Temperature, Body Size and Life History in *Drosophila melanogaster*

Ph.D. Thesis

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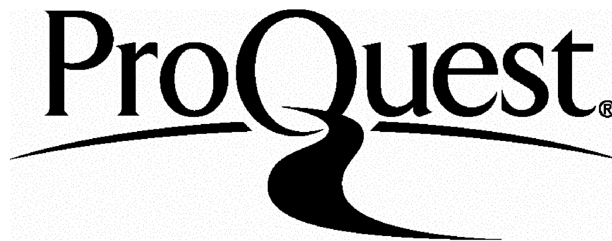

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"To do great work a man must be very idle as well as very industrious"

Samuel Butler

Abstract

Body size in *Drosophila melanogaster* results from the combination of evolutionary genetic and developmental effects, both of which are affected by the thermal environment. Evolution or development at lower environmental temperatures results in increased body size in fruitflies, however, the reasons for these adaptations remain elusive.

To investigate whether larger size is favoured at lower temperature through natural selection on adult males, life-span and age-specific-fertility of males from lines artificially selected for increased and decreased body size were examined at two different temperatures. Larger males were found to be fitter than controls at both temperatures, but the difference in fitness was much greater at the lower experimental temperature. Smaller males did not perform significantly differently from controls at either experimental temperature. These findings suggest that thermal selection for larger adult males is at least in part responsible for evolution of larger body size at lower temperatures in this species.

An investigation into the evolution of plasticity of body size traits was performed. The phenotypic plasticity of body size and its components; cell size and cell number, were examined by rearing populations of flies that had evolved in constant and variable thermal environments at two different experimental temperatures. Plasticity of body size was comparable among all of the populations examined, however, plasticity of both cellular components of body size was significantly greater in flies adapted to variable thermal environments.

The lifespan and fecundity of eight replicated populations of continually mated flies from a cline along the Eastern coast of Australia, when maintained at two different temperatures in the laboratory, was also examined, and no latitudinal trend in either longevity or lifetime fecundity was observed. However, there were some differences in the pattern of fecundity over the lifetime of individuals along the cline.

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1. Introduction

1.1 Spatial Variation

Many species are observed to vary geographically in phenotype, either in response to spatial variation in evolutionary and environmental influences or simply due to random factors.

For example, the European house sparrow (*Passer domesticus*) in North America has been the subject of much study (e.g. Calhoun 1947; Johnston and Selander 1964, 1971). This species was introduced to North America from Europe in 1852, and rapidly spread throughout the continent. As the population spread, evolutionary change occurred within the species, resulting in variation in size and colour. The northernmost populations, the Pacific coast populations, and those from Mexico, all exhibit darker coloration than those from the central areas. The species also exhibits a general trend of larger body size in North, and smaller size in the South (Johnston and Selander 1971). So, in the space of just a hundred generations, the species has developed both increased and decreased size, and darker and lighter coloration than the original population. The observed changes might either reflect some adaptation to the environment that the birds are moving into, or may simply be random genetic variation as the species has expanded. In order to ascertain whether or not the variation is an adaptation to the environment, experiments need to be carried out to determine whether there is an advantage to the observed variation. Such experiments can either be carried out *in situ*, transferring individuals into unfamiliar environments and observing the effect on their fitness, or in the laboratory examining the fitness of individuals in a common environment.

Changes in coloration also typify one of the best known examples of adaptation to environment, that of the peppered moth (*Biston betularia*). Prior to the industrial evolution, only the light coloured, peppered form of the moth was observed. A dark, melanic form was first reported in 1848 near Manchester, and increased in frequency, until it formed more than ninety percent of the population in polluted areas in the mid-twentieth century. In relatively unpolluted areas, however, the light form remained common. Such changes in frequency of the melanic form in the population have been attributed to the action of predators – the melanic form is better camouflaged on the tree trunks of trees in polluted areas, because the soot killed off the lichen that would normally grow on the trees (Kettlewell 1973). The vast increase in frequency of the melanic form over just 50 generations in polluted regions strongly suggests that it is adaptive, as was suggested by Kettlewell's mark-recapture experiments. These experiments have, however, never been successfully repeated, and there has been much criticism of Kettlewell's protocol (e.g. Bishop 1972; Majerus 1998).

If selective predation by birds were the sole factor affecting melanic frequency in populations, it should have resulted in monomorphic populations representing the most cryptic phenotype for each area. However, the melanic form of the peppered moth is common in areas that are relatively unpolluted. For example, in East Anglia, United Kingdom, where pollution is relatively low, the melanic form can be found with a frequency of 80% (Lees 1971). Similarly, populations in urban areas have remained polymorphic, despite strong selection for the melanic morph (Creed *et al.* 1980). Gene flow by migration might account for this observation, and provides one possible explanation for why predicted geographic trends may not be

observed. Observation of spatial variation, and deviations from predicted adaptive geographic patterns, can provide information about gene flow in populations.

Further studies have shown that melanic forms of several species (including pigeons and cats) have increased in frequency in polluted regions despite not suffering increased predation. A potential explanation for this is provided by work on ladybird beetles (*Adalia bipunctata*), in which the melanic form absorbs solar radiation more efficiently in a smoky environment than the non-melanic form (de Jong *et al.* 1996).

The example of the peppered moth also illustrates another property of the study of spatial variation. By studying melanic and non-melanic morphs, the genetics of the adaptation have been investigated, and the coloration has been shown to be entirely due to genotype. Initially, the melanism was thought to be due to just a single gene locus, *carbonaria*, with just two alleles, including a dominant melanism allele. However, subsequent molecular investigation revealed that at least five alleles can be found at the *carbonaria* locus in the peppered moth species, and that their dominance relations are not so simple (Clarke and Sheppard 1964; Lees and Creed 1977; Steward 1977).

Most examples of spatial variation are not as genetically discrete as that of the peppered moth, however. For example, in genetic clines of continuous variables, such as body size, we do not know the exact genotype that produces any specific body size. We can, however, speculate on the genetics responsible for the observed phenotypic variation. Such characters typically express an approximately normal frequency distribution, suggesting the influence of a large number of genes, each of small effect. By comparing the extreme examples of genetic variation, such as the extreme ends of a region of geographic variation, we can experimentally determine

those genes of relatively major effect. In smaller organisms, with shorter generation times, such as *Drosophila*, chromosome substitution experiments can be carried out, to determine the effects of genes on individual chromosomes (e.g. a study of the genetic basis of body size variation along three parallel body size clines; Gilchrist and Partridge 1999). Quantitative locus mapping can then be used to find specific regions of chromosomes containing genes of major effect (e.g. Tanksley 1993; Falconer and Mackay 1996).

For many traits, simply rearing in a different environment can result in changes in phenotype, without any change in the genotype. The effect of phenotypic plasticity upon phenotype can be determined by rearing genetically similar individuals in different environments. For example, as we shall see later in this Introduction, the environment within which an individual is reared in part determines its body size in *Drosophila* species. The contribution of phenotypic plasticity to phenotype is genetically determined, and hence, the degree of phenotypic variation that an individual can express in response to the environment can itself evolve.

The distribution of a species is determined by its ecological attributes, and can be distinguished by the distinction between two terms – the fundamental niche and the realised niche of a species. The area within the tolerance limits endurable by a species is termed its fundamental niche. However, for a number of reasons a species might not occupy its entire fundamental niche, such as competition with other species or the presence of geographic barriers. The realised niche is, hence, that area of the fundamental niche that a species occupies (Hutchinson 1958).

In order to occupy either niche, it may be necessary for a species to express spatial variation to the variations within its environment. Such variations might only occur on a very narrow scale, such as the microvariation observed in soil

composition, or the differences in conditions between an area shaded by a tree and an unshaded area. Broad scale variation occurs over a much larger area, potentially several thousand kilometres in breadth, and is typified by the variation of phenotype of organisms along latitudinal clines, which provide ideal subjects for the study of adaptation. Study of spatial variation can allow us to infer the geographic range that an individual species would be able to occupy, and which environmental factors are responsible for the limitation of the range.

