Poisson sampling. Details of the programmes and algorithms are available on request. Pseudo-data sets consisting of pairs of densities (nymph and adult) were generated in this way, for different values of \( b \) (measure of density-dependent mortality), \( a \) (density independent mortality), and \( k = v/m \) (the negative binomial 'clustering parameter'). The simulation experiments can be used to investigate the effects of altering \( k \). Recall that as \( k \) approaches 1, the negative binomial distribution approaches a Poisson (note that \( \beta = m/k - 1; \alpha = 1/k - 1 \)).

4.4.2 Analysis and results

The unweighted and weighted regression and maximum-likelihood methods described earlier were used to estimate density-dependence. The approach was to compare estimates of \( 'b' \) with the underlying values provided in the simulations. The distribution of estimates from a large number of replicate synthetic data sets (generated with a known \( b, a \) and \( k \)) gives the bias and error associated with the method of analysis. Data sets having a large number of quadrats (say, 100) can be compared with those for which each replicate is of size similar to the smaller field data sets (where \( N \) was often as low as 25). Thus, the simulations were aimed at addressing the following questions:

1. Given \( b=0 \), does the variance in both nymph and adult density create the spurious effect of density-dependent mortality?

2. Given that there is underlying density-dependence, would we expect to detect the effect despite the introduction of random noise?

3. For a given underlying density-dependence, what would be the expected range of estimates?

4. How do the results from the simulated experiments compare with estimates from natural populations?

The values of parameters \( a \) and \( b \) used in the simulation model \( (F = aP - bP^2) \) were compared with the estimates of these parameters from unweighted and weighted regression analysis on the simulated data sets. A comparison of mean \( a \) and mean \( b \) (the mean of 100 replicate runs in all cases) suggests that even unweighted regression gives reasonable estimates of these parameters.
Table 4.3 provides a comparison of weighted and unweighted regression analyses. The underlying parameters used in generating each set of 100 or 25 replicates are described and the mean estimated parameters and their standard deviations provided.

Table 4.4 gives the range of estimates for each data set (out of 100 replicates). The results suggest very little bias even when k is high. Increasing the variance in adult density (k=2), does not alter the reliability of estimates, and nor does an increase in a (cf a=0.4 and a=0.2).

4.4.3 Maximum likelihood

Maximum likelihood estimates were obtained by searching a two-dimensional grid in a-b space, first on a coarse scale - to avoid detecting only a local minimum - and then on a finer scale, to improve the accuracy of estimates. The maximum likelihood estimates of b were compared with the true values for a large number of replicate synthetic data sets, generated with a known b, a and k.

Tables 4.5 and 4.6 summarise the results along with the parameters used to generate each data set. Figures 4.8, 4.9, 4.10 and 4.11 show maximum likelihood support curves. These were obtained by stacking the support curves for each independent replicate. Overall maximum likelihood values of b are provided.

The results suggest that this method is appropriate for inferring density-dependence from the field data sets. This is true even for small data sets. The largest error in b occurs with small sample sizes, and when the variance in adult densities is increased (i.e. if a negative binomial distribution of adults is assumed rather than a Poisson). However, when the underlying b is set to zero in the simulation model, the maximum likelihood estimate of b from the resulting data set is never significantly different from zero; and conversely, if b is set to 0.004 the maximum likelihood estimate is always significantly different from zero. The results suggest that the maximum likelihood estimates of density-dependent mortality in section 4.3, are reliable, and are more accurate than the other methods.

A comparison of estimates from the large and small sampling units is of interest. One would expect the process to be detected only if the sampling units are of the appropriate size (they should match the scale of the density-dependent process). To compare density-dependent regulation, the b’s should be standardised relative to the magnitude of the peak nymph density. The density dependent mortality (bP), calculated from the average values of b and P is 0.10 for the (2m)^2 quadrats and
Table 4.3: Parameter estimates for simulated data sets, using unweighted- and weighted regression. The first column gives details of parameters provided in the simulation (i.e. true values); columns 2 and 4 provide values of $a$ and $b$ as estimated using corresponding methods in column 6 (table continued overleaf).

<table>
<thead>
<tr>
<th>details on data sets</th>
<th>mean estimate of parameters and corresponding standard deviation</th>
<th>methods</th>
</tr>
</thead>
<tbody>
<tr>
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<td>mean</td>
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</tr>
<tr>
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</tr>
<tr>
<td># quadrats in each=100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V/N$ (nymphs)=12</td>
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<td></td>
</tr>
<tr>
<td>$V/N$ (adults)=2</td>
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<td>0.17</td>
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<tr>
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<td>$V/N$ (adults)=1</td>
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<td># quadrats=25</td>
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<td>methods</td>
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<td># quadrats in each-100</td>
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<td>V/M(nymphs)=12</td>
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<td>V/M(adults)=1</td>
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<td>0.023</td>
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<td></td>
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<tr>
<td>V/M(adults)=2</td>
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<tr>
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<td>0.042</td>
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<td># quadrats=100</td>
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<tr>
<td>V/M(adults)=2</td>
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</tr>
<tr>
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<td>0.056</td>
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<td></td>
</tr>
<tr>
<td># quadrats=100</td>
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<td></td>
</tr>
<tr>
<td>V/M(nymphs)=12</td>
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<td></td>
</tr>
<tr>
<td>V/M(adults)=2</td>
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</tr>
<tr>
<td>DATA SET</td>
<td>UNWEIGHTED REGRESSION</td>
<td>WEIGHTED REGRESSION</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>a=0.2, b=0.004 V/M(nymphs)=12, V/M(adults)=2</td>
<td>-0.0002 to 0.013</td>
<td>-0.001 to 0.008</td>
</tr>
<tr>
<td>a=0.2, b=0.0 V/M(nymphs)=12, V/M(adults)=2</td>
<td>-0.002 to 0.005</td>
<td>-0.002 to 0.003</td>
</tr>
<tr>
<td>a=0.4, b=0.004 V/M(nymphs)=8, V/M(adults)=2</td>
<td>0.001 to 0.013</td>
<td>0.001 to 0.008</td>
</tr>
<tr>
<td>a=0.2, b=0.008 V/M(nymphs)=8, V/M(adults)=2</td>
<td>0.001 to 0.006</td>
<td>0.001 to 0.003</td>
</tr>
<tr>
<td>a=0.2, b=0.004 V/M(nymphs)=8, V/M(adults)=1</td>
<td>0.001 to 0.009</td>
<td>0.001 to 0.005</td>
</tr>
<tr>
<td>a=0.2, b=0.004 V/M(nymphs)=12, V/M(adults)=1</td>
<td>0.001 to 0.006</td>
<td>0.001 to 0.004</td>
</tr>
<tr>
<td>a=0.2, b=0.0 V/M(nymphs)=12, V/M(adults)=1 only 25 quads.</td>
<td>-0.005 to 0.03</td>
<td>-0.005 to 0.01</td>
</tr>
<tr>
<td>a=0.2, b=0.0 V/M(nymphs)=2, V/M(adults)=1 only 25 quads.</td>
<td>-0.005 to 0.03</td>
<td>-0.005 to 0.01</td>
</tr>
<tr>
<td>a=0.2, b=0.004 V/M(nymphs)=12, V/M(adults)=1 only 25 quads.</td>
<td>-0.007 to 0.01</td>
<td>-0.003 to 0.007</td>
</tr>
<tr>
<td>a=0.03, b=0.004 V/M(nymphs)=8, V/M(adults)=1</td>
<td>-0.007 to 0.01</td>
<td>-0.003 to 0.008</td>
</tr>
</tbody>
</table>

Table 4.4: Table to show the range of estimates of $b$ (over 100 replicates) obtained using unweighted and weighted regression (column 2 and 3, respectively) for various true parameter values provided in the simulations (column 1).
<table>
<thead>
<tr>
<th>details on data sets</th>
<th>Maximum - likelihood estimate of ( 'b' )</th>
<th>proportion for which ( 'b' ) is NOT significantly different from zero</th>
<th>Range of ( 'b' ) significantly DIFFERENT from zero</th>
</tr>
</thead>
<tbody>
<tr>
<td>a=0.2</td>
<td>b=0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td># replicates=100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># quadrats in each=100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/M(nymphs)= 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/M(adults)= 2</td>
<td>0.0025</td>
<td>0.10</td>
<td>0.0005 - 0.0045</td>
</tr>
<tr>
<td>a=0.2</td>
<td>b=0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td># replicates=100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># quadrats in each=100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/M(nymphs)= 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/M(adults)= 1</td>
<td>0.004</td>
<td>0.00</td>
<td>0.0025 - 0.0044</td>
</tr>
<tr>
<td>a=0.2</td>
<td>b=0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td># replicates=100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># quadrats in each=25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/M(nymphs)= 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/M(adults)= 1</td>
<td>0.0036</td>
<td>0.00</td>
<td>0.002 - 0.0062</td>
</tr>
<tr>
<td>a=0.2</td>
<td>b=0.004</td>
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</tr>
<tr>
<td># replicates=100</td>
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<td></td>
</tr>
<tr>
<td># quadrats in each=25</td>
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<td></td>
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</tr>
<tr>
<td>V/M(nymphs)= 4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>V/M(adults)= 1</td>
<td>0.0036</td>
<td>0.00</td>
<td>0.002 - 0.0057</td>
</tr>
</tbody>
</table>

Table 4.5: Maximum likelihood estimates of density-dependence \( (b) \), for simulated data sets, for which the underlying density-dependence (i.e. \( b \) provided in the simulation) is moderate \( (b=0.004) \). The second column gives the proportion of replicates for which the maximum-likelihood method gives \( b \) NOT significantly different from zero. The third column gives the range of \( b \) NOT significantly different from the underlying value \( (0.004) \). See figure 4.8 and 4.9.
<table>
<thead>
<tr>
<th>details on data sets</th>
<th>Maximum Likelihood estimate of 'b'</th>
<th>proportion for which 'b' is significantly different from zero</th>
<th>range of 'b' not significantly different from zero</th>
</tr>
</thead>
</table>
| a=0.2 
b=0.0 
# replicates=100 
# quadrats in each=100 
V/M(nymphs)=12 
V/M(adults)=1 | 0.00 | 0.02 | 0.0 - 0.0008 |
| a=0.2 
b=0.0 
# replicates=100 
# quadrats in each=25 
V/M(nymphs)=2 
V/M(adults)=1 | 0.0008 | 0.11 | 0.0 - 0.006 |
| a=0.2 
b=0.0 
# replicates=100 
# quadrats=25 
V/M(nymphs)=8 
V/M(adults)=1 | 0.00 | 0.04 | 0.0 - 0.0007 |
| a=0.2 
b=0.0 
# replicates=100 
# quadrats=100 
V/M(nymphs)=12 
V/M(adults)=2 | 0.0008 | 0.09 | 0.0 - 0.0033 |

Table 4.6: Maximum likelihood estimates of density-dependence (b), for simulated data sets, where there is no underlying density-dependence (i.e. b=0.00. The second column gives the proportion of replicates for which b IS significantly different from zero. The third column gives the range of b NOT significantly different from the underlying value (0.00). See figures 4.10 and 4.11.
Figure 4.8: Log-likelihood support curves produced by averaging support curves for each replicate. In all cases, the underlying $b$, (provided in the simulations) was 0.004. Horizontal axis: estimates of the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value. The solid horizontal line shows the maximum likelihood value obtained by when individual maximum likelihoods (for each replicate) are added; dashed line shows the 2-unit support limit.
Figure 4.9: Log-likelihood support curves produced by averaging support curves for each replicate. In all cases, the underlying $b$, (provided in the simulations) was 0.004. Horizontal axis: estimates of the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value. The solid horizontal line shows the maximum likelihood value obtained by when individual maximum likelihoods (for each replicate) are added; dashed line shows the 2-unit support limit.
Figure 4.10: Log-likelihood support curves produced by averaging support curves for each replicate. In all cases, the underlying $b$, (provided in the simulations) was zero. Horizontal axis: estimates of the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value. The solid horizontal line shows the maximum likelihood value obtained by when individual maximum likelihoods (for each replicate) are added; dashed line shows the 2-unit support limit.
Figure 4.11: Log-likelihood support curves produced by averaging support curves for each replicate. In all cases, the underlying $b$, (provided in the simulations) was zero. Horizontal axis: estimates of the parameter 'b' - a measure of density-dependent mortality (see text); vertical axis: corresponding log-likelihood value. The solid horizontal line shows the maximum likelihood value obtained by when individual maximum likelihoods (for each replicate) are added; dashed line shows the 2-unit support limit.
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<tr>
<th>Day</th>
<th>Percentage survival</th>
<th>Mean instar</th>
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<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>15</td>
<td>0.88</td>
<td>0.52</td>
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<td>25</td>
<td>0.85</td>
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</tr>
<tr>
<td>35</td>
<td>0.18</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 4.7: Percentage survival of individuals in 'low-' and 'high-' density cage experiments at Col de la Lombarde (see text for details).

0.35 for the $(10m)^2$ quadrats. This suggests greater density-dependent mortality on the larger scale.

So far I have presented evidence suggesting a density-dependent decrease in numbers between hatching and adulthood from observations on natural populations made on two spatial scales. I have presented simulation experiments that suggest that inferences from the field observation are reliable.

Other lines of evidence for within generation density-dependent mortality come from manipulation experiments at Col de la Lombarde. I will describe these in the next section.

### 4.5 Enclosure experiments

Four $(2m)^2$ enclosures made of wire and wedding veil were set up at Col de la Lombarde, in areas of suitable vegetation (a mix of *Vaccinium myrtillus*, *Vaccinium uliginosum* and herbs). Second instar nymphs were collected from nearby using a pooter, and transferred to the enclosures to establish two low density enclosures, with 20 to 30 nymphs each; and two high density enclosures with 60 nymphs each. Table 4.7 summarises the results. The enclosures showed higher mortality at high densities; however, there was no marked difference in rate of development.

### 4.6 Transfer experiments

Nymphs were collected from sites well away from the study area and were subsequently released into isolated patches of suitable vegetation. This ensured that experimental conditions were as natural as possible, and avoided the complications associated with field enclosures (e.g. artificial shading, and disaster by storm or vandalism).
4.6.1 The sites

Col de la Lombarde provides an excellent area for transfer experiments: there are extensive scree slopes which have isolated or semi-isolated patches of vegetation suitable to *Podisma* (see Figure 4.12). Isolated sites were chosen on the grounds that they would support *contained* populations (sensu Southwood, 1978) suitable for density manipulation. In order to follow the fate of transferred nymphs, the karyotype difference was used as a marker: four of the sites were chosen in the XY region and four in the XO region; nymphs collected from the pure XY area were transferred to the pure XO sites and vice-versa. This arrangement allows four replicates in each of two density categories, and two replicates in each chromosomal area. In this discussion transfer sites situated in the pure XO region will be referred to as *TO* (i.e. *TO*1, *TO*2, *TO*3, *TO*4) and those in the pure XY region as *TY* (i.e. *TY*1, *TY*2, *TY*3, *TY*4).

The sites were chosen early in the season (7-9 July, 1988) and choice was based on the size of the isolated patch and the vegetation present, in particular, on the area of *Vaccinium* cover. The survey included a brief search for native *Podisma* and
Table 4.8: Features of the Transfer Sites. Column six gives distances to the closest vegetation patches.

The presence of grasshoppers was taken as confirmation that the site was suitable. Details of the major features of each site can be found in table 4.8. Vegetation abundance scores for the transfer sites are provided in Table 4.9.

4.6.2 Preparation of the sites

In order to follow the fate of transferred grasshoppers, the sites were cleared of native *Podisma* as far as possible, and replaced with chromosomally marked grasshoppers. Appropriate experimental densities were calculated on the basis of the numbers of natives found in each site during the clearing. Sites were cleared when nymphs were on average second instars, rather than first, to minimize recruitment from subsequent hatchings within sites.

The TY sites were found to be unexpectedly dense, exceeding the highest density found in the extensive surveys described in chapter 3. The most densely populated was TY2, with an estimated 21 grasshoppers per m². This can probably be attributed in part to the presence of *Polygonum alpina* (see discussion), but TY1, TY3, and TY4 in which this species did not occur also had high densities.

Clearing these very high density TY sites until the catch rate dropped to zero was impractical. In any case it was not necessary to clear the sites completely: the numbers remaining and the proportion of chromosomally marked grasshoppers present could be estimated (see below). Native nymphs from each site were collected over a period of between 15 and 30 minutes (depending on densities). Numbers caught were recorded every 5 minutes. Although the numbers diminished with time, they had not dropped to zero after 35 minutes of searching. The dense TY

<table>
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<th>Site</th>
<th>Total area</th>
<th>Area of Vaccinium</th>
<th>Slope</th>
<th>Aspect</th>
<th>Degree of isolation</th>
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<td>24m²</td>
<td>8.8m²</td>
<td>0°</td>
<td>SW</td>
<td>10m</td>
</tr>
<tr>
<td>TO2</td>
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<td>4.7m²</td>
<td>30°</td>
<td>S</td>
<td>10m</td>
</tr>
<tr>
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<td>flat</td>
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</tr>
<tr>
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<td>20°</td>
<td>N</td>
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</tr>
<tr>
<td>TY1</td>
<td>20m²</td>
<td>3.5m²</td>
<td>15°</td>
<td>WSW</td>
<td>4.0-4.5m</td>
</tr>
<tr>
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<td>35m²</td>
<td>10.3m²</td>
<td>10°</td>
<td>N</td>
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<td>49m²</td>
<td>7.6m²</td>
<td>15°</td>
<td>SW</td>
<td>3.0-3.5m</td>
</tr>
<tr>
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<td>19.3m²</td>
<td>3°</td>
<td>WSW</td>
<td></td>
</tr>
<tr>
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<td>Sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TO1</td>
<td>TO2</td>
<td>TO3</td>
<td>TO4</td>
<td>TY1</td>
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<td>0</td>
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<td>16</td>
<td>16</td>
<td>2</td>
<td>13</td>
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<td>Juniperus nana</td>
<td>18</td>
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<td>0</td>
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<tr>
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<td>13</td>
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<td>0</td>
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<tr>
<td>Lic</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>8</td>
<td>4</td>
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<tr>
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<td>33</td>
<td>13</td>
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<td>0</td>
<td>4</td>
<td>19</td>
<td>1</td>
</tr>
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<td>22</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
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<td>4</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>0</td>
</tr>
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<td>Deschampsia</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>9</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>11</td>
</tr>
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<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Sempervivum</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ranunculus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>earth</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Antennaria</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pedicularis</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.9: Vegetation abundance scores for the transfer sites. Each site was subdivided into adjacent quadrats, each \( \approx (2m)^2 \) in area. The species present at the corners of these were recorded. This table gives total scores for each site.
Table 4.10: This table shows the numbers of native *Podisma* per m$^2$ of vegetation caught in 30 minutes for the TO sites and in 45 minutes for the TY sites; the estimated "normal" number per m$^2$ of vegetation (see text); the designated density categories; the estimated numbers remaining after the clearing (per m$^2$ vegetation) and the estimated total number per m$^2$ vegetation after transferring foreign nymphs at the start of the experiment. TY1, TY2 and TY4 were cleared twice before transfer, because these sites were exceptionally densely populated.

sites (TY1, TY2, and TY4) were cleared for a second time, five days later.

### 4.6.3 Estimation of population sizes

Estimation of the original native population size in each site was necessary to provide a standard for calculating suitable experimental densities (these are rough estimates, more accurate measures emerged after the experiment, see below). Original densities were estimated from the numbers of nymphs caught over a series of five minute intervals (a total of 45 minutes). The drop off in numbers with time approximated an exponential decay and the total numbers of nymphs present before clearing were estimated. This also allowed numbers of natives remaining in each site to be estimated (Table 4.10). For those sites that were cleared twice, estimates of population size were calculated from both counts.

### 4.6.4 The density manipulations

The numbers to be transferred were based on an estimate of 'standard' numbers for each site. These were calculated according to the initial native densities in the site; the area of *Vaccinium*; and the densities observed in comparable habitats (as part of the extensive density survey described in chapter 3). Sites were designated 'high' or 'low' density and numbers to be transferred were calculated accordingly:

1. **The TO sites** Sites TO1, TO2 and TO4 had very much lower densities per square metre than would be expected from the density survey (chapter 3). TO3 had a population size similar to that expected from the density survey, possibly
because it was not as isolated as the others. The estimation of standard densities for all TO sites were therefore based on that of TO3. Sites designated High density had their “standard” number increased to densities comparable to the highest densities observed in the survey of natural populations. Low density sites had their numbers matched to those expected in low density areas from the density survey described in Chapter 3 (Table 4.10).

2. The TY sites the standard densities for the TY sites were calculated individually. They were not as isolated as the TO sites, and so in each case the “standard” was equal to the estimated native density. The density surveys (chapter 3) indicated that the standard densities in these sites were higher than would be expected, and so numbers in sites designated high density were not necessarily increased. In low density sites, the standard was halved. Table 4.10 provides a summary.

4.6.5 Collection of grasshoppers to be transferred

Areas for collection were chosen well away from the experimental sites. Second instars were collected for transfer to TO sites (most native nymphs were second instars). The TY sites had more variation in instars, and so both second and third instars were transferred to these. Nymphs were collected (using a pooter to avoid damage) during the day and kept in bulk cages overnight. They were released while still cold and immobile the following morning, thus minimizing ‘agitation dispersal’. Keeping nymphs in bulk cages does not appear to harm them: Podisma survive well in bulk cages for several days.

4.6.6 The mark-release experiment

Fluorescent dust was sprinkled into the bulk cages before releasing the nymphs. This method of marking minimizes handling; it has been used widely in Drosophila with no adverse effects and does not seem to harm Podisma. The mark-release-recapture experiment served to determine the proportion of chromosomally marked (and dusted) nymphs in each site.

Cold and immobile Podisma nymphs were released into the TO sites at 11h30, and each site was scored between 17h15 and 18h15 on the same day. Nymphs were released into the TY sites and recaptured the following morning at 1100h. Table 4.11 provides details on numbers of nymphs released into each site, and the
proportions of marked and unmarked nymphs recaptured. The bright orange dust may have introduced some bias in recapturing and so interpretations should be made with caution. However, surrounding areas were checked briefly for dusted nymphs, which would have been easily visible. The results of this search, together with the recapture in each site, yield some interesting observations which will be discussed below.

### 4.6.7 Successive density scores

Sites were scored for density changes twice during the season. More frequent searches were impractical, unnecessary (since most of the error is likely to be between replicates and not between searches) and would have introduced extra disturbance. The nymphs were caught by hand and kept in tins packed with vegetation for protection. On the first scoring, numbers and instars were recorded at five minute intervals until at least 80% had been caught. The nymphs were subsequently released. On the final count, scoring continued until the numbers dropped to zero; females were released and males were taken for karyotyping. All scoring was done on very comparable, fine days. TO sites were scored 14 days after transfer (29-7-88; average instar 3.2) and 13 days later (11-8-88; average instar 4.5); the TY sites were scored 13 days after the transfer and then 14 days later. The date for the final counts was chosen to allow enough time for most of the female grasshoppers to have mated, but was early enough for the numbers not to have diminished too drastically.

<table>
<thead>
<tr>
<th>Site</th>
<th>Numbers released</th>
<th>Numbers Recaptured</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>marked</td>
<td>unmarked</td>
</tr>
<tr>
<td>TO1</td>
<td>80</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>TO2</td>
<td>70</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>TO3</td>
<td>39</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>TO4</td>
<td>32</td>
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<td>0</td>
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<tr>
<td>TY1</td>
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<td>42</td>
<td>12</td>
</tr>
<tr>
<td>TY3</td>
<td>242</td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>TY4</td>
<td>155</td>
<td>42</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 4.11: Results of the mark-release-recapture experiments.
4.6.8 Density counts and collection from the surrounding areas

A search of the areas surrounding the transfer sites (see Table 4.12) was made on the day of the final counts: numbers were recorded (over a five minute period per \((2m)^2\) vegetation) and all males were collected for subsequent karyotyping. This served several purposes: 1) to investigate whether the transfer sites were isolated or 'contained' areas; 2) to determine the extent to which the grasshoppers dispersed from their release sites and 3) to investigate whether dispersal depended on density.

All males caught during the final counts from the sites and their surrounding areas were collected and dissected the same evening. Their testes were removed and fixed in freshly made 3:1 mixture of ethanol and glacial ethanoic acid. On returning to London, the testes were karyotyped: a few follicles from each testis were stained in propionic orcein and examined under a microscope. The karyotypes described by John and Hewitt (1970) were identified on the basis of at least two or three separate cells in the meiotic metaphase.

The karyotype data from each site and its surrounding areas provides information on the fate of transferred grasshoppers.

4.6.9 Results

Estimates of population size

Table 4.10 gives estimates of the original numbers (of natives) in each site. The TY sites had unusually high densities. The substantial drop in total numbers caught in the second clearing for TY4 and TY1 suggests that the observed decrease with time was not merely due to behavioural response to disturbance. A brief search in the TO sites five days after they were cleared confirmed the very low estimates of residual numbers in these sites.

The recapture

Results on the recapture of foreign dusted nymphs (Table 4.11) should be interpreted with caution: bias introduced by the very bright orange fluorescent dye used could well account for the high proportion of recaptures. The close resemblance between proportions of marked grasshoppers released in the TY sites and proportions recaptured either suggests that this bias is small or (more likely) that the original population sizes were underestimated by the Zippin (1958) method. Using the re-
<table>
<thead>
<tr>
<th>Site</th>
<th>Surrounding area</th>
<th>Distance away</th>
<th>Area</th>
<th>Vegetation type</th>
<th># of Podisma in 5 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>TY1</td>
<td>1</td>
<td>6m</td>
<td>16m²</td>
<td>A</td>
<td>11</td>
</tr>
<tr>
<td>TY2</td>
<td>1</td>
<td>4m</td>
<td>3m²</td>
<td>B</td>
<td>5</td>
</tr>
<tr>
<td>TY2</td>
<td>2</td>
<td>4.5m</td>
<td>15m²</td>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>TY2</td>
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<td>14m</td>
<td>7m²</td>
<td>B</td>
<td>12</td>
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<tr>
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<td>2m</td>
<td>6m²</td>
<td>C</td>
<td>17</td>
</tr>
<tr>
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<td>12m</td>
<td>17m²</td>
<td>C</td>
<td>20</td>
</tr>
<tr>
<td>TY4</td>
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<td>3m</td>
<td>2m²</td>
<td>D</td>
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</tr>
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<td>7m²</td>
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</tr>
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<td>7m²</td>
<td></td>
<td>E</td>
<td>4</td>
</tr>
<tr>
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<td>10m</td>
<td>6m²</td>
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</tr>
<tr>
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<td>10m</td>
<td>48m²</td>
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<tr>
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<td>3</td>
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</tr>
<tr>
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<td>6m</td>
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<td>8</td>
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<td>1</td>
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<td>6m</td>
<td>13m²</td>
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<td>20m²</td>
<td>D</td>
<td>5</td>
</tr>
<tr>
<td>TO4</td>
<td>2c</td>
<td>60m</td>
<td>4.5m²</td>
<td>A</td>
<td>4</td>
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| Vegetation Type | Species Present
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<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>Rhododendron, Vaccinium uliginosum, juncus, Veratrum</em></td>
</tr>
<tr>
<td>B</td>
<td><em>Polygonum alpina, Festuca sp., Veratrum</em></td>
</tr>
<tr>
<td>C</td>
<td><em>Juncus, Rhododendron, Vaccinium myrtillus</em></td>
</tr>
<tr>
<td>D</td>
<td><em>Veratrum, Deschampsia, Crytogamma</em></td>
</tr>
<tr>
<td>E</td>
<td><em>Rhododendron, Luzula</em></td>
</tr>
<tr>
<td>F</td>
<td><em>Juniperus nana, Sempervivum, Juncus</em></td>
</tr>
</tbody>
</table>

Table 4.12: Description of the areas surrounding the transfer sites, and number of *Podisma* caught in five minutes of searching.
Table 4.13: A comparison of population sizes calculated by the Zippin method and the Lincoln-index method

<table>
<thead>
<tr>
<th>Site</th>
<th>Zippin estimate</th>
<th>Lincoln Index estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TY1</td>
<td>140</td>
<td>143</td>
</tr>
<tr>
<td>TY2</td>
<td>103</td>
<td>113</td>
</tr>
<tr>
<td>TY3</td>
<td>304</td>
<td>1078</td>
</tr>
<tr>
<td>TY4</td>
<td>193</td>
<td>727</td>
</tr>
</tbody>
</table>

Table 4.14: The numbers seen in the transplant sites as a proportion of the initial number. This ratio is generally higher for the four low density sites than for the four high density sites. Exact rank sum tests give $P = 5.7\%$ for day 14 and $P = 1.4\%$ for day 27.

capture experiment to estimate population size (by a simple Lincoln-index method, Lincoln,) supports the latter. One might expect the visibility bias to lead to an underestimate of population size, however, densities estimated from the recapture for sites TY3 and TY4 are greater than the original estimates (Table 4.13). Those estimated from TY1 and TY2 are very similar to original estimates.

**Successive density scores**

Two successive density counts for each site allowed a comparison of the proportional drop in numbers between high density and low density sites, for two different time intervals (see Table 4.14).

High density TO sites show a significantly greater proportional drop in density between initial and final counts than their low density counterparts. It appears that this density-dependent effect occurs within the first two weeks (between initial release and the first density count). Although the final two weeks also show this trend in the TO sites, it is as marked: presumably the drop in numbers was substantial enough for density effects to be considerably weakened. The results from, the TY sites are complicated by the uncertain starting densities; the fact that they are less
isolated than the TO sites, and the apparent differences in grasshopper preferences for the sites.

On first inspection, the results on density changes in the TY sites show less convincing evidence of density-dependent regulation. However, it is necessary to interpret the results in the light of individual features of the TY sites (see below). Comparison of TY3 and TY4 show a significantly higher proportional drop in the high density site (TY3) during the first two weeks. Although the drop in density in TY1 (high density) during the first two weeks is low, the overall drop is significantly greater than that of the low density site, TY4. Observed population changes in the other low density site, TY2, are complicated by possible migration into this desirable site and by uncertain starting densities.

The scaling in initial densities in the TO and TY sites was based on native densities and the assumption that the denser TY sites had higher carrying capacities. It is therefore interesting that the TY sites (with consistently higher starting densities than the TO sites) have a proportionally higher overall drop in numbers than do the TO sites.

The karyotype data: fate of transferred grasshoppers

The observed density-dependent reduction in numbers could result from death or dispersal. Karyotype data on males from the sites and from surrounding areas gives information on the fate of transferred grasshoppers. Figure 4.13 shows a decrease in proportion of foreign types with distance from the main site. No foreign types were observed further than 10m from the main sites. There is no evidence for higher dispersal rates from the high density sites (Table 4.15). It seems that distance to the closest surrounding area and its size and suitability are more important. It would be more meaningful to examine the individual features of the transfer sites and their relation to surrounding areas, for a realistic interpretation of the final karyotype distribution.

Individual features of the experimental sites, accuracy of the initial density estimates and the extent of dispersal from the sites are relevant both to the success of the transfer experiments as suitable density manipulation experiments and to possible patterns and mechanisms in density regulation. These will be discussed below.
Figure 4.13: The proportion of chromosomally marked grasshoppers that were recaptured on the final scoring in nearby patches of vegetation, plotted against distance of these patches from the release site. The release (i.e. transplant) sites from which the grasshoppers dispersed are indicated.

<table>
<thead>
<tr>
<th>Site</th>
<th>Density category</th>
<th>Proportion escaped</th>
</tr>
</thead>
<tbody>
<tr>
<td>TY3</td>
<td>high</td>
<td>0.51</td>
</tr>
<tr>
<td>TO1</td>
<td>high</td>
<td>0.00</td>
</tr>
<tr>
<td>TO2</td>
<td>high</td>
<td>0.00</td>
</tr>
<tr>
<td>TY1</td>
<td>high</td>
<td>0.00</td>
</tr>
<tr>
<td>TO3</td>
<td>low</td>
<td>0.65</td>
</tr>
<tr>
<td>TO4</td>
<td>low</td>
<td>0.00</td>
</tr>
<tr>
<td>TY4</td>
<td>low</td>
<td>0.30</td>
</tr>
<tr>
<td>TY2</td>
<td>low</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 4.15: Dispersal from sites: the ‘proportion escaped’ is the proportion of chromosomally marked individuals that were recaptured in one of the surrounding areas (see text).
Population size estimates

The population size estimates for the TO sites were good; the sites had low densities and were easily cleared. The low numbers of unmarked individuals in the recapture experiment verify this. It appears that initial population sizes were underestimated in the TY sites (the bias introduced by the increased visibility of marked grasshoppers, would be expected to result in a smaller estimate of population size and not the observed larger estimates for sites TY3 and TY4). The marked increase in numbers in TY2 from the estimated initial manipulation density to the first rescore could be due, in part, to underestimation of initial sizes but could also result from migration into the site: the site was not well isolated.

Carrying capacities

One could interpret the large differences in native densities between the TO and TY sites as evidence of different carrying capacities. However, it is not obvious that this should be so: the degree of isolation is likely to account for a large proportion of original density differences between sites. The density surveys described in chapter 3 revealed lower average densities in XO regions than XY regions in the same area (for obscure reasons, probably not related to vegetation). The extent to which carrying capacities differ may not therefore be great. The higher proportional drop in densities with time for the TY sites supports this.

Success of the sites as contained areas

For the manipulation experiments to be a success, movement of grasshoppers into and out of the areas should be minimised. Sites were chosen to be as isolated as possible. However, finding sites that were all equally isolated was not possible. Nonetheless, the differences between sites allows speculation on the conditions under which dispersal might occur; factors that might lead to an increase in dispersal; and the extent to which dispersal might act as a density-regulating mechanism.

Dispersal could result from several features of the transfer experiments. Disturbance caused by the introduction of large numbers of individuals into a population is likely to increase the activity of both aliens and residents (Endler, 1977). The aliens might be more likely to move than the established populations (Dobzhansky and Wright 1943; Andrewartha and Birch, 1954; Den Boer, 1971; Crumpacker and Williams, 1973). Increased crowding may increase the dispersal rate.
The release of grasshoppers while they were still cold and immobile was designed to minimise short-term agitation dispersal and the results of the mark-release-recapture experiment indicate that they were successful in this respect. Unfortunately, the fate of transferred grasshoppers could not be followed through time, as this would have necessitated destroying the grasshoppers to determine their karyotype. However, karyotype data from the final collections (both from main sites and their surrounding areas) in conjunction with final density estimates gives some indication as to how successfully contained the sites were. Movement into and out of the sites does not seem to be related to density, but depends rather on the degree of isolation and possibly on the desirability of the closest surrounding vegetation patch. Furthermore the data suggests that any increased activity caused by the introduction of aliens is also not as important to dispersal as the degree of isolation of the sites (and coupled to this, the suitability - or desirability - of surrounding vegetation patches).

**Density-dependent population regulation**

In general, the patterns suggest density-dependent mortality. Table 4.14 shows the numbers seen in the transplant sites as a proportion of the initial number. This ratio is generally higher for the four low density sites than for the four high density sites. Exact rank sum tests give \( P = 5.7\% \) for day 14 and \( P = 1.4\% \) for day 27. Observations on density changes, within each site, through time and proportional differences in these should be interpreted in the light of the above discussions.

The TO sites were generally well isolated, and initial estimates were good. TO3, the least isolated, was a low density site, and it is quite plausible that the density change in this site shows a natural decline. However it was very close to a continuous stretch of desirable vegetation. Dispersal from TO3, as detected from the karyotype data, was high. It is conceivable that there was more-or-less free migration to and from the surrounding area.

A comparison of TY3 and TY4 suggests density-dependent regulation. TY2 with its *Polygonum alpina* should be interpreted with caution: possible migration into the site and the numbers found clustered around the *Polygonum alpina* indicate that it was able to support higher numbers than those dictated by the “high density” category. The final drop in density, in the last two weeks, is analogous to the drop observed in the high density TO sites and TY1 in the first two weeks. One could speculate that high migration into the desirable site converted its 'low density' into
a high density, resulting in the observed density-dependent regulation.

In summary, the transfer experiments generally do suggest density-dependent regulation. The dispersal and fate of transferred grasshoppers show interesting trends. However these should be viewed with caution, because of the limited sample sizes in the final stages of the experiments.

4.7 Discussion

In this chapter, several sources of evidence suggesting a density-dependent decrease in numbers between hatching and adulthood have been presented. Both observations on natural populations made on two spatial scales and manipulation experiments suggest that these are caused by mortality rather than by dispersal. Simulation experiments demonstrate that inferences made from field observations are reliable.

There are several problems with traditional methods of detecting density dependence as discussed in Section 4.2: the methods of analysis can be misleading, for example, if severe non-linearity is introduced by spatial heterogeneity in a subdivided population (Hassel, 1987). *Podisma* is more-or-less continuously distributed and comparisons have been made between sites within this distribution. This approach differs from traditional methods which compare population sizes for a single panmictic population through time.

Another problem with attempting to detect density dependence from observations on a single population through time is that density-dependence may vary on different spatial scales and with site. Freeman and Smith (1990), for example, investigated mortality in the leaf-mining fly *Liriomyza commelinae* at five different sites and three spatial levels (the leaf, the stem and a $(1m)^2$ quadrat) and found that developmental mortality (over three years, or 47 generations) due to parasitoids was not related to population density in leaves or stems but was positively density-dependent in the $1m$ quadrats. Mortality due to other causes was positively dependent on number of mines per leaf at three of the five sites studied and the number of mines per stem at two of the three sites studied, but was independent of density in a $(1m)^2$ quadrat. Their results emphasize the importance of sampling on the appropriate scale if one is to detect density dependence. It is interesting that in the investigation described in Section 4.3 density dependence was observed on two different spatial scales (though it was stronger in the large scale quadrats).

In this investigation I have not addressed the causes of density-dependent mor-
The most obvious question is whether plant-food quality or availability is responsible. Although there seems to be an ample supply of suitable vegetation in the areas investigated at Col de la Lombarde, we cannot rule out this possibility. Plants show qualitative and quantitative chemical variation over space and time, due to both intrinsic (genetic components of the plants) and extrinsic (abiotic and biotic factors such as climate, decomposers, pathogens etc.) factors (Harper, 1977). Clearly the concept of palatability is difficult to define when it involves not only differences between plant species but effects due to plant age and spatial distribution. Preferences can be adequately described only in terms of the relationship between the absolute and relative abundances of all potential foods; the risk of each food and its proportion in the diet. It will probably not remain constant in time or space.

It would be difficult to determine whether food is a limiting factor in *Podisma*. One could use crop- or gut content analyses. However *Podisma* is a polyphagous insect and it would be hard to distinguish whether the contents represented the most recent meal or the preferred food. Enough samples taken at different times might overcome this problem to a certain extent. Controlled experiments on food choice would also be difficult: if offered a range of potential foods, polyphagous insects will tend to concentrate on the most common and only switch to an alternative when the previously common food has been depleted (Harper, 1977). This behaviour is interpreted as due to the formation of search images and may have little to do with food quality. A further complication is that food preferences may change with the stage of development of the grasshoppers.

Another possible mechanism of density-dependent regulation is disease (in the broad sense: pathogens or parasites) or predation. For example, Hassel (1978) reviewed several studies of parasitoid- and predator- searching behaviour, and concluded that over at least part of the range of host densities, enemies spend more time searching where prey are abundant.

In Chapter 3, I discussed the advantage of direct measures of fitness components over laboratory estimates. I discussed attempts at measuring fitness components in the field, and the complications associated with these. In the next chapter I will review a less direct way of measuring fitness components, which is based on Barton’s theory of multilocus clines (Barton, 1983). The quantitative results of his investigation have led to some very useful relations, which allow one to infer parameters such as selection pressures from cline shape. I will describe simulation
experiments aimed at testing these relations.
Chapter 5

Inferences from clines using multilocus analysis - testing the theory using simulation experiments

5.1 Introduction

Patterns of geographic differentiation in species have interested biologists for centuries. The importance of dispersal over geographic continua has long been recognised (see Huxley, 1942; Darwin, 1859; Wallace, 1889; Wright, 1940, 1943, Mayr and Provine, 1980). However, the view after the modern synthesis was that for speciation or even strong geographic differentiation to occur, complete isolation would be required. More recently, the realisation that natural populations are subdivided - in the general sense that not all pairs of individuals in a species are equally likely to mate with each other - has prompted evolutionary biologists to investigate the importance of population structure in evolution. The factors that determine spatial patterns, how these interact and how and under what conditions they might cause local differentiation and speciation have been central to these studies.

Early investigations into the theoretical effects of geographic structure of populations followed two different approaches. The first mathematical analysis of migration and selection was presented by Fisher (1937), who studied the wave of advance of favourable genes. Haldane (1948) developed a closely related equilibrium model of gene flow and selection in a cline, and used it to estimate the intensity of natural selection in the deer-mouse *Peromyscus polionatus*. Haldane (1948) and later Fisher (1950) derived formulae for patterns of gene frequency produced in an infinitely dense population through the interaction between migration and natural selection.
These were modelled by deterministic reaction-diffusion equations. Such equations
have been used widely in biomathematics. Organisms move about in a random way;
when this movement results in some regular change in the statistical distribution
of the whole ensemble, it can be thought of as a diffusion process. Under a vari­
ety of assumptions about individual behaviour, one can derive a continuous model
equation for the global behaviour in terms of the density of the organisms. Fisher
used a one-dimensional diffusion model\(^1\) to describe the wave of advance of an ad­
vantgeous gene, but did not justify it in terms of individual motion. For a rigorous
justification of the diffusion model used by Fisher and Haldane, see Nagylaki (1975).

A slightly different approach from that of Fisher’s and Haldane’s was that of
Wright (1943, 1946) and Malecot (1948) who investigated how the combination of
spatially limited gene flow and sampling drift would cause the differentiation of
demes or neighbourhoods in a subdivided population - a process Wright (1943)
termed “isolation by distance”.

These early investigations formed the foundations of a substantial theory which
has developed over the last two decades, on the effect of population structure on
evolution and population dynamics, and on the behaviour and properties of clines.
Population geneticists have developed a variety of models for these studies. These
are discussed in several good reviews on theoretical population genetics, on the
theory of population structure, and on the theory of clines and hybrid zones (e.g.
Felsenstein, 1976; Endler, 1977; Barton and Clark, 1990; Roughgarden, 1979; Hartl
and Clark, 1989; Barton and Hewitt, 1989).

Until recently the study of hybrid zones was independent of that of clines. Hybrid
zones were investigated more from a taxonomic view-point, of interest because it was
clear that they did not fit into the traditional classification schemes of taxonomists.
Traditional interests in hybrid zone research focus on how they are formed, whether
they are stable or transient and what forces act to modify them. In the early 1960’s
when allopatry was thought to be essential for substantial differentiation to occur, it
was generally accepted that hybrid zones were formed by secondary contact between

\(^{1}\text{Reaction-diffusion models can be generalised to describe, for example, a system of interacting}
\text{species, genotypes or chemicals e.g.}

\[ \frac{\partial u}{\partial t} = f + \nabla (D \nabla u) \quad (5.1) \]

where \( f \) is a vector representing the source; \( u \) is a vector of densities, say and \( D \) is a matrix of
diffusivities (which will be diagonal if there is no cross diffusion among species). Such a reaction-
diffusion system was proposed by Turing (1952) as a model of morphogenesis. Since 1970 these
systems have been studied widely (see Murray, 1989; Okubo, 1980).
forms that had diverged in allopatry but had not reached the point of "good" species. However, models developed by Slatkin (1973) and Endler (1973; 1977) investigated the effects of gene flow and selection on the differentiation of populations, and clearly demonstrated that steep single locus clines could form in the absence of any barrier to gene flow. It became apparent that hybrid zones are synonymous with clines and that the theory of clines maintained by a balance between selection and dispersal could be extended to the analysis of hybrid zones. Since most hybrid zones involve changes in a variety of characters, they consist of a set of parallel clines, and so the theory has been extended to include multilocus systems.

For a clearer understanding of the discussions to follow, it will be advantageous to review the theory of clines in the next section. In Section 5.2.6 I will discuss how hybrid zones relate to this theory, and how the theory of clines has been extended to include multilocus systems for the analysis of hybrid zones.

5.2 The theory of clines: a brief review

Most of the theoretical work on clines has concentrated on "dispersal-dependent" models (see Murray, 1977, 1989; Barton & Hewitt, 1985). These models include those for neutral clines, where the steep gradient caused by the initial mixing of two expanding populations decays with time; waves of advance of an advantageous allele (Fisher, 1937); and clines formed from a balance between dispersal and selection, in which either differences in environment (Haldane, 1948) or selection against heterozygotes (Bazykin, 1969) or recombinants (Bazykin, 1972) maintain a stable cline.

5.2.1 Neutral clines

Consider two expanding populations which meet in a sharp step. Dispersal will produce a gradually broadening cline between them. The change in allele frequency with time can be approximated by a continuous diffusion equation, assuming that genes spread out in some distribution (the equation is a good approximation even if dispersal is not normal) with variance $\sigma^2$ per generation. (Haldane, 1948; Nagylaki, 1975). The dispersal rate $\sigma$ is defined as the standard deviation of the distance between parent and offspring along some axis, and has units of $\text{distance} \times \text{time}^{-1/2}$.

$$\frac{\partial p}{\partial t} = \frac{(\sigma^2/2)}{\partial^2 p/\partial x^2}$$

(5.2)
Consider the shape of a neutral cline which has initial allele frequencies: $p = 1$ when $x > 0$ and $p = 0$ when $x < 0$.

$$p(x_0, t) = \int_{-\infty}^{\infty} g(x_0 - x, t) \theta(x) dx$$

$$= \int_{0}^{\infty} (1/\sqrt{2\pi \sigma^2 t}) \exp\left(-\frac{(x_0 - x)^2}{2\sigma^2 t}\right) dx$$  \hspace{1cm} (5.3)

where $x_0$ and $x$ are separated by $t$ generations of migration. The assumption is that $g(\epsilon, t)$ converges to a normal distribution, even if dispersal in any one generation is not normal (the Central Limit Theorem).

The width of the cline can be calculated from the dispersal rate $\sigma$ and is defined, arbitrarily, as the inverse of the maximum gradient (one could also define cline width to be the distance over which gene frequencies change from some value $p = v$ to $p = (1 - v)$, for example $p = 20\%$ to $80\%$ - see Endler (1977). However these measures are more-or-less equivalent).

Therefore,

$$1/w = \left. \frac{\partial p}{\partial x} \right|_{x=0}$$

$$= 1/\sigma^2 \int_{0}^{\infty} (1/\sqrt{2\pi \sigma^2 t})(y \exp(-y^2/2\sigma^2 t)) dy$$

$$= \frac{1}{\sqrt{2\pi \sigma^2 t}}$$  \hspace{1cm} (5.4)

At generation $t$ the width of the cline will be $w = \sigma \sqrt{2\pi t}$, where $\sigma^2$ is the parent offspring variance: i.e. the width increases with $\sqrt{time}$.

### 5.2.2 Selection at a single locus

Now consider a simple model of selection at a single locus. Suppose we have a haploid population, with allele $A$ having a selective advantage over allele $a$ i.e. suppose the fitness of individuals carrying $A$ is $1+s$ and that of an individual carrying $a$ is $1$. This is the model for the wave of advance of an advantageous allele first investigated by Fisher (1937). This can be applied to a diploid population with individual fitnesses $1 : 1+s : 1+2s$ if $s << 1$. Adding terms for the change in frequency due to selection to the above equation, the change in allele frequency can be approximated by:

$$\frac{\partial p}{\partial t} = (\sigma^2/2)\frac{\partial^2 p}{\partial x^2} + spq \hspace{1cm} (s << 1)$$  \hspace{1cm} (5.5)

The diffusion approximation breaks down for high values of $s$, because higher order terms in $s$ become important, and because changes can no longer be taken to be continuous rather than discrete.
Equation 5.5 has a travelling wave solution. The wave of advance has a range of possible velocities which will depend on the initial conditions. The minimum velocity corresponds to a wave with the smallest possible width, and will be

$$v = \sigma \sqrt{2s}$$

with corresponding width:

$$w = 8 \sqrt{s}$$

On average any fluctuations e.g. those caused by random drift and inhomogeneities in population structure will slow down the wave so it will tend to its minimum velocity (see Stokes, 1976).

### 5.2.3 Stable clines

#### Clines maintained by an ecotone

Various forms of natural selection can stabilize clines. Geographic variation in selection pressures has been studied extensively (e.g. Haldane, 1948; Fisher, 1950; Slatkin, 1973, 1975; May, Endler & McMurtrie, 1975; Endler, 1977). Consider an ecotone, or sharp environmental change separating parental types, with selection $+s$ on one side and $-s$ on the other. Provided that the ecotone does not present a barrier to migration, we will observe a smooth gradient from (say) $p = 0$ to $p = 1$ with the centre of the cline coinciding with the ecotone. A model describing such a system was first developed by Haldane (1948). Equation 5.5 becomes:

$$\frac{\partial p}{\partial t} = \frac{\sigma^2}{2} \frac{\partial^2 p}{\partial x^2} + spq$$

for $x > 0$ and

$$\frac{\partial p}{\partial t} = \frac{\sigma^2}{2} \frac{\partial^2 p}{\partial x^2} - spq$$

for $x < 0$, where $x$ is the geographic distance along the cline, and the centre of the cline is at $x = 0$ (Haldane, 1948; Nagylaki, 1975). The width of this cline will be $\simeq \frac{\sqrt{6}}{s}$.

#### Stable clines maintained by a balance between dispersal and selection against heterozygotes

Bazykin (1969) considered selection, $s$ acting at a single locus, where the two homozygotes each had fitness 1 relative to heterozygotes of fitness $1 - s$. In this model,
the equation describing the change in allele frequency is:

$$\frac{\partial p}{\partial t} = (\sigma^2/2)\frac{\partial^2 p}{\partial x^2} + spq(p - q)$$  \hspace{1cm} (5.10)

where \( p + q = 1 \).

This equation has solution

$$p = \frac{1}{1 + \exp[-(x - x_0)/\sqrt{2\sigma^2/s}]}$$  \hspace{1cm} (5.11)

and the width is \( \sqrt{8\sigma^2/s} \); or equivalently

$$p = [1 + \tanh[\sqrt{s/2}\sigma^2(x - x_0)]]/2$$  \hspace{1cm} (5.12)

(Barton, 1979c and see Bazykin, 1969), where \( x_0 \) is the (arbitrary) centre of the cline.

### 5.2.4 The characteristic scale of selection

In all the cases described above, we have seen that the width of the cline has been described by some variation on \( \sigma/\sqrt{s} \) (where \( \sigma^2 \) is the variance in distance between parent and offspring; \( s \) is proportional to selection, or for a neutral cline, the inverse of time since contact). This is referred to as the "characteristic length" of the cline (Slatkin, 1973). Since \( \sigma \) is the variance of distance moved per time (\( \sigma^2 \sim L^2 T^{-1} \)) and \( \sqrt{s} \) has dimensions \( 1/\sqrt{\text{time}} \), \( l_c \) has dimensions of distance. It describes the typical distance over which selection changes allele frequencies. For example, Slatkin found that the pattern of spatial variation in selection intensities would only result in significant change in gene frequencies over a distance greater than the characteristic length. Therefore migration and the strength of selection set a lower limit on the scale of changes in environment to which a cline will respond.

Any dispersal-dependent cline has width \( w \) (defined as the inverse of the maximum gradient) of the same order as \( l \). For example, the width of a cline maintained by heterozygote disadvantage described above is: \( w = \sigma \times \sqrt{8/s} = 4l \) (Bazykin, 1969). A cline maintained by an ecotone with width \( w = \sigma \sqrt{6/s} \) will have width: \( w = \sqrt{6}l \); and a cline resulting from introgression of neutral alleles for \( T \) generations will have width: \( w = \sqrt{4\pi}l \), where \( l = \sigma \sqrt{T/2} \).

The dispersal-dependent clines described above will all be sigmoid in shape. Differences in the tails can be observed if one transforms the data to a logit scale, because the tails involve extremes in gene frequencies (Fig. 5.1). However, because gene frequencies are low in the tails, one would need very large samples to distinguish them in this way.
Figure 5.1: Clines maintained by a balance between dispersal and selection on a single locus all have similar shapes. Allele frequencies are plotted against distance, on a logit scale $\log_e(p/q)$; the clines are scaled so that all have the same position and width. Letters refer to different models. N: neutral introgression; H: heterozygote disadvantage; E: an ecotone, with fitnesses $1 + s : 1 : 1 - s$ on the left, and $1 - s : 1 : 1 + s$ on the right; D: an ecotone with dominance. Fitnesses are $1 : 1 : 1 - s$ on the left and $1 : 1 : 1 + s$ on the right. D2 refers to the same model, but now the frequency of the regressive homozygote is plotted. Q: stabilising selection on a quantitative trait, with the optimum changing abruptly by $\Delta$ at an ecotone. From Barton and Jackson (in press).
5.2.5 Models which incorporate density effects

Nagylaki (1975) also modelled a single diallelic locus in the absence of genetic drift. He derived a diffusion model of migration and selection in a continuously distributed population and explicitly incorporated the effects of population regulation in his model. Nagylaki (1975) models dispersal using the distribution of progeny at \((y, t+dt)\) from parents at \((x, t)\). This is a model continuous in time and space. Either the stepping stone or the continuous model of population structure can be used to quantify the balance between dispersal and selection. It is not clear which of these is the more realistic, since most natural populations are neither distributed in discrete demes nor continuously. However, the continuous model is easier to analyse, and in any case if \(s << 1\) then the population will be in approximate Hardy-Weinberg equilibrium, changes will be almost continuous in time and the diffusion equation will be a very good approximation (see Nagylaki, 1975). In the stepping stone model, \(\sigma^2 = me^2\), where a fraction \(m\) of individuals are exchanged between demes \(e\) apart each generation, and \(\rho\) is \(N/e\), with \(N\) the number of breeding individuals in each deme. In each generation it is assumed that density is regulated to some definite number independent of local fitness.

In one dimension:

\[
\frac{\partial p}{\partial t} = \left(\frac{\sigma^2}{2}\right)\frac{\partial^2 p}{\partial x^2} + \frac{\partial \sigma^2}{\partial x} \frac{\partial p}{\partial x} + \sigma^2 \left(\frac{\partial \log(\rho)}{\partial x}\right) \left(\frac{\partial p}{\partial x}\right) \tag{5.13}
\]

where \(\sigma^2\) is the variance in progeny position per generation and \(\rho\) is the population density. If selection is weak, and the distribution of dispersal distances not too leptokurtic, only \(\sigma^2\), the variance of the distribution need be known to describe gene flow.

Then combining the effects of selection and dispersal, a single locus cline in one dimension maintained by heterozygote disadvantage, can be described by

\[
\frac{\partial p}{\partial t} = \left(\frac{\sigma^2}{2}\right)\frac{\partial^2 p}{\partial x^2} + \frac{\partial \sigma^2}{\partial x} \left(\frac{\partial p}{\partial x}\right) + \sigma^2 \left(\frac{\partial \log(\rho)}{\partial x}\right) \left(\frac{\partial p}{\partial x}\right) spq(p-q) \tag{5.14}
\]

(Barton, 1979c; Nagylaki, 1975)

5.2.6 The theory of tension zones

We have seen that clines can be formed in several ways. In the absence of selection, mixing of two previously separated genotypes would result in a transient cline, and eventual polymorphism. A wave of advance of an advantageous gene would
similarly result in a transient cline. The two genotypes could meet along an eco-tone, where each genotype would be most suited to its own environment. In this case the hybrids would not be at an advantage in either environment, though they might be at an advantage in the transitional habitat (Moore, 1979). In these cases, hybrids would remain in a stable hybrid zone where the two forms met. Frequency-dependent selection can also result in the formation of a stable cline. For example, stable clines in the warning pattern of unpalatable insects can be maintained by density-dependent predator attacks (Mallet, 1985). A rare pattern will be selected against because predators do not recognise it as being unpalatable. Perhaps the most common cause (see Bazykin, 1969, 1973; Barton and Hewitt, 1989) is selection against recombinant or heterozygous genotypes. If two genotypes have evolved along different paths and then come into contact with each other, they may produce relatively unfit hybrids which leave fewer offspring. An animal migrating into foreign territory is most likely to mate with the resident genotype, producing hybrid progeny which will be selected against. This is likely to produce a stable hybrid zone where the relative strengths of the selection against hybrids and gene flow into the hybrid zone determine its width. Different kinds of selection could act in the same hybrid zone. For example, in *Heliconius* butterflies where the boundaries between different warning patterns are maintained by Mullerian mimicry, there is selection against heterozygotes, recombinants and rare alleles, which all tend to produce patterns that are not recognized as distasteful by predators (Mallet, 1986; Mallet et al, 1990). Selection also acts to favour patterns that are common in other species in the same mimicry ring.

Clines formed by heterozygote disadvantage and frequency-dependent selection can result in the formation of "tension zones" (Key, 1968; Barton and Hewitt, 1985), so called because they are maintained by internal genetic factors, and can move until trapped by barriers to dispersal or low population density (Bazykin 1969; Barton, 1979c). They may also move in response to differences in environment. Ecologically determined clines on the other hand must, by definition, be bound to some environmental gradient and are maintained by that gradient. The distinction between tension zones and ecologically determined clines is important because it determines how the hybrid zone can move i.e. how the sets of genes that distinguish the hybridizing populations compete with each other.

Detailed analysis of hybrid zones has revealed that many of them are tension zones. Evidence comes from several sources. In many cases there is strong evidence
for selection against hybrids. For example, there is clear evidence for selection against rare forms in *Heliconius* and for selection against chromosomal heterozygotes (see Barton, 1980a; Kocher & Sage, 1986; Nichols & Hewitt, 1988; Searle, 1988; Hewitt, Butlin & East, 1987; Mallet and Barton, 1989b; see Barton and Hewitt, 1989). Further evidence comes from the close coincidence of clines at different loci. If the polymorphisms were responding directly to environmental differences this would not be expected. A clear example of this is in the hybrid zone between *Bombina bombina* and *Bombina variegata*, which has clines in allozymes, belly pattern, mating call and mtDNA which differ in position by less than 30% of their average width, and vary in width by at most 27% (Szymura & Barton, 1986). Further, although hybrid zones often follow some environmental difference, this is not inconsistent with their being tension zones. For example, the evolution of tolerance to heavy metals in plants can be accompanied by partial reproductive isolation (MacNair, 1987), which would maintain a tension zone even in a uniform habitat.

Most of the theoretical work on clines has been on single locus models. Since most hybrid zones involve changes in a variety of characters and therefore consist of a set of clines, in order to understand the dynamics of a hybrid zone, it has been essential to develop a theory for the effects of many interacting loci. In the next section I will review the theory of tension zones involving a single locus. This has been based on the theory of a single locus cline maintained by a balance between selection and dispersal, (Section 5.2.3). I will then discuss how this has been extended.

**The dynamics of single locus tension zones**

Following Nagylaki (1975) (see Section 5.2.5) we have seen that a single locus tension zone in one dimension, can be described by

\[
\frac{\partial p}{\partial t} = \left( \sigma^2 / 2 \right) + \left( \partial \sigma^2 / \partial x \right)(\partial p / \partial x) + \sigma^2 (\partial \log(p) / \partial x)(\partial p / \partial x) + spq(p - q) \tag{5.15}
\]

Instead of solving for \( p \) directly, given the above equation, Barton (1979) has derived a function \( H \) of \( p \) which never increases, so that it is minimised at equilibrium.

\[
H \equiv \int_{-\infty}^{\infty} \sigma^2 (\sigma^2 (\partial p / \partial x)^2 + 2\sigma^2 sp^2 q^2) dx \tag{5.16}
\]

The solution to this is either fixation of one or other allele, or Eqn 5.11.

This solution will hold approximately, as long as dispersal, selection against hybrids and population density are more-or-less constant. As selection increases and dispersal decreases, the zone will narrow and it will move to minimize its length.
It will move to low density areas and will be pinned by physical barriers to gene flow. Further, if one allele has a selective advantage, the zone will move in favour of the fitter allele. For example, suppose the fitnesses of genotypes $PP$, $PQ$, $QQ$ are $1 - \alpha s$, $1 - s$ and $1 + \alpha s$ respectively, then

$$\frac{\partial p}{\partial t} = \frac{\sigma^2}{2}(\frac{\partial^2 p}{\partial x^2}) + sp(q-p) + \alpha spq$$

(5.17)

and this has solution

$$p = \frac{1 + \tanh(2x/\omega + \alpha st/2)}{2}$$

(5.18)

where $\omega = \sqrt{\frac{8s}{\alpha}}$ (the equation above is equivalent to the exponential form, see Eqn. 5.11). The cline has the same width, but it will move in favour of the fitter race.

**Multilocus clines**

So far I have reviewed the effects of selection at a single locus. The gametic associations or linkage disequilibria typical of many hybrid zones (see Barton & Hewitt, 1989) suggest the importance of interactions between many loci in the dynamics of hybrid zones (Barton, 1983). Slatkin (1975), using a two-locus model, was the first to investigate the effects of linkage on gene frequency clines, and the role of gene flow in producing linkage disequilibrium between the two loci. Barton has developed an extensive multilocus theory to investigate the effect of gene flow at linked loci (Barton, 1979, 1983). Through this theory, Barton (1982), Szymura and Barton, (1986), Mallet and Barton (1989) and Szymura and Barton (1991) have shown that features of multilocus systems such as linkage disequilibria and cline shape can be used to infer parameters such as selection pressures and rates of gene flow, which are hard to measure in the field. The analyses have been based on stepped clines which show concordant change in biochemical and morphological characters, and strong linkage disequilibria between different genetic markers. These features are typical of many hybrid zones (Barton & Hewitt, 1985; 1989; Szymura & Barton, 1991). The clearest examples are in *Bombina* (see Szymura and Barton, 1991) and *Heliconius* (see Mallet and Barton, 1989a). In this section I will give a brief review of the theory of multilocus clines and how it has been used in the analysis of hybrid zones. In particular, I will concentrate on some of the predictions and inferences made by Szymura and Barton (1991) in analysing the *Bombina* hybrid zone. Then in Section 5.3 I will develop a simulation model for multilocus systems, which will
be used in 5.4 to check the theoretical predictions when some of the underlying assumptions are violated (as they must be in nature), and to expand the theory to cover cases that are analytically intractable.

**Barton’s basic model of multilocus systems**

Barton (1983) has extended the single locus case to a system involving any number \(n\) of loci. He assumes weak heterozygote inferiority, in which an individual heterozygous at \(k\) loci has fitness \((1 - s)^k\), \((s < 1)\) (i.e. fitness is multiplicative), so that the total selective force is \(S = ks\) and the fitness of an individual heterozygous at all \(n\) loci would be approximately \(exp(-S)\). To describe recombination, he assumes the selected loci are evenly distributed along a single chromosome, with recombination \(r\) between neighbouring genes, and a total map length of \(R = (n - 1)r\) \((R\) is assumed small \((R << 1)\), so that multiple crossovers can be ignored). He investigates the behaviour of clines using both a discrete and continuous model of population structure: first he looks at two populations exchanging genes at a very low rate (so that introgressing genes are always rare). He then considers a continuous habitat, where mixing is described by isotropic diffusion in one dimension, the rate of gene flow being \(\sigma^2\) (see Nagylaki, 1975, for derivation).

In this model, the parameters which determine the frequency of an introgressing allele will be the strength of selection against hybrids, \(s\); the dispersal rate of the organism, \(\sigma\) and the number of loci, \(n\). The ratio between selection and recombination \(\theta = s/r\) is of critical importance in determining the strength of disequilibria and hence the shape of the cline.

**Linkage disequilibria and multilocus hybrid zones**

There are various forces which could generate the excess of parental combinations of alleles which is often observed in hybrid zones. For example, one would expect to find disequilibria if selection favours "coadapted" combinations. Lewontin (1974) argued that linkage disequilibria could be used as a sensitive measure of epistasis. However, Li and Nei (1974), Feldman and Christianson (1975) and Slatkin (1975) showed that substantial linkage disequilibria can form as a result of gene flow in a cline, even in the absence of epistasis. Barton (1983) has argued that in multiplicative models of fitness, where \(W = (1 - s)^k\), \((k\), the number of heterozygous loci\), epistasis resulting from deviation from additivity will produce disequilibria of order \(s^2\). Since the strength of interaction will depend on the ratio between epistasis and recombination,
multiplicative fitnesses will generate disequilibria of order \( s^2/r \), which will usually be weak. On the other hand, the disequilibria generated in the centre of a multilocus hybrid zone by the continual immigration from pure populations on either side, and broken down by recombination and segregation in hybrids will be stronger (of order \( s/r \)) than that generated by multiplicative selection in polymorphic populations. Even when there is epistasis, it may generate less disequilibrium than migration when many loci are involved (of order \( s/r \) vs \( s/r)\ln(n) \)). See Li and Nei, 1974, Slatkin, 1975, Barton, 1983).

Consider the linkage disequilibrium \((D)\), generated between locus \( p \) and locus \( u \), by the migration between two demes. \( D \) at time \( t + 1 \) will be proportional to the rate of migration, \( m \), and the difference in allele frequencies between demes, \( \Delta p \), and \( \Delta u \).

It can be shown that when \( m \ll r; \) and \( m \ll 1; \)
\[
D_t \approx m\Delta p_0\Delta u_0 \frac{(1 - 2m)^{2t+1}}{r - 4m} \\
\approx \frac{m\Delta p_t\Delta u_t}{r} \text{ as } t \to \infty
\]
where \( \Delta p_t = (1 - m)^t \Delta p_0 \), \( \Delta u_t = (1 - m)^t \Delta u_0 \), \( m \) is the rate of migration and \( r \) the rate of recombination. If \( 1 >> m >> r \), then
\[
D_t = m(1 - m)\Delta p_0\Delta u_0 \frac{(1 - r)^{t+1}}{4m} \\
\approx \Delta p_0\Delta u_0(1 - r)^{t+1}
\]

For the derivation, see Barton and Jackson (in press). From Equation 5.19 we can see that even if loci are unlinked \((r=1/2)\), \( D \) is not zero. The derivation above was for two discrete demes. Now consider a continuum. Suppose there are clines at locus \( p \) and locus \( u \); then the change in gene frequency can be given by:

\[
\Delta p \simeq \epsilon \partial p/\partial t
\]
and
\[
\Delta u \simeq \epsilon \partial p/\partial t
\]
respectively, where \( \epsilon \) is the deme spacing. The linkage disequilibrium between these two loci can be described by

\[
D \approx (m/r)\Delta p/\Delta u \\
\approx (m/r)\epsilon^2(\partial p/\partial x)(\partial u/\partial x)
\]

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Since \( \sigma^2 = m \epsilon^2 \), the equation to describe disequilibria for coincident clines is:

\[
D \simeq \sigma^2 (\partial p/\partial x)(\partial u/\partial x)/r
\]

The width is defined as \( 1/\text{maximum gradient} \), and since the clines coincide, equation 5.24 becomes:

\[
D \simeq \sigma^2/w^2 r
\]

where \( \sigma^2 \) is the rate of gene flow, \( w \) is the cline width, \( r \) is the recombination rate between the markers involved and \( D \) is the disequilibrium at the centre. This can be derived directly from a continuum model (Barton, 1983).

Thus, gene flow between populations with loci at different allele frequencies will generate correlations or linkage disequilibria (\( D \)) (Li and Nei, 1974). If the populations are fixed for different gene combinations, then when they first mix, they will be in complete disequilibrium. \( D \) will therefore be a maximum at the centre of the zone. Here, each allele is associated with others from its ancestral population so that selection on one locus will act on associated loci even if they are not "linked". (Slatkin, 1975; Barton, 1983; Szymura & Barton, 1986). The allele frequency at a neutral diallelic locus \( i \), for example, will be affected by the linkage disequilibria between all other loci: it will suffer an "effective selection", such that:

\[
\partial p_i/\partial t = \sum_{j=1}^{n} s_j D_{ij}
\]

where \( s_j \) is the selection differential on locus \( j \); \( D_{ij} \) is the disequilibrium between locus \( i \) and \( j \).

This steepens the cline near the centre, the same effect as would be produced by a physical barrier. Linkage disequilibrium can therefore act as a barrier to gene flow (see below). If many genes are involved, the cline will have a steep central region in which disequilibria are strong. On either side, as foreign alleles diffuse through the zone, disequilibria will be broken down; alleles only experience weak selection resulting from their individual effects on fitness and the gradient in allele frequencies will be shallow. This is just the pattern observed in the hybrid zones between \textit{Bombina bombina} and \textit{Bombina variegata} (Szymura and Barton, 1986); the bat \textit{Uroderma bilobatum} (Baker, 1981; Barton, 1982); the grasshopper \textit{Caledia captiva} (Shaw et al 1985) and \textit{Rana pipiens} (Kocher and Sage, 1986). There are exceptions: for example in the zone between \textit{Mus musculus} and \textit{Mus domesticus}, no linkage disequilibrium has been detected; and in \textit{Heliconius} and \textit{Podisma}, linkage
Disequilibrium has been observed, the net selection is strong, and yet there is no step. It could be that fewer loci are involved in these cases (but see Chapter 6).

The shape of multilocus clines

Barton (1986) has shown how to derive an expression which relates the shape of clines for neutral or weakly selected alleles \( u(x) \) to mean fitness \( \bar{W}(x) \). This gives the strength of the barrier to gene flow caused by selection spread over large numbers of loci. Here I will outline Barton's (1986) derivation for an equation expressing the strength of this barrier. More detail can be found in Barton (1986) and Barton and Bengtsson, (1986).

Following Nagylaki (1976), the strength of the barrier \( B \) to the flow of neutral genes say from the right of the barrier across to the left, can be defined as the size of the step relative to the gradient in gene frequency.

\[
B^- = \left( \frac{u_x^+ - u_x^-}{\frac{\partial u}{\partial x}} \right) \tag{5.27}
\]

\[
B^- = \Delta_u / (\partial u / \partial x) \tag{5.28}
\]

where \( u_x^+ \) and \( u_x^- \) are the allele frequencies slightly to the left and right of the barrier at \( x \), where the allele frequency changes abruptly. \( \Delta u = u_x^+ - u_x^- \) is then the size of the step (see Figure 1(c) in Barton & Bengtsson, 1986).

Since \( \Delta u \) is the change of \( u \) across the step, the strength of the barrier to gene flow across a continuous population, \( B \), has dimensions of distance. It can be thought of as the length of unimpeded habitat which would present an equivalent obstacle to the flow of a neutral allele. The effect of the barrier to gene flow depends on the ratio \( B/\sigma \). If two populations which are fixed for alternative neutral alleles meet in a hybrid zone, introgression will be delayed for \( (B/\sigma)^2 \) generations (Barton, 1979b). However, even slightly advantageous alleles will penetrate a localised barrier very rapidly (within \( \approx \log((B/\sigma)^2(\pi s)/2 \) generations).

If we assume that away from the barrier the change in allele frequency can be approximated by a diffusion equation, then:

\[
\frac{\partial u}{\partial t} = (\sigma^2/2)(\partial^2 u / \partial x^2) \tag{5.29}
\]

We have seen in the last section that linkage disequilibrium at the centre of the cline produces a barrier to gene flow. \( D_i \), the linkage disequilibrium between the \( i^{th} \) selected locus and the marker locus is generated by the dispersal of parental
combinations of alleles into the centre of the cline:

\[ \sigma^2 \left( \frac{\partial p_i}{\partial x} \right) \left( \frac{\partial u}{\partial x} \right) \]  

(5.30)

(Li and Nei, 1974; Barton, 1983) and broken down by recombination \((-r_id_i)\), where \(r_i\) is the rate of recombination between the marker and the \(i\)th selected loci.)

Since the marker locus is neutral, no disequilibria are generated by selection. Barton (1983) (equation 5.24) has shown that second order terms in selection can be ignored if \(\theta = s/r << 1\), i.e. selection must be much weaker than recombination. \(D_i\), the disequilibrium between the marker locus \(u\) and locus \(i\) can then be approximated as follows:

\[ D_i \simeq \left( \frac{\sigma^2}{r_i} \right) \left( \frac{\partial p_i}{\partial x} \right) \left( \frac{\partial u}{\partial x} \right) \]  

(5.31)

where \(r_i\) is the rate of recombination between locus \(i\) and locus \(u\).

Changes in overall frequency of the marker locus \((u)\) are caused by gene flow and by selection at loci which are in linkage disequilibrium with the marker locus (see above). If the fitness of each genotype is independent of genotype frequency, then the selection coefficient associated with each selected allele is proportional to the gradient in log mean fitness with respect to the allele.

Hence:

\[ \frac{\partial u}{\partial t} = \frac{(\sigma^2/2)\partial^2 u/\partial x^2}{2} + \sum_{i=1}^{n} 1/2 \frac{\partial \log W}{\partial p_i} \frac{\partial p_i}{\partial x} + O(\theta^2) \]  

(5.32)

(Barton and Bengtsson, 1986). Then substituting Equation 5.31:

\[ \frac{du}{dt} = \left( \frac{\sigma^2}{2} \right) \left( \frac{\partial^2 u}{\partial x^2} \right) + \sum_{i=1}^{n} 1/2 \frac{\partial \log W}{\partial p_i} \frac{\partial p_i}{\partial x} \left( \frac{\sigma^2}{r_i} \right) \left( \frac{\partial u}{\partial x} \right) \]  

(5.33)

The harmonic mean recombination rate \(\bar{r}\) can be used instead of \(r_i\), assuming selection to be spread evenly over the genome. Then the equilibrium solution to Equation 5.33 becomes:

\[ 0 = \left( \frac{\sigma^2}{2} \right) \left( \frac{\partial^2 u}{\partial x^2} \right) + \frac{\sigma^2}{2\bar{r}} \left( \frac{\partial u}{\partial x} \right) \sum_{i} \frac{\partial \log W}{\partial p_i} \frac{\partial p_i}{\partial x} \]  

(5.34)

Simplifying:

\[ 0 = \frac{\partial^2 u}{\partial x^2} + \left( \frac{1}{\bar{r}} \right) \frac{\partial u}{\partial x} \left[ \frac{\partial \log W}{\partial x} \right] = \frac{\partial [(\partial u/\partial x)W^{-1/\bar{r}}]}{\partial x} \]  

(5.35)

where \(W\) is the mean fitness at the centre of the zone, Therefore, \(\partial u/\partial x = \text{constant} \times W^{-1/\bar{r}}\). Since it is independent of \(x\), we can evaluate \(\partial u/\partial x\) at the centre or at the
edge of the zone. Now, $\partial u/\partial x$ at the centre is, by definition = $1/\omega$, where $\omega$ is the width of the cline. $\partial u/\partial x$ evaluated at the edge of the cline is:

$$\frac{\partial u}{\partial x} = \frac{1}{\omega (\bar{W}_H/\bar{W}_P)^{-1/r}}$$

(5.36)

We also have that $B = \frac{\Delta u}{\partial u/\partial x}$, (where $\partial u/\partial x$ is again evaluated at the edge) and since the step, $\Delta u$ is in our case $\approx 1$:

$$B = \omega (\bar{W}_H/\bar{W}_P)^{-1/r}$$

(5.37)

where $\omega$ is the width of the cline; $\bar{W}$ is the mean fitness of individuals at the centre of the cline, $\bar{W}_H/\bar{W}_P$ is the mean fitness of hybrids relative to the population into which genes are introgressing (i.e. the mean fitness of individuals at the centre of the cline) and $r$ is the harmonic mean recombination rate between the marker and selected locus i.e. the strength of the barrier is determined by the net selection against hybrids relative to the rate of recombination. This result is independent of epistasis provided selection is weak.

**Cline width as a function of $s/r$**

The ratio between selection and recombination is of critical importance in determining the behaviour of multilocus systems. Where many genes are involved, and selection is comparable with recombination, disequilibria will induce a sharp step in each cline, flanked by long tails of introgression. The central region of the cline in which disequilibria are strong will have width which depends strongly on the ratio between selection and recombination, $r$. I shall briefly outline Barton’s derivation of the critical importance of the number of loci and the "coupling coefficient", $\theta = s/r$.

Barton (1983) uses scaling arguments to suggest that for a fixed net selection ($s = ns$) when selection is weaker than recombination, the effective pressure on each locus would decline indefinitely as the number of loci increases, whereas when selection is stronger than recombination, there should be strong effective selection on each locus even when very many loci are involved, and the direct effect of each is weak. His analytic results confirm this. He considers two populations fixed for different alleles at $n$ loci, which exchange a fraction, $m$ of their individuals each generation. Heterozygotes are selected against, so that this process is equivalent to the introduction of gametes carrying a set of $n$ deleterious alleles. To start off with these will be in complete disequilibrium, and will be selected against by a pressure $S = ns$, (where the effective selection on each locus is weak ($s << 1$), so that
$(1 - s)^n \simeq \exp(-S)$. Recombination will break the sets of introgressing alleles up into smaller blocks, which will be eliminated more slowly. Clearly, as the number of loci increases, recombination will break the blocks up into finer fragments. This means that for a given overall selection pressure, the effective selection on each gene decreases as the number of loci increases. Barton has shown that when $\log(n) \gg 1$, then if selection is stronger than recombination ($\theta > 1$) selection can hold the proportion of deleterious alleles down to a finite level. However when selection is weaker than recombination ($\theta < 1$), the proportion of alleles on small blocks will make up in aggregate, a large proportion of the population; the allele frequency rises indefinitely, and the effective selection is zero. Thus there are two distinct domains, sharply separated at the critical value $\theta_c = 1$; when coupling is strong, the chromosome acts as the unit of selection $s_{\text{eff}} \simeq S$, i.e. selection pressures on each gene will act together; while when coupling is weak (i.e. if linkage is not tight enough), selection does not act coherently and the genes in a multilocus system will behave independently.

Multilocus theory: observable consequences and interpretation of data from nature

The relations reviewed above, along with genetic data from transects across hybrid zones, can be used to estimate parameters that are hard to measure in the field. This approach has been used by Barton (1982), Szymura and Barton (1986), Mallet and Barton (1989a), Szymura and Barton (1991), where the observed pattern of allele frequencies and linkage disequilibria in clines have led to estimates of the overall strength of selection, the number of genes involved, the rate of individual dispersal and the strength of the barrier to gene flow.

For example, Szymura and Barton (1986; 1991) identified steep clines in six diagnostic allozymes across the Bombina hybrid zone, and argue that these steep gradients are generated by associations with other loci rather than selection on the allozymes themselves. They have found that although the markers are unlinked, they are in strong linkage disequilibrium with each other ($R = D/\sqrt{pquv} = 0.22$ (support limits: 0.15 — 0.29) at the centre of the zone. They calculate the strength of the barrier to gene flow generated by these disequilibria to be $B = 51$ km (support limits: 22 — 81). They interpret this as resulting from reduced hybrid fitness, where: $\frac{W_H}{W_F} = 0.58$, (0.54 — 0.68) and this selection is spread over many loci ($n = 55; (26 - 88)$) (Szymura & Barton, 1991).
Another good example can be found in Mallet and Barton (1989a) where frequency-dependent selection against rare forms maintains clines in *Heliconius*. Here selection and migration can be estimated from a knowledge of dominance, number of genes, gene frequencies and gametic correlations.

In Section 5.4, I will discuss how hybrid zone data can be used to estimate such parameters and I will use computer simulations to check Barton’s analytic results. But first I will describe the program used to simulate a multilocus cline stabilized by a balance between dispersal and selection.

### 5.3 Computer simulation of a multilocus migration-selection cline

#### 5.3.1 Introduction

Computer simulations have been used extensively in population genetics, both to elucidate analytically intractable problems and to test and expand the results and predictions from theoretical investigations, particularly where these have used approximations.

Since the pioneering work of Haldane (1948) and Fisher (1950), there have been numerous models exploring the combined effects of gene flow, selection and drift. Computer simulations have been used regularly in these investigations. For example, Rohlf and Schnell (1971) and Endler (1973) investigate the importance of drift: in particular whether spatially limited gene flow combined with drift can give rise to marked differentiation, (as suggested by Wright, 1943, 1946) and whether it can give rise to an apparently stable cline (Endler, 1977). Slatkin and Maruyama (1975) and Felsenstein (1975) investigated the effects of genetic drift on an ecotone model. These studies have mostly used deterministic models for a single locus (see Felsenstein, 1976, Slatkin, 1973 and Endler, 1977 for reviews), or at most for two or three loci (Slatkin 1975, Bazykin, 1972, Mallet and Barton, 1989). Deterministic models involving several loci are mathematically complex. However, in the last two decades, there have been numerous studies on the effects of many genes in evolution, with reference to polygenic characters. For example there have been many theoretical investigations into the role of mutation in the maintenance of heritable variation for quantitative traits under stabilizing selection (Kimura, 1965; Lande, 1975; Turelli, 1984; Barton, 1986; Latter, 1970; Bulmer, 1972; 1980; Bürger, 1986; Bürger et al, 1988). Authors have used a variety of models for these investigations,
mostly deterministic, in the sense that no random sampling is considered (i.e. the population is effectively infinite). Deterministic models of this kind are at least mathematically tractable, and often provide good approximations to events in nature (although stochastic effects could be important: this raises the question of the extent to which drift matters in evolution (Wright versus Fisher)). In principle, one could allow for stochastic effects in these models, however this necessitates additional assumptions to simplify the mathematics (e.g. Lynch and Hill, 1986). It is therefore of value to combine the mathematical models which allow one to discover general ideas, with simulation studies in which one can check the validity of analytical results using models less restrictive than mathematically tractable ones.