Dobzhansky (1951) proposed that the evolution of diversity is a result of adaptation to habitats that vary in time and space. Traits that confer an advantage in one environment may either prove detrimental in another, or could simply be lost by mutation if they are not advantageous in other environments. This adaptation to environmental factors can precede the diversification of populations into different species (Lewontin 1997). That spatial variation can provide sufficient genetic variation between populations to permit speciation has been confirmed in several studies, such as the observation of "ring" species, such as, the Californian *Ensatina* species of salamander. In the southerly San Diego County, two distinct forms of the species, the blotched *E. klauberi* and the uniformly pigmented *E. eschscholtzii*, behave as separate species with no hybrids (Wake *et al.* 1986). The two forms of salamander can be traced north, one along the eastern side of the San Joaquin Valley, and one along the western side of the valley, meeting again in Northern California and Oregon, where only one morph is found. These studies indicate how expansion over a large area and associated variation due to random gene flow can ultimately lead to parapatric speciation. It follows then that where there is spatial variation due to environmental conditions, the resultant variation may lead ultimately to speciation between populations at the extreme ends of the geographical region occupied.

Investigation of spatial variation can therefore provide information about possible means of speciation.

So, investigation of spatial variation provides a lot of information on a variety of evolutionary phenomena. Examination of such variation can allow us to draw conclusions about the environmental factors influencing fitness, estimate the degree of gene flow within the population, and better understand the geographic range that a species can occupy. Investigation of spatial variation can also allow us to learn more about phenotypic plasticity, genetics of adaptation, and speciation.

1.2 Phenotypic Plasticity

Phenotypic plasticity is a consequence of environmental effects on the phenotypic expression of an individual's genotype (Scheiner 1993), and such phenotypic responses can occur in the short- or long-term.

A phenotypic response to temperature can occur broadly on two different time scales within the lifetime of an individual. The immediate environment around an organism can produce an immediate response; traits that respond this rapidly to the environment are termed labile (Schiener 1993). The other level of response occurs over a longer time scale, taking between minutes and months to occur (Huey and Berrigan 1996), and may either be reversible or non-reversible.

An example of a labile response is provided in ectotherms when they are exposed to a heat shock. When *Drosophila* are exposed to a non-fatal high temperature for a short period, they produce an immediate response by the production of heat shock proteins that counteract the effect of the high temperature (Lindquist 1986).

An example of a reversible longer time scale phenotypic response can be found in fish which, when exposed to lower temperatures, increase the mitochondrial density of their muscles (Guderley and St Pierre 1996). Another example is observed in heterophyllous aquatic plants. Adult foliage in these plants will either develop reversibly into an aerial leaf structure or a submerged leaf structure depending upon its immediate environment (e.g. Wells and Pigliucci 2000). A non-reversible example is the effect of developmental temperature on body size in *Drosophila*, in which lower temperature during development leads to increased body size (e.g. Robertson 1959; Masry and Robertson 1979; David *et al.* 1983; French *et al.* 1998). If temperature alters following the developmental period, it has no further effect on body size.

Determining whether or not responses to environmental variation, such as the labile responses described above are adaptive, is complex, and involves determining whether such responses result in an increase in fitness.

The degree of phenotypic plasticity that an individual can express for a specific trait is itself a genetic trait, which can evolve in response to environmental conditions, as has been observed in numerous species (see section 1.10 of this Introduction).

1.3 Genetic Variation

Spatial variation in phenotype across a geographic range might be due to the establishment, over a number of generations, of a genetic, evolutionary response. Such responses to environment at the genetic level can be observed in numerous species of organism, and have been the subject of much research work.

For example, in *Drosophila melanogaster*, the metabolic rate of flies which had evolved at higher latitudes has been observed to be significantly greater than that of flies which evolved at lower latitudes (Berrigan and Partridge 1997).

Although spatial variation in genotypes may be the result of a response to spatial environmental variation, the observed spatial variation need not necessarily be adaptive. Adaptive explanations can be suggested to explain most observed spatial variation as a response to environmental stimuli. However, such explanations need to be tested to assess their plausibility. Observed spatial genetic variation could be the result of adaptation by natural selection, however, it could also be due to genetic drift or gene flow (Slatkin 1987). If such observed variation were adaptive, then similar spatial genetic variation should be observed in regions with similar environments, and the trends should be repeatable in the laboratory.

1.4 Genetic and Environmental Effects

The observed spatial variation in phenotype in many species is determined both by their genotype and by the environment, by phenotypic plasticity. The contribution of each of these factors can be determined by the use of 'common garden' experiments, in which organisms from different populations are reared in similar environmental conditions, or by reciprocal transplant experiments where pairs of organisms are reared in the opposite environment (Conover and Schultz 1995).

The effects of genetic and environmental factors on phenotype can combine in three ways (Conover and Schultz 1995). Firstly, there can be no covariance between genotype and phenotype, which strongly suggests that the genetic variation is unconnected with the environmental conditions in the area of the spatial variation.

The second type of relationship between genes and the environment is co-gradient variation, in which the genetic effect on the phenotype runs in the same direction to the environmental effect. For example, studies on a geographic cline for voltinism (number of broods per year) in the cricket *Allonemobius socius*, by rearing populations from cline ends using common garden environments which mimicked photoperiod and temperature conditions along the cline, suggested that the length of the growing season was a cue for whether or not females should be univoltine or bivoltine (Bradford and Roff 1995). Genetic differences in the period of egg diapause along the cline were magnified by environmental cues, with populations from regions with a longer growing season tending to adopt a bivoltine strategy. Intermediate females in the genetic cline would either lay direct-developing or diapause eggs depending on environmental cues, which provide a measure of the likelihood of their offspring having sufficient time to develop. Another example is body size variation along latitudinal clines of *Drosophila*, which is believed to be caused by temperature variation with latitude (see section 1.5 of this Introduction). *Drosophila melanogaster* are larger at higher latitudes in the wild, where the environmental temperature is lower. These larger flies are genetically larger, and low developmental temperature acts to make them larger still (James *et al.* 1997). In both of these examples, the genetic effect reinforces the environmental effect on the phenotype.

The third pattern of covariance is counter-gradient variation, in which the genotype acts to oppose the environmental effect on the phenotype. Examples of counter-gradient variation are quite widespread, but can be difficult to uncover because by their very nature counter-gradient effects work against each other to eliminate phenotypic variation along areas of environmental variation. Examples of

counter-gradient variation have been observed in the Atlantic silverside, the striped bass, and the wood frog (Berven 1982b; Conover and Present 1990; Conover *et al.* 1997). In the Atlantic silverside, larval growth rate is genetically faster in high latitude populations. However, the lower environmental temperature at such latitudes acts to slow the individuals' growth rate. Growing seasons are shorter at these higher latitudes, so there is less time available to reach adult size. Attaining a large size by the end of the growing season is important if the fish is to survive the winter (Schultz *et al.* 1998). Hence, at high latitudes, fish are selected to grow faster in a short period of time, in order to attain as large a size as possible before the winter (Conover and Present 1990; Conover *et al.* 1997). Wood frog larvae originating from higher altitudes have a genetically more rapid growth rate too, so that they can achieve metamorphosis earlier and at a larger size. As with the Atlantic silverside, this genetically increased growth rate counteracts the developmental effects of temperature at higher latitudes, which would slow the rate of growth (Berven 1982b).

1.5 Geographic Clines and Dispersal in Ectotherms

The term 'cline' was first proposed by Huxley, to refer to "a gradation in measurable characters" (Huxley 1938), and has been subsequently refined to refer to "a geographic gradient in a measurable character, or gradient in gene, genotype or phenotype frequency" (Endler 1977). Endler's definition was originally only applied to the hybrid zones separating incipient species in parapatric speciation, which often occupy only small geographic areas and may depend upon only small differences in habitat. The definition, however, can apply beyond just hybrid zones, and clines have been observed in other situations, extending over large geographic regions and more

major changes in environmental conditions. For example, a cline can extend over a large area of over 4,000 km, spanning a wide range of latitudes or altitudes, and therefore individuals in the cline experience a wide range of different environmental conditions. The effect of these differing environmental conditions on the phenotypes of the individuals in the cline can either be manifested directly through a developmental response to the environment, through genetic variation along the cline, or some combination of the two. Genetic differences along a cline may be simply due to random genetic drift or migration, or due to natural selection. Significant evidence for a selective cline is provided if repeated evolutionary trends in response to the same spatially varying selection pressure are observed.