A fundamental difficulty in simulating multilocus clines is that although we are primarily interested in deterministic processes, it is not practicable to simulate populations without including random sampling drift. In principle, one could iterate analytic recurrence relations for the gamete frequencies. However, even with as few as ten loci one would need to follow $2^{10} = 1024$ variables! If there were no linkage, and if all loci had equivalent effects on selection, one could follow the frequencies of gametes carrying $0, 1, \ldots, n$ "1" alleles - in other words, one could follow the distribution of the hybrid index without needing to keep track of individual genotypes. However, since we are interested in the effects of linkage, and in the interaction between selected and neutral loci, it is necessary to simulate selection, recombination and gene flow directly, in a finite population, and to follow the genotypes of each individual. Large numbers of genes can be studied; however, random drift causes substantial fluctuations, necessitating a statistical analysis of the results. A particular difficulty is that we wish to compare simulations with theoretical predictions that are valid for weak selection: yet, the effects of drift are most severe when selection is weak.

5.3.2 The basic model for the simulations

The program described below simulates a multilocus cline stabilized by a balance between dispersal and selection. The simulation is aimed at checking Barton's analytical results using a model less restrictive than his mathematically tractable ones. The model used is therefore a direct stochastic simulation of the evolutionary events involved in a multilocus cline stabilized by a balance between selection and dispersal. The model and biological assumptions are described below. The simulation was written in Pascal, and run on a Macintosh SE/30. An annotated copy of the
program is provided in appendix D.1, along with details of the procedures.

Events occur in the following order: movement of haploid gametes, selection, random union of gametes and meiosis. The important parameters for describing multilocus systems are selection, recombination, and the number of loci under selection.

The pure populations on either side of the cline are assumed to have diverged at each of $n$ loci. Since each locus segregates for two alleles (labelled "0" and "1"), each haploid genome (or "haplotype") can be described by a binary number represented in the computer by a set of 16 bit integers. This allows us to save memory and time by using built-in binary operations (see Crosby, 1970).

There have been a variety of models to represent the dispersal of genes. For example a pair of populations could exchange a proportion ($m$) each generation, or a series of "stepping stones" could exchange individuals with their neighbours (e.g. Kimura and Weiss, 1964; Hastings and Rohlf, 1974; Slatkin and Maruyama, 1975; Felsenstein, 1975 and see Endler, 1977 and Felsenstein, 1976) or genes could spread by diffusion through a continuously distributed population (see Nagylaki, 1974; Barton, 1983). The most thoroughly explored of these and the simplest to compute is the one-dimensional "stepping-stone" model developed by Kimura (1953) (and see Kimura and Weiss, 1964). This is the model used here. It consists of a one dimensional linear chain of demes each of size $N$: a proportion $m$ of the gametes from each deme migrates in each generation, and is divided equally between the two neighbours. This proportion is fixed and so must equal $0/2N, 1/2N, \ldots$ in all the simulations described in Section 5.4, $m = 1/2$ to approximate a continuous spatial distribution. When selection is weak, gamete frequencies will change smoothly from place to place.

After migration, haploid gametes unite at random to produce diploids. Selection acts on these diploids, and is followed by meiosis. For each haploid offspring a diploid is chosen as the parent, with probability proportional to its fitness. Thus the number of haploid individuals ($N$) remains constant from generation to generation. Fitness may depend on only a subset of the loci, the rest being neutral markers. The offspring genotype is derived from the two parental haplotypes by re-

---

2The population ("pop") is an array of "haplotypes". A second array ("newpop") stores the new genotype in each deme, after selection, recombination and migration.

3A two dimensional model of population structure would provide a more realistic model. The effects of drift in one and two dimensions are qualitatively different (Malecot, 1948). However, this is not a serious problem here, since we are mainly interested in deterministic processes, which do not depend on the number of dimensions if the hybrid zone is straight.
combination (meiosis): genes are arranged a single chromosome, chiasma positions are random and occur with frequency according to the rate of recombination, r. After recombination, a proportion m/2 of the haploids in each deme migrate between demes. Migrants are chosen randomly.

Although the rate of migration between the demes will typically be m/2, an option is provided in the program, to reduce this rate at the centre of thecline in order to simulate the coincidence of a physical and genetic barrier to gene flow.

The population is started with a sharp step: all demes to the left of centre are fixed for "0" alleles and those to the right are fixed for "1" alleles.

Following the selection scheme used by Barton (1983) and Szymura and Barton (1991) (and which has been investigated by several other authors, see Felsenstein, 1976), an individual heterozygous at n loci would have fitness \((1-ns)\). However, under this model fitness could be negative for large \(s\). To model heterozygote inferiority, an individual heterozygous at \(n\) loci has fitness \(e^{-ns}\) (for small \(s\), \(e^{-ns} \approx (1-s)^n\)).

Multiplicative fitness implies that selection acts independently on each locus: the probability of survival is the product of the separate probabilities of survival due to each locus. In fact, epistasis may be very common in nature. Bazykin (1973) has shown that a tension zone could result from epistasis alone. Accordingly, in the model used here the fitness of a genotype can either be a function of number of heterozygous loci, or a function of degree of departure from a pure genotype (a kind of epistasis: epistasis refers to any non-additive interaction). To model a hybrid zone, there must be an interaction of genes such that the purest phenotypes are of highest fitness. This can be done in a variety of ways (e.g. see Slatkin, 1975, and Mallet and Barton, 1989a). To simulate epistasis in this program, fitness can be modelled either as an inverse Gaussian function (see Fig 5.2) or as a power function. In the Gaussian model, individual fitness is \(W = \exp(-\delta e^{-(x-0.5)^2/2h^2})\). \(\delta\) is set to \(\frac{ns}{1-e^{-1/8h^2}}\), ensuring that fitnesses are comparable across models of epistasis and heterozygote disadvantage; the halfwidth of the region of reduced fitness is an extra parameter (note that \(W = \exp(-s * e^{-(x-0.5)^2/2h^2})\) is used rather than \(W = 1 - se^{-(x-0.5)^2/2h^2}\) to ensure that fitnesses are positive for large \(s\)).

The Gaussian model does not include the case where fitness drops rapidly with a little introgression. Here a power law can be used, where individual fitness is \(1 - (1 - e^{-ns})(4\alpha(1-x))^{\beta}\). Here \(x\) is individual genotype, and \(0 < \beta < \infty\). When \(\beta = 1\), this reduces to a quadratic relation (the Gaussian model with \(h \rightarrow \infty\) will also approach a quadratic model). When \(\beta\) is small, a little introgression greatly
Figure 5.2: Sketch to illustrate the Gaussian fitness function used to model epistasis. Fitness (W) on the horizontal axis; allele frequency (p) on the vertical axis; s is the selection; fitness is described by $W = 1 - se^{-(p-0.5)^2/2h^2}$ where $\sqrt{2}h$ is the half-width.

reduces fitness; when $\beta$ is large, fitness is only affected near $x = 0.5$ (and the fitness function will be very similar in shape to that of the Gaussian model). An individual with $x = 0.5$ has fitness $e^{-ns}$, as above. With one locus this model reduces to heterozygote disadvantage: the parameter $\beta$ has no effect because only genotypes with $x = 0, 0.5$ and 1 exist.

Several tricks are used to speed up the simulations. Fitness depends only on the number of heterozygous loci, or on the number of “1” as opposed to “0” alleles. A table of fitnesses can therefore be compiled at the beginning of the run. Moreover, because the haplotype is stored as a set of integers, a table listing the number of “1” alleles in each 16-bit integer can be compiled, so that the number of “1” bits does not need to be counted up for each generation. Selection of a diploid parent (i.e. a pair of haplotypes) is done by setting a table of cumulative fitnesses: for each deme in each generation, the fitness $W(i,j)$ of each possible diploid pair is calculated and a table $\{0, W(1,1), W(1,1) + W(1,2), \ldots, \sum_{i,j=1}^{2N} W(i,j)\}$ is set up. A random number is drawn from a uniform distribution between 0 and $\sum_{i,j=1}^{2N} W(i,j)$; the point in the table where this lies gives the chosen parent.
Statistics

Since this is a stochastic simulation, many estimates must be made. Strictly speaking, we should take these estimates from many independent replicates. However, since a hundred or more generations must pass before the system settles down, this is not feasible: statistics are therefore recorded at set intervals, after a warm-up period. To the extent that successive estimates are autocorrelated, confidence intervals will be underestimated.

Statistics are calculated from diploids immediately after random union. The key statistics are based on the mean and variance of the “hybrid index”, \( z \) (0 < \( z \) < 1): since a locus can be in one of two states, “0” or “1”, we can assign each locus in the deme a value corresponding to the sum of “1”s (\( \equiv p \)) at that locus, averaged over individuals. The mean of \( z \) is just the mean allele frequency. The variance of \( z \) includes two components: the genic variance, due to heterozygosity at individual loci, and the remainder, due to linkage disequilibria (Bulmer, 1980).

Since the genic variance can be calculated from individual allele frequencies, the average disequilibrium can be found without the need to calculate all \( n(n-1) \) pairwise associations. Suppose the two populations differ in a set of traits, \( z_i \). These might be quantitative traits or Mendelian markers. In the latter case, the three genotypes and a diploid are labelled \( z_i = 0, 1, \) or 2. Data from \( n \) loci or traits can be summarized by a hybrid index \( z = \sum_{i=1}^{n} \alpha_i z_i \). Here this index is scaled so that it runs from 0 for one population to 1 for the other. If there are \( n \) diagnostic marker genes, the appropriate weighting would be \( \alpha_i = 1/2n \), so that \( z \) is just the proportion of alleles derived from one population rather than the other. Assuming Hardy-Weinberg proportions, the variance of \( z \) is:

\[
\text{var}(z) = \sum_{i,j=1}^{n} \alpha_i \alpha_j \text{cov}(z_i, z_j) = \sum_{i=1}^{n} \alpha_i^2 \text{var}(z_i) + \sum_{i \neq j} \alpha_i \alpha_j \text{cov}(z_i, z_j)
\]

(5.38)

This has two components: the first due to variation in each contribution to the index (\( \text{var}(z_i) \)), and the second to covariance between different contributions. Where the index is based on discrete Mendelian markers, these covariances are due to linkage disequilibria: \( \text{cov}(z_i, z_j) = 2D_{i,j} \), and \( \text{var}(z_i) = 2p_i q_i \). The factor of 2 arises because \( z_i \) is the sum over two copies of the \( i^{th} \) gene. With the scaling \( \alpha_i = 1/2n \), we have:

\[
\text{var}(z) = \sum_{i=1}^{n} 2\alpha_i^2 p_i q_i + \sum_{i \neq j} 2\alpha_i \alpha_j D_{ij}
\]
Here, $\overline{D}$ is the average pairwise linkage disequilibrium, $\overline{z}$ is the average of the hybrid index, and $\text{var}(p) = \frac{1}{n} \sum (p_i - \overline{p})^2$ is the variance of allele frequency across the $n$ loci. Since the variance of $z$ and the individual allele frequencies can be easily calculated, Equation 5.39 gives a straightforward way of estimating linkage disequilibria. Approximate confidence limits can be found by using the critical points of the $F_{n-1,\infty}$ distribution to set limits on $\text{var}(z)$, and ignoring uncertainty in the allele frequencies.

To test the theory, we must estimate cline width, average linkage disequilibrium and mean fitness at the centre, and the strength of the barrier to gene exchange. Because averages must be taken over many generations, these must be calculated automatically. Where selection is weak enough that there is no appreciable barrier effect, cline width is estimated by regressing $\ln(p/q)$ against distance, using the region between $\ln(p/q) = -2$ and $+2$. Here, $p$ is the average allele frequency: if the clines at different loci lie in different places, this method will overestimate cline width. Where there is an appreciable barrier, the cline is divided into three regions. Since we expect gradients in allele frequency to be proportional to $W^{1/\gamma}$, the centre is defined as the region where mean fitness is reduced by at least 5% of its maximum drop. Three linear regressions of $z = \ln(p/q)$ against distance are then taken (excluding the edges, where demes are near fixation, and $z$ is outside the range $-5$ to $+5$). The gradient in allele frequency is related to the gradients of these regressions by $(\partial p/\partial x) = pq(dz/dx)$; allele frequency gradients are calculated by extrapolation to the points where these regressions cross.

We expect linkage disequilibrium to be proportional to $(\partial p/\partial x)^2$; since, in simple models, the gradient $\partial p/\partial x$ is proportional to $pq$, the disequilibrium at the centre can be estimated by fitting $D = \alpha(pq)^2$ using least-squares. This gives a better estimate than relying on the maximum $D$, or the single value nearest the centre. However, when the clines are very narrow, no demes have $p \approx 0.5$, and so the disequilibrium estimated by regression may be greater than 0.25 (see Figure 5.13). Mean fitness at the centre is estimated in a slightly different way: because we expect cline shape to depend on the actual mean fitness, we use the actual minimum, averaged over all the sampled generations. Except where selection is very strong, this gives a similar value to that estimated by regression of mean fitness on $pq$.  

\[
= \frac{1}{2n} [\overline{z}(1 - \overline{z}) - \text{var}(p)] + \frac{1}{2}[1 - 1/n]\overline{D} \quad (5.39)
\]
5.3.3 Testing the program

In writing this program, each procedure was tested separately, to ensure that it performed the required task. Random number generators are notoriously unreliable (see Press et. al., 1986). It is therefore important to check procedures where random numbers are employed. The procedure *set_mask* sets up a recombination mask for use in procedure *reproduce* (see above). Figures 5.3 and 5.4 demonstrate that the observed distribution of recombination events agrees very closely with the expected (binomial) distribution.

Results from simulation runs were also tested by comparison with predictions from standard population genetic theory. Genetic drift changes the gene frequencies in a random and unpredictable manner, resulting in the fixation of one or other allele; other evolutionary forces such as selection and migration will tend to change the gene frequencies in a determinate way, pushing them towards some equilibrium value.

Genetic drift

1) If there is no migration, selection or recombination, the effects of drift can be seen by following the mean gene frequency in a population of moderate size over several generations. Starting with an initial gene frequency of 0.5 in the population, the gene frequency was recorded every 10 generations for 100 generations. Five replicate populations were recorded, each of which had a population size of 50 haploid individuals. The results are illustrated in Figure 5.5, with the mean frequency of foreign genes plotted on the vertical axis, and time in generations on the horizontal axis.

Linkage disequilibrium

The mixing of populations with loci at different allele frequencies generates gametic correlations (or linkage disequilibrium) (Li and Nei, 1974; Feldman and Christiansen, 1975) In the extreme case, where the populations are fixed for different gene combinations, the mixture will be in complete disequilibrium. For example, in the centre of a cline high $D$ will result from migration from either side of the barrier. Demes in the tails will have lower $D$.

Theory predicts that gametic frequencies change from generation to generation as a function of $r$ in the absence of selection, mutation and drift in the following
Figure 5.3: The frequency of recombination events for ten loci: (a) $r=0.5$, 100 replicates; (b) $r=0.3$, 200 replicates; (c) $r=0.1$, 200 replicates; (d) $r=0.01$, 200 replicates.
Figure 5.4: The frequency of crossovers in 200 replicates, $r=0.1$

Figure 5.5: Testing drift: five replicates each with population size $N = 50$. Mean frequency of foreign genes is plotted against time in generations.
$X_{1,t+1} = X_{1,t} - rD_t$

$X_{2,t+1} = X_{2,t} + rD_t$

$X_{3,t+1} = X_{3,t} + rD_t$

$X_{4,t+1} = X_{4,t} - rD_t$

(5.40)

and $D_{t+1} = (1 - r)D_t$.

In a large random mating population, with no selection or migration, linkage disequilibrium will decay at a rate $(1 - r)^t$.

In the test, 12 demes of size 50 haploid individuals with 2 genes each were simulated. The demes had starting frequency $P = 0.5$. (Half the individuals were fixed for indicator variable 1 representing gene type $P$ and the other half fixed for indicator variable 0 at both loci - representing gene type $Q$.) There was no selection or migration. Figure 5.6 shows the decay of linkage disequilibrium with time, relative to its initial value ($D_0$), a) when $r = 0.05$ and b) when $r=0.2$: for both values of $r$ the observed rates are not significantly different from the expected rates.

**Migration**

1. **Following the change in allele frequency.** Migration has an averaging effect, bringing all gene frequencies to a common value, without actually changing the overall gene frequency. If allele frequencies do not change much between generations or demes, they can be taken to be roughly continuous, and the change in allele frequency with time can be approximated by diffusion:

$$\frac{\partial p}{\partial t} \approx \left(\frac{\sigma^2}{2}\right)\left(\frac{\partial^2 p}{\partial x^2}\right)$$

(5.41)

This equation has solutions which depend on the initial conditions. In the special case, where two populations fixed for different neutral alleles meet in a sharp step, the step gradually collapses, and its shape at time $t$ can be found by supposing that the initial distribution is made up of a series of spikes at $y = 0$ up to infinity:

$$P(x, 0) = \begin{cases} 1(x < 0), 0(x < 0) \\ = \int_0^\infty (\partial(x - y))dy \end{cases}$$

(5.42)
Figure 5.6: The decay of linkage disequilibrium when (a) \( r=0.2 \) and (b) \( r=0.05 \).
Figure 5.7: Changing cline width with time, m=0.1.

Then each spike spreads out in a normal distribution, and the net shape is the integral:

\[ P(x, t) = \int_0^\infty \exp\left(-\frac{(x-y)^2}{2\sigma^2t}\right) \frac{1}{\sqrt{2\pi\sigma^2t}} \, dy \]  

(5.43)

Thus as the step collapses, one expects the width of the cline to increase with \( \sqrt{2\pi\sigma^2t} \), or equivalently, in the 1-D stepping stone model: with \( \sqrt{2\pi mt} \) (here the variance in parent offspring distance is simply the migration rate \( m \)).

With starting frequencies in a step, 5 replicates consisting of 12 demes each with 50 haploid individuals, were simulated. The rate of migration, \( m \) was 0.1; there was no selection or recombination. Figure 5.7 shows the change in cline width after 100 generations.

2. Selection, migration and drift: the stepping stone model. The decrease of genetic correlation with distance in the stepping stone model can be used to test the migration procedure. This phenomenon Wright called 'isolation by distance' (Wright, 1943). It describes local differentiation of gene frequencies in demes, resulting from random genetic drift due to the fact that the distance of individual migration is usually much smaller than the distribution range of the entire population. This local differentiation can be investigated in terms of change in correlation with distance (Malecot, 1975) or in terms of \( F_{st} \). For small \( m \), the predicted \( F_{st} \) is \( \frac{1}{2N\sqrt{2ms}} \). For large \( m \), \( F_{st} \) will be somewhat reduced. For example when \( m=0.5 \), the predicted \( F_{st} \) will be \( F_{st} = \frac{1}{2N\sqrt{2ms}=1.072} \) (from Kimura and Weiss, 1964). In the tests initial individual allele frequencies were set to \( p = 0.5 \), then with balancing selection (heterozygote advantage), \( s = -0.05 \) and with \( m = 0.5 \), \( F_{st} \) values were calculated from five replicate runs for four different population sizes:
N = 12; N = 24; N = 50; and N = 100 haploid individuals. In Figure 5.8 we see that for $N \gtrsim 18$ the observed $F_{st}$ is not significantly different from the predicted value.

**Selection**

1. **Heterozygote disadvantage.** Since $1/\omega \propto \sqrt{s}$, we can test selection by observing the width of the cline for different values of $s$. Cline width can be defined in a variety of ways: it could be defined as the distance over which gene frequencies change from some value $p = v$ to $p = (1 - v)$, for example $p = 20\%$ to $p = 80\%$ (see Endler, 1977). A more-or-less equivalent definition of cline width is 1/maximum gradient (see 1.2.1), and this is the definition that has been used in the theory of clines discussed in 1.2). Since for a cline maintained by heterozygote disadvantage, $p = \frac{1}{1 + e^{-x/\omega}}$ and $q = \frac{e^{-x/\omega}}{1 + e^{-x/\omega}}$; $p/q = e^{x/\omega}$ and $\ln(p/q) = \frac{4s}{\omega}$, on the logit scale, the allele frequency in a deme is $\frac{4s}{\omega}$ (i.e. $\ln(p/q) = \frac{4s}{\omega}$), where $\omega = \frac{8m}{\omega}$ and $x$ is the distance from the centre of the cline. A good estimate of the width of the cline can be obtained from the slope of the cline on a logit scale. Figure 5.9 shows the observed widths and predicted widths for increasing selection, $s$.

2. **Epistasis: the Gaussian model.** The shape of the cline can also be used to check epistasis. The predicted width of the cline with the Gaussian model of epistasis will be a function of migration, $m$; selection, $s$; the halfwidth of the region of reduced fitness, $h$; and the number of loci under selection, $n$:

$$\omega = \sqrt{\frac{m}{\tilde{s}}} \sqrt{\frac{2}{(1/8) - (5/24)e^{-1/(8h^2)} + (1/12)e^{-1/(32h^2)}}}$$

(5.44)

(Barton and Jackson, 1991). Here, $\tilde{s} = \frac{n^2}{1 - e^{-1/(8h^2)}}$ and is set to ensure that selection is comparable across models for heterozygote disadvantage and epistasis. Figure 5.10
shows the results of a test in which five replicates each with 50 individuals were simulated, with $m = 0.5$, $n = 2$, $r = 0.5$ and $s = 0.1$ for different values of $h$ (halfwidth). We can see that as the halfwidth narrows with the Gaussian model of epistasis, there is an increased discrepancy between observed and predicted cline widths. This discrepancy is not surprising: under this model, one would not expect a straight line on the logit scale (except near the centre of the cline). The regression method was used to estimate the width (see above), using only the three central demes (values spanning zero on the logit scale.)

In tests in which five replicates were simulated with 50 haploid individuals, $m = 0.5$, $h = 0.25$, $n = 2$, $r = 0.5$ for different values of $s$, the observed widths were not significantly different from those predicted by theory (see Figure 5.11).

In conclusion, these tests demonstrate that the program is performing as it should.

### 5.4 Checking the theoretical predictions

How do the frequencies of the various genotypes found in hybrid zones allow us to infer parameters such as gene flow, selection pressure, the number of loci under selection and the ease with which alleles cross from one gene pool to another? Since the mechanism of selection has little effect on the shape of clines, we can make

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4This leads to an interesting question: how does one distinguish a stepped pattern resulting from epistasis from one resulting from a barrier to gene flow?
Figure 5.10: The width of the cline for different half-widths $h$ under the Gaussian model. Note that $\hat{s} = \frac{ns}{1-exp(\theta h^2)} \approx \frac{ns}{\theta h^2}$. Therefore as $h \to 0$ $\hat{s} \to \infty$, giving the odd behaviour when $h = 0.1$. Strong "effective" selection $\hat{s}$ would be required.

Figure 5.11: The width of a cline with Gaussian epistasis.
inferences without needing to know just how selection is operating. We have seen that if selection is not too strong, cline shape does not depend on the local population structure: gene flow through a continuous habitat and across a grid of demes or “stepping stones” can both be approximated by diffusion (Nagylaki, 1975). The effect of gene flow then depends on a single parameter, $\sigma$, defined as the standard deviation of the distance between parent and offspring, measured along a linear axis.

### 5.4.1 Cline shape

We have seen that cline shape depends only weakly on the way selection acts. On a logit scale a cline maintained by selection against heterozygotes follows a straight line, with slope $\partial p/\partial x = 4/\omega$; other models give clines which approach a straight line on a logit scale, though complete dominance results in clear deviation from this pattern (Fig. 5.1) (see Mallet, 1986; Barton and Jackson, in press).

However, one might expect interactions amongst genes to distort the shape of a set of clines. A simple model of epistasis has been investigated (see above), in which the fitness $W$ of an individual depends on the fraction of alleles from each of the hybridizing populations $x$ and $1 - x$:

$$W(x) = 1 - s(4x(1 - x)\beta)$$  \hspace{1cm} (5.45)

I have used this model rather than the Gaussian, because the Gaussian does not include cases where fitness drops rapidly with a little introgression. When $\beta = 1$, Eqn. 5.45 reduces to a quadratic relation (Fig. 5.12). When $\beta$ is large (upper curves in Fig. 5.12: $\beta = 4$ and 16), only individuals with genotype close to $x = 50\%$ suffer; in contrast, when $\beta$ is small (lower curves in Fig. 5.12: $\beta = 1/4$ and 1/16), a little hybridization greatly reduces fitness. With one locus, this model reduces to heterozygote disadvantage: the parameter $\beta$ has no effect, because only genotypes with $x = 0, 0.5$ and 1 exist.

With several loci, variations in $\beta$ changes the fitness of backcross genotypes; one would expect that when $\beta$ is large, so that backcrosses are almost as fit as pure individuals, the cline would be shallow at the edges (where unfit genotypes with $x = 50\%$ are rare), and steeper at the centre. Conversely, when $\beta$ is small, selection between pure and backcross genotypes will be strong at the edges, and relatively weaker near the centre, where all hybrids have low fitness. This pattern is seen when large numbers of loci interact (see Fig. 5.12, which shows results for eight loci), though large distortions are only seen when $\beta \gg 1$. However the type of
Figure 5.12: (a) In this model, individual fitness, $W(x) = 1 - s(4x(10x))^\beta$, where $x$ is the fraction of alleles derived from one of the hybridising populations. The curves are for $\beta = 1/16, 1/4, 1, 4$ and 16. Figures (b) and (c) show the shapes of clines with two and eight loci respectively; each shows curves for $\beta = 1/16, 1/4, 1, 4, 16$ plotted using a logit transform, and scaled to have the same width. With two loci (b), epistasis has little effect on cline shape. With eight loci (c), clines are stepped when $\beta > 1$; however, epistasis still has little effect when $\beta < 1$. Curves for large $n$ are similar to those for $n = 8$. Cline shapes were calculated using the diffusion approximation and ignoring linkage disequilibrium (accurate when selection is weak).
epistasis has remarkably little effect when just two loci interact (Fig. 5.12).

Thus strong distortions can be produced by epistasis, but only if a moderate to large number of loci interact in their effects on fitness, if the effect is such that only individuals close to the central genotype have reduced fitness, and if one is observing the loci that are actually under selection.

5.4.2 Cline width

The simplest parameter to measure from a hybrid zone is its width, defined as $1/\text{maximum gradient}$. We have seen that a variety of models of selection on single loci or quantitative traits produces clines with similar shapes. In all cases discussed, the width of the cline is proportional to $\sigma/\sqrt{s^*}$ (where $s^*$ is the difference in mean fitness between populations at the centre and the edge of the cline). This robust relation should allow one to infer selection pressures from cline widths and dispersal rates. However there are difficulties in measuring selection and dispersal in the field (see Barton and Hewitt, 1985; Barton and Jackson, in press). Without knowing either of these parameters, the relation is not very useful. It is therefore desirable that we have some way of estimating these directly.

5.4.3 Linkage disequilibria

In this section I will discuss how gametic associations can be used in conjunction with cline widths to estimate dispersal and selection. Barton (1986) has shown how the strength of linkage disequilibria can be calculated by balancing the rate of influx against the rate of recombination (see above and also Barton and Jackson, in press). In a continuous population in which dispersal is approximated by diffusion, the strength of the association between alleles is given by:

$$D = \frac{\sigma^2}{r} \frac{\partial p}{\partial x} \frac{\partial u}{\partial x}$$

$$= \frac{\sigma^2}{r} \omega_p \omega_u \text{at the centre} \quad (5.46)$$

($\equiv$ Equation 5.25) where $\sigma^2$ is the rate of gene flow, $\omega$ is the cline width, $r$ is recombination rate between the markers. We can estimate $r$ from a direct genetic investigation into the number of chromosomes and chiasmata. This means that we can estimate dispersal from cline width (which is easy to measure) and disequilibria. Linkage disequilibria can be estimated by maximum likelihood or from the variance in hybrid index (for details see Barton and Jackson, in press and see Lewontin, 1988 for a discussion of alternative methods).
To determine how robust these estimates are, we need to consider the various assumptions made in deriving the above relation. \( D = \sigma^2/rw^2 \) holds for small \( s \). When selection is increased, the diffusion approximation breaks down, because second order terms in \( s \) become important (see Mallet and Barton, 1989a). However we would expect the approximation to be more accurate as the number of loci increase. Barton (1983) has shown that the migration term becomes very large relative to terms in selection as the number of loci increase. The prediction in Equation 5.46 has been tested against simulations. Figure 5.13 shows results for hybrid zones maintained by heterozygote disadvantage or epistasis, with eight unlinked loci. The observed linkage is compared with the value expected from the cline width predicted by weak selection theory (straight line), and with that expected from the cline width actually seen in simulations (thinner curve). There is good agreement with prediction from the observed width, even when selection is strong, and even when there is epistasis: the values in the two graphs are similar. Thus, dispersal rates could be estimated from observed linkage disequilibria, without the need to know how the clines are maintained. When selection is strong, linkage disequilibria cause the clines to become narrower than expected, and in turn, to generate more disequilibrium. This accounts for the discrepancy between the predictions in Fig. 5.13. When selection is weak, and the clines are maintained by heterozygote disadvantage, disequilibrium is somewhat weaker than expected. This may be because random drift scatters the clines to different locations, weakening their interaction. The effect is not seen with epistasis, since this tends to pull the clines together. Similar comparisons with just two loci show a similar pattern, though the confidence limits are wider, and there is less steepening of the clines with strong selection.

### 5.4.4 Cline shape and barriers to gene flow

Cline shape can be used to infer selection pressures. A cline with a sharp central step flanked by shallow tails of introgression is typical of many hybrid zones. We have seen that if we explain such a pattern by the action of linkage disequilibrium at the centre (Barton, 1983; Szymura and Barton, 1991, and see above), then the shape of the cline (expressed as barrier strength, \( B \)) depends on the mean fitness:

\[
B/\omega = W^{-1/\tau}
\]  

(5.47)

The advantage of this remarkably general relation is that we can measure the width of the zone \( \omega \) from allele frequencies; \( \tau \) can be calculated from the number of chromo-
Heterozygote disadvantage: 
n=8 r=0.5

Epistasis: 
n=8 r=0.5 beta=1

Figure 5.13: Comparisons between observed linkage disequilibrium, and that expected from a balance between dispersal and recombination. (a) gives results for selection against heterozygotes, (b) is for epistasis with \( \beta = 1 \) (i.e. \( W = 1 - 4s(1 - x) \)). There were 40 demes, each of \( 2N = 50 \) haploid individuals; selection acts on eight unlinked genes. The disequilibrium \( (D) \) at the centre is plotted on a log scale, against the strength of selection at each locus \( (s) \). Haploid gametes migrate after meiosis, and before selection; disequilibrium is measured immediately after meiosis, and so the predicted value is \( m(1 - r)/rw^2 \) (see Section 5.3.2). The straight line shows the prediction, given the width calculated using a weak selection approximation (\( w = \sqrt{8m/s} \) for heterozygote disadvantage (a), and \( w = \sqrt{64m/15s} \) for epistasis (b)). The thinner line shows the prediction made from the actual width observed in the simulations: deviations between the two lines are due to differences between observed and expected widths. Observed disequilibria are shown with 95% confidence intervals; data are accumulated from generations 100 to 400 in each run, and recorded every 20 generations (apart from \( s = 0.005 \), for which generations 400 to 1000 were used).
somes and chiasmata, and the barrier strength $B$ can be estimated from the shape of the cline: it is determined by the ratio between the step size and the external gradient. Thus we can use the cline shape to find the total selection pressure acting on the hybrid zone. Since Equation 5.47 was derived on the assumption that is weak, it may not be safe to extrapolate to the strong selection pressures needed to produce strong barriers. Predictions have been checked against simulations of clines maintained by heterozygote disadvantage and by epistasis (Figure 5.14). Selection acts on 16 loci, spaced evenly along a single chromosome. These alternate with 16 neutral marker loci; the recombination rate between adjacent loci is 5%, giving a map length of $31 \times 0.05 = 1.55$ Morgans. This is a much shorter map than is usual, however, because the barrier increases with the number of loci, but decreases with map length, this choice may give results typical of selection on more loci.

The observed ratio between the gradients at the centre and edge fits reasonably well with the theory (shown by the solid line in Fig. 5.14) when selection is weak. Although there are apparently some significant deviations, the confidence limits shown on the graphs may be too narrow. This is because the frequency of the neutral alleles changes slowly as a result of random drift, so that successive estimates are somewhat autocorrelated. When selection is strong, allele frequencies change abruptly between adjacent demes, and so all demes are close to fixation for one or other parental genotype. The mean fitness therefore never declines below about 0.5. The barrier to gene flow continues to become stronger with increasing selection against hybrids, even though the mean fitness does not decrease further. In *Bombina*, the observed ratio of gradients is 0.11 (limits 0.09–0.21); in this range, the simulations show that there is good agreement between simulations and theory.

In this chapter I have described the theory of clines and some of the predictions have been tested. In the next chapter, I will describe a field investigation into gene flow across a physical barrier. The predictions from Barton’s multilocus theory on barriers to gene flow can be combined with direct measures of cline shape and dispersal to investigate the effect of a physical barrier and the shape of the cline, where it coincides with a physical barrier, can be used as an indirect measure of reduced hybrid fitness. I will outline how the theoretical predictions on barriers to gene flow could be tested using simulation program described in this chapter.
Figure 5.14: The ratio between the gradient in allele frequency at neutral loci at the edge of the hybrid zone, and at the centre, plotted against selection on each locus: a small ratio indicates a strong barrier to gene flow. In (a), selection acts against heterozygotes and in (b), there is epistasis with $\beta = 1$. Observed values are from simulations of 16 selected loci, alternating with 16 neutral markers, with recombination of $r = 0.05$ between adjacent loci. There are 40 demes (except for $s = 0.025$ where there are 80). Statistics are calculated every 50 generations, from generations 400 to 800; the slopes on either side are averaged. The solid line gives the predicted ratio, $W^{1/r}$; the harmonic mean recombination rate $r$ between neutral and selected loci is 0.1782 here.
Chapter 6

Barriers to gene flow

6.1 Introduction

Because the biological species is defined as a group that cannot exchange genes with another such group (see Chapter 1), the nature and effects of barriers to gene flow are central to an understanding of the process of speciation. In a continuous habitat, the flow of genes through a population can be described by the flux, which is the total rate of increase in allele frequency in some region:

\[ J = \int \frac{\partial p}{\partial t} dx \]  

(6.1)

Since the change in allele frequency can be approximated by diffusion:

\[ \frac{\partial p}{\partial t} = \frac{\sigma^2}{2} \frac{\partial^2 p}{\partial x^2} \]  

(6.2)

where \( \sigma^2 \) is the variance in offspring position produced per generation (see Section 5.2.1), combining Equations 6.1 and 6.2 gives:

\[ J \Delta p = \left( \frac{\sigma^2}{2} \right) \int_0^\infty \frac{\partial^2 p}{\partial x^2} dx \]

\[ = \left( \frac{\sigma^2}{2} \right) \frac{\partial p}{\partial x} \]  

(6.3)

Thus gene flux is proportional to the gradient in gene frequency: the steeper the gradient, the greater the flow. Barriers to gene flow could be physical, for example the flow could be impeded by a local reduction in density or dispersal (Barton and Hewitt, 1985). Since tension zones tend to move to regions of low density and dispersal (Bazykin, 1969; Hewitt, 1975; Barton, 1979; and see Section 5.2.6) one would expect them to be associated with such a barrier. Gene flow can also be impeded by a reduction in density resulting from reduced hybrid fitness: the "hybrid sink" effect (Barton, 1980b; Barton and Hewitt, 1985). Or, the barrier
could be due to the selection maintaining the clines: in Section 5.2.6, we saw that as genes diffuse through a hybrid zone, linkage disequilibria will be generated. This results in strong induced selection on each locus which produces a sharp gradient and in turn stronger disequilibria, and a stronger effective selection. In either this case, or where the flow meets a local physical barrier, a sharp step will be produced. The size of the step, \( \Delta p \), at the centre of the cline will be proportional to the gradient on either side and the strength of the barrier can be estimated by the shape of the cline \( B = \frac{\Delta p}{\partial p/\partial x} \) (see Section 5.2.6 and Nagylaki, 1976). \( B \) has the dimensions of distance: its magnitude relative to the dispersal rate \( \sigma \) will determine the delay to the spread of genes across the barrier. Neutral alleles will be delayed substantially by such a barrier \( (T \approx (B/\sigma)^2) \), whereas alleles with only a small selective advantage \( s \), will penetrate very rapidly \( (T \approx \frac{\log[(B/\sigma)^2 \pi s/\sigma]}{2s}) \); Barton (1986). For example, Barton has estimated that in Bombina the barrier strength is \( \equiv 51\text{km} \). Such a barrier would delay neutral alleles for \( \approx 2,700 \) generations, but would only delay an allele with advantage 1% for \( \approx 200 \) generations (Barton, 1986).

We have seen how a barrier produced by selection leads to an expression for \( B \) in terms of the harmonic mean recombination rate, cline shape and mean fitness: \( B = \omega(W_{11}/W_{10})^{-1/r} \) (Barton 1986, and see Section 5.2.6). Szymura and Barton (1986) have used this expression to interpret data from Bombina. They show how the barrier can be explained by a reduction in mean fitness at the centre of the hybrid zone to \( \approx 58\% \), and that selection must act on many genes \( (\approx 55) \) (see Section 5.4).

In the next section I will describe a field investigation into the shape of the chromosomal cline in Podisma pedestris, where it coincides with a stream, and the effect this has on gene flow. The results of this investigation raise an interesting question relating to the joint effects of a physical barrier and one resulting from linkage effects. This will be discussed in Section 6.3.

### 6.2 The effect of a physical barrier on the hybrid zone in Podisma pedestris

#### 6.2.1 Summary of previous work

The position of the hybrid zone between the chromosomal races of Podisma pedestris has been described in Chapter 2. It has been surveyed in detail at Tende and at Seyne, where there are no physical barriers and there is a smooth transition between
the two karyotypes over a distance of about 800m. Surveys at Lac Autier (Currie, 1992) and at Col de la Lombarde (see below), however, have shown that where the zone coincides with a small stream, the karyotype changes over only a few metres. In this section, I describe the effect of the physical barrier at Col de la Lombarde. Fig. 6.3 shows a map of the area, and photographs of the site are provided in Figs. 6.1 and 6.2.

In 1988 Cox (1989) estimated the shape of the cline in this region by finding the best fit to data on chromosome frequency. A stepped pattern was observed, from which the strength of the barrier to gene flow was inferred: the barrier strength can be approximated by \( B = \Delta p / (\partial p / \partial x) \), where \( (\partial p / \partial x) \) is the gradient in gene frequency and \( \Delta p \) the size of the step (see section 5.2.6). From detailed measurements
Figure 6.2: Marking out the grid in 1989.
of dispersal she was also able to obtain a direct estimate of the extent to which the stream acts as a barrier. Her results suggested that there is too much movement across the stream for the sharp step in the chromosomal cline to be due to the physical barrier alone. Cox also found that there is a rapid drop in the frequency of foreign types immediately adjacent to the stream, after which it changes more gradually. A cline of this shape could result if there were strong selection close to the stream and weaker selection further away. It could be that selection acts strongly on the whole chromosome in the first few generations of hybridisation, while all the selected loci still segregate together. Since reduced dispersal will increase such associations, this suggests that the physical barrier might have the effect of amplifying the barrier due to selection against hybrids. Although significant, Cox’s results were based on assumptions about density and on only 4 observed movements across the stream. The site was revisited in 1989 to expand her investigation. The results described in section 6.2.3 support her conclusions.

Since Cox has not yet published her investigation, with her permission, I will briefly describe the experiments in Section 6.2.2.

6.2.2 Cox’s investigation

Dispersal measurements

Two transects were established 40 metres apart, (transect E and F in Fig 6.3), with the stream running through the centre of each and each extending for 215 to 275 metres on either side. This defined four areas: E+ and F+ on the west (or positive) side of the stream (up-valley to XY side), and E- and F- on the east (or negative) side (down-valley to XO side) (see Fig 6.3) For convenience, in the discussions to follow I will refer to the two sides of the stream as the West and East sides respectively.

Dispersal was measured using mark-release-recapture experiments. A total of 1025 Podisma were marked individually, using a 3-spot, 10-colour code. Each of the 4 areas was searched in a zig-zag manner, starting at the stream edge and working away from it, until 150 individuals had been caught and marked (this meant searching for between 40 and 100 metres from the stream). In order to minimize disturbance, individuals were released immediately after marking, as close as possible to where they had been found. The distance from the point of release to one of 2 fixed points on the transect was measured. Three or four days after marking and again 3 or 4 days later, each area was searched to determine the new position
Table 6.1: Summary of distances moved in each area: $F^+$, $F^-$, $E^-$, $E^+$. $E + F$ refers to $E$ and $F$ combined; "+" and "-" refer to the west and east side of the stream respectively. $n$ is the number of movements observed and $\bar{x}$ is the mean distance moved per day and $\sigma$ is the standard deviation of distance moved per day.

<table>
<thead>
<tr>
<th></th>
<th>$F^+$</th>
<th>$F^-$</th>
<th>$E^-$</th>
<th>$E^+$</th>
<th>$E + F$</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>3.723</td>
<td>4.682</td>
<td>5.124</td>
<td>4.467</td>
<td>4.593</td>
<td>4.035</td>
<td>5.255</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>3.187</td>
<td>4.928</td>
<td>4.213</td>
<td>4.083</td>
<td>4.128</td>
<td>3.601</td>
<td>4.596</td>
</tr>
<tr>
<td>$n$</td>
<td>144</td>
<td>94</td>
<td>215</td>
<td>104</td>
<td>453</td>
<td>248</td>
<td>205</td>
</tr>
</tbody>
</table>

The average movement was about 5.3m per day, and $\sigma$ was estimated to be 21.1$m/generation^{1/2}$. This is remarkably similar to other measures of dispersal (Barton and Hewitt, 1982; Nichols, 1984; Mason, 1989; Currie, 1992). Local differences in dispersal were observed, but there was no significant difference between males and females.

Density measurements

No record was kept of the numbers of unmarked *Podisma* so that it was not possible to estimate density from the mark-release-recapture experiments. Instead, densities were estimated by searching each of 25 vegetation-types (see Cox, 1989) in zig-zag
walks. This method gives a rough idea of numbers present. From the number observed on walks, density was estimated to be between 0.087 and 0.579 *Podisma* per m².

**The strength of the barrier estimated from observations on dispersal**

To determine the extent to which the stream acts as a barrier to dispersal, we need to work out the observed flux, $J$ across the stream. From this we can estimate the observed barrier strength provided by the stream.

The observed *flux* is the number of individuals that cross one metre of stream in a generation, relative to the density of individuals (per m²) in the area to which they cross. In other words, a flux $J$ corresponds to the replacement of the native population by immigrants in a strip $J$ metres wide.

Cox found that four movements out of the observed 453 were across the stream (over a 3 day period). Over a twenty-day adult life-span, this corresponds to the replacement of the native population in a strip $J = 2.4m/gen$ wide. This was found to be significantly fewer than the number that moved across a strip of habitat of equal width where there is no physical barrier. If movements are expressed as the proportion of local population size $N$ per 1m², then $2.4* N$ moved across the stream per generation whereas $20.3* N$ moved across the same width of unimpeded habitat, in a control experiment (see Cox, 1989). *Podisma* moved significantly further in the direction away from the stream than towards it, as one might expect if the stream is acting as a barrier.

**The shape of the cline**

To estimate cline width and position at $E$ and $F$, Cox first divided the area into 25 regions, each of a specific vegetation type (see Cox, 1989). Since pilot investigations suggested that the zone was narrow where it coincided with the stream most collections were made within 50 metres of the stream. However extra samples were taken at intervals of 50 to 60 metres to a distance of 215 to 275 m from the stream on either side. After the dispersal experiments had been completed, between 7 and 40 adult males were collected from each region. The observed pattern of fused chromosome frequencies as well as data from further years work are shown in Figure 6.3. Cox tried fitting both a *tanh* curve and an exponential decay to the data, and found that
a simple exponential decay gave the best approximation in this area.

\[ A \exp(-x/I) \]  \hspace{1cm} (6.5)

where \( I \) gives the rate of decay i.e. the scale over which allele frequencies change. Note that since the chromosome frequency is low, a \( \tanh \) curve can be approximated by an exponential decay: the \( \tanh \) model with \( \omega = 800m \) is approximately an exponential decay with \( I = 200m \).

**Estimating the barrier strength from cline shape**

Since the barrier strength can be approximated by \( B = \Delta p/(\partial p/\partial x) \), where \( \partial p/\partial x \) is the gradient in frequency and \( \Delta p \) the size of the step, then assuming exponential decay, the shape of the cline can be approximated by:

\[ \left( \frac{\Delta p}{B} \right) \exp(-x/I) \]  \hspace{1cm} (6.6)

Barton (pers. comm.) used maximum likelihood to obtain estimates of \( I \), and \( B \). He estimated the following best fit parameters: \( B = 270m \), \( I = 70m \) (support limits 45 – 125) on the East side (\( \Delta L = 10.61^1 \) with 16df (ns: P=17%)); and \( B = 3,100; \) \( I = 200m \) (support limits 83 – \( \infty \)), on the West side (\( \Delta L = 8.81(\text{ns}) \)) (the allele frequencies on the west side are lower, so the data give less information). Setting \( I = 200m \) in the model for the East side of the stream (to correspond to a cline width of 600m), the barrier needed to maintain the observed sharp step at the stream here would be 1200m (limits: 800-1600).

Given the observed movements across the stream, can one explain the pattern of karyotype frequencies by the presence of the stream acting as a physical barrier? We have calculated the observed flux, \( J = 2.4m/\text{gen} \): it now remains to use this to estimate the observed barrier strength.

Substituting Eqn. 6.3 into \( B = \frac{\Delta p}{\partial p/\partial x} \), \( B \approx \frac{\sigma^2}{2J} \), where \( B \) has units: \( \frac{\text{distance}^2 \cdot \text{time}^{-1}}{\text{distance} \cdot \text{time}^{-1}} = \text{distance} \).

We have seen that the observed flux is \( J = 2.4m/\text{gen} \). A flux of this magnitude represents a barrier of \( B = \frac{\sigma^2}{2J} = 83m \). But we estimated above that a barrier of 1200m would be needed to maintain the observed sharp step at the stream. Even given the very small numbers seen moving across, the barrier is significantly weaker than is required to account for the step. The chance of seeing four or more

\(^1\)2\( \Delta L \) is a \( G \) statistic, roughly \( \chi^2 \) distributed.
movements given a Poisson distribution with expectation $4(83/1200) = 0.28$ is $P = 0.02$.

This suggests that the step in the cline is not being maintained by the physical barrier alone, an interpretation supported by detailed observation of cline shape. There is a sudden drop in frequency of the foreign karyotype within 50 to 60 m from the stream after which it becomes more gradual (see Fig. 6.7). A cline of this shape could be maintained if there were strong selection close to the stream, and weaker selection further away. This could result from strong selection acting on the chromosome in the first few generations of hybridisation, while it is still associated with the other selected loci. On this interpretation, the pattern of introgression depends on the number of loci under selection and the ratio between selection and recombination, $\theta = s/r$ (see section 6.3). Barton has developed a model to describe the joint effects of a physical barrier and selection due to linkage disequilibrium. This is reviewed in section 6.3.

Although Cox has demonstrated that the barrier is significantly weaker than is required to account for the step, the measurement of the barrier was based on 4 grasshoppers crossing the stream, and on assumptions about density. In 1989 the site was revisited to check and expand Cox’s investigation. The primary aim was to obtain more detailed information on the pattern of densities on either side of the stream at the site investigated by Cox. The effect of dispersal across the stream can only be interpreted if the density of the population into which the grasshoppers move is known. Ideally, one would have replicate sites along the stream. Local patterns of density and dispersal along with cline width could tell us how the pattern of density affects the step and whether the step is larger where there is more movement. Unfortunately, on revisiting the area in 1989, we found Cox’s site to be the only suitable site: elsewhere, the habitat on either side of the stream was too sparsely populated to obtain large enough karyotype samples or significant density estimates. However, we did obtain more information on the pattern of density and dispersal at the site investigated by Cox, and the results support her conclusions.

### 6.2.3 A further investigation

**Methods**

In 1989, the stream was mapped in detail: Fig 6.3 shows the site investigated by Cox in 1988 and the grid established to extend her investigation in 1989. A subdivided
grid allows detailed information on density and dispersal. Grids 20m by 70m were established on either side of the stream, and were each sub-divided into fourteen \(\approx (10m)^2\) areas. Measurements were made from the stream edge, and so, because of the shape of the stream, the subdivisions were not always square.

Estimates of density and dispersal were obtained from mark-release-recapture experiments. Each \(\approx (10m)^2\) area was assigned a two-colour code used to mark grasshoppers found within the area (see Table 6.2). Marking with oil paint has been used widely in dispersal experiments on *Podisma* with no adverse effects. On the first day (5/8/89) of the mark-release-recapture experiment, each \(10m^2\) square was divided into four \(\approx 2.5m^2\) areas each of which was searched by two scorers for 2.5 minutes. Individuals were caught into a tin cushioned with vegetation, and numbers of each instar (mostly adults) were recorded. Individuals were marked with small oil paint dots, according to the colour code shown in Table 6.2. Since the grasshoppers could lose a dot or even an entire leg during the experiment, both legs were marked to avoid ambiguity on subsequent scorings. Immediately after marking, individuals were released into the centre of the \(2.5m^2\) area in which they were found, thus minimising disturbance. Four days later (9/8/89), each \(2.5m^2\) area was searched again for 2.5 minutes and numbers of marked and unmarked grasshopper recorded. Again, all individuals caught were marked with small dots in the colour code of the square in which they were found, but this time on either side of the thorax. In addition, an extra four of the \((10m)^2\) areas \((B0^-, F0^-, B0^+, FO^+)\) in the grid were searched and individuals marked (see Fig 6.4). A further search 6 days later (15 or 16 /8/89) completed the experiment.

Each area was scored by two scorers. For the initial capture (5/8) and the first re-capture (9/8), scorers referred to here as \(K\) and \(N\) searched all areas. Because of bad weather, the second scoring had to be split between two days (15/8 and 16/8) and two pairs of scorers (\(K\) and \(N\) on 15/8 and \(D\) and \(A\) on 16/8). Table 6.3 summarises the differences in the abilities of scorers to see grasshoppers. The abilities of individuals within a pair can be compared, but since different areas were searched by each pair a comparison between pairs is not valid.

**Population size**

The Lincoln index is the simplest way to estimate population size. If \(r\) individuals are marked and released in a given area and if on a subsequent scoring \(n\) individuals are caught of which \(m\) are marked and \((n - m)\) are unmarked, then the chance of catching
Figure 6.3: a: the hybrid zone follows the stream (solid line on right) to the river in Vallon d'Orgials (top left solid line). Filled circles, pure XY samples; open circles, pure XO samples. The rectangle is the area detailed in b. Distances are in metres. The coordinates are the same as those in Fig. 3.1. b: This shows the frequency of the chromosomal fusion in the region in which density and dispersal were estimated. The overlay shows the position of the grid established in 1989 (crosses) relative to Cox's 1988 transects “E” and “F”.

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**XO Side (East of stream)**

<table>
<thead>
<tr>
<th>A1</th>
<th>B1</th>
<th>C1</th>
<th>D1</th>
<th>E1</th>
<th>F1</th>
<th>G1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0</td>
<td>B0</td>
<td>C0</td>
<td>D0</td>
<td>E0</td>
<td>F0</td>
<td>G0</td>
</tr>
<tr>
<td>A1</td>
<td>B1</td>
<td>C1</td>
<td>D1</td>
<td>E1</td>
<td>F1</td>
<td>G1</td>
</tr>
</tbody>
</table>

**Stream**

| A0 | B0 | C0 | D0 | E0 | F0 | G0 |

**XY Side (West of stream)**

| A1 | B1 | C1 | D1 | E1 | F1 | G1 |

Figure 6.4: Sketch to show grid layout. In Table 6.2 the east side of the stream is coded “-” and the west side “+”.

Figure 6.5: An adult male *Podisma* marked with oil paint for the mark-release-recapture experiment.
### Table 6.2: Colour codes for (10m)² areas.

Areas in which grasshoppers were not coded could not be used for density estimates, but gave extra information on dispersal.

<table>
<thead>
<tr>
<th>area</th>
<th>colour code</th>
<th>legs marked(5/8)</th>
<th>thorax marked(9/8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0⁻</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B0⁻</td>
<td>red-yellow</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>C0⁻</td>
<td>white-yellow</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>D0⁻</td>
<td>yellow-yellow</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>E0⁻</td>
<td>green-yellow</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>F0⁻</td>
<td>red-white</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>A1⁻</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1⁻</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1⁻</td>
<td>white-white</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>D1⁻</td>
<td>yellow-white</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>E1⁻</td>
<td>green-white</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>F1⁻</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A0⁺</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B0⁺</td>
<td>red-green</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>C0⁺</td>
<td>white-green</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>D0⁺</td>
<td>yellow-green</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>E0⁺</td>
<td>green-green</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>F0⁺</td>
<td>red-red</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>A1⁺</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1⁺</td>
<td>not coded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1⁺</td>
<td>white-red</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>D1⁺</td>
<td>yellow-red</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>E1⁺</td>
<td>green-red</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

### Table 6.3: Table to show differences in the abilities of scorers.

Numbers of marked and unmarked grasshoppers seen on the initial capture and two subsequent scorings are provided. Numbers of adult females (F), adult males (M) and nymphs (N) seen by each scorer are provided. Score 1 and score 2 refer to the first and second recaptures.

<table>
<thead>
<tr>
<th>scorer</th>
<th>unmarked</th>
<th>marked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>init. nos.</td>
<td>score 1</td>
</tr>
<tr>
<td></td>
<td>F  M  N</td>
<td>F  M</td>
</tr>
<tr>
<td>K</td>
<td>30 27 12</td>
<td>34 38</td>
</tr>
<tr>
<td>N</td>
<td>25 30 13</td>
<td>37 34</td>
</tr>
<tr>
<td>D</td>
<td>6  15</td>
<td>0  0</td>
</tr>
<tr>
<td>A</td>
<td>21 12</td>
<td>4  0</td>
</tr>
</tbody>
</table>

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a marked grasshopper is estimated as simply \( m/r \). The estimated population size, \( \hat{N} \), is then \( \frac{n}{m/r} \). The problem with this method is that it assumes that there is no mortality or loss by dispersal—clearly unrealistic for Podisma. A more robust method for estimating population size is Jolly’s method (Jolly, 1965). To use this, the site must be scored twice. Let the number of individuals that were released initially and re-captured on both scorings be given by \( n_{11} \); the number released initially and re-captured on the first scoring only, by \( n_{10} \); the number initially released and re-captured on the second rescoring only by \( n_{01} \) and the number initially released and never seen again by \( n_{00} \). Population size on the first scoring day is then estimated by the ratio of the number of unmarked individuals caught on the first recapture to the chance of catching a marked individual. The fraction of individuals present at the second scoring which were also seen on the first scoring is taken as the probability of seeing a grasshopper.

The Jolly method takes into account loss by death and dispersal, but cannot give an estimate on the second day of scoring (in the analyses described here, I have pooled data from different scorers, so that the abilities of different scorers are irrelevant).

Densities were calculated for each \( \approx (10m)^2 \) area individually. However, numbers in most of the \( (10m)^2 \) areas were too low so it was more informative to pool data to obtain estimates on average population size on either side of the stream. The adult population density on the West side was estimated to be 0.14 per \( m^2 \); and on the East side \( \hat{N} = 0.165 \) per \( m^2 \).

Since there is little dispersal from the area as a whole (see Table 6.5) and since mortality is likely to be low, the Lincoln index method may give a more accurate estimate of population size on either side of the stream.

On the west side of the stream population size (\( \hat{N} \)) estimated from the first recapture was 0.812/\( m^2 \), and from the second recapture 0.920/\( m^2 \). On the east side \( \hat{N} \) estimated from the first recapture was 0.680/\( m^2 \) and from the second, 1.347/\( m^2 \).

**Dispersal**

We are most concerned with the extent to which the stream impedes dispersal, and whether there is too much movement across the stream for the observed step to be due to the physical barrier alone. We will also be interested in whether there is any bias in direction of movement. For example, is there a tendency for individuals to move downhill, or away from the stream? Unfortunately, since most sites along
Table 6.4: Estimates of population sizes. "—" indicates that numbers were too small to get an estimate (either $n_{10}$ or $n_{11}/n_{10}$ was zero).

The stream were very sparsely populated, we do not have enough data to answer questions on whether local density differences influence dispersal and what effect such differences might have on cline shape, as originally hoped.

Dispersal is measured in terms of variance in movement per unit time. To estimate the variance in movement, we need to make some assumption about the distribution of movements. If the population were homogeneous and movements uncorrelated, then the resulting distribution would be normal. Most experiments on dispersal in insects show a leptokurtic distribution, with an excess of long distance migrants (Endler, 1977; Taylor, 1978; Pielou, 1977; Roughgarden, 1979). However, in *Podisma* the distribution is very close to normal (Barton and Hewitt, 1982; Nichols, 1984). Although even a few long distance migrants may be very important to the effects of dispersal, in this investigation, time was limited, and the need to measure movements over a large enough distance to include long distance migrants had to be balanced against the need to detect a reasonable proportion of those grasshoppers released. We were primarily interested in two things: to get better density estimates; and to get a good estimate of numbers crossing the stream. For both of these aims it was more important mark a large number of grasshoppers and thus obtain large samples, than to mark grasshoppers with individual colour codes- which would give more detail on dispersal, but is very time consuming. Cox had already done an extensive study on dispersal in the same area, in which she marked grasshoppers individually and measured distances exactly. She estimated

<table>
<thead>
<tr>
<th>site</th>
<th>$\hat{N}$ for day 2 per m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jolly</td>
</tr>
<tr>
<td>$C0^+$</td>
<td>-</td>
</tr>
<tr>
<td>$C1^+$</td>
<td>-</td>
</tr>
<tr>
<td>$D0^+$</td>
<td>0.18</td>
</tr>
<tr>
<td>$E0^+$</td>
<td>-</td>
</tr>
<tr>
<td>$D1^+$</td>
<td>-</td>
</tr>
<tr>
<td>$E1^+$</td>
<td>0.10</td>
</tr>
<tr>
<td>$C0^-$</td>
<td>0.64</td>
</tr>
<tr>
<td>$C1^-$</td>
<td>-</td>
</tr>
<tr>
<td>$D0^-$</td>
<td>-</td>
</tr>
<tr>
<td>$E0^-$</td>
<td>-</td>
</tr>
<tr>
<td>$E1^-$</td>
<td>0.28</td>
</tr>
</tbody>
</table>
dispersal to be $\approx 20m/\text{gen}^{1/2}$ which agrees well with several other studies. It was therefore not necessary to repeat her extensive investigation. The grid design and colour-coding methods described above allowed us to mark a reasonable number of individuals in the limited time available, and yet get some information on dispersal. By searching a grid 80 metres by 70 metres thoroughly there is a reasonable chance of detecting movements of up to about 40 metres, and a good chance of detecting individuals that cross the stream.

I include calculations on the rate of dispersal for the sake of completeness, here, but this is rather rough, and in the discussions to follow I have used Cox's more accurate estimate instead. Table 6.5 gives the lower limit for the observed distances moved from each $(10m)^2$ area by grasshoppers recaptured on day 4 and day 11. From these, one can calculate the estimated distances moved per day and get a lower limit of the rate of dispersal (see Appendix E.1). This was calculated using the same method as Cox (see above) as $\sigma^2 = 12m/\text{gen}^{1/2}$. However, since Cox's estimate for dispersal is more accurate, in the analyses below I have assumed dispersal is $20m/\text{gen}^{-1/2}$.

Is there bias in direction of movement?

G-tests\(^2\) were used to determine if there is a bias in movement with respect to the stream. Table 6.6 summarises the data.

The number of individuals moving upstream is not significantly different from the number moving downstream ($G_1 = 2.336 - \text{ns}$). The difference in the observed number of movements towards and away from the stream are also not significant ($G_1 = 0.466$).

Dispersal across the stream, flux and the barrier strength

Out of 160 observed movements (recaptures) only five were across the stream. Two individuals moved from East to West and 3 from West to East, over a period of 11 days. The actual number of grasshoppers which move from one side of the stream to the other can be estimated using either the Jolly- or the Lincoln index method. Since the Jolly method can only give an estimate of the chance of seeing a grasshopper on the first scoring, I assume that the probability of seeing a grasshopper is constant between days (this seems reasonable here: the total number of grasshoppers seen

\(^2\)G = 2 \sum \text{obs.ln(oss/exp)}
Table 6.5: (a): This table gives lower limits of distances moved from the initial release points (1989 data). The distances moved are correct to within 5 metres. These are observed rather than actual movements which may be larger (see text). (b) Numbers of observed movements. The estimated distances moved per day are provided.
Table 6.6: (a) Numbers of movements observed in either direction. (b) The estimated average distance moved per day in either direction, in metres. (c) The observed number of movements towards and away from the stream. (d) The estimated average distance moved per day towards and away from the stream.

On successive scores were very similar on the west side of the stream (85 vs. 86) though on the east side total numbers were 110 vs. 77. Pooling the data on the West side of the stream, the chance of seeing a grasshopper, \( \theta = n_{11}/n_{00} = 7/10 = 0.7 \) and on the East side, \( \theta = 6/11 = 0.54 \) (overall, \( \theta = 13/21 = 0.62 \)). Over a period of 11 days, \( s_1 = 3 \) moved from the West side into a strip 30m long on the East side and \( s_2 = 2 \) moved from East to West side into a strip 30m long, over 11 days. Since the chance of seeing a grasshopper is, on average, \( \approx 0.6 \), an estimated \( 2.5 \times 1.667 = 4.7 \) will cross the stream into a strip 30m long over 11 days - i.e. \( J = 0.14 \) for every metre. This corresponds to an estimated 0.26 in a 20 day generation time. To calculate the flux we need to know the density per m\(^2\) into which these grasshoppers move. The average density estimated above was \( \hat{N} = 0.15m^{-2} \). Therefore \( J = 0.26m^{-1}.gen^{-1}/0.15m^{-2} = 1.7 \). Now \( J = (\sigma^2/2)(\partial p/\partial x) = \frac{\sigma^2}{2B} \) (see above); therefore \( B = \frac{\sigma^2}{J} \) and if we assume that \( \sigma^2 = 20m/gen^{1/2} \) then \( B \approx 117m \).

Using the Lincoln index method to estimate the actual number of grasshoppers that cross the stream, the barrier strength \( B \approx 107m \). Both these estimates are remarkably similar to Cox's (\( B \approx 83m \) - see above), giving confidence that these estimates are close to the true value.
Discussion

The survey at Col de Lombarde has shown that in continuous habitat the two chromosomal races are separated by a smooth cline $\approx 800m$ wide $(p = 1/(1 + \exp(4x/w))$. At the edges, the frequency of introgressing chromosomes decreases as $\exp(-\lambda x)$ with $\lambda^{-1} \simeq 200m$. However, there is a sharp change over only a few metres where the zone coincides with a stream. Beyond this step, the karyotype frequency changes in the same way as elsewhere.

The observations made by Cox in 1988 and by me in 1989, both suggest that the stream is not a strong enough barrier to account for the observed size of the step. The results from the 1989 investigation (where density estimates were more accurate) suggest a somewhat stronger barrier strength than that estimated by Cox, but still an order of magnitude weaker than that needed to explain the sharp step. Strong selection on the chromosome could result in a stepped pattern despite a weak barrier. However if this were the case, we would not expect the smooth cline seen elsewhere (but note that Barton (1983) found that observed selection against hybrids was too strong to be consistent with the smooth cline near Tende). This apparent paradox might be resolved if a physical barrier is amplified by selection against hybrids (see below).

In Section 5.2.6 I discussed the strength of a barrier caused by selection at many loci; how this can be used to estimate the net reduction in hybrid fitness and how, combined with measures of linkage disequilibria, it can give estimates of the number of genes responsible for reproductive isolation between hybridizing species. Recently, Barton (unpublished) has extended this theory to investigate the interaction between a physical barrier and multilocus selection. In section 6.3, I will review the approximations developed by Barton and will interpret the data from the field experiments using these.

6.3 The effect of a physical barrier on a cline: a review of the theory

6.3.1 The model

The model follows from the theory on the behaviour of multilocus clines (see Barton (1983; 1986); Barton and Bengtsson (1986) and see Section 5.2.6). There is a single chromosome with $N$ equally spaced loci. Genes flow across a physical barrier separating populations almost fixed for alternative alleles at each of the $N$ loci.
The genes then diffuse into the new population. There is at most one crossover per generation. The chance of a crossover between adjacent loci is \( r \); the net map length, \( r(N - 1) \). Selection of strength \( s \) acts against introgressing alleles, such that an individual heterozygous at \( j \) loci has fitness \( (1 - sj) \), and the total selection pressure is \( S = Ns \). The strength of the barrier is measured by the ratio between the step in allele frequency and the gradient: \( B = \Delta p / \Delta x \). Assuming \( \Delta p \approx 1 \), the influx gives the boundary condition that \( \partial p(N, x) / \partial x = 1/B \) at \( x = 0 \) (\( p(N, x) \) is the frequency of a chromosome with \( N \) loci). Introgressing alleles are assumed to be rare. Matings between hybrids can therefore be ignored, and since it is assumed that there is at most one crossover per generation, foreign alleles will be found in single blocks. Most of the disequilibrium in the tails of the cline is generated in the centre and diffuses out (disequilibrium generated by dispersal will be negligible because alleles are rare: \( D_{ij} \approx \sigma^2(\partial p_i/\partial x)(\partial p_j/\partial x)/r_{ij} \approx 0 \) if \( p_i, p_j \) are small, \( p_i \) is the frequency of chromosomes carrying blocks of \( i \) alleles).

Under a variety of assumptions, which will be discussed more fully below, Barton derives expressions for the average allele frequency, \( \bar{p} \) and the standardized linkage disequilibria, \( R = D_{ij}/\sqrt{p_ip_jq_iq_j} \). In this section, I will briefly review the results of Barton’s theoretical investigation.

The average allele frequency is given by:

\[
\bar{p}(x) = \frac{1}{N} \sum_{i=1}^{N} i p(i, x)
\]  

(6.7)

Here, \( p(i, x) \) gives the frequency of chromosomes carrying different numbers of foreign alleles. When introgressing alleles are very rare, the linkage disequilibrium between two loci is approximated by the frequency of chromosomes carrying foreign alleles at both loci. The standardised disequilibrium is \( R = D_i/\sqrt{p_ip_jq_iq_j} = D/\bar{p} \), where \( \bar{p} \) is the average allele frequency, since \( p \) is the same at all loci. Then consider loci separated by \( rk \) map units. There are \( N - k \) such pairs and a block of length \( i \) contains \( i - k \) such pairs:

\[
R(k, x) = \frac{D(k, x)}{\bar{p}(x)} \\
= \frac{(N/(N - k)) \sum_{i=k+1}^{N} (i - k) \cdot p(i, x)}{\sum_{i=1}^{N} i \cdot p(i, x)} \\
= \frac{(N/(N - k)) \sum_{i=k+1}^{N} \frac{C(i, x)}{\sum_{i=1}^{N} C(i, x)}}{\sum_{i=1}^{N} C(i, x)}
\]

(6.8)

\((C(i, x) \approx \bar{p}(x) : \text{Eqn. 6.7 above}).

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When \((s/r = \theta << 1)\) the average allele frequency approaches \(\bar{p} = m/s\) (as one would expect for selection at a single locus). When selection is increased, associations with other loci reduce the degree of introgression. The strength of the barrier increases with the number of loci.

Barton derives expressions for the frequency of chromosomes carrying blocks of \(i\) alleles: \(p(i, X)\), (here \(X\) is distance measured relative to the characteristic scale, \(l = \sigma/\sqrt{2s}\), in terms of the barrier strength, the linkage disequilibria between locus \(i\) and locus \(j\) and a mixture of exponentials decaying at rates \((\lambda_1 \ldots \lambda_{N-1})\), which represent the break-up of blocks of foreign alleles of length \(i\).

Thus:

\[
p(i, X) = \sum_{j=i}^{N} (d(i, j)/B\lambda_j)\exp(\lambda_j X)
\]

where \(\lambda_j^2 = [(1 + \theta)j - 1]/\theta\) gives the rate of decay of the \(j^{th}\) component. The problem is to find the coefficients \(d(i, j)\).

Recombination breaks up blocks of genes and selection eliminates them. Large blocks are lost most rapidly, so that the linkage disequilibrium between loosely linked loci decays much faster than allele frequency. At large negative \(X\), the linkage disequilibrium between loci separated by \(k\) intervals decays with \(\exp(\lambda_{k+1} X)\), \((\lambda_{k+1} > 1)\) whereas average allele frequency decays with \(\exp(X)\). Thus the standardised disequilibrium decays with \(\exp((\lambda_{k+1} - 1)X)\) and should be negligible for large \(X\).

The average allele frequency decays at a rate which depends on the average size of introgressing blocks and so will decay faster near the barrier than away from it. Thus the effect of multilocus selection on average allele frequency can be summarised by the allele frequencies near the barrier and far from it. If loci segregate independently, the allele frequency adjacent to the barrier would be: \(\bar{p}(0) = (l/B)\). Selection on linked loci reduces \(\bar{p}(0)\) by the factor shown in Fig 6.6. This factor decreases with the number of loci and with the ratio between selection and recombination \((\theta)\). For large \(\theta\), it tends to \(1/\sqrt{N}\). The average allele frequency far from the barrier is also reduced. For \(X << -1\), \(\bar{p}(0) \approx (ld(1,1)/B)\exp(X)\) instead of \((l/B)\exp(X)\). Thus \(d(1,1)\) gives the factor by which multilocus selection reduces gene frequency far from the barrier, and hence the factor \(B/B^*\) by which it amplifies the barrier to gene flow. Again, this increases with the number of loci and with the ratio between selection and recombination. Even if there is an appreciable frequency near the barrier (\(\bar{p}(0)\)), the rate of gene flow far from the barrier may still
be greatly reduced.

An important result of Barton's theoretical analysis is that the result of selection on many loci reducing the influx of genes across a hybrid zone depends mainly on the strength of selection relative to recombination, $\theta = s/r$. In Section 6.2.2, we saw that the shape of the cline in *Podisma* where it coincides with a stream, suggests very strongly that the effects of the physical barrier on the flow of the chromosome are being amplified by the effects of association with other selected genes in the first few generations of hybridisation. We saw in section 5.2.6 that the width of the cline (and therefore the strength of the barrier) is a function of $\theta = s/r$. So one can calculate from the pattern of chromosome frequencies, and the size of the step relative to observed rates of movement, the strength of selection needed to explain the step.

**Reanalysing the data**

The sharp step in the chromosomal cline across the stream could be due to a physical barrier to dispersal, disrupting the cline maintained by selection against heterozygotes. However, this requires a physical barrier an order of magnitude stronger than is measured directly from grasshopper movements as discussed above. However, a model in which the physical barrier is augmented by selection against introgressing alleles at 100 loci, and in which the direct measures of barrier strength and the width seen in an adjacent unimpeded transect (combining data from Cox and the 1989 data, $B = 95m$ and $W = 600m$) are used, is substantially more likely ($\Delta \log(L) = 10.53$) than a model with no genetic barrier, the same width and a strong physical barrier (to account for the size of the step): $B = 950m$ for flow to the left and 1535m for flow to the right. This implies a ratio between selection and recombination of $S/R = 0.89$ (2-unit support limits 0.68 – 1.24) against introgression to the left, and 0.55 (0.45 – 0.72) against introgression to the right. The net barrier would then be 7400m on the left and 2360m on the right.

Using this model, and assuming (arbitrarily) that there are 100 loci under selection, the best fit parameters (using maximum likelihood) to Cox's data are: $l = 150m$, $B = 65m$ and $s/r = 0.73$ This gives $\Delta L = 7.21$, a significant improvement in likelihood over the parameter estimates obtained if we assume a simple exponential (for one extra degree of freedom). The barrier is a bit weaker than that measured (see above) but the figures are consistent with $l = 200m$; $B$ in the range 83m (Cox's estimate) to 107m (my estimate) and $s/r = 0.85$. Figure 6.7 shows the
Figure 6.6: (a) The average allele frequency adjacent to the barrier, $p(0)$, relative to the value which would be found in the absence of linkage disequilibrium ($l/B$). (b) The same ratio, plotted against the number of loci, $N$. (c) The ratio between the average allele frequency far from the centre ($p(X)$ for $X >> l$) and the value which would be found in the absence of linkage disequilibrium ($l/B \exp(X)$). This is the factor by which multilocus selection strengthens the initial barrier; it is denoted by $B/B_{eff}$. (d) The same ratio, plotted against the number of loci, $N$. Figure by N.H. Barton.
Figure 6.7: The frequency of the chromosomal fusion plotted on a logit scale. The frequency changes in a sharp step across the stream. Straight lines show the best fit if one assumes that the physical barrier to dispersal simply disrupts a cline maintained by selection against chromosomal heterozygotes. The model in which the physical barrier is augmented by selection against introgressing alleles at 100 loci predicts a further change just near the stream (curved lines). Figure from Barton and Jackson (in press).

best fit to Cox's data combined with data collected in 1989 and 1990.

Hence the step can be accounted for by \( \theta \) of the same order as that in Bombina. However, if the ratio of selection to recombination is this high, we would expect to find a step in the absence of a barrier. Could it be that there is a critical \( \theta \), such that selection is weak enough to result in a smooth cline where the habitat is continuous, but large enough that the effects of linkage will be revealed in the presence of reduced gene flow caused by a physical barrier. Simulation experiments could be used to investigate this question.

Suggestions for simulation experiments

Simulation experiments could be based on the model described in Chapter 5. To simulate a physical barrier coinciding with the centre of the cline, simply reduce the rate of migration between the two demes in the centre. We have seen that the observations at Col de la Lombarde suggest a strong net selection (limits \( s/r = 0.43 - 0.85 \)). With such strong linkage effects, one would expect a step in the absence of a barrier however there is no evidence of this. Further, based on the magnitude of hybrid inviability (see Chapter 2) and the number of chiasmata, Barton (1983)
estimated that \( S = 2.4 \) and \( R = 9.3 \) i.e. \( s/r = 0.26 \). All this suggests that the physical barrier is having the effect of "amplifying" the genetic barrier.

The approximations reviewed in 6.3 were derived under a variety of assumptions. Simulation experiments could also be used to check that these approximations hold when the assumptions are violated, as they must be in nature.

1. In order to obtain linear equations, Barton ignores interactions between hybrid genotypes. This means that the rate of introgression has to be low. If there are large numbers of weakly selected loci, as is likely, immigration must be very low. Barton has pointed out however, that individuals will have a detectably hybrid genotype even when only a few diagnostic markers are studied and even where the average frequency of any one marker is low. It is therefore important to check the accuracy of his approximations as allele frequencies increase.

2. In the model effects of different loci add, so that epistatic interactions are ignored. Simulations could be used to check the effects of epistatic interactions.

3. Although selection can be strong, \( S = Ns \), in the additive model, must be less than one for fitness to be positive. Further, local gene flow is approximated by diffusion, and we have seen that the diffusion approximation breaks down for large \( S \). The accuracy of the approximation needs to be checked for large \( S \).

4. Only a single cross-over can occur each generation. Since the effect of recombination depends on how the foreign genes are distributed along the chromosome, one would need to follow all possible blocks of foreign alleles. Following higher order disequilibria is not feasible for more than a few loci- since it involves a sum over \( \approx 2^n \) terms: this is why Barton assumes that the chromosome is short enough for only one crossover to occur in each generation. In the simulations, multiple cross-overs could be allowed.

In summary, the investigation described in this chapter suggests a useful approach to measuring fitness and the number of genes under selection. Across either side of the valley at Col de la Lombarde the cline is wide, but inbetween the chromosome frequencies change abruptly across a small stream. The dispersal experiments showed that this is not simply because few grasshoppers cross the stream: observed movements across the stream were too frequent to be consistent with the sharp step.
The most likely explanation is that although selection on the chromosome fusion itself is weak (there is introgression for hundreds of metres to either side), there is strong overall selection against hybrids. This argument is supported by the sharp drop in karyotype frequency immediately away from the stream. Such a pattern is expected if selection acts strongly to eliminate genes when they first cross, but acts more weakly once they have recombined in the new background. Indeed, the theoretical model in which selection is spread over 100 genes, with selection per map unit $S/R = 0.89$, fits significantly better than a single locus model ($\Delta \log(L) = 10.53$).
Chapter 7

The position of the hybrid zone

7.1 Introduction

What determines the position of a hybrid zone? In Section 5.2.6 I discussed the different ways in which clines can be formed. Ecologically determined clines are bound to some environmental gradient and are maintained by that gradient. An obvious question therefore, is whether a hybrid zone coincides with a barrier or environmental discontinuity. Hunt and Selander (1973), for example, have found that the hybrid zone between *Mus musculus* and *Mus domesticus* is associated with a climatic gradient in Denmark. Another example is the Australian grasshopper, *Caledia captiva* in which the Moreton and Torresian races meet close to the 30% contour of the coefficient of variation of rainfall (Shaw and Wilkinson, 1980; Shaw et al., 1985).

Hybrid zones between many different pairs of species or sub-species often appear to coincide geographically (known as “suture zones”: Remington, 1968; Haffer, 1969). The observed concordance has been interpreted as evidence for secondary contact between populations that were isolated in refugia during the Pleistocene glaciation. The idea here is that different species respond to the same changes in population structure, and so end up with parallel distributions.

As discussed in Section 5.2.6, while ecologically determined clines are bound to some environmental change, clines maintained by heterozygote disadvantage or by frequency-dependent selection can result in the formation of tension zones, maintained by internal genetic factors rather than by external selection differentials. This means that they are mobile and can be perturbed from their initial position by a variety of forces (Bazykin 1969; Barton, 1979c; and see chapter 5). In a homogeneous environment, a race with a selective advantage will move the zone forward in favour of the superior type. Tension zones will tend to move to areas of low population
density and will tend to be pinned by physical barriers to gene flow. Other forces will also be important: local differences in habitat type, population structure and local barriers to gene flow will affect the position of a tension zone (Barton 1979c). There have been very few studies investigating the position of tension zones in nature and how they relate to population structure and habitat. One example can be found in the investigation by Moore and Buchanan (1985) on the hybrid zone between red- and yellow- shafted Flickers on the Great Plain of the U.S.A. They investigated whether this hybrid zone is associated with a population density trough: it does not appear to be, although sample sizes are rather small.

The investigation described in this chapter was motivated by the results of the extensive surveys on the pattern of densities across two transects at Col de la Lombarde as well as across transects at Col de Fenestre; Vallée des Merveilles and Jas du Plan Tendasque (see Chapter 3). These surveys show that the XY race is consistently more numerous, and that the XO sites have densities which are on average similar to those of hybrids. This is a surprising result: in a homogeneous environment, we would expect a consistently larger XY population size to push the zone forward and eventually eliminate the less dense XO race. This is because populations at the centre of the zone would receive more immigrants from one side than from the other, so that the frequency of introgressing alleles would rise. (Hewitt, 1975; Barton, 1979c; and Chapters 1 and 5). We would therefore expect to find tension zones trapped by regions of low density, flanked on either side by denser populations. Indeed, over most of its length at Tende (Barton and Hewitt 1981a) and Seyne (Nichols and Hewitt, 1986) the zone is found to follow such low density areas.

In this chapter, I will investigate whether the position of the zone at Col de la Lombarde is consistent with the asymmetric pattern of densities: would one expect the cline to move to a new position? Should the XY race swamp the XO race? This question can be addressed by comparing the observed position of the zone with that expected. The expected position can be found either analytically, using a potential method (Barton, 1979a,c; Barton and Hewitt, 1989; see Section 7.2), or by simulation (Nichols, 1984; see Section 7.4.3). Barton (1979a,c) and Barton and Hewitt (1981a) used this approach to investigate the nature and position of the zone near Tende and Nichols (1984) applied it to the region near Seyne. It will be useful to summarise their investigations in Section 7.3 before describing a similar study at Col de la Lombarde. In Section 7.4, computer simulations will be used to
estimate the position from the pattern of densities. Note that I will be investigating whether density patterns alone can explain the position: this approach assumes that dispersal is constant (perhaps not too bad an assumption because if dispersal varies it is likely to do so in the same way as density). To estimate the position from the pattern of densities, it is necessary to have reliable density estimates over a large area spanning the hybrid zone (approximately twice the width of the zone). The population density survey described in chapter 3 provides density estimates in several 2 * 2 m² quadrats, placed in a specific vegetation type, and scattered along transects spanning the zone. However, this survey did not include measurements in less hospitable areas. For example, large patches of scree and inhospitable vegetation could affect the position of the zone in this region. Barriers to dispersal will also be important: we have seen the effect of the stream in this area on dispersal and on the shape of the cline (chapter 6). Moreover, the quadrat surveys described in Chapter 3 were biased, in that sites were chosen to have high density and suitable vegetation. It would therefore be rash to draw any conclusions regarding the position of the zone or its stability from these estimates.