In equilibrium theory, gene flow by dispersal will tend to counteract the effects of selection gradients along a cline, and thereby, make the clines less distinct and broadening their width (Endler 1977). The dispersal distribution of a population is therefore important in determining the width of a dispersal-selection cline.

The degree of dispersal in populations along a cline can be affected by a number of factors. Firstly, the population density can affect the degree of dispersal, with increased dispersal observed when population density is higher (Endler 1977).

Dispersal rate also varies across a population range. Crumpacker and Williams (1973) found a far greater dispersal rate in peripheral regions of the range of *Drosophila pseudoobscura* than Dobzhansky and Wright (1943) did in more central regions. Even then, movement into a new area does not always result in the establishment of the genes of the dispersed individuals. Studies on small mammals (Crowcroft and Rowe 1963; Selander 1970) and in Lepidoptera (Ehrlich 1961, 1965; Gilbert and Singer 1973) provide evidence that dispersed individuals are much less likely to reproduce successfully than natives.

Continuously distributed populations of organisms are relatively rare. Organisms are often found in clumps around a favourable microclimate, separated by regions of lower population density (e.g. Andrewartha and Birch 1954; Ehrlich 1961, 1965), and organisms will be inclined to move further over the unfavourable regions (Endler 1977). In other words, the distance between populations can influence estimations of gene flow by dispersal - longer dispersal distances will be observed where populations are widely spaced, and shorter distances in closely spaced populations.

Early attempts to quantify dispersal involved carrying out marked release-recapture experiments (e.g. Dobzhansky and Wright 1943; Andrewartha and Birch 1954; Den Boer 1971; Crumpacker and Williams 1973), typically finding figures of around a hundred metres per day for dispersal rate. For example, in one study, *Drosophila pseudoobscura* were observed to move an average of 133 metres on the first day following release, and an average of just 90 metres on each subsequent day (Dohzhansky and Wright 1943). Recent field studies, however, have found that the same fruitfly species is capable of moving 10 kilometres in 24 hours over a desert (Jones *et al.* 1981), and as fast as 15 kilometres in 15 hours (Coyne *et al.* 1982), though such movements are rare. Subsequent studies have used genetic methods to quantify gene flow between populations (e.g. Slatkin 1981, 1985; Singh and Rhomberg 1987), and the results are consistent with the fact that rapid long-distance dispersal can occur between widely spaced populations.

Nonetheless, such rapid long-distance dispersal between populations is relatively rare, and lower estimates of dispersal rate of *Drosophila* of the order of tens of metres are far more frequently observed. It therefore seems reasonable to conclude that adaptations observed in populations sampled from along long-

established clines of *Drosophila* reflect adaptation to local environmental conditions only in the immediate area of the cline from which they were sampled.

Latitudinal clines have been observed in numerous characters for several different species of ectotherms across the world. To confirm that these clines are genetic, rather than developmental, in nature, individuals from along the cline have to be removed from the natural environment and reared in a single control environment, and examined to see whether the observed phenotypic cline persists. If the phenotypic trend remains, upon rearing in the control environment, then this confirms that there is a genetic component to the cline. The presence of a genetic component in several clines in response to the same spatially varying selection pressure can be seen as evidence that the cline is maintained by natural selection.

There follows an account of traits observed in clines of ectotherms.

1.5.1 Body Size in Ectotherms

Body size, as inferred from various morphological measurements, has often been investigated along geographic clines, and latitudinal clines of body size characters have been observed in a wide variety of ectotherm species. Larger body size has been consistently observed in populations that have evolved at higher latitudes in several species; *Apis mellifera* (honey bee) (Alpatov 1929); *Musca domestica* (house fly) (Bryant 1977); *Myrmeleon immaculatus* (ant lion) (Arnett and Gotelli 1999); *Scottolana canadensis* (a crustacean copepod) (Lonsdale and Levinton 1985); and *Littorina obtusata* (an intertidal snail) (Trussell 2000).

Altitudinal clines have been observed in a number of species too, with larger larval body size observed at higher altitudes, as well as at higher latitudes, in the frog

species *Rana climitans* and *Rana sylvatica* (Berven *et al.* 1979; Berven 1982b; Berven and Gill 1983; Riha and Berven 1991). The similarity of these responses suggests that a similar environmental cue is responsible for both the latitudinal and altitudinal variation in body size.

1.5.2 Growth Rate in Ectotherms

Growth rate is a crucial factor affecting the early fitness of an organism, as a rapid growth rate will allow an individual to achieve maturity earlier. However, rapid growth rate is associated with a higher juvenile mortality rate, as is observed in male birds and mammals relative to slower-developing females (Clutton-Brock *et al.* 1985). If a more rapid growth rate is associated with a higher mortality rate, then it would follow that a higher growth rate would be disadvantageous when food is in short supply.

Latitudinal clines of growth rate have been observed in several ectothermic species, with increased growth rate at higher latitudes; *Myrmeleon immaculatus* (ant lion) (Arnett and Gotelli 1999); *Scottolana canadensis* (a crustacean copepod) (Lonsdale and Levinton 1985); and several species of fish (*Morone saxatilis* (striped bass) (Conover *et al.* 1997); *Fundulus heteroclitus* (mummichog) (Schultz *et al.* 1996); and *Menidia menidia* (Atlantic silverside) (Conover and Present 1990)). As with body size, altitudinal and latitudinal clines of growth rate in the frog species *Rana climitans* and *Rana sylvatica* have been observed, with more rapid growth rate observed at higher latitudes and altitudes (Berven *et al.* 1979; Berven 1982b; Berven and Gill 1983; Riha and Berven 1991).

1.5.3 Development Time in Ectotherms

Development time, like growth rate, will greatly influence the fitness of an individual, since individuals with shorter development times will reach maturity before those with longer development times. However, reduced development time can be associated with increased pre-adult mortality in *Drosophila* (Chippindale *et al.* 1997). Development time has been observed to decrease at higher latitudes in the ant lion (*Myrmeleon immaculatus*) (Arnett and Gotelli 1999). Similarly, in the frog species *Rana climitans* and *Rana sylvatica*, individuals from both higher altitudes and latitudes develop more rapidly (Berven *et al.* 1979; Berven 1982b; Berven and Gill 1983; Riha and Berven 1991).

1.5.4 Growth Efficiency in Ectotherms

The efficiency with which an individual can use limited resources to grow will obviously greatly affect its fitness. Growth efficiency, defined as the total weight gain per unit of food consumed, has been most thoroughly investigated in fish species, and has been observed to increase with latitude in the Atlantic silverside (*Menidia menidia*) (Present and Conover 1992), such that a fish from a higher latitude can grow more efficiently than one from a lower latitude. A cline of growth efficiency would, in part, explain the observed latitudinal cline in growth rate in the Atlantic silverside (Conover and Present 1990), however, food consumption has also been observed to increase with latitude (Present and Conover 1992; Billerbeck *et al.* 2000).

1.5.5 Metabolic Rate of Ectotherms

The metabolic rate of organisms influences all of the traits observed in the latitudinal clines described above, and a study of metabolic rate along these clines would be extremely useful in understanding the observed trends. Very few studies have been carried out to date rearing individuals from along clines at a common temperature, to confirm genetic contribution to the observed clines (see review by Garland and Adolph 1991). In the Atlantic silverside, oxygen consumption has been observed to be greater in individuals adapted to higher latitudes, but only at some experimental temperatures (Billerbeck *et al.* 2000). At lower temperatures, no difference in oxygen consumption was observed between populations from different latitudes. This suggests that there is counter-gradient selection acting on metabolic rate, such that elevated oxygen consumption of the high latitude populations is eliminated by environmental temperature.

1.5.6 Egg Size in Ectotherms

Latitudinal variation in egg size has been observed in several ectotherm species, with individuals from higher latitudes producing larger eggs; the water strider *Aquarius remigis* (Blanckenhorn and Fairbairn 1995) and the wood frog *Rana sylvatica* (Berven 1982a).

1.6 Geographic Clines in *Drosophila*

By far the majority of studies investigating clinal variation in ectotherms has been carried out on fruit fly species belonging to the *Drosophila* genus. *Drosophila* are found world-wide, and express clinal variation in numerous characters across geographical regions. The ubiquity of the *Drosophila* genus, and particularly the genetically well-understood species *Drosophila melanogaster*, makes it an ideal model organism for investigation of clinal variation.