A larger scale survey across all habitat types in the area is required. In 1989 the habitat in a 1Km² area at Col de la Lombarde was mapped; and in 1990 a population density survey and a finer scale vegetation survey were undertaken in a grid within this area. These surveys will be described in Sections 7.4.1 and 7.4.2. Densities were estimated from direct counts; however, it is difficult to obtain accurate estimates over a large area in this way (see Chapter 4). Nichols (1984) investigation suggests a way of predicting the position of the zone without the need for extensive counts: he used aerial photographs to identify habitat types and inferred densities from these. In Section 7.4.2, I will use the vegetation map at Col de la Lombarde to infer densities over a finer scale than was possible with the density counts. It will also be possible to compare the two methods, since the vegetation and density surveys cover the same area.

7.2 The effects of an asymmetric barrier to gene flow

If the environment were homogeneous, one would expect a consistently larger XY population size to push the zone forward and eventually eliminate the less dense XO race. In fact, the distribution of vegetation types and consequently the pattern
of grasshopper densities at Col de la Lombarde is complicated, and its effect on the position of the zone will be investigated using simulations (Section 7.4.3). However we have seen that the stream coinciding with the hybrid zone produces a strong barrier to gene flow (Chapter 6) which might prevent the zone from pushing forwards. Since the asymmetric densities suggest that the flow from XY to XO is greater than that from XO to XY, one must consider an asymmetric barrier: under what conditions would one expect the zone to be stable? Barton (unpublished) has used a potential method\(^1\) to find the critical asymmetry at which one type would swamp the other. Suppose that flow from left to right is given by \([dp/dx]_L B_L = (p_L - p_R)\), and in the opposite direction by \([dp/dx]_R B_R = (p_L - p_R)\) (where \(p_R\) and \(p_L\) refer to allele frequency just to the right, and just to the left of the barrier respectively). To ensure that \(H\) is at a minimum at equilibrium, Barton gives different weights to the left and right hand sides. The potential \(H\) is proportional to:

\[
H = \left(\frac{B_a}{B_L}\right) \int_{-\infty}^{0} \left[\frac{\partial p}{\partial x}\right]_L^2 + \left(\frac{pq}{2}\right) dx/2 + \left(\frac{B_a}{B_R}\right) \int_{0}^{\infty} \left[\frac{\partial p}{\partial x}\right]_R^2 + \left(\frac{pq}{2}\right) dx/2 - \frac{(p_L - p_R)^2}{2B_h}
\]

where \(B_a\) is the arithmetic mean, \((B_R + B_L)/2\) and \(B_h\) is the harmonic mean, \(2/B_h = 1/B_R + 1/B_L\). Here distance is scaled to \(l = \sqrt{\sigma^2/2s}\) (i.e. \(\omega/4\)). With this definition,

\[
\frac{\partial H}{\partial t} \propto - \left(\frac{B_a}{B_L}\right) \int_{-\infty}^{0} \left[\frac{\partial p}{\partial t}\right]^2 dx - \left(\frac{B_a}{B_R}\right) \int_{0}^{\infty} \left[\frac{\partial p}{\partial t}\right]^2 dx
\]

Thus, \(H\) always decreases, and so will be at a minimum at equilibrium.

The methods set out in Barton (1979c) give the general solution and conditions for stability in unscaled units: the equilibrium only exists if \(B_R/\omega - B_R > 4B_L/\omega > 4B_R/\omega\). If the barrier is weak, it must be almost symmetrical for a stable hybrid zone. If it is strong, the constraint is milder: the zone is always stable if \(B_R\) and \(B_L\) are both greater (\(\omega/4\)).

Thus it appears that a stable hybrid zone can be maintained in the face of an asymmetric barrier, provided that the above conditions are met.

Can we predict the position of the zone from density alone? (If not, it could be that the zone is not in equilibrium, that dispersal varies from place to place, or that dispersal varies with \(B^*\).) In the next section, I will describe previous investigations that have used this approach. Figure 7.4(a) shows the position of the zone at Col de

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\(^1\)recall that the potential \(H\) is a functional of \(p(z)\), which, for a cline maintained by heterozygote disadvantage at a single locus, will be minimised at equilibrium (see chapter 5)

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la Lombarde. Note that the zone is straight and that despite the density differences, it is probably pinned by a barrier (the stream) in the central section of the contact between the two races (we saw in the last chapter that the net strength of the barrier to gene exchange is strong and can easily hold the cline). Above this region the cline is broad and seems free to move, however there are patches of scree which might suffice to pin it (Fig 7.3). This will be investigated below. At both Tende and Seyne, the zone bulges and is probably pinned by barriers to either side. In these areas it is the curvature that balances the density difference (Nichols, 1984).

7.3 Previous studies

7.3.1 Barton's investigation

Barton (1979a) found a bulge in the cline at Tende. To investigate this pattern further, he constructed a crude density map over a large area in which sites were graded zero to three in increasing order of abundance. He estimated abundances by walking through some sites for a few minutes, and by using these estimates to infer abundances at other sites in similar habitat (see Figure 7.1). The abundance estimates were used along with data on karyotype frequencies in the area, to calculate the potential, $H$, for various possible zone positions. He found that the position is influenced by two components: a tendency for the bulge in the cline to be pushed back by surrounding populations, and a region of high density running along the zone (see Figure 7.1). The total potential, $H_T$, defined as the sum over different contours (from the zone centre to contours of $p$) is: $H_T = \int 4\bar{p}^2\sigma^2 p^2 q^2 dx$ where $x$ is the distance of the chromosome frequency contour from the centre of the zone; $p$ is the chromosome frequency along the contour, $\bar{p}^2$ is the mean square population density along the contour and $l$ is the length of the contour. There is a contribution to the potential from density and from contour length variations. The results of such calculations for the cline positions (see Table 13.1 and Fig 13.2 Barton, 1979a) suggest that the combination of the two factors produces a weak equilibrium about 300m ahead of the observed cline position. However, the cline should not bulge north-east as much as it does: indeed the XO race should have swamped the XY race east of Roche Rousse.

Barton suggested several possible explanations:

1. The data may be inaccurate.
Figure 7.1: This map shows the distribution of *Podisma* near Tende (for a map of the area see Fig 3.6). Sites are graded from "0" to "2" in increasing order of abundance (figure from Barton and Hewitt, 1981a).
2. Dispersal (σ) could vary from place to place.

3. The cline may be a wave of advance: the spread of the XY karyotype may be delayed along the cliffs south of Fontanalbe, but unimpeded in the forest to the east. It would have taken \( \approx 300 \) years for the bulge to develop to its present size. However, we have seen strong evidence to suggest that the cline is maintained by genetic incompatibilities.

4. If the cline is maintained by selection acting on many loci, its position would be determined by the effect of population structure on the most strongly selected of these. Hence the narrow clines would perceive more local equilibria and might then be stabilized.

5. There is some interaction between the environment and reduced hybrid fitness affecting the cline position.

6. The bulge could be explained if there were a selective imbalance between the two types. However, Barton (1979a) has argued that this would simply send the cline ahead of its present position (A gradient \( H' \) corresponds to a selective imbalance \( S = \frac{ew H'}{H} \)). At Tende the cline is at a point where \( H'/H = 0.0016m^{-1} \), corresponding to \( S = 0.17\% \).

Barton did not take drift into account in his investigation, and there is evidence that it could affect the position of the zone: Nichols (1984) found that drift alters the position of the zone, making it longer than it would otherwise be. These results are supported by an investigation by Rouhani and Barton (1987a). I will discuss the possible importance of drift later.

### Nichols’ investigation

Nichols and Hewitt (1986) observed that, as at Tende, the cline runs along an area of low population density over most of its length near Seyne. However, in one section it runs through an area of very high density area (Figure 7.2). Nichols (1984) used a computer simulation to estimate the expected position of the cline. He mapped the pattern of gene frequencies and estimated densities in a 1560m by 780m grid, sampled every 60m. Density estimates were made by recording numbers seen in walks at 60m per minute. Each \( 60m^2 \) in the survey was considered a separate deme in the simulation. He simulated a single locus two allele system, with heterozygote
disadvantage. Selection, drift and migration were iterated directly, in that order. The karyotype frequency after migration was given by:

\[ p_x = \frac{p_x(1-g)N_x + (1/4)g \sum N_i p_i}{(1-g)N_x + (1/4)\sum N_i} \]  

(7.3)

where \( p_x \) is the karyotype frequency before gene flow in population \( x \); \( p_i \) is the karyotype frequency in one of the four surrounding populations \( N_i \), \( N_x \) the number of individuals in one of the surrounding populations \( x \); and \( g \) is the proportion that migrate each generation. To model genetic drift, individuals to produce the next generation were chosen randomly from a Poisson distribution (approximated by a normal distribution for \( N_x \) greater than 20). Parameter values used in the simulations came from field and laboratory estimates. The best estimate of dispersal at Seyne was \( 15m/gen^{1/2} \); selection maintaining the cline was estimated at \( s = (8\sigma^2/w^2) = 0.25\% \) (where \( \sigma \) is the dispersal rate and \( w \) is the width of the zone) (Barton, 1979c, Nichols, 1984; and see Chapter 5).

To estimate population size (\( \rho \)) in each deme, Nichols used the relation: \( N_e = -ln(2s)/F_{st} = 4\pi N_d m \). Here, \( N_e \) is the effective population size. (In fact this relation comes from \( F_{ST} = \frac{-\log(2s)}{4\pi\rho s^{2}} \) (Malecot, 1948). This is approximately true for a cline so that \( \rho \) can be estimated from \( F_{ST} \) (note that \( N_e \neq 4\pi Nm \): neighbourhood size is not the same as effective population density or number).

If the deviations of karyotype frequencies from a smooth cline are assumed to be due to genetic drift, and to be independent, then they can be used to estimate \( F_{st} \): \( F_{st} = var(P)/P_e(1-P_e) \), where \( var(P) \) is the variance of the observed karyotype frequency away from the expected smooth cline, \( P_e \) (see Barton and Hewitt, 1981a). The proportion of individuals migrating between adjacent demes in a stepping stone model: \( m = g/2 \) depends on the dispersal rate, \( \sigma \) and the distance between demes, \( e \): \( \sigma = \epsilon/\sqrt{m} \). Since \( \sigma = 15m/gen^{1/2} \) and \( \epsilon = 60m \), \( m = 0.0625.gen^{-1} \). For a rough estimate of \( N_d \), Nichols substituted Barton and Hewitt’s (1983) estimate of \( F_{st} \), 0.014 (note that this estimate is from a different area) giving \( N_e = -ln(2s)/F_{st} = 378 \) and \( N_d = 378/4\pi \times 0.0625 \approx 482 \). \( N_d \) is \( \approx 100 \) times the number actually seen in a 60m square.

Nichols ran the simulation using these parameter values and an initial gene frequency distribution equal to that observed. After 1000 generations, the cline had not moved from its initial position. To investigate the effect of population structure further, he changed the observed densities to lower values in the simulation. This time, the XY race pushed forwards. Thus the observed position of the zone in
Figure 7.2: A contour map of the *Podisma* density near Seyne (for a map of the area see Fig. 3.4). The centre of the cline and the density per (10m)$^2$ are indicated (figure from Hewitt, 1988).
this high density area can be explained by details of the population structure: the position of the zone is determined by the effects of drift, via the absolute density and not by the relative density alone. Nichols results agree well with the expected position of a cline maintained by a balance between dispersal and selection against hybrids.

In summary, the evidence based on field measures of population structure at Tende and Seyne is ambiguous: on the one hand Barton found that where the zone was free to move the bulge in the XY distribution was larger than could be accounted for by density patterns; on the other, Nichols' simulations predicted that the zone is held in position through the high density area by the pattern of densities and the effects of drift, supporting the theory that it is maintained by reduced hybrid fitness. The discrepancy could be because Barton's density estimates were not as accurate as Nichols' and possibly because Barton did not take account of drift.

An investigation at the Col de la Lombarde site, with its asymmetric density, will provide an interesting comparison.

7.4 Predicting the position of the zone at Col de la Lombarde

7.4.1 The density survey

The area chosen for this investigation is shown in Fig. 7.3 and 7.4. This region had already been well mapped for the survey described in chapter 3; there was adequate information on the position of the zone (and extra samples were collected during this survey); and it includes the transect 1 sites, where the asymmetric density pattern was originally observed (chapter 3). Individuals are pure for the XO karyotype, about 400 metres to the right of the stream, and pure for the XY karyotype about 400m to the left. The zone follows the stream in the valley (Figure 7.4) (where it is likely to be pinned in position). To extend the investigation to an area where the zone would be free to move, and to include populations pure for each race, one would need to cover an area of about $1Km$ by $500m$. Time limits how exhaustively one can sample such an area. I have discussed the difficulties involved in obtaining accurate estimates over a large area in chapter 4. Here, as there, I was interested in the peak adult density (since dispersal is mostly by adults), and so the entire area had to be searched in at most 2 weeks (the average adult survival time). It seemed most appropriate to sample the area using a $(10m)^2$ quadrat: this scale
<table>
<thead>
<tr>
<th>Habitat type</th>
<th># Quadrats</th>
<th>Mean density</th>
<th>Std. error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13</td>
<td>14.10</td>
<td>3.64</td>
</tr>
<tr>
<td>B</td>
<td>23</td>
<td>2.75</td>
<td>0.84</td>
</tr>
<tr>
<td>C</td>
<td>65</td>
<td>0.55</td>
<td>0.84</td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td>20.01</td>
<td>4.11</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
<td>15.32</td>
<td>3.69</td>
</tr>
<tr>
<td>F</td>
<td>12</td>
<td>27.51</td>
<td>3.26</td>
</tr>
</tbody>
</table>

Table 7.1: Statistics on densities in each habitat type. The habitat types are described in Table 7.2 and their distribution is shown in Figure 7.10.

is of the same order as the dispersal range, and the calibration data in Chapter 4 suggests that two scorers searching a (10m)$^2$ area for ten minutes will allow a reliable density estimate. Barton (1979c) has shown that for an organism living in discrete demes, variations in density over distances which are short in comparison with the width of the cline are unlikely to be important to the movement of the zone, though this ignores drift. If small scale variations were important, then the scale over which we measure densities would matter. Nonetheless, since the habitat types which are likely to affect *Podisma*'s density distribution (see chapter 3) typically vary over a distance of about 100m or greater (see Figure 7.10), one should obtain adequate density estimates in this area by spacing the quadrats 60m apart. A grid 420m by 960m with 136 quadrats each spaced 60 metres apart covers the region of interest. Since about 40% of this area is covered in pure scree, where scoring was not necessary, we could easily obtain density estimates for each quadrat in two weeks.

Accordingly, a grid of (10m)$^2$ quadrats, each spaced approximately 60 metres apart was established by measuring out parallel transects 'A' to 'H' each 60 m apart and extending for about 960m across the region of interest (Figure 7.10: distortions are due to the steep topography). A quadrat was established every 60m along each transect and searched for 10 minutes, by two scorers (K. Jackson and N. Barton). The scoring methods were the same as those described in Chapter 4: individuals were counted into a tin and released into the centre of the quadrat at the end of the scoring period. Since the region had been well mapped in 1988 and 1989, it was possible to measure the transects relative to known points (control points) on

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By pure scree, I mean scree with no vegetation at all. *Podisma* is not found in these areas, and so we can assume that the density is zero. However, note that the scree marked in figure 7.10, consists of pure scree interspersed with patchy scree in which between 1% and 30% of a (10m)$^2$ quadrat could consist of vegetation, and should therefore be scored. It is these quadrats that account for the "scree" score in Table 7.1.
Figure 7.3: This photograph shows the region in which the grid was established. The centre of the hybrid zone coincides with the stream; this is shown on the overlay, together with the approximate bounds of the grid in which densities were measured in 1990.
Figure 7.3: This photograph shows the region in which the grid was established. The centre of the hybrid zone coincides with the stream; this is shown on the overlay, together with the approximate bounds of the grid in which densities were measured in 1990.
Figure 7.4. To avoid confusing a geographic trend in densities with a time-related one (which could result from adult mortality), the quadrats were established and scored in the following row order: E, C, A, D, G, H, B, F. There is no evidence that the time of day effects observed numbers (see chapter 3). However, to be sure to avoid any bias, quadrats in rows A, C, E, and G were scored (in order) from quadrat 1 to 17; while in rows B, D, F and H quadrats were scored in the opposite direction (i.e. from 17 to 1). Numbers of individuals seen in each quadrat corrected for instar in Appendix F.2 (based on data from Chapter 3, see Fig. 3.20).

7.4.2 The vegetation survey

One possible way around the difficulty of obtaining accurate estimates over a large area is to infer densities from habitat type. Aerial photographs and satellite images can be useful aids for mapping habitat types over a large area. Nichols (1984), for example, was able to use aerial photographs to distinguish habitat types at Seyne (see above). Landsat images have been used to detect large scale patterns in conservation studies. For example Avery and Haines-Young (1990) used the near infra-red band of the multi-spectral scanner (MSS) successfully to predict numbers of dunlin *Calidris alpina* in northern Scotland.

The low resolution of the MSS (79m by 79m) restricts its use in smaller-scale ecological investigations. The *Thematic Mapper* (TM) has a higher ground resolution (30m by 30m) and has been used to identify habitat types for the caribou (Thompson et al, 1980), the nesting kestrel (Lyon, 1983) and the ruffed grouse (Palmeirim, 1988). Virdee (1991) used the TM to investigate the relation between habitat and density of the grasshopper *Chorthippus parallelus*, across the hybrid zone near Col du Pourtalet in the Pyrenees. She was able to show that the hybrid zone coincides with unsuitable habitat type and that high-density habitats are fragmented, implying that *Chorthippus* must be patchily distributed in these areas.

I investigated the use of satellite images to distinguish habitat types at Col de la Lombarde. Data from the TM were used to generate a false colour image of a 12Km by 12Km area including the region of interest near Col de la Lombarde (Figure 7.5). The satellite data were viewed and analysed at ITE, Monkswood using S600 and I²S software. The image was corrected to match the UTM projection of an IGN map of the region. Bands 4, 5 and 3 were assigned to the red, green and blue intensity respectively. Rock is identifiable as grey-blue and vegetation appears in orange and red shades. The distribution of scree and pine (dark orange or red) can be seen
Figure 7.4: (a) The frequency of the chromosomal fusion over the entire area near Col de la Lombarde. Distances are in metres. The rectangle is the area detailed in (b). (b) The frequency of the fusion across the region in which densities were measured in 1990; this is marked by the inner rectangle (see Figure 7.10 and 7.3).
clearly. However, note the strong effect of illumination on slopes of different aspect. The different shades of orange in the area of interest suggested that it should be possible to distinguish habitats other than just “scree”, “pine” and a “grass-herb” mix. Thus regions of known vegetation (from the surveys described in chapters 3 and 4) were identified and used as “training” areas for seven classes: (1) scree; (2) lush vegetation dominated by *Vaccinium myrtillus* and *Vaccinium uliginosum* (typical of the *transect 1* sites described in chapter 3); (3) pine; (4) water or shade (since these are indistinguishable on a colour-coded image); (5) dry areas with sparse vegetation (mostly bare-earth but with a sparse mixture of herbs and *Vaccinium uliginosum*; (6) grass (dominated by *Festuca peniculata*) and (7) areas in which there is a mix of herbs and grasses interspersed with rock.

To classify, the program uses all seven bands to divide the seven-dimensional space into regions. Each region corresponds to a class and is defined by a range of intensity values for each of the bands. The boundaries between regions depend on the degree of clustering of points correspond to classes, as well as the Cartesian separation of the classes in the seven-dimensional intensity space. To get a reasonable estimate of the variance in intensity values within a class, the training areas need at least 12 pixels covered by a unique habitat. Because of the patchy nature of the environment in this area, it is hard to find suitable training areas. Pixels were 20m by 20m (resampled from 28.5m by 28.5m) and areas larger than 12 pixels (i.e. \( \approx 85 \text{m by 114m} \)) are seldom uniform. Another problem is that a given habitat type seems to vary in appearance on the false-colour image (see Figure 7.5), probably due to changes in illumination and the proportion of rocks. When all pixels in the image were classified using the training areas described above, no distinction could be made between types (2: lush vegetation) and (6: grass). This is not surprising, since there are patches of type (2) in an area dominated by (6) and vice-versa (see chapter 3). In the colour-coded image displayed in Figure 7.6, types 1, 3, 4, and 5 can be distinguished; 2 and 6 have been lumped and so this class is somewhat too broadly defined. A further problem is caused by “mixed pixels”. For example, at the borders of scree in Figure 7.6, pixels consist of a mixture of rock and vegetation and are classified as *water* or *shade* (dark blue). These problems as well as the highly variable illumination resulting from the steep topography in this area, make an automated classification inappropriate. An interpretation of the false colour image seems more reliable, but here the resolution is too poor to be of much use in distinguishing habitat types in the 420m by 960m area. However, the false-colour
Figure 7.5: The LANDSAT false-colour image of the site at Col de la Lombarde. The overlay shows the region in which densities were measured in detail (rectangle).
Figure 7.5: The LANDSAT false-colour image of the site at Col de la Lombarde. The overlay shows the region in which densities were measured in detail (rectangle).
image (figure 7.5) does show a regional distribution of habitat types: notice the
darker-orange colour in areas covered predominantly by *Festuca peniculata* (mostly
on the XO side) relative to that on the XY side. I will return to this observation
later.

So how best to classify the habitat? Since it was possible to distinguish six
different habitat types by eye, from suitably high vantage points (which had been
mapped using a theodolite the previous year), it was more appropriate simply to
map the habitat types directly. Accordingly, in 1989, the habitat types shown in
Figure 7.10 were sketched from the vantage points, and subsequently measured out
more accurately relative to appropriate mapped points, using a tape measure and
compass. Further refinements were made in 1990 when the grid for the density survey
was established. Surprisingly few modifications were necessary. The six different
habitat types are shown in Figures 7.7, 7.8 and 7.9 and a detailed description of
each is given in Table 7.2.

To infer densities from habitat, it was first necessary to obtain reliable estimates
of expected densities from each. These came from the density survey described
above: the grid in Figure 7.10 covers the same area as the habitat survey. Although
it was not difficult to distinguish between the habitat types described in Table 7.2
by eye, in order to quantify the differences, percentage cover of the major vegetation
types, rock, scree and other features were recorded for each quadrat in the density
survey. A description of the habitat in each quadrat is provided in Appendix F.1.
A principle component analysis3 (PCA) of this data (Figure 7.11) suggests that
quadrats do cluster into distinct types. There is some overlap, however this is
expected. Some of the quadrats fall into the 'wrong' vegetation type, but this
probably reflects the patchy distribution of habitat types on this scale, since within
each of the six habitat types one would expect small patches more typical of at least
one of the other five habitat types (for example, it is not uncommon to find patches
of vegetation in a scree slope, say, or patches of *Vaccinium* in an area dominated by
grass: see chapters 3 and 4). Table 7.1 provides statistics on densities in each type.
The estimates provided in this table were used to infer densities from the vegetation
map in a grid with samples spaced 25m apart.

Thus there are two different sets of data on population density: 1) a 1Km² grid
in which samples spaced 60 metres apart are density estimates from direct counts

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3Since habitat types are quite distinct and since only 19 "species" were distinguished in this
vegetation survey, a TWINSpan analysis (such as that used in Chapter 3) would be no more
informative than a simple PCA.
Figure 7.6: The LANDSAT colour-coded image of the sites at Col de la Lombarde (classification derived from the Thematic Mapper). Grey = scree (1); Green = Vaccinium myrtillus, Vaccinium uliginosum, grass (2) and (6); red = pine (3); dark blue = water or shade (4); yellow = dry areas with sparse vegetation (5); dark green = a mix of herbs and grasses interspersed with rock (7); (see text).
Figure 7.6: The LANDSAT colour-coded image of the sites at Col de la Lombarde (classification derived from the Thematic Mapper). Grey = scree (1); Green = Vaccinium myrtillus, Vaccinium uliginosum, grass (2) and (6); red = pine (3); dark blue = water or shade (4); yellow = dry areas with sparse vegetation (5); dark green = a mix of herbs and grasses interspersed with rock (7); (see text).
Figure 7.7: Letters refer to habitat types. These are described in detail in Table 7.2. Dominant features: A: rock and *Juniperus nana*, B: *Festuca peniculata*. 
Figure 7.8: Letters refer to habitat types. These are described in detail in Table 7.2. Dominant features: C: scree with small patches of vegetation, D: grasses with *Veratrum album*.
Figure 7.9: Letters refer to habitat types. These are described in detail in Table 7.2. Dominant features: E: grasses, F: grass mix, herbs and *Vaccinium uliginosum*. 
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<td>0</td>
<td>0</td>
<td>10</td>
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<td>90</td>
<td>0</td>
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</table>

Table 7.2: A classification of habitat types is shown in Fig. 7.10. Numbers here correspond to approximate percentage cover of major distinguishing features (listed in the left hand column) in a randomly chosen 40m by 40m area within each habitat type (A-F).
Figure 7.10: Map of the habitat types. The coordinates shown at the edges correspond to those in Figure 7.4. Six different types were distinguished: (A) brown = vegetation dominated by rock and dwarf pine. (B) orange = dominated by Festuca peniculata. (C) grey = scree. (D) light green = dominated by grasses with Rhododendron and Veratrum. (E) yellow = dominated by grasses. (F) dark green = dominated by a mix of grass, herbs and Vaccinium uliginosum. The road is marked in white. The overlay shows the grid of quadrats used for the density survey. The road, path (dashed lines) and stream (solid line) are shown.
Figure 7.10: Map of the habitat types. The coordinates shown at the edges correspond to those in Figure 7.4. Six different types were distinguished: (A) brown = vegetation dominated by rock and dwarf pine. (B) orange = dominated by Festuca pniculata. (C) grey = scree. (D) light green = dominated by grasses with Rhododendron and Veratrum. (E) yellow = dominated by grasses. (F) dark green = dominated by a mix of grass, herbs and Vaccinium uligonosum. The road is marked in white. The overlay shows the grid of quadrats used for the density survey. The road, path (dashed lines) and stream (solid line) are shown.
Figure 7.11: Projection of the habitat scores onto the first and second principal component axes. Quadrats have been coded A to F according to the habitat type in which they are found (see Fig. 7.10). Each letter represents one or more quadrats. "Pure scree" quadrats have been left out of the analysis, since they would dominate the first principal component. Minimum convex polygons have been drawn to enclose clusters (excluding obvious outliers).
in \((10m)^2\) quadrats (Appendix F.2) and 2) a 1Km\(^2\) grid in the same area, in which samples spaced 25 metres apart are estimates inferred from vegetation type.

7.4.3 The computer simulations

Barton (unpublished) has developed a program to predict the position of a cline from density data. The program takes data on population density and observed allele frequencies, which can be either from a grid or from arbitrary sample points. The densities are interpolated onto a grid of demes which is used in the simulations. The population is started either with the hybrid zone running in some chosen position, or using the observed karyotype frequencies (again, interpolated onto the grid). The grid of demes can then be treated in one of three ways. In the simulations described below, a single locus two allele system, with heterozygote disadvantage is modelled, and migration, population regulation, drift and selection are iterated directly, in that order. Nearest-neighbour migration between demes is simulated. In a grid of demes spaced \(\epsilon\) apart, migration, \(m\), can be approximated by diffusion: provided that changes are gradual (see Chapter 5), and if we ignore drift, \(\sigma^2 \approx mc^2\) (\(\sigma^2\) is the variance in parent-offspring distance). Thus in the continuous limit, the grid spacing should not matter, and the proportion of individuals migrating between demes, \(m\), depends on the dispersal rate, \(\sigma\); and the spacing between demes, \(\epsilon\). In the program, migration rates in the \(x\) and \(y\) directions are calculated separately (since they could differ if the intervals on the grid in the \(x\) and \(y\) directions are not equal). The allele frequency, \(p^*\), in a deme after migration is:

\[
p^*_i = \frac{\rho_{ix}p_{ix}(1 - m_x - m_y) + m_x^2 \rho_{ix-1}p_{ix-1} + m_x\rho_{ix+1}p_{ix+1} + m_y^2 \rho_{iy-1}p_{iy-1} + m_y\rho_{iy+1}p_{iy+1}}{\rho(1 - m_x - m_y) + m_x^2 \rho_{ix-1} + m_x\rho_{ix+1} + m_y^2 \rho_{iy-1} + m_y\rho_{iy+1}}
\]

\((7.4)\)

\(\rho\) is the density in a deme (i.e. at a point on the grid); \(m_x, m_y\) are the migration rates in the \(x\) and \(y\) directions respectively, and \(p\) is the allele frequency in a deme. Subscripts \(ix\) and \(iy\) refer to the \(i\)th deme in the \(x\) and \(y\) directions respectively. Since migration will have a smoothing effect on densities, it is necessary to include population regulation in the simulation. This is done simply by setting the \(\rho\)'s equal to the original (interpolated) density in each deme, every generation. Thus the migration procedure affects allele frequencies, but not densities. Drift is modelled by choosing a random sample from the \(2N\) genes in each deme to produce the next generation. For small \(Np\), genes are chosen from a Poisson distribution; if \(Np\) is
large, they are chosen from a normal distribution.

Three variables are observed: \( A \), the area occupied by the XY type (i.e. the integral of \( p \) over the area weighted by \( \rho \)); \( H \), the potential; and \( L \), the log likelihood of the current model. The potential, \( H \) decreases under migration and selection (see Chapter 5: strictly it must only decrease in the continuous limit, and may fluctuate because of drift). The likelihood is the probability of observing the data, given the simulated parameters and taking into account the fact that allele frequencies fluctuate around their average values (the allele frequency is assumed to have a \( \beta \) distribution), with parameters estimated from accumulated runs of the simulation. For each point in the simulation grid, the mean and variance of allele frequency, sampled over a number of generations, is calculated. For example in the simulations described below after an adequate 'warm-up' period (2000 generations), statistics are calculated every 50 generations for 8000 generations: thus providing 120 'replicates'. The mean and variance are then interpolated across to each sample point and are compared with the data to give the likelihood of the current model (i.e. given the simulation parameters, what is the probability (or likelihood) of observing the data?). By varying parameters and comparing the log likelihood of the models one can find the best fitting combination of dispersal, selection and drift.

In the simulations described below, I have tested parameter combinations within a reasonable range of those estimated from field and laboratory investigations. Several different estimates of dispersal suggest that \( \sigma \approx 20m/\text{gen}^{1/2} \) (Barton and Hewitt, 1982; Nichols, 1984; Currie, 1992; Mason, 1989, chapter 4). Selection against chromosomal heterozygotes has been estimated by \( s = (8\sigma^2/w^2) \approx 0.005 \) (Barton 1979a) and the calibration data in Chapter 4 provide a reliable estimate of the actual density in each \((10m)^2\) area. The scale on which significant variation in density actually occurs in nature, as well as the importance of small-scale variation on the position of the zone, will affect the results. We have seen that to decide on a scale over which to sample the area, it was necessary to judge the scale over which variation in density would be likely to affect the position of the zone. In the simulations demes are arranged in a grid and since the data are not, it is necessary to interpolate densities from the data onto the simulation grid. One must also decide on an appropriate smoothing scale. This scale depends on how smooth one believes the surface to be. If densities vary greatly over small distances, the smoothing scale could make a considerable difference to the results of simulation: for example a narrow line of zero density constituting an absolute barrier to gene flow would become permeable
if smoothed. We also need to choose an appropriate spacing between demes in the simulation grid. Again we must take into account the actual scale over which significant variation occurs in nature (though clearly the scale over which we sampled will set a lower limit on the resolution possible in the simulation). The dispersal rate, $\sigma$, sets a limit on the grid spacing (see below) and will be important in determining a reasonable smoothing scale. The appropriate grid spacing and smoothing distance will be discussed in more detail below.

**Converting densities**

The raw data on population densities consist of numbers at particular points, given by the X and Y coordinates corresponding to the appropriate point on Figure 7.4(b). For the 'count' data, these points correspond to the middle of each of the $(10m)^2$ quadrats and for the data inferred from vegetation type, they correspond to the centre of each $25m^2$ area. The densities are the observed numbers (corrected for instar) seen per $(10m)^2$. These are corrected to actual number per $m^2$ by multiplying by the parameter 'dconv': the ratio between the effective density per $m^2$ and the densities observed or inferred per $(10m)^2$. The calibration data described in chapter 4 suggests that in ten minutes two scorers see approximately $1/3$ of the actual number present in a $(10m)^2$ area, in ten minutes. Thus 'dconv' should be $4 \approx 0.03$. Note that since decreasing "dconv" reduces the densities in each deme, this parameter can be used to investigate the effect of drift on the position of the zone. Table 7.3 shows the effect of altering "dconv" for a given $\epsilon$ and smoothing distance. Note that the area occupied by the XY race does not change much; nor does the likelihood except when "dconv"=0.001: surprisingly this value gives the best fit to the data (this may be an artefact since drift causes the likelihood to fluctuate greatly when density is low). This result contrasts with that of Nichols (1984) who found drift to be important and it may be that drift has little effect here, because the zone is straight. Rouhani and Barton (1987a) showed that a curved zone should bulge outwards as a result of drift, tending to increase its length. However, a straight zone should not be affected since movement does not affect its length.

---

4There should be an additional factor since effective population size is in fact $(2/3)N$ (in a sex-linked model). However, since we do not know what $N_e$ is relative to the actual density, and since in any case, errors in density measurements are likely to be larger than those introduced by ignoring this factor, I have not included it here.
Densities estimated from the grid of quadrats.

Figure 7.12: Density was measured in two ways. First, numbers of adults were counted in each of the 136 $(10m)^2$ quadrats. Second, vegetation type was scored, and densities were extrapolated from the average numbers seen in such vegetation. This gave coverage of the whole area, rather than a fraction. The patterns from the two methods are similar.
Table 7.3: Testing the effect of drift: the parameter “dconv” sets the ratio of actual to effective population size. “Area” is the area occupied by the XY race after 8000 generations; the observed area is about 353000m² and the maximum area is about 600000m²; \( \sigma = 20m \), smoothing scale=50m; grid spacing=70m and selection is 0.0025 for all runs.

<table>
<thead>
<tr>
<th>dconv</th>
<th>Area</th>
<th>Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.060</td>
<td>440356</td>
<td>-255.08</td>
</tr>
<tr>
<td>0.030</td>
<td>440542</td>
<td>-253.94</td>
</tr>
<tr>
<td>0.020</td>
<td>440582</td>
<td>-256.33</td>
</tr>
<tr>
<td>0.005</td>
<td>419949</td>
<td>-211.71</td>
</tr>
<tr>
<td>0.001</td>
<td>497342</td>
<td>-212.00</td>
</tr>
</tbody>
</table>

Grid spacing

Since the model is for nearest-neighbour migration only, there is a lower limit on grid spacing: it must be larger than the dispersal rate \( \sigma \): \( m = \sigma^2 / \epsilon^2; \epsilon = \sigma / \sqrt{m} \) and since \( m \leq 0.5, \epsilon \geq \sigma \sqrt{2} \). Migration can be approximated by diffusion provided changes are gradual and drift is negligible: thus the spacing between demes should not affect the position of the zone in the continuous limit and with high density. However, if random fluctuations are important, the grid spacing will matter: for example a coarse grid could smooth out local barriers. The grid spacing will also influence the effect of drift.

Table 7.4 shows the effect of grid spacing on the area covered by the XY type for different “dconv”. The appropriate grid spacing will be towards the lower end of this range, since \( \sigma \approx 20m \). Grid spacing does not seem to affect the position of the zone much, but the difference in likelihood between models is significant: when “dconv” = 0.03 or 0.005, the most likely value is 70. When “dconv” = 0.001, it is 50 (see Table 7.4). This is probably because grid spacing affects the pattern of random fluctuations.

Smoothing scale

To interpolate densities onto the grid for the simulations, the program smooths densities from the raw data within a given radius (the smoothing distance) of each point on the grid. The contribution of each sample to the density at the grid point is weighted according to a Gaussian function, with half width equal to the smoothing distance. If there are no data within the smoothing scale, the nearest point is used. The program also uses the nearest data point if the smoothing parameter is set to
Table 7.4: The effect of grid spacing on the area occupied by the XY race after 8000 generations, \( s = 0.0025; \sigma = 20m; \) smoothing scale is 50m for all runs. “A” is the area in \( Km^2 \) and “L” is the log-likelihood of the model.

<table>
<thead>
<tr>
<th>Grid spacing</th>
<th>( d_{conv}=0.030 )</th>
<th>( d_{conv}=0.005 )</th>
<th>( d_{conv}=0.001 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>L</td>
<td>A</td>
</tr>
<tr>
<td>30</td>
<td>436884</td>
<td>-254.98</td>
<td>473360</td>
</tr>
<tr>
<td>50</td>
<td>440543</td>
<td>-253.94</td>
<td>420893</td>
</tr>
<tr>
<td>70</td>
<td>427205</td>
<td>-207.07</td>
<td>437320</td>
</tr>
<tr>
<td>80</td>
<td>432008</td>
<td>-282.08</td>
<td>442392</td>
</tr>
<tr>
<td>100</td>
<td>435852</td>
<td>-330.17</td>
<td>445389</td>
</tr>
</tbody>
</table>

Table 7.5: The effect of smoothing scale on the position of the zone. In all cases \( "d_{conv}\) = 0.03 zero. It is more likely that densities interpolated from a number of data points over a sensible smoothing scale would reflect true densities than if there were no smoothing and density estimates were from single samples. The appropriate smoothing scale depends on one’s belief as to the actual pattern (see above): it should be smaller than the width of the zone; smaller than the typical scale over which the habitat types change, but on a scale similar to or greater than that over which samples were taken; and it should be of the same order as the expected dispersal distance. An appropriate smoothing scale would be \( \approx 50m \). Table 7.5 shows the effect of smoothing distance on the position of the zone. Note that setting this parameter to zero results in a very poor fit. Interestingly Nichols(1984) did not smooth in his simulations. I have re-analysed the data from Seyne (see Fig. 7.13): Fig 7.14 show the results with no smoothing: the zone disappears. This contradicts Nichols’ (1984) results, a contradiction not yet resolved.
Starting position

In the simulations described here, the population is started with the observed karyotype frequencies (interpolated onto the grid) - see Fig. 7.15. The karyotype frequency data are provided in Appendix A.1 (methods of collection and preparation have been described in chapter 2). The zone is likely to be held securely at its top edge by the stream (see figure 7.4). I have tried simulating both with the top edge fixed and with no edges fixed: apart from the stream there are no obvious barriers that might hold the zone in position. Table 7.6 shows the effect of starting the simulations with the position to the left of its actual position (-200 on the x axis) or to the right (+600 on the x axis). Despite very different initial areas (-200000 and +600000) the zone moves more-or-less to the correct (observed) position (after 8000 generations they move to 473679 and 483775 respectively: the areas are not exactly the same because these figures are still subject to random variations).

7.5 Results and discussion

Table 7.7 shows the effect of varying $s$. Note how the area occupied by the XY race drops with decreasing $s$. The best fit is with $s = 0.0025$, somewhat lower than that estimated by Barton ($\approx 0.005$). Simulations in which densities come from direct counts and those in which densities are inferred from vegetation type give similar results; though simulations using the vegetation data give a slightly better fit. This suggests that inferring densities from vegetation type in this area is reliable, and can be used to predict the position of the zone. If we start with allele frequencies equal to those observed and assume that the zone is held securely at its top end by the stream, then the zone stabilises, and does so in more or less the correct (observed) position (it moves forward slightly but not to a position significantly different from that observed) (see Figures 7.16 and 7.17). If on the other hand, we do not fix the

<table>
<thead>
<tr>
<th>Starting position</th>
<th>Area after 8000 generations</th>
<th>Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>-200</td>
<td>473680</td>
<td>-285.98</td>
</tr>
<tr>
<td>600</td>
<td>483775</td>
<td>-288.56</td>
</tr>
</tbody>
</table>

Table 7.6: The effect of varying the initial position of the zone. $s = 0.0025; \sigma = 20m$; smoothing=50m; grid spacing=50m. The actual area occupied by the XY type is $\approx 353000m^2$; the maximum area is 600000$m^2$. 
Figure 7.13: Nichols data from Seyne interpolated onto the simulation grid: (a) observed allele frequency. The intensity of stippling is proportional to the frequency of the fused chromosome. (b) observed density. Here the intensity of stippling is proportional to density.
Figure 7.14: Predicting the position of the zone at Seyne (data from Nichols, 1984). Parameters in this simulation are the same as those used by Nichols (1984): selection, $s = 0.0025$; dispersal, $\sigma = 15.0m$; grid spacing = 60m; "dconv"=0.029; no smoothing. The zone disappears.
Figure 7.15: Allele frequencies at Col de la Lombarde interpolated onto the simulation grid: the intensity of stippling is proportional to the frequency of the fused chromosome. The maximum and minimum X and Y coordinates for the simulation grid correspond to those in Figures 7.4 and 7.10.

At the top end, the XY race swamps the XO race (Figure 7.18), as one might expect from the pattern of densities - unless selection is unrealistically high ($s = 0.02$).

Of the simulations described here, the best fit is with a grid spacing of 70m; a smoothing scale of 50m; $s = 0.0025$, and $\sigma = 20m$. One might expect multiple solutions since more than one combination of selection and dispersal could stabilise the zone. However, it is encouraging that the best fit is with parameters close to those expected from field and laboratory investigations. Selection is somewhat lower than that estimated by Barton (1979a) from the width of the cline at Tende ($s=0.0025$ here vs. 0.005 at Tende - see Chapter 2). This could be because the scree sharpens the cline here (see Chapter 6) so that less selection is needed to maintain it. It is also encouraging that if we start with the zone away from its actual position, it moves forward to more or less the correct position.

In conclusion, the simulation results suggest that provided the zone is pinned by the stream at its top end, we can predict its position from the pattern of densities. It seems that a combination of the stream at the top and the extensive scree slope on the left (Figure 7.10) - see Section 7.2 - stabilise the hybrid zone, despite the marked difference in density between the two races in this region. This result is consistent with the theory and provides further evidence that the cline is maintained by genetic
Figure 7.16: Col de la Lombarde data showing the best fit when densities are from counts. The top edge has been fixed; selection, $s = 0.0025$; dispersal, $\sigma = 20.0$; grid spacing $= 50$; “dconv=0.03; smoothing $= 50.0$. (a) shows densities interpolated onto the simulation grid. The intensity of stippling is proportional to density. (b) shows the allele frequencies after 8000 generations. $T =$ time in generations, $L=$ log-likelihood, $H$ is the potential (see text). The actual area occupied by the XY type is $\approx 353000m^2$; the maximum area is $600000m^2$. The area occupied by the XY type after 8000 generations has increased from about $353000m^2$ to a new stable area of $440000m^2$. 
Figure 7.17: Col de la Lombarde data showing the best fit when densities are inferred from vegetation. The top edge has been fixed. Selection, $s = 0.0025$; dispersal, $\sigma = 20.0$; grid spacing $= 50$; "dconv" $= 0.03$; smoothing $= 50.0$. Coordinates on the grids correspond to those in Figs. 7.4 and 7.10. (a) shows densities interpolated onto the simulation grid. The intensity of stippling is proportional to density. (b) shows the allele frequencies after 8000 generations. $T$ = time in generations, $L$ = log-likelihood, $H$ is the potential (see text). The actual area occupied by the XY type is $\approx 353000m^2$; the maximum area is $600000m^2$. 

\[ T = 8000 \text{ Likelihood} = -307.98 \]
\[ T = 8000 \text{ Weighted area} = 379695.2 \]
\[ T = 8000 \text{ H} = 784146.5 \]
Figure 7.18: Col de la Lombarde data when the top edge is not fixed. (a) $s = 0.005$, $\sigma = 20.0$, grid spacing = 50m, “dconv”=0.03; smoothing = 50. (b) $s = 0.02$, $\sigma = 20$, grid spacing = 50; smoothing = 50. If the top edge is not fixed, the XY race swamps the XO race (a); unless selection, $s$ is unrealistically high (b) (but even here the XY race has pushed forward to cover most of the area). The likelihood of this model is significantly lower than when the top edge is fixed.
Table 7.7: This table shows the effect of varying selection (s). For all runs 
"dconv"=0.03; the smoothing distance = 50m; dispersal (σ)=20m and grid spacing 
= 50m. In all cases the zone stabilizes: the potential drops and then stabilizes. “A” 
is the weighted area after 8000 generations (the actual area is 353000m² and the 
maximum is 600000m²); “L” is the likelihood of the model. The best fit is with 
s = 0.0025: this is less than that expected from deterministic calculations, probably 
because the barrier sharpens the cline.

incompatibilities. However, it does not rule out the possibility that one karyotype 
has some selective advantage (of order less than 0.0025) over the other. This raises 
two questions:

1. Does the chromosomal fusion have an advantage?

2. Does the XY race have an advantage due to other loci, and if so why have the 
favourable alleles (responsible for increased density) not spread?

I will discuss these questions in the next chapter.
Chapter 8
Synthesis

8.1 A summary of results

The extensive surveys on the pattern of densities across the two transects at Col de la Lombarde, as well as across transects at Col de Fenestre, Vallée des Merveilles and Jas du Plan Tendasque (Chapter 3) showed that the XY race is consistently more numerous than the XO race, and that the XO sites have densities which are on average similar to those of hybrids. This is a surprising result in view of the stability of the zone (see Chapter 2): in a homogeneous environment, we would expect the consistently larger XY population size to push the zone forward and eventually eliminate the less dense XO race. This is because populations at the centre of the zone would receive more immigrants from one side than from the other, so that the frequency of introgressing alleles would rise (Hewitt, 1975; Barton, 1979c; see Chapters 1 and 5).

Another interesting result to emerge from the density surveys was a density-dependent decrease in numbers between hatchlings and adults. Observations on natural populations made on two spatial scales, and manipulation experiments suggest that these are caused by mortality rather than by dispersal. The simulation experiments showed that inferences made from the field observations are reliable.

The approach used to detect density dependence is different from traditional time-series studies. Most of the literature on populations dynamics concerns single panmictic populations, and most of the methods for detecting density-dependence use comparisons across time. Here I inferred density-dependence by making comparisons between sites (i.e. comparisons across space rather than time), for a continuously distributed organism rather than a single panmictic population.

One of the aims of my research was to compare components of fitness between genotypes in natural populations of Podisma pedestris. In Chapter 3 I discussed
the advantage of direct measures of fitness components over laboratory estimates. I described measurements of fitness components in the field and the complications associated with these. We saw, for example, how density-dependent effects complicate the interpretation of measures of survival and, possibly of fecundity. In Chapter 5 I reviewed a less direct way of measuring fitness components, based on Barton's theory of multilocus clines. I discussed how the quantitative results of Barton's investigation have given relations which allow one to infer parameters such as selection pressures from cline shape, and described simulation experiments which show good agreement with his theoretical predictions.

In Chapter 6 I described a field investigation into gene flow across a physical barrier at Col de la Lombarde. The predictions from theory on barriers to gene flow combined with direct measures of cline shape and dispersal suggested that although selection on the chromosomal fusion itself is weak, there is strong overall selection against hybrids. Indeed, a theoretical model in which selection is spread over 100 genes, with selection per map unit \( S/R = 0.89 \), fits the data significantly better than a single locus model.

The asymmetric pattern of densities at Col de la Lombarde motivated an investigation into whether the position of the zone in this region is consistent with this imbalance. In Chapter 7 the observed position of the zone can be compared with that expected either analytically (Section 7.2) or by simulation (Section 7.4.3). The simulation results suggest that provided the zone is pinned by the stream at its top end, its position can be predicted from the pattern of densities. It seems that a combination of the stream and the extensive scree slope stabilise the hybrid zone, despite the marked differences in density between the two races in this region. This result is consistent with the theory, and provides further evidence that the cline is maintained by genetic incompatibilities, rather than by an environmental gradient.

### 8.2 Competition between chromosomes and between chromosome races

At the end of Chapter 7 I raised two questions: (1) Does the chromosomal fusion have an advantage? (2) Does the XY race have an advantage due to other loci, and if so, why have the favourable alleles (responsible for increased density) not spread? Before addressing these questions, it will be useful to briefly discuss some relevant investigations on chromosomal evolution and the probability of "peak shifts".
In discussions to follow, I will assume that the XO and XY races are at two different “adaptive peaks” or stable equilibria. In Chapter 1 I discussed Wright’s (1931; 1980) “shifting balance” theory of evolution, in which adaptation within a species occurs by shifts between demes at different stable equilibria. These result in some reproductive isolation and may allow the population to reach well adapted combinations of genes. Tension zones are closely related to this model. One can think of them as steep clines which have formed between races fixed at different equilibria (Barton, 1979a; Rouhani and Barton, 1987a): if an ancestral population evolves into two different and incompatible equilibria, then when populations at these different equilibria meet, selection will maintain the equilibria. If migration between adjacent demes is low, then the demes will retain a distinctive combination of selected alleles (Karlin and McGregor, 1972). When the diverged populations meet in a continuous habitat, a smooth stable tension zone forms (Rouhani and Barton, 1987a). The movement of tension zones can give insights into the ways species evolve better adapted genotypes.

The probability of the establishment and spread of underdominant chromosomal rearrangements has been investigated as an example of Wright’s “shifting balance” (Lande, 1985; Barton and Rouhani, 1991). Closely related species differ in their karyotype and yet chromosomal polymorphisms are rare, and when they do occur, are usually separated by narrow hybrid zones (White, 1978a). The traditional explanation is that meiotic non-disjunction causes partial sterility and that, since the rare form will be selected against, new chromosomal arrangements can only be established by random drift in small isolated demes (Wright, 1941; Lande, 1979). However, Barton and Rouhani (1991) modelled a continuous population with homogeneous structure, and showed that as long as an underdominant chromosomal rearrangement has a selective advantage in the homozygous form and if neighbourhood size is sufficiently low, it can become established despite free gene flow.

Once established, how would an underdominant chromosomal rearrangement spread? Barton and Rouhani (1991) summarise three modes:

1. Spread by extinction and recolonisation. For example, in Lande’s (1979; 1985) model, populations are subdivided into discrete demes and migration between demes is low \((m << s)\). Under these conditions if one adaptive peak results in a lower rate of extinction or a higher rate of colonisation, that peak will tend to spread and such interdemic selection will increase the chance of fixation and expected time to fixation.
2. Deterministic spread. For example, Rouhani and Barton (1987a) and Barton and Rouhani (1991) show that a better adaptive peak can spread deterministically in a constant population structure if the new peak has sufficient advantage over the old one.

3. Spread by stochastic transitions. Lande (1985) compared the spread of a new peak through a subdivided population by a sequence of stochastic transitions with spread by random extinctions and recolonisation, and found that a new peak will spread by increasing the rate of emigration and colonisation, and by reducing the threshold frequency above which the new karyotype will be fixed, but that the new karyotype will gain more of an advantage through individual than interdemic effects, as long as stochastic transition is frequent relative to extinction and recolonisation.

Whether spread is deterministic, by stochastic transition or by extinction and recolonisation will depend partly on the extent to which the population occupies discrete demes, or is continuously distributed with homogeneous population structure. Most real populations are likely to be somewhere inbetween. An underdominant rearrangement that is advantageous in the homozygous form is most likely to arise in regions of low neighbourhood size, in a manner analogous to that described by Barton and Rouhani’s model, but will then be unable to move out into more densely populated regions. Occasional extinctions and recolonisations (e.g. after climatic changes) may allow the rearrangement to spread throughout the entire range (in a manner analogous to that described by Lande, 1979, 1985).

The historical evidence for the origin and spread of the Podisma pedestris XY race and the present distribution of the two races has been discussed in some detail by Hewitt (1975) and Nichols and Hewitt (1986) (see Chapter 2). Podisma probably established in central Europe in the late Tertiary and extended southwards to the Alps with the palearctic ice ages. Podisma’s range would have been fragmented and rejoined as the climate warmed and cooled in regular fluctuations and the interglacial and glacial periods. The chromosomal fusion probably became fixed in a small population isolated during these changes, somewhere to the south west of the Alpes-Maritimes, and spread as the population expanded with further climatic change (Nichols and Hewitt, 1986). As the ice retreated, the ridge of the Alpes Maritimes would have been the last strip to be recolonised. Here, the two races met forming a hybrid zone. The zone is likely to have formed several times with
population expansion from refugia during interglacial periods. It will have formed most recently when the ice sheets retreated after the last glaciation and in some places from refugia on mountain peaks after the post-glacial climatic optima (Hewitt, 1988).

The present position of the zone is consistent with the theory that it is maintained by genetic incompatibilities. On a large scale, the zone has not moved far from where it formed: the cline follows the alpine ridge closely (see Fig. 2.4). On a finer scale the zone is trapped by local regions of low density or low dispersal, for example it tends to run along cliffs, streams, grazed areas or other inhospitable regions. Sometimes it bulges outwards (Barton, 1979c, Nichols and Hewitt, 1986), due to the local distribution of grasshoppers: pressure of numbers counteracts the contraction of the tension zone. At Col de la Lombarde the zone is trapped despite a marked difference in density on either side. This is also consistent with the theory. One would not necessarily expect the XY race to spread, simply because it has higher densities, or even if it had a selective advantage in relative fitness. Crow et al (1990) argued that very low rates of migration are sufficient to overcome selection, but as Barton (in prep.) points out, this merely reflects the power of migration relative to selection and is not due to the higher fitness of the new peak: a new peak will only spread if it has sufficient advantage over the old one. Furthermore it is important to note that while the movement of tension zones is deterministic, it depends on a variety of forces which can be absorbed into a potential\(^1\) (Barton, 1979c): the potential, \(H\), is a functional of the complete configuration of a tension zone and incorporates (1) the effects of a fitness difference (2) the effects of varying density or dispersal (3) the effect of a density difference on either side. Thus the tension zone can be trapped, despite a difference in density, by other forces, as observed in Chapter 7.

But why is there a consistent difference in density on either side of the zone? There are several possibilities. The density difference could simply reflect chance associations with ecological differences. Since the quadrats in the density survey of Chapter 3 were chosen in similar habitat types, any large-scale environmental heterogeneities and spatial patterns were excluded from the data. Such a difference could well result in corresponding large-scale patchiness in \(Podisma\) density. The extent to which the chosen quadrats are continuous with the surrounding vegetation, or

\(^1\)\(H\) is analogous to \(\overline{W}\) but is not identical to it. We can describe the two pure races as being at different adaptive peaks, and we can define the movement of a tension zone in terms of the spread of one peak at the expense of the other, however, the movement is not up a fitness surface unless one redefines fitness to include the effects of gene flow and population regulation.
are patches of isolated habitable units; the nature of the surrounding vegetation and whether the quadrats are in a generally inhospitable area would all be of importance to distribution patterns. If the chosen quadrats are isolated and desirable units but in a generally inhospitable area, their densities could be a function of the densities in the surrounding areas, and an associated low recruitment rate from these. The satellite image of the Col de la Lombarde region does show regional distribution of habitat types: areas covered predominantly by *Festuca peniculata* (which appears as a darker orange in the false-colour image (Fig 7.5) are mostly on the XO side.

However, the density trends in geographically distinct areas (6 different transects) are the same (Chapter 3), and although I do not have detailed vegetation maps of these regions, the range of habitats in each area was somewhat different, arguing against chance association.

It could be that the chromosomal fusion is directly responsible for increased densities, but this is inconsistent with the fact that the cline is much wider than the dispersal range (arguing for weak selection) (see Chapter 2). It is possible that other genes are responsible for increased density in the XY race and that the fusion is interacting epistatically - or simply coincides - with these. However, this possibility raises a paradox. Although the position of the chromosomal cline can be explained by barriers and reduced hybrid fitness (despite the density difference), if other loci are responsible for the larger numbers in the XY race, one might expect the genes conferring higher density to spread quickly from one race to the other.

One possibility is that the two races evolved different population dynamics as adaptations to different environments while isolated. The hybrid zone is not obviously associated with any environmental discontinuity, however we cannot rule out the possibility that on a broad scale, the XO and XY races are adapted to different habitats. Such broad scale adaptations have been observed in *Bombina bombina* and *Bombina variegata* (Szymura and Barton, 1986, 1991) and in the Torresian and Moreton races of *Caledia captiva* (Shaw and Wilkinson, 1980; Shaw et al., 1985), for example. In both these examples the zones are maintained largely by selection against hybrids and are not at any obvious ecotones. Suppose there is a similar pattern in *Podisma* (a broad scale habitat difference in the XO and XY ranges) and that the two adaptive peaks have evolved not only different carrying capacities (*K*) but also different responses to crowding depending on the habitat (note that this is not considered in traditional models of density-dependent selection). For example, suppose the fitness of each race depends on density in the way shown in Fig. 8.1.
Figure 8.1: In this model, in which fitness ($W$) is a function of population size ($N$), at equilibrium ($W = 2$) $N$ will evolve to the highest carrying capacity $K$. In *Environment 1* the XY race has the highest $K$ while in *Environment 2* the XO race does.

The dotted line shows fitness, $W$, when the populations are stable (i.e. $W = 2$ by definition). In environment 1 (a), the XY race has a higher carrying capacity ($K$) at $W = 2$, whereas in environment 2 (b), it is the XO race that has the highest $K$. If fitness ($W$) is a function of population size ($N$), the highest carrying capacity ($K$) will evolve (e.g. see Roughgarden, 1971, 1979) and so in *environment 1* we would find $N = K_{XY}$ ($K$ of the XY race), whereas in *environment 2* one would find $N = K_{XO}$ (at equilibrium), thus accounting for the observed density difference.

Another possibility is that the difference in numbers in the XY race and XO race is caused by incompatible sets of genes. Suppose that there is no environmental difference, but that the set of genes responsible for $K$ in one race (adaptive peak) are incompatible with those in the other: i.e. suppose that the fitness in each race depends not only on density, but also on allele frequency at the same and other loci. Here one would not necessarily expect the two races to evolve to the same equilibrium population density after contact, since the genes conferring higher density could be prevented from spreading by the breakup of "co-adapted" sets in hybrids.

The investigations described here suggest interesting implications for the evolution of population size and competition between races. In Chapter 3 we saw that genes that increase population size do not necessarily increase fitness. Even if the genes do increase fitness, one would not necessarily expect them to spread: the genes
responsible for increased density may be incompatible or the two forms may have evolved different population dynamics as adaptations to different habitats. This raises a general question: to what extent is divergence into incompatible races accompanied by differences of ecological significance? We have seen that the zone can easily be trapped by local barriers (Chapter 7) and so it is unlikely to move far from where it first formed in the broken alpine habitat. The races are likely to be incompatible at a large number of genes (Chapter 6). All this suggests that extinction of one race through competitive displacement by the other is unlikely.

In the long term, variation in density and dispersal, in the extreme, extinctions and recolonisations (e.g. after changes in climate) may shift the position of the zone. If the races are adapted to different habitats on a broad scale, then a change in habitat could alter its position. The investigation by Barton and Rouhani (1991) suggests that in general, in a more or less continuously distributed organism, an advantageous chromosomal rearrangement is most likely to become established in regions of low neighbourhood size (< 40, say) over a sufficiently large area (> $2l = 1.6Km$ in Podisma). If neighbourhood size varies gradually, the new type will spread over the whole species range (in Podisma density must double over no more than 500m; Barton, 1979a). However, the rearrangement is likely to be held back by physical barriers causing sharp changes in neighbourhood size (in Podisma, the main ridge of the Alps). Then the rate of chromosomal change will depend on the frequency with which small refugial populations spread across the whole species range. In general, whether or not a chromosomal rearrangement will become fixed over the entire species range, and its rate of fixation will depend on its advantage as well as the long term dynamics of the population.
References


Barton N.H. On the spread of new gene combinations in the third phase of Wright's "shifting balance". (in prep.)


Brunet E. 1986. *Locating and mapping a chromosomal cline in the alpine grasshopper Podisma pedestris*. Honours project, University College London.


Cox F. 1989. Analysis of a Narrow Hybrid Zone in the Alpine Grasshopper Podisma pedestris. Honours project, University College London.


Dobzhansky T. 1951. Experiments on sexual isolation in Drosophila X Reproductive isolation between D. pseudoobscura and D.
persimilis under natural and laboratory conditions. *Proc. Nat. Acad. Sci. (USA)* 37: 792-796


J. Wiley and Sons, New York.


Lande R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. *Genetics* **99**: 541-553.


SAS. 1985. *System for statistical analysis (version 6).* SAS Institute, Cary NC, USA.


Appendix A

Appendices for Chapter 2
A.1 List of the karyotype data

Summary of all Col de Lombarde data, measured on a coordinate system constructed from a theodolite survey of the region (origin at B2, and baseline running between B2 and B11).

Includes:

- Eugene Brunet's sites 900.. 1984
- Tim Wickham's data 2000.. 1987
- Danny Hewitt's grid 2100.. 1987
- Quadrats: CR, KJ, NB, FC 2200.. 1988 (includes Flora's stream sites)
- Col de Fenestre: NB, JSJ 2300.. 1988
- Adam Leibowitz's sites 3100.. 1989
- Adam Leibowitz's sites 4000.. 1990
- K. Jackson's grid 4100.. 1990

Numbers and codes after F. Cox's stream sites (2200..) refer to distance from stream, and code, from Cox (1989).

Global coordinates, and TW's coordinates have been measured for 4 landmarks:

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The codes after Danny Hewitt's sites (21..) refer to his grid points (A0 to Q5) (D. Hewitt, 1988).

MEANS

Coordinates:
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-2160 330
-2235 410.
-2435 2000.
-4600 4210
-2110 230.
-1995 80.
-1660 -50
-1580 -335
-1685 -530
-1580 -960

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END
Appendix B

Appendices for Chapter 3

B.1 Vegetation species list

Alphabetic listing of plants occurring at Col de la Lombarde and at Seyne.

The most commonly occurring herbs and grasses found in the study areas. In some cases it was not possible to identify plants down to species level for example if plants were not in flower or if identification required detailed examination of roots.

**Trees:**

*Pinus sp*

**Shrubs and herbs:**

*Achillea erba rotta* (Compositae)
*Achillea millefolium* (Compositae)
*Achillea odorata* (Compositae)
*Adenostyles leucophyllos* Rchb. (Compositae)
*Alchemilla alpina* (Rosaceae)
*Allium saxatile* Bieb. L. (Liliaceae)
*Antennaria dioica* Gaertner. (Compositae)
*Arnica montana* L. (Compositae)
*Armenia sp.* Willd. (Plumbaginaceae)
*Asperula hexaphylla* All. (Rubiaceae)
*Aster alpinus* L. (Compositae)
*Campanula rotundifolia* (Campanulaceae)
*Centaurea montana* (Compositae)
*Centaurea uniflora* (Compositae)
*Cirsium spinosissimum* Scop. (Compositae)
*Cryptogamma crispa*
*Dianthus gratianopolitanus* Vi11. (Caryophyllaceae)
Eriophorum scheuchzeri L. (Cyperaceae)
Erigeron uniforus L. (Compositae)
Euphrasia alpina Lmk. (Scrofulariaceae)
Galium verum L. (Rubiaceae)
Galium helveticum L. (Rubiaceae)
Gentiana campestris L. (Gentianaceae)
Gentiana kochiana (Gentianaceae)
Gentiana verna (Gentianaceae)
Geranium sylvaticum (Geraniaceae)
Hieracium alpinum L. (Compositae)
Hieracium pilosella L. (Compositae)
Hugueninia tanacetifolia Reichenb. (Cruciferae)
Hypericum richeri (Vil1.) (Guttiferae)
Jasione montana (Campanulaceae)
Juniperus nana Willd.
Lathyrus L. (Leguminosae)
Lotus alpinus (Leguminosae)
Lotus corniculatus (Leguminosae)
Meum athamanticum (Miller) (Umbelliferae)
Myosotis alpina (Boraginaceae)
Myosotis silvatica (Boraginaceae)
Nigritella nigra Rich. (Orchidaceae)
Nigritella cornelliana Soo. (Orchidaceae)
Pedicularis tuberosa (Scrofulariaceae)
Pedicularis sylvatica (Scrofulariaceae)
Phleum pratense
Plantago alpina (Plantaginaceae)
Polygonum alpina (Ranunculaceae)
Polygonum bistorta L. (Ranunculaceae)
Pulsatilla sp. Miller. (Ranunculaceae)
Ranunculus sp. L. (Ranunculaceae)
Rumex alpinus L. (Polygonaceae)
Rumex acetollesa L. (Polygonaceae)
Rhododendron ferrugineum
Santolina (Compositae)
Saxifraga sp. (L. Saxifragaceae)
Sempervivum arachnoideum (Crassulaceae)
Senecio incanus L.
Silene acaulis L. ssp. exscapa J. Braun
Silene vulgaris (Caryophyllaceae)
Solidago sp. (Compositae)
Stellaria sp. (L. Caryophyllaceae)
Thymus pulegioides (Labiatae)
Trifolium alpinum
Urtica dioica
Vaccinium myrtillus
Vaccinium uliginosum
Veratrum album L. (Liliaceae)
Veronica fruticans (Jacq. Scrophulariaceae)
Veronica acinifolia (Scrophulariaceae)
Viola sp. (Violaceae)
Viola biflora sp. (Violaceae)
Viola nummularifolia sp. (Violaceae)

Grasses:
Anthoxanthum odoratum (L. Gramineae (Pooid))
Agrostis sp. (L. Gramineae (Pooid))
Carex sempervirens (L. Crassulaceae)
Deschampsia flexuosa (Pal. Gramineae (Pooid))
Eriophorum scheuchzeri L. (Famille : Cyperacees)
Euphrasia alpina Lmk. (Famille : Scrofulariacees)
Festuca sp. (L. Gramineae (Pooid))
Festuca peniculata (L. Schinz and Thell)
Juncus trifidus (L. Juncaceae)
Luzula campestris (DC. Juncaceae)
Nardus stricta (L. Gramineae)
Poa sp. (L Gramineae (Pooid))
Trisetum sp. (Pers. Gramineae (Pooid))
Seyne, 1987

First division

Vacc. ulig. 1-8

Vacc. myrt. 1-5

Veronica spp. 2

Hieracium spp 2

Lotus spp 2

Myosotis spp 1

Carex semp.2

Lotus spp. 1

Alch. 1

Vacc. myrt. 6

earth 3

polygonum bistorta 1

Veronica spp 1

Figure B.1: Hierarchy of indicator species. “1” to “8” are measures of abundance: the number of times a species occurred at a randomly chosen “point” (see text). “Vacc. ulig.” = Vaccinium uliginosum; “Carex semp.” = Carex sempervivum; “Vacc. myrt.” = Vaccinium myrtillus; “Alch.” = Alchemilla spp.. A list of “preferential” species found in the TWINSPAN groups “a” to “j” in this hierarchy are provided in Table B.1.

B.2 Vegetation analysis using TWINSPAN

TWINSPAN (Hill, 1979) uses successive divisions of ordination. First it constructs a classification of the quadrats. Using this, the program obtains a classification of the species according to the degree to which they are confined to particular groups of quadrats. These classifications are then used to obtain an ordered two-way table: the ordering is aimed to exhibit the relation between species and samples as clearly as possible. It is this ordering that distinguishes TWINSPAN from standard ordination methods. In TWINSPAN, odd species are placed into an anomalous position (a standard ordination would typically place odd species in an intermediate position). The indicator ordination is based on a small number of the most strongly differential species - designed to provide a simple criterion for re-identification of the groups. Preferential species are those that are likely to occur in a group.

TWINSPAN is a “divisive classification” (Hill, 1979): it starts with the whole dataset and makes successive divisions of quadrats judged to be most dissimilar.
Table B.1: Seyne “preferential” species.

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</tbody>
</table>

Figure B.2: Col de la Lombarde, 1987. Hierarchy of indicator species for the first and second TWINSPLAN dichotomies. “1” = species present; “2” = species sparse; “3” = moderate cover; “4” = species abundant; “5” = species dominant. Few preferential species were listed: Branch “a” - none listed; branch “b” - none listed; branch “c” - Luzula, 2; Carex 1; Vacc. myrt. 2; Deschampsia 4; Festuca spp. 2,3; branch “d” - earth 2,3; branch “e” - none listed; branch “f” - Vacc. myrt. 2,3.
Col de la Lombarde, 1988

Figure B.3: Twinspan analysis on 1988 quadrat data: hierarchy of indicator species. This figure only includes divisions that result in at least one group consisting of more than 6 quadrats. In the General Linear Models described in Section 3.2.5 only groups with more than 14 quadrats were assigned a category. Since all further dichotomies in the TWINSPLAN analysis split off groups of fewer than 4 quadrats, they have not been included in the figure. \( n \) = the number of quadrats in a group; 1 = species present in all quadrats in the group; 2 = species occurs commonly in the quadrats; 3 = species is very abundant/dominant; Dian = Dianthus spp.; Vacc. Myrt. = Vaccinium myrtillus; Vacc. ulig. = Vaccinium uliginosum; Rhod. = Rhododendron spp.; Ant = Antennaria spp.; Sax. = Saxifraga spp.. A list of preferential species for groups 'a' to 'r' is provided in Table B.2.
Table B.2: Col de la Lombarde, 1988, “preferential” species.

This method of classification has the advantage over agglomerative methods in that in the latter atypical quadrats have a strong effect on the first round (Hill, 1979; Gauch, 1982).

The divisions are made by an “ordination-space partitioning method”: the data are ordinated using one-dimensional reciprocal averaging (a version of principal component analysis (PCA)).

In reciprocal averaging ordination, the quadrats and species are ordinated simultaneously; scores are assigned to each quadrat and to each species so as to maximize the correlation between quadrat scores and species scores. The axis is broken at the centroid giving a crude partition of the ordinated swarm. Reciprocal averaging is repeated at least once with species quantities weighted to emphasize the influence of especially useful diagnostic species (known as the “two-way indicator species analysis”). The process results in the dendrogram with its branches having similar points close to each other.
### B.3 Estimating effects using the SAS General Linear Models procedure

The SAS general linear model procedure (see Freund et al, 1986) computes several sums of squares. Each SS can be expressed as the difference between the regression sum of squares for two models (complete and reduced models). This approach relates a given SS to the comparison of two regression models. The GLM procedure is useful in that it can analyse general linear models that include categorical terms and polynomials. The procedure is also particularly useful for analysis of variance for unbalanced data, where a direct computation of sums of squares would fail. Dummy variables are used, which produces models that are overspecified (i.e. they contain more parameters than can be uniquely estimated).

#### The dummy variable model

For the implementation of the dummy variable model, the analysis of variance model:

\[ y_{ij} = \mu + \tau_i + \epsilon_{ij} \]  

(B.1)

\(y_{ij}\) is the j'th measurement in the i'th group; \(\mu\) is the reference value; \(\tau_i = \mu_i - \mu\) (\(\mu_i\) = population mean of the i'th group); \(\epsilon_{ij}\), random error with mean zero and variance \(\sigma\) is rewritten as a regression model:

\[ y_i = \beta_0 x_0 + \beta_1 x_1 + \ldots + \beta_t x_t + \epsilon_{ij} \]  

(B.2)

(where \(\beta_0 = \mu; \beta_i = \tau_i\); and the dummy variables, \(x_0 ... x_t\), are defined as follows: \(x_0\) is always 1; \(x_1\) is 1 for observations in group 1, zero otherwise; \(x_2\) is 1 for observations in group 2, zero otherwise ... \(x_t\) is 1 for observations in group t, zero otherwise.

The normal equations \((X'X)\hat{\beta} = X'Y\) are equivalent to the set:

\[
\hat{\beta}_0 + \hat{\beta}_1 = \bar{y}_1 \\
\vdots \\
\hat{\beta}_0 + \hat{\beta}_2 = \bar{y}_2 \\
\hat{\beta}_0 + \hat{\beta}_t = \bar{y}_t 
\]  

(B.3)

Since there are only \(t\) equations, there is obviously no unique solution for \((t+1)\) estimates: \(\hat{\beta}_0, \hat{\beta}_2 ... \hat{\beta}_t\). This follows from the fact that the reference parameter \(\beta_0\) is arbitrary (and so the model is said to be overspecified): the \(X'X\) matrix describing the
set of normal equations is 'singular'. Clearly, the first row of the \( X'X \) is equal to the sum of the other \( t \) rows. Since overspecified models yield singular \( X'X \) matrices, they require special methods to produce useful estimates and tests. Because the \( X'x \) matrix is not of full rank, it has no unique inverse. So GLM computes a generalised inverse \((X'X)^{-1}\) and uses it to compute a regression estimate: \( b = (X'X)^{-1}X'Y \) (a generalised inverse of a matrix \( A \) is any matrix \( G \) such that \( AGA = A \)). The different generalised inverses would lead to different solutions to the normal equations and would have different expected values: \( E(b) = (X'X)^{-1}X'XB \). It is therefore important to understand what is being estimated by a particular solution. Fortunately not all computations in regression analysis depend on the particular solution obtained. e.g. the error sum of squares does not change w.r.t \((X'X)^{-1}\) and is given by \( SSE = Y'(1 - X(X'X)^{-1}X')Y \). The model sum of squares is therefore not dependent on the particular generalised inverse obtained.

SAS GLM uses a 'restriction method', so called because it defines one of the \( \tau_i \) (i.e. the difference between mean and \( \mu_i \)) to be zero so that \( \mu \) becomes the mean of that group and the corresponding restriction on the solution to the normal equations is to require that \( \tau_i = 0 \) this leads to a unique set of values for the remaining set of estimates: \( \hat{\mu}, \hat{\tau}_1, ... \hat{\tau}_{t-1} \) (because \( \hat{\tau}_1 \) is dropped from the linear model and therefore the column corresponding to \( \hat{\tau}_1 \) is dropped from the X matrix).

Computing generalised inverse by partitioning of a singular matrix:

\[
X'X = \begin{bmatrix}
A_{11} & A_{12} \\
A_{21} & A_{22}
\end{bmatrix}
\]  \hspace{1cm} (B.4)

where \( A_{11} \) is \( k \) by \( k \) and of rank \( k \), then \( A_{11}^{-1} \) a generalised inverse of \( X'X \) is:

\[
\begin{bmatrix}
A_{11}^{-1} & \theta_{12} \\
\theta_{21} & \theta_{22}
\end{bmatrix}
\]  \hspace{1cm} (B.5)

each \( \theta_{ij} \) is a matrix of zeros of the same dimension as \( A_{ij} \).

This approach for obtaining generalised inverse can be extended indefinitely by partitioning a singular matrix into several sets of matrices as shown above. The resulting solution to the normal equations \( b = (X'X)^{-1}X'Y \) has zeros in the positions corresponding to the rows filled with zeroes in the \((X'X)^{-1}\). The resulting solution is therefore regarded as a biased estimate of \( \beta \). However since I am interested only in relative effects and analysis of variance, the solutions are very useful.

Since \( b \) is not unique, neither in general is a linear function \( Lb \). However, there is a class of linear functions called estimable functions with the following properties:1) the vector \( L \) is a linear combination of rows of \( X \) 2) \( Lb \) and its variance are invariant
Table B.3: The estimates of relative effects of each variable resulting from GLM were used to adjust densities. This table gives adjustments calculated from the least squares estimated solution to the best fit linear model to the data, using GLM Type IV SS (see Appendix B.3). V=vegetation category, G=ground cover category, T=topography category.

through all possible generalised inverses: so that \( Lb \) is unique and is an unbiased estimate of \( L\beta \). Analogous to the full rank case, the variance of an estimable function \( Lb \) is given by: \( V(Lb) = (F'(X'X)^{-1}L')\sigma^2 \). This expression is used for the statistical inferences in Table 3.10.

Note that because the data is not of "full rank", there is no way of obtaining contributions to density. Instead, one has to consider effects of each variable. In the SAS GLM this is done by using generalised inverse for solving the normal equations (as described above). The SAS solution estimates an intercept for the model and parameters for all variables. The intercept ensures least squares residuals for the model with the given alignment (that is where the last variable \( \mu \) is set to zero in each case, as described above).
Appendix C

Appendices for Chapter 4

C.1 Calibration of the large-scale quadrats

Mark-release-recapture experiments were used to estimate the proportion of the total numbers present that are caught in the first ten minutes of searching by two scorers. Six of the \((10m)^2\) quadrats were chosen for calibration. The initial search had indicated that some quadrats had higher densities than others (for obscure reasons) and so calibration sites were chosen to include the lowest- and highest-density quadrats. These were Grid 4: B1, C2; Grid 3: B1; Grid 2: C2; Grid 1: B1, C2. An additional six quadrats were chosen for calibration at the time of final scoring: Grid 1: E3, D3; Grid 2: D1, A0; Grid 4: A1, B0. Each site was searched by two scorers for ten minutes. Individuals were counted into a tin which had been lightly dusted with rust-coloured fluorescent dust (see Chapter 3) and at the end of the ten minute search period, were scattered around the quadrat. Approximately four hours later the sites were re-scored for ten minutes each and numbers of marked and unmarked individuals recorded. The results are summarized in Table C.1.

There was no significant difference in the number of nymphs or adults caught on the first and second captures \((G = 7.25, \text{ns} \text{ and } G = 7.34, \text{ns} \text{ respectively})\).

The Lincoln index method was used to estimate population size \((\hat{N})\): if \(r\) individuals are marked and released in a given area and if, on a subsequent scoring, \(n\) individuals are caught of which \(m\) are marked and \(n-m\) are unmarked, then the chance of catching a grasshopper is estimated as \(m/r\). The estimated population size, \(\hat{N}\) is then \(n/mr\). This method assumes no loss by dispersal or mortality, which seems reasonable here. Pooling the data on nymphs: \(r = 256\) and \(m = 95\), therefore the chance of catching a marked grasshopper is 0.37. Since \(n = 261\), \(\hat{N} = 703.32\) and the fraction caught in ten minutes is approximately 0.36. Pooling the data on adults: \(r = 169\) and \(m = 80\), therefore the chance of catching a marked grasshopper
<table>
<thead>
<tr>
<th>SITE</th>
<th># caught, marked and released</th>
<th># recaptured unmarked</th>
<th>Total recaptured</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Nymphs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grid 4 B1</td>
<td>27</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Grid 4 C2</td>
<td>32</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Grid 3 B1</td>
<td>12</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Grid 2 C2</td>
<td>55</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>Grid 1 C2</td>
<td>68</td>
<td>44</td>
<td>25</td>
</tr>
<tr>
<td>Grid 1 B1</td>
<td>62</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>(b) Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grid 1 E3</td>
<td>34</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Grid 1 D0</td>
<td>42</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Grid 2 D1</td>
<td>47</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>Grid 2 A0</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Grid 4 B0</td>
<td>15</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Grid 4 A1</td>
<td>24</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>

Table C.1: The calibration data

is 0.473. Since \( n = 162 \), \( \hat{N} = 342.49 \) and the fraction caught in ten minutes is about 0.493.

Is there a difference in the proportion caught in low density and high density quadrats? Table C.2 summarizes the data.

The difference between the proportion seen in high and low density sites is not significant: for nymphs \( G = 0.064 \); for adults \( G = 0.0147 \).

<table>
<thead>
<tr>
<th>High density</th>
<th>Proportion</th>
<th>Low density</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Nymphs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grid 2 C2</td>
<td>0.439</td>
<td>Grid 4 B1</td>
<td>0.357</td>
</tr>
<tr>
<td>Grid 1 C2</td>
<td>0.362</td>
<td>Grid 4 C2</td>
<td>0.324</td>
</tr>
<tr>
<td>Grid 1 B1</td>
<td>0.364</td>
<td>Grid 3 B1</td>
<td>0.300</td>
</tr>
<tr>
<td>(b) Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grid 1 E3</td>
<td>0.486</td>
<td>Grid 2 A0</td>
<td>0.333</td>
</tr>
<tr>
<td>Grid 1 D0</td>
<td>0.538</td>
<td>Grid 4 B0</td>
<td>0.600</td>
</tr>
<tr>
<td>Grid 2 D1</td>
<td>0.390</td>
<td>Grid 4 A1</td>
<td>0.577</td>
</tr>
</tbody>
</table>

Table C.2: Estimated proportion of (a) nymphs and (b) adults that are seen in the ten minute scoring period in the high and low density calibration sites.
C.2 Polynomial regression analysis

The SAS procedure for polynomial regression was used to build an appropriate polynomial model, by sequential fitting of equations with higher order terms (to 4th order). In most cases a no-intercept model\(^1\) with linear and quadratic terms gave the best fit, and higher order terms did not improve the fit.

While polynomial regression is useful as an approximation, this method assumes that the variances are normally distributed: an assumption which is unlikely to be correct for these field data sets.