The existence of similar clines in different continents allows for the comparison between the continents, and thereby permits investigation of the adaptive nature of the clines. Variation along latitudinal clines for body size, development time, egg size and ovariole number have all been unambiguously observed along clines of *Drosophila melanogaster* in different continents. The observed variation along clines persists when laboratory studies are carried out, rearing lines from different latitudes in a common environment, implying that the variation is genetic in nature, rather than due to environmental cues. The fact that similar genetic clines for these phenotypic characters are observed in different continents implies that the observed clinal variation is due to adaptation to their environment, rather than simply genetic drift. This observation has been confirmed by Gockel *et al.* 2001 in a study which investigated whether there was a correlation between phenotypic variation and presumably neutral molecular variation, in an east Australian cline of *Drosophila melanogaster*. This study found no such correlation, and hence strongly suggested that the observed phenotypic variation was shaped by selection pressure.

The phenotypic characters observed to vary with latitude might either be due to a pleiotropic phenomenon, where selection has acted on just a single trait that is

correlated to the other characteristics, or the independent action of selection on each of the traits individually. To determine whether or not natural selection acts upon a single trait alone is extremely challenging. Lines produced by artificial selection for the specific trait, and lines collected from nature which express genetic differences for that trait, will all exhibit changes in other traits, rendering the role of the specific trait under investigation impossible to disentangle. One unambiguous way to investigate the importance of a specific trait is to manipulate the trait by artificial selection, while ensuring that potentially confounding factors remain unaffected by the selection (see Chapter 3 introduction).

1.6.1 Body Size in *Drosophila*

Geographic clines of body size have been observed throughout the world in several different species of *Drosophila*, with individuals that have evolved at higher latitudes achieving a larger body size than those that have evolved at lower latitudes. Such latitudinal variation of body size has been found in *D. robusta* (Stalker and Carson 1947), *D. simulans* (David and Bocquet 1975b; Watada *et al.* 1986; Capy *et al.* 1993), *D. subobscura* (Misra and Reeve 1964; Huey *et al.* 2000), *D. kikkawai* (Karan *et al.* 1998c; Parkash *et al.* 1999), *D. obscura* (Pegueroles *et al.* 1995), as well as in *D. melanogaster*.

In *Drosophila melanogaster*, latitudinal variation in body size has been observed throughout the geographic range of the species. Latitudinal clines of body size have been observed in Australia (James and Partridge 1995), South America (van't Land 1997), western Europe and Africa (David and Bocquet 1975b), Japan

(Watada *et al.* 1986), North America (Coyne and Beecham 1987) and eastern Europe and central Asia (Imasheva *et al.* 1994).

One study has observed a different relationship between body size and latitude from these latitudinal clines, however. In a central continental North American cline of *D. melanogaster*, larger flies were found in central latitudes, with smaller flies at both cline ends (Long and Singh 1995).

1.6.2 Development Time in *Drosophila*

Development time, the time taken for an adult fly to develop from an egg, has also been observed to vary genetically with evolution at different latitudes in *Drosophila*. In laboratory selection experiments, selection for increased body size was correlated to an increase in development time (Partridge and Fowler 1993; Partridge *et al.* 1999).

In latitudinal clines of *Drosophila*, however, development rate has been observed to vary, with flies from higher latitudes developing faster, and therefore achieving a larger adult size in a shorter period of time. This observation has been made for both Australian (James and Partridge 1995) and South American (van't Land *et al.* 1999) clines. These observations show a correlation between size and development time opposite to that observed in artificial selection experiments.

1.6.3 Egg Size in *Drosophila*

Egg size has been found to vary along latitudinal clines of *D. melanogaster* from both South America and Australia, with larger eggs being laid by female flies from higher latitudes (Azevedo *et al.* 1996). Egg size itself is an important life-history characteristic, giving an indication of maternal investment in offspring, and is highly correlated with offspring fitness (Azevedo *et al.* 1997).

1.6.4 Ovariole Number in *Drosophila*

Ovariole number in *Drosophila* is an important life-history characteristic, as it is correlated to the daily rate of egg laying in female flies, although the relationship is non-linear (David 1970; Bouletreau-Merle *et al.* 1982). Ovariole number has been observed to be greater in lines that have evolved at higher latitudes.

Latitudinal clines in ovariole number in *D. melanogaster* from Australia, Europe, Africa, America and Japan have been observed (David and Bocquet 1975a,b; Watada *et al.* 1986; Azevedo *et al.* 1996). Clines for ovariole number in *D. simulans* in Europe, Africa and America (David and Bocquet 1975b; Watada *et al.* 1986; Capy *et al.* 1993) and *D. kikkawai* in India (Karan *et al.* 1998c; Parkash *et al.* 1998) have also been observed.

1.6.5 Starvation Resistance and Fat Content in *Drosophila*

There is significant evidence for latitudinal variation in traits related to environmental stress, such as starvation resistance, which is in turn strongly correlated with fat content in *Drosophila* (David *et al.* 1975; Zwaan *et al.* 1991;

Zwaan *et al.* 1995a,b), although metabolic rate variation can affect this correlation (Hoffmann and Parsons 1989).

A summary of research into desiccation and starvation resistance has been carried out by Hoffmann and Harshman (1999). Work on latitudinal variation in starvation and fat content has been largely restricted to species of *Drosophila*. Latitudinal clines in starvation resistance have been found in populations of *Drosophila melanogaster*, *D. ananassae*, *D. kikkawai*, and *Zaprionus indianus* in India, with higher starvation resistance occurring at lower (tropical) latitudes (Karan *et al.* 1998a; Karan and Parkash 1998). Other studies in India have also found latitudinal trends in starvation resistance in smaller numbers of populations of *Drosophila melanogaster* (Shamina *et al.* 1993), *D. kikkawai* (Parkash and Vandna 1994), *D. bipectinata* and *D. malerkotliana* (Parkash *et al.* 1994).

There is little evidence for latitudinal clines of starvation resistance outside of these Indian clines, although inter-population differences have been observed in other countries (e.g. Da Lage *et al.* 1990). Also, there is weak evidence for a latitudinal cline of starvation resistance in an Eastern Australian cline of *Drosophila melanogaster*, but only in one sex (Hoffmann *et al.* 2001). A recent study examining starvation resistance and fat content along a South American cline found no evidence for a latitudinal cline in either trait (Robinson *et al.* 2000).

1.6.6 Desiccation Resistance in *Drosophila*

Like starvation resistance, desiccation resistance is associated with environmental stress. Much of the research into desiccation resistance has been carried out on the same populations and species as starvation resistance (Shamina et al. 1993; Parkash

et al. 1994; Parkash and Vandna 1994; Karan *et al.* 1998a; Karan and Parkash 1998). Clines of desiccation resistance were observed in all of these populations to run in the opposite direction to those of starvation resistance, with desiccation resistance higher with higher (temperate) latitudes. Also, like starvation resistance, clines in desiccation resistance have so far only been found in India. A recent study found no evidence for desiccation resistance in an Eastern Australian cline of *Drosophila melanogaster* (Hoffmann et al. 2001).

1.6.7 Longevity in *Drosophila*

Remarkably little research has been carried out on differences in longevity along clines of *Drosophila.* One study reports a difference in longevity between the extreme ends of a North American cline of *D. melanogaster*, though provides no data to support this observation (Matzkin and Eanes, unpublished data, reported in Schmidt *et al.* 2000).

There is, however, significant evidence pointing to a relationship between stress resistance and longevity in *Drosophila melanogaster*, which implies that where a cline of stress resistance traits has been observed, we might expect to find a cline of longevity. Long-lived selection lines have been observed to be more resistant to both starvation and desiccation stress (Rose and Archer 1996). Long-lived mutant lines also tend to be more stress resistant (Lin and Benzer 1998), and selection for stress resistance can lead to increased longevity (Rose *et al.* 1992; Hoffmann and Parsons 1993). However, these trends are not always observed. One set of *D. melanogaster* lines selected for increased starvation resistance, were not observed to show a correlated change in longevity (Harshman *et al.* 1999). Similarly, one study of long-

lived lines, did not show increased stress resistance (Force *et al.* 1995), and another study observed a correlated change in stress resistance in response to selection for reduced longevity, but not increased longevity (Zwaan *et al.* 1995b). This is clearly a field that requires more attention, and there is no evidence to suggest whether or not wild lines showing stress resistance express greater longevity.