\(^1\)A no-intercept model was used, because if the peak nymph density is zero then the adult density will also be zero
<table>
<thead>
<tr>
<th>DATA SET</th>
<th>VARIABLES USED IN POLYNOMIAL REGRESSION MODEL</th>
<th>F-RATIO FOR VARIABLE</th>
<th>PARAMETER ESTIMATES FOR APPROPRIATE MODEL</th>
<th>STATISTICAL SIGNIFICANCE (F-VALUE AND SIGNIFICANCE)</th>
<th>R-SQUARE AND ADJUSTED R-SQUARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRID1</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>37.85</td>
<td>0.43</td>
<td>117.048</td>
<td>0.975 (0.97)</td>
</tr>
<tr>
<td>GRID3</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>16.29</td>
<td>0.75</td>
<td>54.002</td>
<td>0.95 (0.93)</td>
</tr>
<tr>
<td>GRID4</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>11.31</td>
<td>0.55 (0.81)</td>
<td>36.19</td>
<td>0.92 (0.89)</td>
</tr>
<tr>
<td>LOW88</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>1.44</td>
<td>0.31 (0.4)</td>
<td>11.56</td>
<td>0.62 (0.57)</td>
</tr>
<tr>
<td>LOXY88</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>7.35</td>
<td>0.59</td>
<td>35.38</td>
<td>0.89 (0.87)</td>
</tr>
<tr>
<td>LOW89</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>1.08</td>
<td>0.42</td>
<td>6.304</td>
<td>0.56 (0.47)</td>
</tr>
<tr>
<td>LOXY89</td>
<td>PEAK, PEAK<em>2, PEAK</em>3, PEAK*4</td>
<td>3.07</td>
<td>1.224 (0.18)</td>
<td>16.109</td>
<td>0.83 (0.78)</td>
</tr>
</tbody>
</table>

Table C.3: Polynomial regression analysis: a summary. The first column describes the variables used in the model, provided in the order in which they were sequentially added. The corresponding F-statistics for each variable are provided in the second column. F ratios were obtained by dividing TYPEI sum of squares (which represents the contribution of each independent variable to the regression sum of squares as that variable is added to the model), by the residual mean square. The third column describes parameter estimates for the most appropriate model, for example, if the most appropriate model is $aP + bP^2$, estimates of $a$ and $b$ for this model will be provided. The fourth column provides the statistical significance of the entire model, along with its significance level (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; not significant). The last column gives the $R^2$ statistic (the coefficient of determination) and in brackets the adjusted $R^2$, adjusted (because the $R^2$ statistic can suggest a good fit simply by adding more variables to the model) according to: $ADJ - R^2 = 1 - (1 - R^2)(n-1)/(n-m-1)$, where $m$ is the number of regression parameters and $n$ is the number of observations in the data set.
Appendix D

Appendices for Chapter 5
D.1 The program

D.1.1 Program listing

02/01/92 22:15

program multilocus (input, output);

uses
utilities, functions, graphics, multilocus_procs; (global constants and types are in multilocus_procs)

var
out_file, bf, cricket_file: Text;
out_fname, list_fname, pict_fname, fname_root, batch_fname, cricket_fname: string;
batch: boolean;
ans, ans2, ans3: string;
pop, newpop: popptrtype;
s, dopt, rec, mutn, mig, migbarr, hwidth, beta: real;
ninit, nfix, g: integer;
demo: demepltype;
pbar, pvar, dbar: tdemeptrtype;
allOne, allZero: haplotype; (haplotypes corresponding to all ones or all zeroes)
ip, iq, i1, i2, i3, i4, h: haplotype;
d: demetype;
i: indtype;

procedure askstuff;
var
ib: blocktype;
gg: genetype;
begin
write('The program runs several replicates; each is run for tmax generations.');
write('Statistics are recorded every dt gens, after an initial delay of twarm gens');
writeln('tmax, dt, twarm ?
');
readln(tmax, dt, twarm);

write('Haploid individuals migrate. Haploids come together at random to produce diploids. Selection acts on these diploids. For each haploid in the next generation,
');
write('a diploid is chosen, with probability proportional to the fitness of that');
write('genotype. The haploid is generated by recombination from the diploid parent.');
write('Would you like some of the genes to be neutral ?
');
readln(ans);
nneut := 0;
seln_mask := allOne;
if ans = 'y' then
begin
write('For each gene, type y if it is to be neutral, n otherwise 
');
for gg := 0 to ngenes - 1 do
begin
write('Gene ', gg : 4, '; neutral ?
');
readln(ans);
if ans = 'y' then
begin
nneut := nneut + 1;
putgene(gg, 0, sein_mask);
end
end;
end;
nsel := ngenes * nneut;
writeln('The genes are on a chromosome, with r between each. Quicker to use \( r = 0.5 \) (no linkage)');
writeln('Recombination, mutation ?');
readln(rec, mutn);

writeln('nmig individuals migrate (half in each direction); at the centre, there is a barrier.');
writeln('across which nmigbarr migrate. If there is no barrier, set nmig=nmigbarr');
writeln('nmig (a multiple of 2), nmigbarr (a multiple of 2)');
readln(nmig, nmigbarr);

repeat
  writeln('Would you like Gaussian epistasis ("e"), power epistasis ("pe"), heterozygote disadvantage ("h"), or stabilising seln ("s") ?');
  readln(ans);
until (ans = 'h') or (ans = 's') or (ans = 'e') or (ans = 'pe');
epistasis := (ans = 'e');
epistasisp := (ans = 'pe');
hetdis := (ans = 'h');
stabilising := (ans = 's');
if epistasis then
  begin
    writeln('s (epistatic), 1/e gene freq halfwidth for epistasis (try 0.25) ?');
    readln(s, hwidth)
  end;
if epistasisp then
  begin
    writeln('s (epistatic), beta ?');
    readln(s, beta)
  end;
if hetdis then
  begin
    writeln('s (against each heterozygous locus) ?');
    readln(s)
  end;
if stabilising then
  begin
    writeln('s, Dopt (strength of stabilising seln, and change in optimum: \( W = \exp(-s(z-Dopt/2)^2/2) \)) ?');
    readln(s, dopt)
  end;
if epistasis or hetdis or epistasisp then
  begin
    repeat
      writeln('There are several possible starting frequencies; type 1, 2, 3 or 4');
      writeln(' 1: all loci in a deme at the same frequency, so the mean is at the optimum');
      writeln(' 2: all loci fixed; all demes the same, and the mean at the midpoint');
      writeln(' 3: all loci fixed; mean at optimum, and as far as possible, loci the same across the centre');
      writeln(' 4: all loci fixed; mean at optimum, but different states across the centre');
      readln(initfrequencies)
    until (initfrequencies >= 1) and (initfrequencies <= 5);
  end;
writeln('Seed for random numbers ?');
readln(seed);
if (tmax div dt > maxsamples) then
writeln('TOO MANY SAMPLES (max=\text{maxsamples}: 3, \text{\})');
mig := nmig / ninds;

end;

procedure askstuff_batch;
var
  ib: blocktype;
  gg: genetype;
  bt: bittype;
begin
  readln(bf, tmax, dt, twarm);
  readln(bf, ndemes, ninds, ngenes);
  ndip := ninds * (ninds + 1) div 2;
  nblocks := 1 + ((ngenes - 1) div 16);
  for ib := 1 to nblocks do
    allOne[ib] := -1; (* in binary, this is all ones *)
  for ib := 1 to nblocks do
    allZero[ib] := 0; (* in binary, this is all zeroes *)

  '{Would you like some of the genes to be neutral? }'
  readln(bf, ans);
  nneut := 0;
  sein_mask := allOne;
  if copy(ans, 1, 1) = 'y' then
    begin
      for gg := 0 to ngenes - 1 do
        begin
          read(bf, bt);
          putgene(gg, bt, sein_mask);
          if bt = 0 then
            nneut := nneut + 1;
        end;
      readln(bf)
    end;
  nsel := ngenes - nneut;
  readln(bf, rec, mutn);
  readln(bf, nmig, nmigbarr);

  '{Would you like epistasis ("e"), heterozygote disadvantage ("h"), or stabilising sein ("s") ?}'
  readln(bf, ans);
  ans := copy(ans, 1, 1);
  epistasis := (ans = 'e');
  epistasisp := (ans = 'p');
  hetdis := (ans = 'h');
  stabilising := (ans = 's');
  if epistasis then
    readln(bf, s, hwidth);
  if epistasisp then
    readln(bf, s, beta);
  if hetdis then
    readln(bf, s);
  if stabilising then
    begin
      readln(bf, s, dopt);
    end;

  '{Initialisation method: 1 to 4}'
  readln(bf, initmethod);
  if hetdis or epistasis or epistasisp then
    readln(bf, initfrequencies);
  readln(bf, seed);

  if (tmax div dt > maxsamples) then
    writeln('TOO MANY SAMPLES (max=\text{maxsamples}: 3, \text{\})');
mig := nmig / ninds;
procedure set_listfile (var fl: text);
begin
  writeln(fl, 'Simulating a multilocus cline: ', date, ', ', time);
  writeln(fl, ' ndemes=', ndemes : 4, ' tmax=', tmax : 4, ' dt=', dt : 4);
  writeln(fl, ' ninds=', ninds : 4, ' ngenes=', ngenes : 4, ' nmig=', nmig : 4);
  writeln(fl, ' nmigbarr=', nmigbarr : 4, ' mig=', mig : 8 : 6);
  if initfrequencies = 1 then
    writeln(fl, 'Start with a step');
  if initfrequencies = 2 then
    writeln(fl, 'Start with half individuals fixed at all loci for "1", half for "0", in all demes');
  if initfrequencies = 3 then
    writeln(fl, 'On average start with individual gene frequencies in each deme at p=0.5');
  if initfrequencies = 4 then
    writeln(fl, 'Start with a cline');
  if initfrequencies = 5 then
    writeln(fl, 'Start with one individual in each deme fixed for "1" all others fixed for zero');
  if epistasis then
    writeln(fl, 'Epistasis: s=', s : 8 : 6, ' halfwidth for epistasis=', hwidth : 8 : 6);
  if epistasisp then
    writeln(fl, 'Epistasis: s=', s : 8 : 6, ' beta=', beta : 8 : 6);
  if hetdis then
    writeln(fl, 'Heterozygote disadvantage: s=', s : 8 : 6);
  if stabilising then
    writeln(fl, 'Stabilising selection: s=', s : 8 : 6, ' change in optimum: ', dopt : 8 : 2);
  if initmethod = 1 then
    writeln(fl, 'Start with all loci in a deme at the same frequency; mean at the local optimum');
  if initmethod = 2 then
    writeln(fl, 'Start with all loci fixed at 0 or 1; all demes the same, and mean at the overall midpoint');
  if initmethod = 3 then
    writeln(fl, 'Start with all loci fixed at 0 or 1; mean is at the local optimum, but as far as possible, loci are in
    the same state on either side');
  if initmethod = 4 then
    writeln(fl, 'Start with all loci fixed at 0 or 1; mean is at the local optimum, and loci are assigned 0 or 1
    independently on either side');
  writeln(fl, 'rec=', rec : 8 : 6, ' mutn=', mutn : 8 : 6, ' seed=', seed : 4);
  writeln(fl, 'Output file corresponding to the above parameters has root: ', fname_root);
  writeln(fl, 'Batch file was: ', batch_fname);
end;

procedure initrep;
var
  rvalue, exfreq, beta; x, tanh, middeme: real;
  i: indtype;
  ib: blocktype;
  m_mask: haplotype;
  g: genetype;
  d, demeno: integer;
  plo, phi, exp: real;
  nhi, nio, nmid: integer;
begin
  t := 0;
  [a bit roundabout, but safest to set haplotypes to zero first]
  for d := 0 to ndemes + 1 do
    for i := 1 to ninds do
      begin
        pop^[d, i] := AllZero;
        newpop^[d, i] := AllOne;
      end;
  if stabilising then
    begin
      plo := 0.5 - dopt / (4 * ngenes); [lower eqbrm under stabilising selection]
      plo := min(max(plo, 0), 1);
    end;
\[
\phi := 0.5 + \frac{d_{opt}}{4 \cdot n_{genes}}; \text{(upper eqbrm under stabilising selection)} \\
\phi := \min(\max(\phi, 0), 1); \\
\text{if } \text{initmethod} = 1 \text{ then (set all loci to } \phi \text{ or } p_{lo}) \\
\text{begin} \\
\text{mutation_mask returns a mask with 1 at probability } p_{lo} \text{ or } \phi \\
\text{for } d := 0 \text{ to } n_{midleft} \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, mutation_mask(plo), } d, i); \text{ [genes set to 1 or 0 with probability } p_{lo}] \\
\text{for } d := n_{midright} \text{ to } n_{demes} + 1 \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, mutation_mask(\phi), } d, i); \text{ [genes set to 1 or 0 with probability } \phi] \\
\text{end; } \\
\text{if } \text{initmethod} = 2 \text{ then [set } n_{genes'}p \text{ loci to 1, rest to 0]} \\
\text{begin} \\
n_{mid} := \text{round}(n_{genes} \cdot (p_{lo} + \phi) / 2); \\
m_{mask} := \text{allZero}; \\
\text{if } n_{mid} > 0 \text{ then} \\
\text{for } g := 0 \text{ to } n_{mid} - 1 \text{ do} \\
\text{putgene(g, 1, } m_{mask}); \\
\text{for } d := 0 \text{ to } n_{midleft} \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, } m_{mask}, d, i); \text{ [genes set to 1 or 0 with probability } p_{lo}] \\
\text{for } d := n_{midright} \text{ to } n_{demes} + 1 \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, } m_{mask}, d, i); \text{ [genes set to 1 or 0 with probability } \phi] \\
\text{end; } \\
\text{if } \text{initmethod} = 3 \text{ then [set } n_{genes'}p_{lo} \text{ loci to 1, rest to 0]} \\
\text{begin} \\
n_{lo} := \text{round}(n_{genes} \cdot p_{lo}); \\
m_{mask} := \text{allZero}; \\
\text{if } n_{lo} > 0 \text{ then} \\
\text{for } g := 0 \text{ to } n_{lo} - 1 \text{ do} \\
\text{putgene(g, 1, } m_{mask}); \\
\text{for } d := 0 \text{ to } n_{midleft} \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, } m_{mask}, d, i); \text{ [genes set to 1 or 0 with probability } p_{lo}] \\
n_{hi} := \text{round}(n_{genes} \cdot \phi); \\
m_{mask} := \text{allZero}; \\
\text{if } n_{hi} > 0 \text{ then} \\
\text{for } g := 0 \text{ to } n_{hi} - 1 \text{ do} \\
\text{putgene(g, 1, } m_{mask}); \\
\text{for } d := n_{midright} \text{ to } n_{demes} + 1 \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, } m_{mask}, d, i); \text{ [genes set to 1 or 0 with probability } \phi] \\
\text{end; } \\
\text{if } \text{initmethod} = 4 \text{ then [set } n_{genes'}p_{lo} \text{ loci to 1, rest to 0; choose loci independently on either side]} \\
\text{begin} \\
m_{mask} := \text{mutation_mask(plo)}; \\
\text{for } d := 0 \text{ to } n_{midleft} \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, } m_{mask}, d, i); \text{ [genes set to 1 or 0 with probability } p_{lo}] \\
m_{mask} := \text{mutation_mask(\phi)}; \\
\text{for } d := n_{midright} \text{ to } n_{demes} + 1 \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, } m_{mask}, d, i); \text{ [genes set to 1 or 0 with probability } \phi] \\
\text{end; } \\
\text{end; } \\
\text{else if } \text{initfrequencies} = 1 \text{ then } \\
\text{begin} \\
\text{for } d := 0 \text{ to } n_{midleft} \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, allZero, } d, i); \text{ [all genes set to zero]} \\
\text{for } d := n_{midright} \text{ to } n_{demes} + 1 \text{ do} \\
\text{for } i := 1 \text{ to } n_{inds} \text{ do} \\
\text{puthap(newpop, allOne, } d, i); \text{ [all genes set to one]} \\
\text{end; } \\
\text{end; }
en d  
d e ls e  i f initfrequencies = 2 th e n  
be gin  
for d := 0 to ndemes + 1 do  
be gin  
for i := 1 to (ninds div 2) do  
puthap(newpop, allZero, d, i); {all genes set to zero}  
for i := (ninds div 2) + 1 to ninds do  
puthap(newpop, allOne, d, i) {all genes set to one}  
end  
end  
d e ls e  i f initfrequencies = 3 th e n  {set all loci to exp}  
be gin  
exp := 0.5;  
{mutation_mask returns a mask with 1 at probability exp}  
for d := 0 to ndemes + 1 do  
for i := 1 to ninds do  
puthap(newpop, mutation_mask(exp), d, i); {genes set to 1 or 0 with probability exp}  
end  
d e ls e  i f initfrequencies = 5 th e n  
be gin  
for d := 0 to ndemes + 1 do  
be gin  
for i := 2 to ninds do  
puthap(newpop, allZero, d, i); {all genes set to zero}  
puthap(newpop, allOne, d, 1) {when i = 1, all genes set to one}  
end;  
end;  
end;  

p ro c e d u r e  draw_cline;  
va r  
cx, cy: real;  
gg: genetype;  
dd: demepltype;  

p ro c e d u r e  draw_axes (xO, yO, xmin, xmax, ymin, ymax, dx, dy, cx, cy: real; xlab, ylab, plotlab: string);  
{xO,yO: origin;}  
{xmin,xmax,ymin,ymax:range;}  
{dx,dy:interval for axis labels;}  
{cx,cy:character size;}  
{xlab,ylab,plotlab:labels}  
va r  
i: integer;  
x, y: real;  
be gin  
set_window(xmin - (xmax - xmin) / 4, xmax - (xmax - xmin) / 8, ymin - (ymax - ymin) / 4, ymax + (ymax - 
ymin) / 8);  
set_char_size(cx, cy);  
mov e(x0, ymin); {draw axes}  
l ine(x0, ymax);  
mov e(xmin, y0); {draw axes}  
l ine(xmax, y0);  
fo r i := 0 to round((xmax - xmin) / dx) do{draw axis labels}  
be gin  
x := xmin + i * dx;  
if x <> x0 then  
be gin  
mov e(x, y0);  
l ine(x, y0 - cy / 2);  
mov e(x - cx, y0 - 2.5 * cy);  
WriteDraw(x : 3 : 1)  
end;  
end;
end;
for i := 0 to round((ymax - ymin) / dy) do
begin
  y := ymin + i * dy;
  if y <> y0 then
  begin
    move(x0, y);
    line(x0 - cx / 2, y);
    move(x0 - 4.5 * cx, y - cy / 2);
    WriteDraw(y : 3 : 1)
  end;
end;
move((xmax - x0 - cx * length(xlab)) / 2, y0 + 4 * cy);
WriteDraw(xlab);
move(xmin - (xmax - xmin) / 4, (ymin + ymax) / 2);
WriteDraw(ylab);
move((xmin + 3 * xmax) / 4 - cx * length(plotlab) / 2, ymax + 3 * cy);
WriteDraw(plotlab);
end;

procedure update_stats;
{updates the list of statistics: zbar,Vgenic,Vdiseq,wbar for neutral and selected loci,}
{collected every dt generations}
var
d: demepltype;
g: genetype;
wbar: real;
begin
  sample := sample + 1;
  if nneut > 0 then
  begin
    for d := 0 to ndemes + 1 do
    begin
      zbar_neut[sample, d] := zbar(pop, d, true);
      Vgenic_neut[sample, d] := genic_variance(pop, d, true);
      Vdiseq_neut[sample, d] := total_variance(pop, d, true) - Vgenic_neut[sample, d];
      Vtotal_neut[sample, d] := total_variance(pop, d, true);
    end;
    Dmax_neut[sample] := Dmax(Vgenic_neut, Vdiseq_neut, sample, nneut);
    width_neut[sample] := width(zbar_neut, sample, nneut);
  end;
  if nsel > 0 then
  begin
    for d := 0 to ndemes + 1 do
    begin
      find_wbar(pop, d, wbar, varw);
      zbar_sel[sample, d] := zbar(pop, d, false);
      Vgenic_sel[sample, d] := genic_variance(pop, d, false);
      Vdiseq_sel[sample, d] := total_variance(pop, d, false) - Vgenic_sel[sample, d];
      Vtotal_sel[sample, d] := total_variance(pop, d, false);
      wbar[sample, d] := wbar;
      varw[sample, d] := varw;
    end;
  end;
end;
Dmax_sel(sample) := Dmax(Vgenic_sel, Vdiseq_sel, sample, nsel);
width_sel(sample) := width(zbar_sel, sample, nsel);

if (nsel > 0) and (nneut > 0) then (only makes sense to calculate a barrier with neutral and selected loci)
begin
calculate_barrier(step_neut, slope_left_neut, slope_mid_neut, slope_right_neut, wbarmin, wbarreg,
zbar_neut, wbar, sample, nneut);
step_neut(sample) := step_neut;
slope_left_neut(sample) := slope_left_neut;
slope_right_neut(sample) := slope_right_neut;
slope_ratio_left(sample) := slope_left_neut / slope_mid_neut;
slope_ratio_right(sample) := slope_right_neut / slope_mid_neut;
width_barr_neut(sample) := 1 / slope_mid_neut;
wbarmin(sample) := wbarmin;
wbarreg(sample) := wbarreg;
end;
end;

{..................................................}
procedure write_cricket (full: boolean);
{Writes out a list of data in cricket graph format;
{  if full is set, lists all the individual logit transformed allele frequencies.}
{};
{Note: update-stats must already have been run, to set up the data}
var
d: demotype;
g: genetype;
wbar, vw, sdw, sdd, sdstep, sdslrr, sdslrl, rb: real;
begin
if sample = 0 then
begin
writeln(cricket_file, 'Overall means after ', dt : 8, ' generations: samples every ', dt : 4, ' gens, starting at ',
' max = ', tmax : 6);
if nneut > 0 then
begin
    sdd := sqrt(variance1(Dmax_neut, nsamples));
    sdw := sqrt(variance1(width_neut, nsamples));
    write(cricket_file, ' Neutral loci:');
    writeln(cricket_file, ' Dmax ', Dmax_neutA[0] : 10 : 6, ' sd ', sdd : 10 : 6);
    writeln(cricket_file, ' 95% limits +/- ', 2 * sdd / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
    writeln(cricket_file, ' width ', width_neutA[0] : 10 : 6, ' sd ', sdw : 10 : 6);
    writeln(cricket_file, ' 95% limits +/- ', 2 * sdw / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
end;
if nsel > 0 then
begin
    sdd := sqrt(variance1(Dmax_sel, nsamples));
    sdw := sqrt(variance1(width_sel, nsamples));
    write(cricket_file, ' Selected loci:');
    writeln(cricket_file, ' Dmax ', Dmax_selA[0] : 10 : 6, ' sd ', sdd : 10 : 6);
    writeln(cricket_file, ' 95% limits +/- ', 2 * sdd / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
    writeln(cricket_file, ' width ', width_selA[0] : 10 : 6, ' sd ', sdw : 10 : 6);
    writeln(cricket_file, ' 95% limits +/- ', 2 * sdw / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
end;
end;
if (nsel > 0) and (nneut > 0) then
begin
write(cricket_file, ' Barrier:');
calculate_barrier(step_neut, slope_left_neut, slope_mid_neut, slope_right_neut, wbarmin, wbarreg,
zbar_neut, wbar, sample, nneut);
end;
end;

writeln(cricket_file, '95% limits +/- 2 * sdslrl / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
write(cricket_file, 'slope ratio right', slope_ratio_right[0] : 10 : 6, ' sd ', sdslrl : 10 : 6);
writeln(cricket_file, '95% limits +/- 2 * sdslrl / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
write(cricket_file, 'net slope ratio ', (slope_ratio_right[0] + slope_ratio_left[0]) / 2 : 10 : 6, ' sd ',
sqrt((sqr(sdslrl) + sqr(sdslrl)) / 2) : 10 : 6);
writeln(cricket_file, '95% limits +/- 2 * sqrt((sqr(sdslrl) + sqr(sdslrl)) / 2) / sqrt(2 * nsamples) : 10 : 6, 2 * nsamples : 6, ' samples');
write(cricket_file, 'slope ratio right slope_ratio_rightA [0] ; 10 : 6, ' sd ', sdslrl : 10 : 6);
writeln(cricket_file, '95% limits +/- 2 * sdslrl / sqrt(nsamples) : 10 : 6, nsamples : 6, ' samples');
write(cricket_file, 'net slope ratio ', (slope_ratio_right[0] + slope_ratio_left[0]) / 2 : 10 : 6, ' sd ',
sqrt((sqr(sdslrl) + sqr(sdslrl)) / 2) : 10 : 6);
writeln(cricket_file, '95% limits +/- 2 * sqrt((sqr(sdslrl) + sqr(sdslrl)) / 2) / sqrt(2 * nsamples) : 10 : 6, 2 * nsamples : 6, ' samples');
write(cricket_file, 'Check that there are no zero entries above');
write(cricket_file, 'Harmonic mean r is ', rb : 8 : 4, ' mean minimum fitness is', wbarmin*[0] : 8 : 4, ' expected slope ratio is ', power(wbarmin*[0], 1 / rb) : 10 : 6);
end
end else
writeln(cricket_file, 'Generation ', t : 8);
if nneut > 0 then begin
writeln(cricket_file);
writeln(cricket_file, 'Data on ', nneut : 4, ' neutral loci');
write(cricket_file, 'Deme', chr(9));
if full then for g := 0 to ngenes - 1 do if (getgene(g, sein_mask) = 0) then
write(cricket_file, ' gene', g : 3, chr(9));
 writeln(cricket_file, ' mean z', chr(9), ' logit z', chr(9), ' Vgenic ', chr(9), ' Vdiseq ');
for d := 0 to ndemes + 1 do begin
write(cricket_file, d : 4, chr(9));
if full then for g := 0 to ngenes - 1 do if (getgene(g, sein_mask) = 0) then
write(cricket_file, getgenefreq(pop, d, g) : 8 : 4, chr(9));
write(cricket_file, zbar_neutA [sample, d] : 8 : 3, chr(9));
write(cricket_file, logit(zbar_neutA [sample, d] / (2 * nneut), 5) ; 8 : 4, chr(9));
write(cricket_file, Vgenic_neutA [sample, d] : 8 : 3, chr(9));
write(cricket_file, Vdiseq_neutA [sample, d] : 8 : 3);
writeln(cricket_file);
end;
writeln(cricket_file, 'Dmax, width:');
write(cricket_file, Dmax_neutA [sample] : 10 : 6, chr(9), width_neutA [sample] : 10 : 2);
if nsel > 0 then begin
writeln(cricket_file, 'Step size, left slope, right slope, central width, slope ratio (left), slope ratio (right), wbar (min), wbar (by regression)');
end;
writeln(cricket_file);
end;
if nsel > 0 then begin
writeln(cricket_file);
writeln(cricket_file, 'Data on ', nsel : 4, ' selected loci');
write(cricket_file, 'Deme', chr(9));
if full then for g := 0 to ngenes - 1 do if (getgene(g, sein_mask) = 1) then
write(cricket_file, ' gene', g : 3, chr(9));
 writeln(cricket_file, ' mean z', chr(9), ' logit z', chr(9), ' Vgenic ', chr(9), ' Vdiseq ', chr(9), ' Wbar ', chr(9), ' varW ');
for d := 0 to ndemes + 1 do begin
write(cricket_file, d : 4, chr(9));
if full then
procedure set_sample_zero;
{Sets data for the fictional sample zero to the overall mean}
var
d: demotype;
i: indtype;
g: genetype;
begin
if nneut > 0 then
begin
Dmax_neut[0] := mean(Dmax_neut, nsamples);
width_neut[0] := mean(width_neut, nsamples);
for d := 0 to ndemes + 1 do
begin
zbar_neut[0, d] := mean(zbar_neut, d, nsamples);
Vgenic_neut[0, d] := mean(Vgenic_neut, d, nsamples);
Vdiseq_neut[0, d] := mean(Vdiseq_neut, d, nsamples);
Vtotal_neut[0, d] := mean(Vtotal_neut, d, nsamples);
end;
end;
if nsel > 0 then
begin
Dmax_sel[0] := mean(Dmax_sel, nsamples);
width_sel[0] := mean(width_sel, nsamples);
for d := 0 to ndemes + 1 do
begin
zbar_sel[0, d] := mean(zbar_sel, d, nsamples);
Vgenic_sel[0, d] := mean(Vgenic_sel, d, nsamples);
Vdiseq_sel[0, d] := mean(Vdiseq_sel, d, nsamples);
Vtotal_sel[0, d] := mean(Vtotal_sel, d, nsamples);
wbar[0, d] := mean(wbar, d, nsamples);
end;
end;
if (nsel > 0) and (nneut > 0) then
begin
step_neut[0] := mean(step_neut, nsamples);
slope_left_neut[0] := mean(slope_left_neut, nsamples);
slope_right_neut[0] := mean(slope_right_neut, nsamples);
width_barr_neut[0] := mean(width_barr_neut, nsamples);
wbarwin[0] := mean(wbarmin, nsamples);
wbarreg[0] := mean(wbarreg, nsamples);
slope_ratio_left[0] := mean(slope_ratio_left, nsamples);
slope_ratio_right[0] := mean(slope_ratio_right, nsamples);
end;
end:
{ ******************************************* }

procedure draw_zbar;
var
i: integer;
begin
  pane(0, ndemes + 1, -logitmax, logitmax, 0.525, 0.95, 0.55, 0.75, true, true, false, ' Mean Z (logit scale)');
  if nneut > 0 then
    begin
      move(0, logit(zbar_neut*[sample, 0] / (2 * nneut), logitmax));
      for i := 1 to ndemes + 1 do
        line(i, logit(zbar_neut*[sample, i] / (2 * nneut), logitmax))
    end;
  if nsel > 0 then
    begin
      move(0, logit(zbar_sel*[sample, 0] / (2 * nsel), logitmax));
      for i := 1 to ndemes + 1 do
        line(i, logit(zbar_sel*[sample, i] / (2 * nsel), logitmax))
    end;
end;

procedure draw_wbar;
  var
    i: integer;
  begin
    pane(0, ndemes + 1, 0, 1, 0.525, 0.95, 0.3, 0.5, true, true, false, ' Mean fitness');
    move(0, wbar*[sample, 0]);
    for i := 1 to ndemes + 1 do
      line(i, wbar*[sample, i])
  end;

procedure draw_pind;
  var
    i, g: integer;
  begin
    pane(0, ndemes + 1, -logitmax, logitmax, 0.525, 0.95, 0.05, 0.25, true, true, false, ' Allele frequencies (logit scale)');
    for g := 0 to ngenes - 1 do
      begin
        move(0, logit(getgenefreq(pop, 0, g), logitmax));
        for i := 1 to ndemes + 1 do
          line(i, logit(getgenefreq(pop, i, g), logitmax))
      end;
  end;

procedure draw_Vd;
  var
    i: integer;
    vexp: real;
  begin
    if stabilising and (mutn > 0) then
      if s > 0 then
        vexp := 4 * ngenes * mutn / s
      else
        vexp := 0
    else
      vexp := ngenes / 2;
    pane(0, ndemes + 1, -vexp / 2, 2 * vexp, 0.05, 0.475, 0.34, 0.54, true, true, false, ' Disequilibrium variance');
    if stabilising and (mutn > 0) then
      begin
        move(0, vexp);
        line(ndemes + 1, vexp)
      end;
    move(0, 0);
    line(ndemes + 1, 0);
  if nneut > 0 then
    begin
      // code continues...
    end;
move(0, Vdiseq_neut*[sample, 0]);
for i := 1 to ndemes + 1 do
  line(i, Vdiseq_neut*[sample, i])
end;
if nsel > 0 then
  begin
    move(0, Vdiseq_selA*[sample, 0]);
    for i := 1 to ndemes + 1 do
      line(i, Vdiseq_selA*[sample, i])
  end;
end;

procedure draw_Vg;
var
  v: integer;
  vexp: real;
begin
  if stabilising and (mutn > 0) then
    vexp := 4 * ngenes * mutn / s
  else
    vexp := ngenes / 2;
  pane(0, ndemes + 1, 0, (5 / 2) * vexp, 0.05, 0.475, 0.07, 0.27, true, true, false, 'Genic variance');
  if stabilising and (mutn > 0) then
    begin
      move(0, vexp);
      line(ndemes + 1, vexp)
    end;
  if nneut > 0 then
    begin
      move(0, Vgenic_neutA*[sample, 0]);
      for i := 1 to ndemes + 1 do
        line(i, Vgenic_neutA*[sample, i])
    end;
  if nsel > 0 then
    begin
      move(0, Vgenic_selA*[sample, 0]);
      for i := 1 to ndemes + 1 do
        line(i, Vgenic_selA*[sample, i])
    end;
end;

procedure draw_parameters;{onto screen}
const
  vex = 0.018;
  vcy = 0.032;
  var
    cx, cy: real;
    err: integer;
    line: string;
begin
  set_user_window(0, 1 / vex, 0, 0.2 / vcy, 0.02, 1, 0.55, 0.75);
  cx := 1;
  cy := 1;
  set_char_size(cx, cy);
  move(0, 4);
  WriteDraw('Multilocus program: t=', t : 5, ' date, time);
  move(0, 3);
  if stabilising then
    WriteDraw('Stabilising selection: change in optimum=', dopt : 8 : 4);
  if epistasis then
    WriteDraw('Epistasis: width=', hwidth : 8 : 4);
  if epistasisp then
    WriteDraw('Epistasis: beta=', beta : 8 : 4);
if hetdis then
  WriteDraw('Heterozygote disadvantage');
  move(0, 2);
  WriteDraw('s=', s : 7 : 4, ' r=', rec : 7 : 4, ' mu=', mutn : 8 : 6, ' m=', nmig / ninds : 6 : 3);
  move(0, 1);
  WriteDraw('ninds-
   ninds : 6, ' ngenes*', ngenes : 4, ' ndemes*', ndemes : 4);
end;

@ @ }
procedure update_drawing;
begin
  set_viewport(0, 1.05, -0.05, 0.74);
  draw_parameters;
  draw_zbar;
  draw_wbar;
  draw_pind;
  draw_Vd;
  draw_Vg;
end;

begin
  writeln('Heap is ', Memavail : 10);
  new(pop);
  new(newpop);
  new(cumwptr);
  new(nptr);
  new(pbar);
  new(pvar);
  new(dbar);
  new(zbar_neut);
  new(Vgenic_neut);
  new(Vdiseq_neut);
  new(Vtotal_neut);
  new(zbar_sel);
  new(Vgenic_sel);
  new(Vdiseq_sel);
  new(Vtotal_sel);
  new(wbar);
  new(varw);
  new(countbitstable);
  new(Dmax_neut);
  new(Dmax_sel);
  new(width_neut);
  new(width_sel);
  new(step_neut);
  new(slope_left_neut);
  new(slope_right_neut);
  new(width_barr_neut);
  new(slope_ratio_left);
  new(slope_ratio_right);
  new(wbarmin);
  new(wbarreg);

  writeln('Heap is ', Memavail : 10);

  write('Do you want to read parameters from a file ? '); readin(ans);
  batch := (ans = 'y');
  if batch then
    begin
      batch_fname := OldFileName('Parameter file ? ');
      reset(bf, batch_fname);
readln(bf, fname_root);
end
else
fname_root := NewFileName('File for output and pictures ?');
while not (copy(fname_root, 1, 3) = 'END') do
begin
if batch then
  askstuff_batch
else
  askstuff;
  initialise;
  nfix := 0;
end;

[i use one root for all the file names]
pict_fname := concat(fname_root, '.pic');
[out_fname := concat(fname_root, '.list');]
[rewrite(out_file, out_fname);]
[set_listfile(out_file);]
[cricket_fname := concat(fname_root, '.cricket');
 rewrite(cricket_file, cricket_fname);
 set_listfile(cricket_file);
 set_lastmask;
 set_countbitstable;
 set_fitness_table(s, hwidth, beta, dopt);
 set_ntable;
 initialrep;
 swappop(pop, newpop);
 repeat
  t := t + 1;
  [ShowText;write('Generation ', t : 6, ', ');writetime;]
  [i haven't bothered creating "dummies": i just have no migration beyond the ends]
  migrate(pop, newpop);
  swappop(pop, newpop);
  reproduce(pop, newpop, rec, mutn);
  swappop(pop, newpop);
  if (t >= twarm) and (t mod dt = 0) then
  begin
    [write('Updating stats for generation = ', t : 2);writetime;]
    update_stats;
    update_drawing;
    write_cricket(true) [write data in cricket graph format]
    [beware: writing out individual freqs gives too much output with many genes]
  end;
  until t = tmax;
  nsamples := sample;
  [dump_allele_frequencies(false);]
  [don't bother with logit transformed output]
  set_sample_zero;
  [write_stats;]
  sample := 0;[this is a trick to draw the overall means, which are stored in element 0]
  update_drawing;
  SaveDrawing(pict_fname);
  write_cricket(true); [write data in cricket graph format]
  [close(out_file);]
  close(cricket_file);
  if batch then
    readln(bf, fname_root)
  else
    fname_root := 'END';
end;
dispose(pop);
dispose(newpop);
dispose(cumwptr);
dispose(nptr);
dispose(pbar);
dispose(pvar);
dispose(dbar);
dispose(zbar_neut);
dispose(Vgenic_neut);
dispose(Vdiseq_neut);
dispose(Vtotal_neut);
dispose(zbar_sel);
dispose(Vgenic_sel);
dispose(Vdiseq_sel);
dispose(Vtotal_sel);
dispose(wbar);
dispose(varw);
dispose(countbitstable);
dispose(Dmax_neut);
dispose(Dmax_sel);
dispose(width_neut);
dispose(width_sel);
dispose(step_neut);
dispose(slope_left_neut);
dispose(slope_right_neut);
dispose(width_barr_neut);
dispose(slope_ratio_left);
dispose(slope_ratio_right);
dispose(wbarmin);
dispose(wbarreg);

dispose(dmbar);
end.
unit multilocus_procs;

interface

uses
  functions;

const
  checksein = false;
  checkmigr = false;
  maxinds = 100;
  maxp = 5050;  { (maxinds+1) * maxinds/2  .. remember to change}
  maxdemes = 100;
  maxdemespl = 101;  {maxdemes plus 1  ..remember to change}
  maxmig = 50;
  maxsamples = 120;
  maxt = 1000;  {max generations: only used for testing sel & rec on 2 loci}
  maxblocks = 6;
  maxgens = 96;  {maxblocks*16}
  maxgens2 = 192;  {2*maxgens}
  logitmax = 5;  {bounds on the logit function}

type
  bittype = 0..1;
  haplotype = array[1..maxblocks] of integer;
  genetype = 0..maxgenes;
  demetype = 1..maxdemes;
  demetypep = 0..maxdemespl;
  indtype = 0..maxinds;
  poptype = array[0..maxdemespl, 1..maxinds] of haplotype;
  popptrtype = *poptype;
  choosetype = array[0..maxdemespl, 1..maxmig] of indtype;
  dtdemetype = array[0..maxsamples, 0..maxdemespl] of real;
  dtdemeptrtype = *dtdemetype;
  dtttype = array[0..maxsamples] of real;
  dtttritype = *dtttype;
  realpvector = array[0..maxp] of real;
  realpvectortritype = *realpvector;
  intpvector = array[0..maxp] of integer;
  intpvectortritype = *intpvector;
  realtypetype = array[1..maxt] of real;
  realindvectortype = array[0..maxgens] of real;
  blocktype = 1..maxblocks;
  countbitstabletype = array[0..32767] of 0..15;
  countbitstableptrtype = *countbitstabletype;
  rec_masktabletype = array[1..15] of integer;

var
  t, dt, sample, nsamples, seed: integer;
  ninds, nming, nmingbar: indtype;
  ndemes, nmingle, nmingright: demetype;
  ngenes, nsel, nneut: genetype;
  ndip: integer][(n+1)/2  - the number of distinct diploid genotypes]
  fitness_table: array[0..maxgens2, 0..1] of real;
  {array holding the fitnesses of individuals with i hets. or i ones}
  countbitstable: countbitstableptrtype;
  {array holding the number of bits in an integer from 0 to 32767}
  lastmask: integer;
  cumwptr: realpvectortritype;
  zbar_neut, zbar_sel, Vdiseq_neut, Vdiseq Sel, Vgenic_neut, Vgenic sel, Vtotal_neut, Vtotal Sel, wbar, varw:
    dtdemeptrtype;
  Dmax_neut, Dmax sel, width_neut, width sel: dttritype;
  step_neut, slope_left_neut, slope_right_neut, slope_mid_neut, wbarmin, wbarreg: real;
  step_neut, slope_left_neut, slope_right_neut, width_barr_neut, wbarreg, wbarmin, slope_ratio_left.
slope_ratio_right: dtptrtype;
nptr: intvecptrtype;
step, hetdis, epistasis, stabilising, epistasisp: boolean;
ininitmethod, initfrequencies, twarm, tmax: integer;
sein_mask: haplotype; {determines whether or not an allele is selected}