1.6.8 Fecundity in *Drosophila*

Only one study has investigated lifetime fecundity of *Drosophila* from tropical and temperature populations, and concluded that *Drosophila* from tropical populations have decreased fecundity in laboratory conditions relative to temperate populations (Bouletreau-Merle *et al.* 1982). However, this study only examined two unreplicated populations from France and North Africa, and hence these results might not reflect an overall latitudinal trend (see Chapter 5).

1.6.9 Metabolic Rate in *Drosophila*

When measured at high temperatures, the metabolic rate of *Drosophila melanogaster* has been observed to be higher in populations that have evolved at higher latitudes (Giesel *et al.* 1991; Berrigan and Partridge 1997). It has also been observed that the metabolic rate of high latitude populations is more sensitive to increases in temperature (Giesel *et al.* 1991). This higher metabolic rate in high latitude populations could go some way to explaining differences in growth rate and efficiency at different latitudes. However, differences in growth rate between

populations have been found to be unrelated to differences in metabolic rate (de Moed *et al.* 1998).

1.6.10 Molecular Evidence of Clines in *Drosophila*

There are several pieces of molecular evidence that confirm genetic variation along clinal populations of *Drosophila melanogaster*; namely chromosome inversions, allozyme frequencies, and DNA sequence polymorphism.

The species shows a high degree of polymorphism for chromosome inversions (Mourad and Mallah 1960; Watanabe 1967; Singh and Das 1990). Clines in their frequency have been found throughout the world, and the repeatability of the clines suggests that there is an adaptive value to them.

One inversion in particular, *In(2L)t*, has been found to be higher in frequency at higher latitudes in parts of the world; in Japan (Inoue and Watanabe 1979), Australasia (Knibb *et al.* 1981), India (Singh and Das 1990) and South America (van't Land *et al.* 2000). However, frequency of the *In(2L)t* inversion in the United States has been found to be higher both in more northerly (Stalker 1976) and southerly (Mettler *et al.* 1977) populations. Most evidence points to a world-wide cline in *In(2L)t* frequency, however the evidence is less persuasive than for phenotypic traits. Additionally, inversions are lost rapidly when lines are stored in laboratory conditions (Inoue 1979). This suggests that the inversions are not related to clinal genetic variation in phenotypic traits, which are maintained even after a long period of laboratory culture.

Clines in allozyme frequency, particularly *Adh* and *α-Gpdh* have been observed (Vigue and Johnson 1973; Oakeshott *et al.* 1982). There is significant

linkage disequilibrium between these loci and the *In(2L)t* inversion (Langley *et al.* 1974; Mukai *et al.* 1974; Alahiotis *et al.* 1976; Watanabe and Watanabe 1977; Van Delden and Kamping 1989). Although evidence suggests that the clines in inversion and allozyme frequency are linked (Voelker *et al.* 1978; Oakeshott *et al.* 1982; Knibb 1983), inversion clines do not correlate with the entire span of the allozyme cline (Voelker *et al.* 1978; but see Knibb 1983).

Polymorphic variation at five SNP (single nucleotide polymorphism) sites of the *Drosophila methuselah* (*mth*) gene has been observed along a North American cline of *Drosophila melanogaster* (Schmidt *et al.* 2000). This gene was examined, as it is a candidate gene implicated in ageing, and some evidence had been found to suggest variation in longevity along this cline (Matzkin and Eanes, unpublished data, reported in Schmidt *et al.* 2000).

1.6.11 Summary of Clinal Traits in *Drosophila*

A large number of traits vary clinally in species of *Drosophila*, and it is particularly noticeable that flies from higher latitudes seem to be at an unconditional advantage. Flies adapted to higher latitudes are larger, achieve this larger size in a shorter development time, lay larger eggs, and have more ovarioles. Since all of these traits would be advantageous to a fly living at any temperature, this implies that selection must be acting to prevent the evolution of these traits in populations at low latitudes. However, despite many experiments, little evidence has been found for fitness trade-offs in clines of *Drosophila*. No evidence has been found for variation in competitive ability along clines (James and Partridge 1998). However, body size of high latitude populations was observed to be more sensitive to population density and temperature

increases, suggesting that the efficient growth of high latitude populations might be disrupted, to an extent, by stressful conditions such as higher density and temperature. If wild populations of *Drosophila* from low latitudes typically experience high densities and temperatures, then this might go some way to explaining the observed clines. Further investigation into conditions experienced by *Drosophila* in the wild would help determine the factors that maintain the cline.

1.7 Environmental Variation along Latitudes

The observed genetic variation in several characteristics along latitudinal clines is an adaptation to environmental cues. Numerous environmental factors vary with latitude, including temperature, humidity, rainfall, food availability, levels of competition, day length, levels of predation and number of generations per breeding season.

The evidence implicating temperature as the main factor in the evolution of latitudinal variation is quite significant, since similar variations are observed along altitudinal clines and with seasonal variation (Stalker and Carson 1948, 1949; Tantawy 1964; Berven 1982b). Temperature is correlated both with altitude and season, as well as with latitude. Temperature has also been observed to have a major effect on the development of ectotherms (Atkinson 1994).

In order to confirm that temperature is the main factor responsible for the observed evolution of latitudinal variation, laboratory selection experiments have to be carried out in which environmental temperature alone is manipulated, while all other factors are controlled. Such experiments involve organisms being kept under controlled temperature conditions for a large number of generations. These

experiments have been repeated several times, and present evidence confirming that temperature alone has significant and comparable effects on several of the traits observed in latitudinal and altitudinal clines.

1.8 Laboratory Thermal Selection in *Drosophila*

The effect of laboratory thermal selection in *Drosophila* is very similar to that observed in latitudinal clines. *Drosophila melanogaster* and *Drosophila pseudoobscura* both increase in size genetically when allowed to adapt to lower temperatures in the laboratory (Anderson 1966; Cavicchi *et al.* 1985; Partridge *et al.* 1994a). Replicate selection lines were used in these studies to confirm that the observed differences between lines, which were adapted to different temperatures, were due to selection in response to environmental cues, and not genetic drift. A non-replicated study using *D. willistoni* also found an increase in size with evolution at lower temperatures (Powell 1974).

This genetic increase in body size with adaptation to lower temperatures of *D. melanogaster* and *D. pseudoobscura* was associated with a decrease in development time (Anderson 1966; Partridge *et al.* 1994b; James and Partridge 1995), such that flies can develop to reach a larger body size in a shorter period of time. Egg size is also greater in lines of *D. melanogaster* that have adapted to lower environmental temperatures (Azevedo *et al.* 1996). Lines adapted to low temperatures have increased larval growth efficiency relative to lines that have evolved at higher temperatures (Neat *et al.* 1995), just as lines from higher latitudes have greater larval growth efficiency than those reared at lower latitudes (Robinson and Partridge 2001).

All four of these traits (body size, development time, egg size and larval growth efficiency) respond in the same way to laboratory thermal selection as they do in natural geographic clines. This strongly implies that temperature is involved in the evolutionary response to latitudinal variation.

A further study investigated fecundity and longevity of replicated populations of *Drosophila melanogaster* that had been adapted to two different thermal environments in the laboratory (Partridge *et al.* 1995). This study found that flies expressed increased fecundity and longevity when reared and tested at the thermal environment to which they were adapted, relative to the lines adapted to the other thermal environment.

1.9 Developmental Effect of Temperature

In addition to the evolutionary effects of temperature, the temperature at which an ectotherm develops influences its phenotype. The variation in phenotype that a single genotype can produce in response to different environmental conditions is known as phenotypic plasticity (Bradshaw 1965). The range of phenotypes that can be adopted by a single genotype in response to a specified set of environments is termed its norm of reaction (Woltereck 1909; Stearns 1989).

Alteration of developmental temperature has been observed to affect several phenotypic characters in ectotherms.

1.9.1 Developmental Temperature and Body Size

The temperature at which an ectotherm develops can have a very significant effect on its size, with body size increasing at lower rearing temperatures (see Ray 1960 and Atkinson 1994 for reviews, or individual studies on species; e.g. ciliates (Lee and Fenchel 1972); protozoa (Finlay 1977; Rogerson 1981); an intertidal snail (Trussell 2000); *Drosophila* sp. (David *et al.* 1983, 1994; Scheiner and Lyman 1989; Partridge *et al.* 1994a; Noach *et al.* 1996)). Ray (1960) examined the effect of altering developmental temperature on seventeen species of ectotherm, including several species of *Drosophila*, and found that 75% of the species increased in size at lower rearing temperatures. Atkinson (1994) surveyed the effect of rearing temperature in 109 studies of ectotherms, plants and protists, and found evidence for a developmental effect of temperature on body size in over 80% of the organisms examined.

All species of *Drosophila* examined have shown an increase in body size with development at lower environmental temperatures. However, the actual shape of the relationship between temperature and body size is parabolic, with body size decreasing again if rearing temperature is reduced below a certain point (e.g. in *Drosophila melanogaster* and *Zaprionus indianus* (David *et al.* 1994; Karan *et al.* 1998b, 1999a,b)).

The increase in body size in ectotherms with rearing at lower temperatures is similar to the pattern observed in endotherms, known as Bergmann's rule (Bergmann 1847). Bergmann's rule states that, in warm-blooded organisms, lower environmental temperature leads to the evolution of a genetically larger body size, and therefore a lower relative surface area, reducing heat loss through the skin. The application of

this rule to ectotherms is extremely questionable, particularly in the case of small ectotherms, such as *Drosophila*. Small ectotherms cannot thermoregulate, and due to their size, will readily adopt the temperature of their surroundings, so the explanation for size increase at lower temperature to avoid heat loss is insufficient (Stevenson 1985).

The vast majority of evidence suggests that increases in body size in response to developmental temperature in ectotherms can be attributed to an increase in cell size. Cell size has been found to increase with increasing rearing temperature in the nematode *Caenorhabditis elegans* and in fish (van Voorhies 1996). In *Drosophila melanogaster*, body size increase in response to decreased temperature is achieved through an increase in cell size, with little or no change in cell number (studies examining wing area: Alpatov 1930; Robertson 1959; Delcour and Lints 1966; Masry and Robertson 1979; Cavicchi *et al.* 1985; Partridge *et al.* 1994a,b; de Moed *et al.* 1997; James *et al.* 1997; other body parts: R. Azevedo, L. Partridge and V. French, unpublished data).

1.9.2 Developmental Temperature, Development Time and Growth Rate

In ectotherms, in general, development time increases and growth rate decreases at lower developmental temperatures, such that at lower rearing temperatures ectotherms reach a larger adult size, but take longer to achieve that size (Atkinson 1994). Growth rate has been observed to slow at lower environmental temperatures in *Fundulus heteroclitus*, *Morone saxatilis*, *Menidia menidia* and *Rana sylvatica* (Conover and Present 1990; Riha and Berven 1991; Schultz *et al.* 1996; Conover *et al.* 1997). Development time has been observed to increase with decreasing

temperature in several ectothermic species including *Drosophila melanogaster*, *Sepsis cynipsea* and *Scathophaga stercoraria* (Schultz *et al.* 1996; James *et al.* 1997; Blanckenhorn 1999).

1.9.3 Developmental Temperature and Growth Efficiency

Body size and development time both increase when ectotherms are reared at lower temperatures, so an individual organism gets larger, but takes longer to achieve that size. If lower temperatures led to increased growth efficiency, this would allow a larger size to be achieved at lower temperatures. In the Atlantic silverside, growth efficiency is greater at lower temperatures (Present and Conover 1992). Larval growth efficiency in *Drosophila melanogaster* has also been observed to be higher at lower environmental temperatures (Robinson and Partridge 2001).

1.10 Plasticity of Body Size in *Drosophila*

The observed plasticity of body size in response to environmental temperature could be adaptive, if the fitness of the different phenotypes vary with the environment, or could be a non-adaptive, passive effect. Determining whether or not observed phenotypic plasticity is adaptive is extremely difficult (Gotthard and Nylin 1995), since it must be shown to confer an advantage to the plastic organism.

Were the phenotypic plasticity adaptive, this would allow a single genotype to maintain its fitness in a range of environments (Bradshaw 1965; Thompson 1991). Several models argue that, under varying environmental conditions and in the absence of genetic restraints, reaction norms should evolve towards a response that

gives an optimal phenotype in each environment (Via and Lande 1985; de Jong 1990, 1999; Gomulkiewicz and Kirkpatrick 1992; Gavrilets and Scheiner 1993).

Two lines of evidence go against the idea that larger adult body size is an adaptation to lower temperature in *Drosophila.* Firstly, if larger adult size were adaptive at lower temperatures, then temperature during the pre-adult growth period would act as a cue to predict adulthood thermal conditions, as observed in temperature-dependent sex-determination. However, temperature does not act as a developmental switch for determining adult body size in *Drosophila.* Instead, there is a cumulative effect of temperature during growth on body size, with no evidence for a particular sensitive period (Masry and Robertson 1979; David *et al.* 1983; French *et al.* 1998). Secondly, if there was an optimal adult size for each temperature, then we might expect all genotypes to use a combination of genetic body size and plasticity to achieve that optimum. However, this is not what is observed. The adult body size achieved by full growth at a specific temperature differs between genotypes that have evolved at different latitudes or different temperatures in the laboratory.

The reaction norm of body size in response to temperature in *D. melanogaster* could evolve in nature, because genetic variation for phenotypic plasticity has been demonstrated (Scheiner and Lyman 1989; David *et al.* 1994; Noach *et al.* 1996), and artificial selection for increased and decreased phenotypic plasticity has been successfully carried out (Scheiner and Lyman 1991). However, the norm of reaction of body size in *Drosophila* appears not to evolve in any consistent way, either in latitudinal clines (e.g. James *et al.* 1997; Morin *et al.* 1999), or in laboratory thermal selection lines (e.g. Partridge *et al.* 1994a). The main evolutionary response of body size to temperature, both in nature and in the

laboratory, is in mean body size, with at most minor effect on the degree of plasticity.

1.11 Outline of Thesis

In this thesis, I investigate the relationship between body size and temperature in *Drosophila*, using lines that have been artifically selected for increased and decreased body size, lines that have adapted to thermal regimes in the laboratory, and populations originating from along a cline in Eastern Australia. My aim was to increase our understanding of the relationship between body size and temperature, and to investigate other traits along latitudinal clines.

In Chapter 3, I investigated whether larger body size is favoured at lower temperature through the action of natural selection, at least in part, on adult male fitness. Life span and age-specific fertility of male flies were examined in lines that had been artificially selected for increased and decreased body size. A similar study (McCabe and Partridge 1997) had previously found that larger body size led to increased female fitness, and so these results establish whether male body size merely changes because it is correlated to female body size, or because changes in body size also influence adult male fitness.

In Chapter 4, I investigated the evolution of plasticity of body size components, by examining the norm of reaction of cell size and cell number, as well as body size itself, in populations of *Drosophila melanogaster* that had been adapted to constant thermal regimes or thermal regimes which varied between two temperatures, when reared at two different temperatures. This investigation examined whether levels of plasticity were any greater in individuals that had been exposed to

variable thermal regimes. In addition to examining body size components, I also examined larval development time in the lines adapted to constant and variable thermal environments.

In Chapter 5, I examined the longevity and fecundity of populations collected from along a cline in Eastern Australia, which had previously been shown to exhibit a cline for body size (W. J. Kennington, pers. comm.) and a weak cline for starvation resistance (Hoffmann *et al.* 2001). These results established whether adaptive variation along the cline led to genetic differences in longevity. Additionally, the results confirm whether or not increased starvation resistance in wild populations is correlated with increased longevity.

2. General Materials and Methods

2.1 Populations of *Drosophila melanogaster*

2.1.1 Body size selection lines (Chapter 3)

These lines were used to investigate the effect of selecting for body size on male fitness. The body size selection lines were produced from a random-bred, wild-type population collected in Dahomey (now Benin), West Africa, in 1970. This population was maintained in cage culture at 25°C, until 1996, when nine egg samples were taken from the population. These nine samples were used to establish three replicate large-, control- and small-sized lines by artificially selecting for wing area, while maintaining a constant cell area, in accordance with the pattern of latitudinal variation in size (James *et al.* 1995).