function rand (range: real): real;
function rand_highres (range: real): real;
function gethap (var p: popptrtype; d: demeptype; i: indtype): haplotype;
function getgene (g: genotype; x: bittype; var h: haplotype);
function set_countbitstable;
procedure swappop (var p, np: popptrtype);
procedure migrate (var pop, newpop: popptrtype);
procedure reproduce (var pop, newpop: popptrtype; rec, mu: real);
procedure set_countbitstable;
procedure set_lastmask;
function mutation_mask (mm: real): haplotype;
procedure set_ntable;
procedure set_fitness_table (sel, hwidth, beta, dopt: real);
procedure find_wbar (var pp: popptrtype; d: demeptype; var wb, varw: real);
procedure writetime;
function zbar (var pp: popptrtype; d: demeptype; neutflag: boolean): real;
function genic_variance (var pp: popptrtype; d: demeptype; neutflag: boolean): real;
function meanx1 (var x: dtptrtype; nn: integer): real;
function variance1 (var x: dtptrtype; nn: integer): real;
function mean (var x: dtptrtype; d: demeptype; nn: integer): real;
function variance (var x: dtptrtype; d: demeptype; nn: integer): real;
procedure write_array (var out_file: text; x: dtptrtype; tt, nf, nd: integer);
procedure initialise;
function bar (sm: haplotype; rec: real): real; {harmonic mean recombination between }
{neutral and selected loci}
procedure regress (var xb, yb, b: real; x, y: dtptrtype; nn: integer; originflag: boolean);
function Dmax (var Vg, Vd: dtptrtype; ns, ng: integer): real;
function width (var zb: dtptrtype; ns, ng: integer): real;
procedure calculate_barrier (var step, slope_left, slope_mid, slope_right, wbmin, wbreg: real; var zb, wb: dtptrtype; ns, ng: integer);

implementation

function rand (range: real): real;
{random number from 0 to range}
begin
  rand := (0.50000762939 + random / 65536) * range; {works on the Mac}
end;

function rand_highres (range: real): real;
{random number from 0 to range; higher resolution}
var
  tr: longint;
begin
  tr := random + bsl(random, 16); 
  rand_highres := (0.5 + tr / 4294967296) * range; {works on the Mac}
end;

function gethap (var p: popptrtype; d: demeptype; i: indtype): haplotype;
{gets haplotype for deme d, individual i from the array p}
begin
  gethap := p^[d, i]
end;

function getgene (g: genotype; h: haplotype): bittype;
var
k: integer;
begin
  k := g \div 16;
  if odd(bar(h[k + 1], (g - k \cdot 16))) then
    getgene := 1
  else
    getgene := 0;
end;

function getgenefreq (var pp: popptrtype; d: demeptype; g: genetype): real; {for a deme}
  var
    sum: real;
    i: indtype;
  begin
    sum := 0;
    for i := 1 to ninds do
      sum := sum + getgene(g, gethap(pp, d, i));
    getgenefreq := sum / ninds
  end;

procedure puthap (var p: popptrtype; h: haplotype; d: demeptype; i: indtype);
begin
  p[d, i] := h
end;

procedure putgene (var g: genetype; x: bittype; var h: haplotype);
{(This works. At first sight, one should be able to use bset and bclr. However, these require }
{var variables, and so don't work. )
  var
    k: integer;
  begin
    {need care here, because bsl returns a longint}
    k := 1 + g \div 16;
    if x = 1 then
      h[k] := BitOr(h[k], LoWrd(bsl(1, (g \mod 16))))
    else
      h[k] := BitAnd(h[k], BitNot(LoWrd(bsl(1, (g \mod 16)))));
  \} h is an array of integers, where each integer represents 0 to 15 genes
end;

procedure swappop (var p, np: popptrtype);
  var
    tptr: popptrtype;
  begin
    tptr := p;
    p := np;
    np := tptr;
  end;

procedure transfer (var pop, newpop: popptrtype; mfromleft, mfromright, mtoleft, mtoright: indtype; demeno: demeptype; ninds: integer);
  var
    offset: integer;
    i: indtype;
    h: haplotype;
  begin
    if mfromleft + mfromright <> mtoleft + mtoright then
      writeln('ERROR in transfer: asymmetric migration');
    if mfromleft > 0 then
      for i := 1 to mfromleft do
        puthap(newpop, gethap(pop, demeno - 1, i), demeno, i);
    if mfromright > 0 then
      for i := ninds downto ninds - mfromright + 1 do
        puthap(newpop, gethap(pop, demeno + 1, i), demeno, i);
    offset := mtoright - mfromleft;
for i := mfromleft + 1 to ninds - mfromright do
  puthap(newpop, gethap(pop, demeno, i + offset), demeno, i);
end;

procedure migrate (var pop, newpop: popptrtype);
var
d: demetype;
i, k, kb: indtype;
begin
  k := round(nmig / 2);
  kb := round(nmigbarr / 2);
  if ndemes = 2 then
    begin
      transfer(pop, newpop, 0, k, 0, k, 0, ninds);
      transfer(pop, newpop, k, kb, k, kb, 0, ninds);
      transfer(pop, newpop, kb, k, kb, k, 0, ninds);
      transfer(pop, newpop, k, 0, k, 0, 3, ninds);
    end;
  if ndemes = 3 then
    begin
      transfer(pop, newpop, 0, k, 0, k, 0, ninds);
      transfer(pop, newpop, k, kb, k, kb, 0, ninds);
      transfer(pop, newpop, kb, k, kb, k, 0, ninds);
      transfer(pop, newpop, k, k, k, k, 3, ninds);
    end;
  if ndemes > 3 then
    begin
      transfer(pop, newpop, 0, k, 0, k, 0, ninds);
      transfer(pop, newpop, k, k, k, k, 1, ninds);
      if nmidleft - 1 > 1 then
        for d := 2 to nmidleft - 1 do
          transfer(pop, newpop, k, k, k, k, d, ninds);
      if nmidright + 1 < ndemes then
        for d := nmidright + 1 to ndemes - 1 do
          transfer(pop, newpop, k, k, k, k, d, ninds);
      transfer(pop, newpop, k, k, k, ndemes, ninds);
      transfer(pop, newpop, k, 0, k, 0, ndemes + 1, ninds);
      transfer(pop, newpop, kb, k, kb, nmidright, ninds);
    end;
  end;
end;

function countupbits (i: integer): integer;{important not to have var here}
var
sum, g: integer;
begin
  sum := 0;
  for g := 0 to 15 do
    begin
      if odd(i) then
        sum := sum + 1;
      i := bsr(i, 1);
    end;
  countupbits := sum
end;

procedure set_lastmask;
var
k, kmax: integer;
begin
  lastmask := 1;
  kmax := ((ngenes - 1) - ((nblocks - 1) * 16));
  if kmax = 15 then [this avoids an obscure overflow problem]
lastmask := -1

else
    for k := 0 to kmax - 1 do

{the maximum number in the last block}
    lastmask := 1 + bsl(lastmask, 1);
{lastmask is 00001111..., designed to mask superfluous bits in the last block}
end;

procedure set_countbitstable;
var
    k: integer;
begin
    writeln('Setting up countbitstable..');
    for k := 0 to 32767 do
        countbitstable[k] := countupbits(k)
end;

function countbits (h: haplotype): integer;
var
    sum1, ic, imax: integer;
begin
    sum1 := 0;
    for ic := 1 to nblocks do
        begin
{slightly simpler to do this, rather than use two loops}
        if ic = nblocks then
            begin
            h[ic] := BitAnd(h[ic], lastmask) ;{lastmask (set by set_lastmask)}
                {ensures that the extra parts of h are zero}
            h[ic] := BitAnd(h[ic], seln_mask[ic]);
                if h[ic] < 0 then
                    sum1 := sum1 + countbitstable[*32768 + h[ic]] + 1
                else
                    sum1 := sum1 + countbitstable[*h[ic]]
            end;
        countbits := sum1
        end;

function counthets (h1, h2: haplotype): integer;
var
    ic: integer;
    temp: haplotype;
begin
    for ic := 1 to nblocks do
        temp[ic] := BitXor(h1[ic], h2[ic]);
    counthets := countbits(temp)
end;

function countones (h1, h2: haplotype): integer:
begin
    countones := countbits(h1) + countbits(h2)
end;

function search (var t: realvecptrtype; x: real; nn: integer): integer;
{Looks for the position of x in a table of reals:t*[0]=0,t*[j]=t*[j-1]+w[j].}
{0<x<t*[max]; returns j if t*[j-1]<x<=t*[j]}
var
    i, imax, imin: integer;
begin
    if nn <= 1 then
        search := 1
    else
        begin
            imax := nn;
            imin := 0 ;{set the initial interval; x must lie between t*[imax] and t*[imin]}
            repeat
                i := search (t, x, nn)
            until (i = 0) or (i = nn)
        end;
function search_integer (var t: intvecptrtype; x; integer; nn: integer): integer;
{Looks for the position of x in a table of integers: t[0]=0, t[i]-t[i-1]=w[i].}
{0<x<t[imax]; returns j if t[j-1]<x<=t[j].}
var
  i, imax, imin: integer;
begin
  if nn <= 1 then
    search_integer := 1
  else
  begin
    imax := nn;
    imin := 0; {set the initial interval; x must lie between t[imax] and t[imin].}
    repeat
      i := (imax + imin) div 2;
      if x > t[i] then
        imin := i
      else
        imax := i
      until imax = imin + 1;
      if x = t[imin] then
        search_integer := imin
      else
        search_integer := imax
    end;
  end;
end;

procedure select (var pop: popptrtype; d: demeptype);
var
  i1, i2: indtype;
  w: real;
  p, p2, l, count1, count2: integer;
  ftjndex : bittype;
begin
  p := 0;
  if d < nmidright then
    ftjndex := 0
  else
    ftjndex := 1;
  cumwp^p[p] := 0; {initialising cumulative fitness probability table}
  for l := 1 to ninds do {allow selfing}
    for i2 := l to ninds do
      begin
        if hetdis then
          w := fitness_table[countheats(gethap(pop, d, i1), gethap(pop, d, i2)), ft_index];
        if epistasis then
          w := fitness_table[countones(gethap(pop, d, i1), gethap(pop, d, i2)), ft_index];
        if epistasisp then
          w := fitness_table[countones(gethap(pop, d, i1), gethap(pop, d, i2)), ft_index];
        if stabilising then
          w := fitness_table[countones(gethap(pop, d, i1), gethap(pop, d, i2)), ft_index];
        if i1 = i2 then
w := w / 2;
(allow for the fact that only the lower diagonal is searched)
p := p + 1;
cumwptr[p] := cumwptr[p - 1] + w
end;
end;

procedure find_wbar(var pp: popptype; d: deme1type; var wb, varw: real);
var
i1, i2: indtype;
ft_index: bittype;
w, smw, ssqw: real;
begin
if d < midsright then
  ft_index := 0
else
  ft_index := 1;
  smw := 0;
  ssqw := 0;
for i1 := 1 to ninds do
  for i2 := 1 to ninds do
    begin
      if heteris then
        w := fitness_table[countheats(gethap(pp, d, i1), gethap(pp, d, i2)), ft_index];
      if epistasis then
        w := fitness_table[countones(gethap(pp, d, i1), gethap(pp, d, i2)), ft_index];
      if epistasis then
        w := fitness_table[countones(gethap(pp, d, i1), gethap(pp, d, i2)), ft_index];
      if stabilising then
        w := fitness_table[countones(gethap(pp, d, i1), gethap(pp, d, i2)), ft_index];
        smw := smw + w;
        ssqw := ssqw + sqr(w)
    end;
    wb := smw / sqr(ninds);
    varw := (ssqw / sqr(ninds)) - sqr(wb);
end;

procedure swapind(var a, b: indtype);
var
z: indtype;
begin
z := a;
a := b;
b := z
end;

procedure set_ntable;[this table returns the position in the table of diploids of individual i]
var
i: integer;
begin
nptr[0] := 0;
for i := 1 to ninds do
  nptr[i] := ninds * i - ((i * (i - 1)) div 2);
end;

procedure parents(var i1, i2: indtype);
var
i, j: integer;
r: real;
begin
i := search(cumwptr, rand_highres(cumwptr*[ndip]), ndip);
[nptr*[i-1]<rand<=cumwptr*[i]]
i1 := search_integer(nptr, i, ninds);
i2 := ninds - nptr*[i] + i;
[decoding i1,i2 from i]
if \( \text{rand}(1) > 0.5 \) then
\[
\text{swapind}(i_1, i_2);
\]
end;

**function** mutation\_mask (mm: real): haplotype;
\{returns an integer which has 1's with probability \( \mu \)\}
\[\text{var} \]
ib: blocktype;
h: haplotype;

**function** one\_mutation\_mask (mu: real; ng: integer): integer;
\{returns an integer which has 1's with probability \( \mu \)\}
\[\text{var} \]
temp: longint;
g: integer;

begin
if \( \text{rand\_highres}(1) < \mu \) then
\[
\text{temp} := 1
\]
else
\[
\text{temp} := 0;
\]
if \( \text{ng} > 1 \) then
\[\text{for } g := 2 \text{ to } \text{ng} \text{ do} \]
begin
\[
\text{temp} := \text{bsl}(\text{temp}, 1);
\]
if \( \text{rand\_highres}(1) < \mu \) then
\[
\text{temp} := \text{temp} + 1
\]
end;

one\_mutation\_mask := \text{LoWrd}(\text{temp})
end;

\[\begin{align*}
\text{begin} \\
h[nblocks] := \text{one\_mutation\_mask}(\text{mm}, (\text{ngenes} - 1) \mod 16 + 1); \\
\text{if } nblocks > 1 \text{ then} \\
\text{for } \text{ib} := 1 \text{ to } nblocks - 1 \text{ do} \\
\hspace{1em} h[\text{ib}] := \text{one\_mutation\_mask}(\text{mm}, 16); \\
\text{mutation\_mask} := h;
\end{align*}\]

**procedure** reproduce (var pop, newpop: popptrtype; rec, mu: real);
\[\text{var} \]
d, q1, blk: integer;
jim, il, i2: indtype;
r\_mask, sonnyjim, m\_mask: haplotype;
rec\_masktable: rec\_masktabletype;

**procedure** set\_recmasktable (var rm: rec\_masktabletype);
\[\text{var} \]
k: integer;

**function** rec\_mask (j: integer): integer;
\{rec\_mask(j) returns a mask 0001111... with j 1s, j between 1 and 15\}
\[\text{var} \]
i2, nn: integer;

begin
\[\text{nn} := 1; \]
if \( j > 1 \) then
\[\text{for } i2 := 2 \text{ to } j \text{ do} \]
\[\hspace{1em} \text{nn} := 1 + \text{bsl}(\text{nn}, 1); \]
\[\text{rec\_mask} := \text{nn}; \]
end;

begin
\[\text{for } k := 1 \text{ to } 15 \text{ do} \]
\[\text{rm}[k] := \text{rec\_mask}(k); \]
\[\text{end};\]
procedure set_mask (var mask: haplotype; r: real);
var
ib: blocktype;
oldbit: boolean;
begin

begin

begin

begin

begin

begin

begin

begin

begin

begin

begin

begin

begin

gethap(pop, d, i2[blk]));
{mutate if necessary}
if mu > 0 then
begin
m_mask := mutation_mask(mu);
for blk := 1 to nblocks do
sonnyjim[blk] := BitXor(sonnyjim[blk], m_mask[blk])
end;
{put the new individual into newpop}
puthap(newpop, sonnyjim, d, jim);
end
end;

procedure set_fitness_table (sel, hwidth, beta, dopt; real);
var
i, imax: integer;
s_epi, c_epi: real;

function fitness (n: integer; s, hw, bta, zopt: real): real;
{if gaussian epistasis, power epistasis or stabilising, then n is countones, else n is counthets}
{These definitions ensure that with heterozygote disadvantage or epistasis, the least fit}
{individual has fitness exp(-n s). s has a different interpretation with stabilising selection}
begin
if epistasis then
fitness := c_epi * exp(-s_epi * exp(-sqr(n / (2 * nsel) - 0.5) / (2 * sqr(hw))));
if epistasisp then
fitness := 1 - (1 - exp(-s * nsel)) * power(4 * (n / (2 * nsel)) * (1 - (n / (2 * nsel))), bta);
if hetdis then
fitness := exp(-s * n);
if stabilising then
fitness := exp(-s * sqr(n - zopt) / 2);
end;

begin
if epistasis then
begin
s_epi := nsel * sel / (1 - exp(-1 / (8 * sqr(hwidth))));
c_epi := exp(s_epi * exp(-1 / (8 * sqr(hwidth))));
imax := 2 * nsel
end;
if hetdis then
imax := nsel;
if epistasisp then
imax := 2 * nsel;
if stabilising then
imax := 2 * nsel;
for i := 0 to imax do
fitness_table[i, 0] := fitness(i, sel, hwidth, beta, nsel - dopt / 2);{left of centre}
for i := 0 to imax do
fitness_table[i, 1] := fitness(i, sel, hwidth, beta, nsel + dopt / 2);{right of centre}
end;

procedure writetime:
var
d: DateTimeRec;
begin
GetTime(d);
writeln(* ', d.hour : 2, ' : ', d.minute : 2, ' : ', d.second : 2)
end:

function zbar (var pp: popptrtype; d: demep1type; neutflag: boolean): real;
var
g: genotype;
sum: real;
begin

sum := 0;
for g := 0 to ngenes - 1 do
  if (neutflag and (getgene(g, seln_mask) = 0)) or (not neutflag and (getgene(g, seln_mask) = 1)) then
  begin
    p := getgenefreq(pp, d, g);
    sum := sum + 2 * p * (1 - p);{I assume diploidy here}
  end;
end;

genic_variance := sum
end;

function genic_variance (var pp: popptrtype; d: demeptype; neutflag: boolean): real;
var
  g: genetype;
  p, sum: real;
begin
  sum := 0;
  for g := 0 to ngenes - 1 do
    if (neutflag and (getgene(g, seln_mask) = 0)) or (not neutflag and (getgene(g, seln_mask) = 1)) then
      begin
        p := getgenefreq(pp, d, g);
        sum := sum + 2 * p * (1 - p);{I assume diploidy here}
      end;
  end;
  genic_variance := sum
end;

function total_variance (var pp: popptrtype; d: demeptype; neutflag: boolean): real;
var
  k: indtype;
  g: genetype;
  p, sum, sum2, zind: real;
begin
  sum := 0;
  sum2 := 0;
  for k := 1 to ninds do
    begin
      zind := 0;
      for g := 0 to ngenes - 1 do
        if neutflag then
          zind := zind + getgene(g, ppA[d, k]) * (1 - getgene(g, seln_mask))
        else
          zind := zind + getgene(g, ppA[d, k]) * getgene(g, seln_mask);
      sum := sum + zind;
      sum2 := sum2 + sqr(zind);
    end;
  total_variance := 2 * ((sum2 / ninds) - sqr(sum / ninds)){assuming diploidy}
end;

function meanl (var x: dtptrtype; nn: integer): real;
var
  i: integer;
  sm: real;
begin
  sm := 0;
  for i := 1 to nn do
    sm := sm + x[i];
  if nn > 0 then
    meanl := sm / nn
  else
    meanl := 0
end;

function variance1 (var x: dtptrtype; nn: integer): real;
var
  i: integer;
  mn, sm: real;
begin
  mn := meanl(x, nn);
  variances1 := sum([x[i] - mn] / nn)
multilocus procs

sm := 0;
for i := 1 to nn do
sm := sm + sqr(x[i] - mn);
if nn > 1 then
  variance1 := sm / (nn - 1)
else
  variance1 := 0
end;

function mean (var x: dtemepttype; d: demeptype; nn: integer): real;
  var
  i: integer;
  sm: real;
begin
  sm := 0;
  for i := 1 to nn do
    sm := sm + x[i, d];
  if nn > 0 then
    mean := sm / nn
  else
    mean := 0
end;

function variance (var x: dtemepttype; d: demeptype; nn: integer): real;
  var
  i: integer;
  mn, sm: real;
begin
  mn := mean(x, d, nn);
  sm := 0;
  for i := 1 to nn do
    sm := sm + sqr(x[i, d] - mn);
  if nn > 1 then
    variance := sm / (nn - 1)
  else
    variance := 0
end;

procedure initialise;
  var
    d: demeptype;
    j: indtype;
    t: integer;
begin
    nmidleft := (ndemes div 2);
    nmidright := (nmidleft + 1);
    randseed := seed;
    sample := 0;
    for t := 0 to tmax div dt do
    begin
        Dmax_sel[t] := 0;
        Dmax_neut[t] := 0;
        width_sel[t] := 0;
        width_neut[t] := 0;
        step_neut[0] := 0;
        slope_left_neut[0] := 0;
        slope_right_neut[0] := 0;
        width_barr_neut[0] := 0;
        slope_ratio_left[0] := 0;
        slope_ratio_right[0] := 0;
        wbarmin[0] := 0;
        wbarreg[0] := 0;
        for d := 0 to ndemes + 1 do
        begin
            zbar_neut[t, d] := 0.0;
        end;
    end;
Vgenic_neut[t, d] := 0.0;
Vtotal_neut[t, d] := 0.0;
Vdiseq_neut[t, d] := 0.0;
zbar_sel[t, d] := 0.0;
Vgenic_sel[t, d] := 0.0;
Vtotal_sel[t, d] := 0.0;
Vdiseq_sel[t, d] := 0.0;
end
end;

function rbar (sm: haplotype; rec: real): real;
{ Calculates the harmonic mean recombination rate between neutral and selected loci; }
{ sm is the "selection mask", which determines whether or not the gene is selected; }
{ the function takes into account multiple crossovers, using Haldane's mapping function. }
var
  nn, ns, g, kn, ks: integer;
  sum: real;
begin
  { count the number of neutral and selected loci; }
  { could use nneut and nsel, but safer to recalculate }
  nn := 0;
  ns := 0;
  for g := 0 to ngenes - 1 do
    if getgene(g, sm) = 0 then
      nn := nn + 1
    else
      ns := ns + 1;
  { sum over all neutral loci }
  sum := 0;
  for kn := 0 to ngenes - 1 do
    if getgene(kn, sm) = 0 then
      for ks := 0 to ngenes - 1 do
        if getgene(ks, seln_mask) = 1 then
          sum := sum + 2 /(1 - power((1 + 2 * rec), abs(kn - ks)));
      rbar := nn * ns / sum;
end;

procedure regress (var xb, yb, b: real; x, y: dptrtype; nn: integer; originflag: boolean);
{ finds the regression of y on x; if originflag, this is forced through the origin; }
{ otherwise, y = yb + b*(x-xb) }
var
  x0, y0, sxx, sxy: real;
  k: integer;
begin
  sxx := 0;
  sxy := 0;
x0 := meanl(x, nn);
yb := meanl(y, nn);
  if originflag then
    x0 := 0
  else
    x0 := xb;
  if originflag then
    y0 := 0
  else
    y0 := yb;
  for k := 1 to nn do
    begin
      sxx := sxx + sqr(x[k] - x0);
      sxy := sxy + (x[k] - x0) * (y[k] - y0);
    end;
  if sxx > 0 then
b := sxy / sxx
else
begin
  writeln('ERROR in regress: sxx=0');
b := 0
end;
end;

function Dmax (var Vg, Vd: dtdemeptrtype; ns, ng: integer): real;
var
  k: integer;
slope, pq2bar, Dbar: real;
pq2, D: dtptrtype;
begin
  new(D);
  new(pq2);
  if ng > 1 then
  begin
    for k := 1 to ndemes do
      begin
        pq2[k] := sqrt(Vg[ns, k] / (2 * ng));
        D[k] := Vd[ns, k] / (2 * ng * (ng - 1));
        end;
    regress(pq2bar, Dbar, slope, pq2, D, ndemes, true);
    Dmax := slope / 16
  end
  else
    Dmax := 0; \{ one or no genes \}
  dispose(D);
  dispose(pq2);
end;

function width (var zb: dtdemeptrtype; ns, ng: integer): real;
\{ This function searches for points at which ln(p/q) is -2 and +2; it then measures the \}
\{ width by calculating a regression over this range. The algorithm is not foolproof, and \}
\{ should be checked against a few graphs. If the system does not span the required range, \}
\{ zero is returned \}
const
  Imax = 2;
var
  k, kmin, kmax: deme1type;
  deme, lp: dtptptrtype;
  slope, zcritmin, zcritmax, demebar, lpbar: real;
begin
  new(deme);
  new(lp);
  zcritmax := 2 * ng / (1 + exp(-Imax));
zcritmin := 2 * ng / (1 + exp(Imax));
kmin := ndemes + 1;
repeat
  kmin := kmin - 1
until (kmin = 1) or (zb[ns, kmin] < zcritmin);
kmax := 0;
repeat
  kmax := kmax + 1
until (kmax = ndemes) or (zb[ns, kmax] > zcritmax);
if (kmin = 1) or (kmax = ndemes) then
  width := 0
else
  begin
    for k := kmin to kmax do
      begin
        lp[k - kmin + 1] := logit(zb[ns, k] / (2 * ng), 5);
        deme[k - kmin + 1] := k
        end;
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regress(demebar, lpbar, slope, deme, lp, kmax - kmin + 1, false);
width := 4 / slope
end;
dispose(deme);
dispose(lp);
end;

procedure calculate_barrier (var step, slope_left, slope_mid, slope_right, wbmin, wbreg: real; var zb, wb: dtttype; ns, ng: integer);
{ This calculates barrier strength, from the average shape of a set of neutral loci. The method 
is complicated, and may not be entirely reliable. }
{}
{ First, the mean fitness (wb) is searched to find its minimum (wbmin near kmid). Then, the 
points at which mean fitness is reduced by 5% of its maximum drop are calculated. Outside 
this region (kleft, kright), hitchhiking should be negligible. The slope of the clines to left 
and right are calculated, using least-squares regression on logit transformed data. Only the 
region which is not fixed is used (ie, abs(logit) < 5. The slope at the centre is found by 
regression of logit transformed data, either between kleft, kright, or over 5 demes, whichever 
is the smaller. The barrier strengths for the two directions are step/slope_left and step/slope_right }
var
k, kleft, kright, kfarleft, kfarright, kmid, kmidleft, kmidright: integer;
lp, deme, pq, wtemp: dtttype;
zbmin, zmax: real;
pqbar, wbbarmid, sl: real;
lpbarleft, demebarleft, slleft: real;
xleft, xright, zleft, zright, lpbarmid, demebarmid, slmid: real;
lpbar_right, demebar_right, sl_right: real;
begin
new(lp);
new(deme);
new(pq);
new(wbtemp);
{ set minimum and maximum zb }
zbmin := 2 * ng / (1 + exp(5));
zmax := 2 * ng / (1 + exp(-5));
{ find position of minimum wbar }
for k := 1 to ndemes do
  wtemp[k] := wb[n, k];
kmid := 1;
wblem := 1;
for k := 1 to ndemes do
  if wtemp[k] < wrblem then
    begin
      kmid := k;
      wblem := wtemp[k]
    end;
{ find minimum mean fitness by regression on pq }
for k := 1 to ndemes do
  pq[k] := (zb[n, k] / (2 * ng)) * (1 - zb[n, k] / (2 * ng));
regress(pqbar, wbbarmid, sl, pq, wtemp, ndemes, false);
wblem := wbbarmid + (0.25 - pqbar) * sl;
{ find left edge of region of reduced fitness }
kleft := kmid + 1;
repeat
  kleft := kleft - 1
until (kleft = 0) or (wbbarmid > 1 - (1 - wblem) / 20);
if kleft = 0 then
  writeln('ERROR in calculate_barrier: can\'t find left edge of low wb region ');
{ find right edge of region of reduced fitness }
kright := kmid - 1;
repeat
  kright := kright + 1
until (kright = ndemes + 1) or (wbbarmid > 1 - (1 - wblem) / 20);
if kright = ndemes + 1 then
  writeln('ERROR in calculate_barrier: can\'t find right edge of low wb region ');

(find far left edge, where fixation is reached)
kfarleft := 0;
repeat
  kfarleft := kfarleft + 1
until (zbA[ns, kfarleft] > zbmin) or (kfarleft >= kleft - 3);

(find far right edge, where fixation is reached)
kfarright := ndemes + 1;
repeat
  kfarright := kfarright - 1
until (zbA[ns, kfarright] < zbmax) or (kfarright >= kright + 3);

(find leftmost point to count central regression)
if (kmid < kleft) > 3 then
  kmidleft := kmid - 3
else
  kmidleft := kleft;

(find rightmost point to count central regression)
if (kright - kmid) > 3 then
  kmidright := kmid + 3
else
  kmidright := kright;

(set up leftmost regression)
for k := kfarleft to kleft do
begin
  demeA[k - kfarleft + 1] := k;
  lpA[k - kfarleft + 1] := logit(zbA[ns, k] / (2 * ng), 5);
end;
regress(demebar_left, Ipbar_left, sl_left, deme, lp, kleft - kfarleft + 1, false);

(set up middle regression)
for k := kmidleft to kmidright do
begin
  demeA[k - kmidleft + 1] := k;
  lpA[k - kmidleft + 1] := logit(zbA[ns, k] / (2 * ng), 5);
end;
regress(demebar_mid, Ipbar_mid, sl_mid, deme, lp, kmidright - kmidleft + 1, false);

(set up rightmost regression)
for k := kright to kfarright do
begin
  demeA[k - kright + 1] := k;
  lpA[k - kright + 1] := logit(zbA[ns, k] / (2 * ng), 5);
end;
regress(demebar_right, Ipbar_right, sl_right, deme, lp, kfarright - kright + 1, false);

(These regressions are on a logit scale (z). What is wanted are dp/dx, not dz/dx.)
(to find these, the relation dp/dx = pq dz/dx = \exp(z)/\sqr{1+\exp(z)} dz/dx is used.)
(This means that the “centre” of the barrier must be defined. I take it to be the point in the )
(called regression at which p=0.5, z=0: an implicit assumption of symmetry here)
(}
(}

(A modification: slopes are calculated at the point where the regressions intersect)
xmid := demebar_mid - Ipbar_mid / sl_mid;
xright := (Ipbar_mid + Ipbar_right + sl_right * demebar_right - sl_mid * demebar_mid) / (sl_right - sl_mid);
yleft := (Ipbar_mid + Ipbar_left + sl_left * demebar_left - sl_mid * demebar_mid) / (sl_left - sl_mid);
zipt := Ipbar_left + (xleft - demebar_left) * sl_left;
zright := Ipbar_right + (xright - demebar_right) * sl_right;
slope_left := (exp(zleft) / \sqr{1 + \exp(zleft)}) * sl_left;
slope_mid := sl_mid / 4;
slope_right := (exp(zright) / \sqr{1 + \exp(zright)}) * sl_right;
step := 1 / (1 + \exp(-zright)) - 1 / (1 + \exp(-zleft));

dispose(deme);
dispose(lp);
dispose(pq);
 disposedown;
end;

end.
D.1.2 Programming methods

General considerations

An array can not occupy unlimited size in Pascal: each array can occupy a maximum of 64K. We therefore make use of pointers. To use operators on binary numbers, a haploid genome can be represented by an integer that translates to at most 16 bits in binary code. Genotypes are stored in an array of integers, as discussed in 5.3.2. The functions that translate sets of integers to and from genotypes are:

'gethap' : returns a haploid represented by a single integer from the relevant population arrays.
'getgene' : For a given haploid, getgene returns a gene value: 1 or 0 for a locus.
'putgene' : alters the value at a locus- it puts the required change (x) in the locus position.
'puthap' : puts the altered haploid back into its population arrays.
'countbits' : counts the number of 1's

Procedure askstuff

Each run is for tmax generations. Statistics are recorded every dt generations after an initial delay of twarm generations. Other parameters required are: the number of demes; the number of individuals in each deme; the number of genes under selection; the number of haploid individuals that migrate between demes in one generation (nmig) The number that migrate is required rather than the migration rate, (the migration rate is simply nmig/ninds) this avoids the problem of having to round in procedure migrate; only whole individuals migrate.

Migration

The procedure transfer determines which individuals are to migrate between demes.

Each deme has a proportion of its individuals replaced by individuals in the corresponding position from the deme to its left, and similarly a proportion will be replaced with individuals from the deme to the right. This is best illustrated by the following examples: Suppose a proportion $m/2$ of the individuals in a deme migrate to the deme on the left, and a proportion $m/2$ to a deme on the right where there is no barrier, and that a reduced proportion, $m_b/2$ migrate between demes separated by a barrier. Consider deme C in Fig D.1. The proportion shaded in deme B constitutes migrants from the left and will replace the corresponding shaded portion.
Figure D.1: Sketch to illustrate how the stepping-stone model of migration has been simulated. Demes are represented by rectangles A - H. Shaded areas represent the proportion of individuals that are “replaced” in a deme by migrants from the deme to the left; stippled areas represent the proportion replaced from the deme to the right. The solid line represents a physical barrier, which reduces the number of migrants (see text).

in deme C. Similarly, the stippled portion from deme D will replace the stippled portion in deme C, and constitutes migrants from the right. Note that moving (say) the top \((m/2)\) will not cause bias because the positions of individuals in the array are random. The method restricts the number that migrate to \((0, 2/n, 4/n \ldots)\) and there is no random element in \(n\).

Now suppose there is a barrier to gene flow, (this option will be used in Chapter 6) represented by a solid line in the figure. Migrants from deme E to F will be reduced to \(m_b/2\) (represented by the smaller shaded portion in deme E). Similarly, only \(m_b/2\) individuals will migrate from F to E (stippled region in F). Since the proportion migrating across the barrier is reduced, the proportion of non-migrants in demes adjacent to the barrier is \(m/2 + 1\) to \(ninds m_b/2 + offset\) (i.e. \(m/2 - m_b/2\); see procedure migrate in the program).

Reproduction

To simulate reproduction, for each of the \(N\) individuals in a deme, two diploid parents must be chosen, and from these a recombined haploid descendant must be produced. To describe how this is done, I will use the same notation as that which
Figure D.2: Diagrammatical representation of the cumulative fitness table. In this example diploids: 1, 2, . . . , 10 have fitnesses 5, 3, . . . , 1 units respectively.

appears in the program provided in appendix D.1. It will be useful to refer to the program. $i$ refers to a haploid individual in generation $t$, where each deme has $i = 1$ to $ninds$ individuals. $i_1$ and $i_2$ refer to two haploids from generation $t$ that constitute a diploid parent, $i$ (see Fig D.3). $cumwptr[i]$ refers to a table giving the fitnesses of every possible pair of haploids ($i_1 * i_2$) each corresponding to a diploid parent. Actually, this is expressed as a cumulative distribution, where the spacing between successive diploids will have magnitude equal to the fitness of the diploid (see Fig D.2).

To choose parents randomly, but with probability depending on their fitnesses, for each parent we simply choose a random number with range equal to that of the cumulative fitness table. Then, if the random number has a value greater than the cumulative fitness of diploid $i$ but less than or equal to the cumulative fitness of diploid $i$, the diploid chosen as parent is $i$.

We could search through all $i_1*i_2$ combinations until $cumwptr[i] < rand \leq cumwptr[i+1]$. However, this would be extremely inefficient: we would have to do this twice for every haploid descendant. There would also be a potential problem with the random number generator which has only 64K locations. Instead the fitness of each diploid is calculated first, and stored in a table. Setting up a table of diploids in this way saves time: we can simply find the position of $i$ in the table of diploids and then decode $i_1$ and $i_2$: procedure set.ntable returns the position in the table of diploids of a haploid individual ($nptr[i]$), and the function search.integer searches for $i_1$ in this table (binary search). $i_2$ is then simply $ninds*nptr[i1]+i$ (refer to program).

To create a recombined haploid individual, $i$, for the next generation, we make use of a recombination mask: $rec.mask$ consists of a string of zero’s and one’s where the transition from one to the other represents the position of a chiasma. This mask is used in procedure recombine to create recombined individuals using operators on
Figure D.3: Diagrammatical representation of the table of diploids.

binary numbers.
Appendix E

Appendices for Chapter 6

E.1 Estimating dispersal

From the mark-release-recapture data, for each $(10m)^2$ colour-coded area, we can use the Jolly method to obtain the fraction of marked grasshoppers present at the first scoring ($m_1$). If we assume that the chance of seeing a grasshopper is constant, then we can infer the fraction present at the second scoring ($m_2$). Then $1 - m_1$ will be the fraction lost after the first scoring and $1 - m_2$ the fraction lost after the second scoring. Some of those lost will have been found in one of the other $10m^2$ areas in the grid, at a known distance (to within 5 metres) from their home area. To obtain a lower limit for the distance moved, we would then assume that the remainder of those lost went only just outside the searched area; and that individuals found in other $10m^2$ areas dispersed the least possible distance from their home area (again we could use the Jolly method to estimate the actual number by counting as marked, any painted individual that occurs in the desired radius to estimate $\theta = n_{11}/n_{10}$). We would also have to assume that the loss was not due to mortality. I have included a table of observed and actual numbers remaining in each $10m^2$ area and lost from the entire grid.
Table E.1: The *observed* and *actual* fraction of those individuals initially released that remain in a 10m\(^2\) area. Actual numbers were calculated using the Jolly method.
### Appendix F

#### Appendices for Chapter 7

**F.1 Percentage cover of major species in each quadrat**

Column 1: quadrat number, Column 2: numbers refer to habitat types on Figure 7.10 - 1 = A, 2 = B, 3 = C, 4 = D, 5 = E, 6 = F. Columns 3-21 give the approximate percentage cover of the 19 most common species/features found in quadrats. Column 3 = herbs; 4 = grasses; 5 = *Juniperus nana*; 6 = *Vaccinium uliginosum*; 7 = *Festuca pennulata*; 8 = bare earth; 9 = *Vaccinium myrtillus*; 10 = *Rhododendron*; 11 = scree; 12 = *Veratrum album*; 13 = rock; 14 = nettles; 15 = *Nardus stricta*; 16 = Carex; 17 = *Festuca sp.*; 18 = *Ranunculus sp.*; 19 = *Deschampsia*; 20 = thistle; 21 = *Rumex sp.*. Species occurring 3 times or less in the dataset were excluded from the PCA (Figure 7.11).

<table>
<thead>
<tr>
<th>Quadrat</th>
<th>Herbs</th>
<th>Grasses</th>
<th><em>Juniperus nana</em></th>
<th><em>Vaccinium uliginosum</em></th>
<th><em>Festuca pennulata</em></th>
<th>Bare Earth</th>
<th><em>Vaccinium myrtillus</em></th>
<th><em>Rhododendron</em></th>
<th>Scree</th>
<th><em>Veratrum album</em></th>
<th>Rock</th>
<th>Nettles</th>
<th><em>Nardus stricta</em></th>
<th>Carex</th>
<th><em>Festuca sp.</em></th>
<th><em>Ranunculus sp.</em></th>
<th><em>Deschampsia</em></th>
<th>Thistle</th>
<th><em>Rumex sp.</em></th>
</tr>
</thead>
</table>
### F.2 Density estimates from counts

Table of densities for direct counts. “D” is the estimated adult density corrected for instar in a (10m)^2 quadrat. First and second columns give the X and Y coordinates in Fig. 7.4. The fourth column gives the quadrat numbers.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>D</th>
<th>Quadrat</th>
</tr>
</thead>
<tbody>
<tr>
<td>-140</td>
<td>235</td>
<td>14.1</td>
<td>a1</td>
</tr>
<tr>
<td>-95</td>
<td>235</td>
<td>32.9</td>
<td>a2</td>
</tr>
<tr>
<td>-25</td>
<td>250</td>
<td>45.6</td>
<td>a3</td>
</tr>
<tr>
<td>85</td>
<td>225</td>
<td>12.41</td>
<td>a4</td>
</tr>
<tr>
<td>125</td>
<td>190</td>
<td>21.33</td>
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Appendix G

Data provided in microfiche form

The following data are provided in microfiche form in a pocket at the back of this thesis:

1. Density and instar scores for each quadrat on each day of scoring at Seyne and Col de la Lombarde in 1987, Col de la Lombarde in 1988 and Col de la Lombarde, Vallée des Merveilles, Col de Fenestre and Tende in 1989. OBS = observation number; QUAD = quadrat number; DAY = day of scoring; DEN = observed density; KARY = karyotype, 1=XY, 2=XO, 3=mixed; MEANLEN = mean length of individuals in the quadrat; MEANSTG = mean stage of individuals in the quadrat; CORDEN = density corrected for the effects of habitat and instar; ASPECTS = aspect of the quadrat; SLOPE = slope of the quadrat; GP = vegetation type as classified by TWINSPLAN; ASPDIV = the topography code; LTYPE = the ground cover code.

2. Peak nymph density and adult (final) density scores for the (10m)$^2$ quadrats, Col de la Lombarde, 1989.
K.S. Jackson, Ph.D. Thesis: Errata

p16  line 20  “myotic” should be “meiotic”
p19  line 16  “Orr’s” should be “Orr”
p24  line 2  “Appenines” should be “Apennines”
p47  line 7  “Nichols” should be “Nichols’”
p48  Fig. 3.1 Caption “The dotted line shows the stream, the solid line shows the river in the valley”
p92  line 4  “fromation” should be “formation”
p94  line 17  “parolatina” should be “pardolina”
p138  line 2  “Lincoln” should be deleted
p169  line 13  “z” should be “z”
p196  line 10,16,17  “2.5m^2” should be “25m^2”
p219  line 9  “percieve” should be “perceive”
p234  Fig. 7.2 Caption “habitat” should be “habitat”