For each replicate line, the wing area and cell area of 25 pairs of flies were measured in each generation. For the control lines, 10 males and 10 females were selected at random to be the parents of the next generation. For the large- and small-sized selection lines, flies with the ten largest or smallest wing areas were selected as the parents of the next generation, while ensuring that the mean cell area of the pairs was not significantly different from the mean cell area of the controls. Each replicate line was propagated by setting up 100 first instar larvae in a bottle of 70ml of medium seeded with live yeast. Selection was continued for eight generations, after which the lines were maintained in bottle culture at 18°C (see McCabe & Partridge, 1997; McCabe *et al.* 1997).

2.1.2 *Scarlet* population (Chapter 3)

This population was used as a competitor stock for the males of the body size selection lines (Section 2.1.1). The population was produced by crossing flies from two populations bearing the recessive mutant marker *scarlet* in an approximately Dahomey genetic background, three generations before the experiment, and then allowing the progeny to mate randomly. The original two *scarlet* populations were produced by the back-crossing of a *scarlet* mutant into a Dahomey background in 1988. Following the production of the lines, they were maintained in population cage culture at 25°C.

2.1.3 Thermal selection lines (Chapter 4)

These lines were used to investigate body size, cell size, cell number and larval development time, and the plasticity of these traits, in flies which had evolved under constant and variable temperature regimes.

The thermal selection lines also originated from the collection of *Drosophila melanogaster* made in Dahomey, West Africa in 1970. In September 1994, twelve cages were established from samples taken from the Dahomey population, and randomly allocated to four different thermal treatments. The first two treatments were constant temperatures of 18 and 25°C. Two variable temperature regimes were also initiated, experiencing temperatures of both 18 and 25°C. These cycling lines spent less time at 25°C to compensate for the increased rate of living at this higher temperature. One cycling regime changed temperature each day (short cycle), with

10 hours at 25°C and 14 hours at 18°C. In the other cycling regime (long cycle), flies spent 7.2 weeks at 25°C and 10 weeks at 18°C.

2.1.4 Australian populations (Chapter 5)

These lines were used to investigate the fecundity and longevity of populations along a latitudinal cline in Eastern Australia.

The eight Australian populations were derived from a series of populations collected in January and February 2000 by J. Gockel, W. J. Kennington and L. Partridge (University College London) and A. A. Hoffmann (La Trobe University, Melbourne) along a latitudinal range from 16.09°S to 43.09°S (Table 2.1). In order to standardise for factors other than latitude, the collection sites were chosen to be at similar longitudes and altitudes, and at similar distances from the sea. The longitudes of the collection sites varied between 145.10°W and 153.49°W, and the altitude of all collection sites was less than 100m. Between five and eight isofemale lines were maintained per collection site in vials at 25°C for a year before the experiment was carried out.

Virgin flies were collected from each isofemale line and randomly mated, in bottle culture. After two generations of out-breeding, the resulting populations were used in the experiment.

Table 2.1 *The locations and latitudes of the collection sites of the Australian populations*

Population	Location	Latitude (°S)
MEG	Cape Tribulation, Queensland	16.09
TOW	Townsville, Queensland	19.29
ROC	Rockhampton, Queensland	23.40
COO	Coolangata, Queensland	28.14
BEL	Belmont, New South Wales	33.00
MIL	Milton, New South Wales	35.31
YY	Yin Yan, Victoria	37.55
HUO	Huonville, Tasmania	43.09

2.2 Rearing Methods

2.2.1 Culture media

2.2.1.1 Standard food (ASG)

85g sugar

60g maize meal

20g dried yeast

10g agar

25ml of 10% Nipagin solution in ethanol

1 litre of water

This medium was used for maintaining bottle populations and also used for standard density rearing.

2.2.1.2 Grape juice medium

50g agar

600ml grape juice

1 litre water

42.5ml of 10% Nipagin solution in ethanol

This medium was used for collecting larvae in laying pots, as they can easily be seen on the surface of the medium.

2.2.2 Stock Maintenance

2.2.2.1 Bottle stocks (body size selection lines, Australian populations)

Flies were transferred to a fresh 1/3 pint bottle containing 70ml of ASG medium. The flies were given a few hours to lay a moderate density of eggs (enough to produce up to 300 emergees) on the surface of the medium, before being removed from the bottle. This process was repeated every 14 days at 25°C, and every 28 days at 18°C, by which time the majority of flies had eclosed.

2.2.2.2 Cage stocks (*scarlet* populations, thermal selection lines)

Three bottles containing 70ml of ASG medium were added each week to each cage, and the three oldest bottles were removed. The number of bottles maintained in a cage depended upon the temperature at which the cage was maintained. Cages maintained at 25°C were kept on a four-week cycle, so that there were always 12 bottles in the cage. Cages maintained at 18°C were kept on a six-week cycle, with 18 bottles in the cage.

The short cycling thermal selection line cages were kept on a five-week cycle, with 15 bottles in the cage. The long cycling thermal selection line cages were kept on a four-week cycle when they were at 25°C, and on a six-week cycle when they were at 18°C, and the number of bottles in the cages was adjusted accordingly.

2.3 General Methods

2.3.1 Fly handling

When the genotype of flies was examined, or flies were manipulated, they were anaesthetised by using carbon dioxide. However, when flies were handled within three hours of eclosion, carbon dioxide was avoided as it can cause bubbling of the gut, reducing fitness. When flies were handled within three hours of eclosion, such as the collection of virgin flies in the experiments described in Chapters 3 and 4, they were anaesthetised by placing them on a glass surface cooled with ice.

2.3.2 Standard density culture using vials (Chapters 3 and 4)

Adult flies were placed in laying pots containing grape juice medium with a dab of live yeast on the surface. After an acclimatisation period of 24 hours, flies were transferred onto fresh medium for a pre-lay period of one hour to encourage laying of any retained eggs. Flies were then transferred to fresh medium for 3 hours at 25°C, or 6 hours at 18°C, for egg collection.

Larvae were then transferred to vials containing approximately 7ml of standard (ASG) medium, using a mounted needle. A standard, uncrowded, larval density of 50 first instar larvae was used in the experiments described in Chapter 3, and of 30 first instar larvae was used in those described in Chapter 4.

2.3.3 Standard density culture using bottles (Chapter 5)

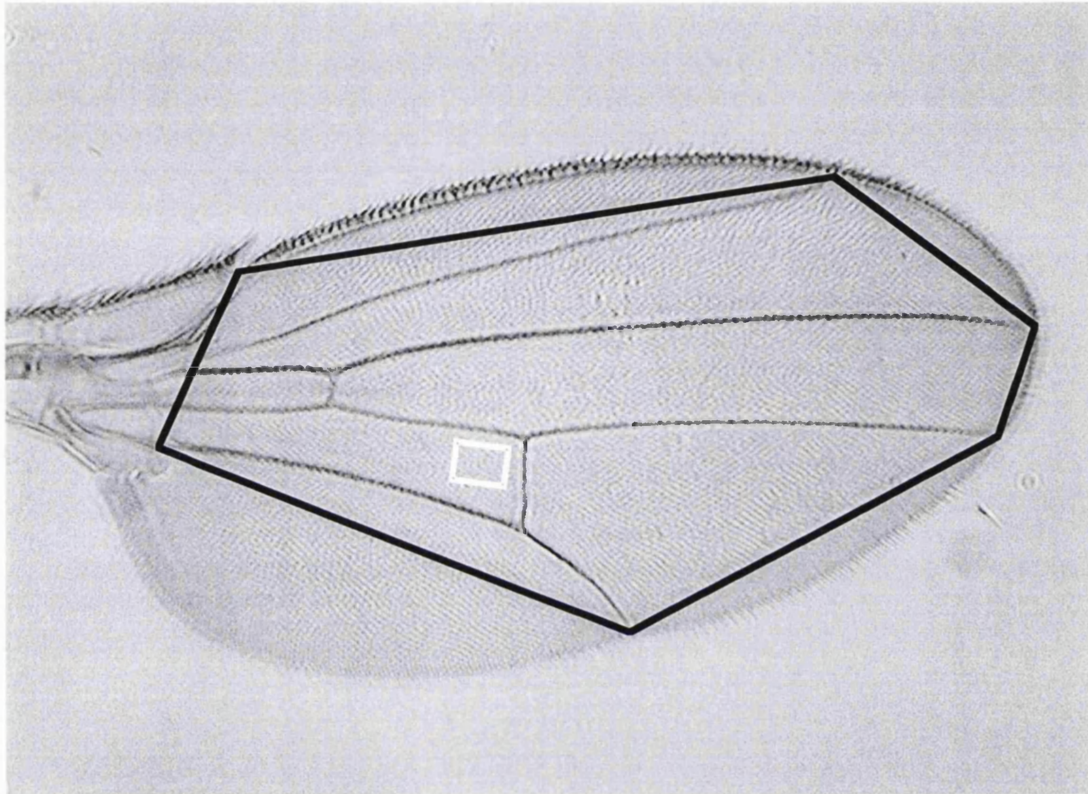
Adult flies were placed in laying pots containing grape juice medium with a dab of live yeast on the surface. After an acclimatisation period of 24 hours, flies were transferred onto fresh medium for a pre-lay of one hour to encourage laying of any retained eggs. Flies were then transferred onto fresh medium for 3 hours at 25°C, or 6 hours at 18°C, for egg collection.

Eggs were then washed off the grape juice medium using PBS (phosphate buffered saline) solution. 2.8ml of the resulting suspension of eggs was then transferred using a Gilson pipette into bottles containing 70ml of standard (ASG) medium. This produced a standard density of approximately 250 to 300 flies per bottle.

2.3.4 Wing area measurement

Adult flies were collected using carbon dioxide anaesthesia, and transferred into Eppendorf tubes to be frozen. The left wings of adult flies were removed, fixed onto slides with propanol, and mounted using Aquamount. Images of the wings were captured on a PowerMacintosh 8600/200 computer with a video camera attached to a compound microscope at 50x magnification. A good approximation of wing area was measured by calculating the area enclosed by six points around the edge of the wing, four of which were points where veins intersected with the edge of the wing (the black line in Fig 2.1). Wing area was calculated using Object Image 1.60p software (by Norbert Vischer, based on the public domain NIH Image program, available at http://simon.bio.uva.nl/object-image.html).

Fig 2.1 *Fly wing. The black line indicates the area used to measure wing area. The white rectangle indicates the area within which measurements of cell density were taken.*



2.3.5 Cell area and cell number measurement

Since each cell in the wing blade produces a bristle (trichome), these can be used to count cells, and thereby provide a measure of cell density. To assess the cell density in fly wings, two images in an area of the wing (within the white rectangle in Fig 2.1) were captured on a PowerMacintosh 8600/200 computer using a video camera attached to a compound microscope at x400 magnification. The total number of trichomes within each image was counted and the average cell density of the two images was used, in conjunction with the area of the whole wing, to calculate an index of cell area and cell number for each wing.

Two images were taken because of minor variation within this region of the wing. Images were captured using Object Image 1.60p software (by Norbert Vischer, based on the public domain NIH Image program, available at http://simon.bio.uva.nl/object-image.html).

2.4 Statistical Analysis

All statistical analysis was performed using JMP 3.2.2 for the Macintosh (SAS 1997)

3. An interaction between environmental temperature and genetic variation for body size for the fitness of adult male *Drosophila melanogaster*

3.1 Abstract

Genetic variation of body size along latitudinal clines is found globally in *Drosophila melanogaster*, with larger individuals encountered at higher latitudes. Temperature has been implicated as a selective agent for these clines, because the body size of laboratory populations allowed to evolve in culture at lower temperatures is larger. In this study, we investigated the hypothesis that larger size is favoured at lower temperature through natural selection on adult males. We measured lifespan and age-specific fertility of males from lines of flies artificially selected for body size, at two different experimental temperatures. There was an interaction between experimental temperature and body size selection for male fitness; large-line males were fitter than controls at both temperatures, but the difference in fitness was greater at the lower experimental temperature. Smaller males did not perform significantly differently from control males at either experimental temperature. The results imply that thermal selection for larger adult males is at least in part responsible for the evolution of larger body size at lower temperatures in this species. The mechanisms responsible require further investigation.

This work has already been published as 'Increased body size confers greater fitness at lower experimental temperature in male* Drosophila melanogaster*' (Reeve, M. W., K. Fowler and L. Partridge. 2000. Journal of Evolutionary Biology 13:836-844.)

3.2 Introduction

Latitudinal clines of numerous phenotypic traits, including body size, have been observed in a wide range of ectotherms (see Introduction, section 1.5), and specifically in *Drosophila* species (see Introduction, section 1.6). Temperature is strongly implicated as the major factor responsible for the evolution of these traits along the cline (see Introduction, section 1.7). However, the identity of the target or targets of selection are not clear, and body size *per se* may not be the target of selection. Body size may either evolve directly in response to temperature, or as a correlated response to thermal selection on another trait that responds to temperature.

To determine whether thermal selection acts on body size, this trait must be manipulated without such correlated responses to thermal selection, and the fitness effects examined. Neither lines produced by laboratory thermal selection (e.g. Partridge *et al.* 1995), nor lines from nature expressing genetic difference in size (e.g. Tantawy and El-Helw 1970), can be used to infer the effects of thermal selection on body size *per se*. Such lines will exhibit differences in other aspects of adaptation to temperature, from which the role of body size cannot be disentangled. Investigation of the effect of body size using rearing temperature to produce variation in size (e.g. Zamudio *et al.* 1995; Nunney and Cheung 1997) is also potentially problematic,

because rearing temperature affects other traits, such as growth efficiency, that influence adult fitness at different temperatures.

One way to approach the problem is to manipulate body size by artificial selection. One study has done this, manipulating body size by selecting for increased and decreased wing area (McCabe and Partridge 1997). In these selection lines cell size was kept constant, in accordance with the pattern of latitudinal variation in size (see Introduction, section 1.9.1). Artificial selection can itself result in correlated responses, but they are in general different from those seen with thermal selection on body size. Neither preadult development time (Azevedo *et al.* 1997) nor growth efficiency (Pelage, McCabe and Partridge, unpubl. data) were altered by artificial selection. These selection lines therefore did not show the pattern of correlated responses to body size change seen with latitudinal and thermal selection (James and Partridge 1995). McCabe and Partridge (1997) demonstrated that the fitness of the adult female stage of life history in *Drosophila melanogaster* is implicated in the evolution of larger size at lower temperature. They found a strong interaction between body size selection and environmental temperature for both survival and lifetime reproductive success, with larger females living relatively longer and producing relatively more offspring than controls at the lower experimental temperature (McCabe and Partridge 1997).

No comparable investigation has been carried out on the adult male stage of the life history. Since the physiology and behaviour of female and male *D. melanogaster* are very different, we might not expect the relationship between their fitness and their body size to respond in the same way to environmental temperature. There are precedents for interactions between sex and temperature in the determination of adult fitness in *Drosophila* (Vieira *et al.* 2000). Males produce

many small gametes, while females produce relatively few large ones, so males are capable of fertilising more eggs than are produced. Males hence compete for females, and are therefore under strong selection to find and court females. The main cost of reproduction in male *Drosophila* is behavioural (Cordts and Partridge 1996), whereas that in females is associated with egg-production and the consequences of mating (e.g. Maynard Smith 1958; Lamb 1964; Chapman *et al.*, 1995). In *Drosophila* there is a quite pronounced sexual dimorphism, with female flies significantly larger than males. Thermal selection on adult body size might therefore be expected to differ between the sexes. Body size of both sexes evolves in response to latitude and laboratory thermal selection, but male size could be a correlated response to selection on females, since there is a strong genetic correlation between the size of the two sexes (Cowley and Atchley 1990).

I have therefore tested the hypothesis that larger size evolves at low temperatures in part through selection on adult males. I looked for an interaction between effects of genetically determined body size and environmental temperature for male longevity and fertility. The replicated selection lines had been artificially selected for increased and decreased wing area (McCabe *et al.* 1997). The selection lines expressed correlated responses in thorax length (McCabe *et al.* 1997), and adult dry weight (Azevedo *et al.* 1997), confirming that the selection on wing size conferred differences in body size. In order to examine male fitness traits, the selected males were competed with a standard, mutant-marked, random-bred competitor strain, to produce a biologically realistic set of measures of adult male competitive reproductive success.

3.3 Materials and Methods

3.3.1 Selection Lines and Competitor Stock

The body size selection lines and *scarlet* competitor stock were used in this experiment (see General Materials and Methods, sections 2.1.1 and 2.1.2 respectively).

3.3.2 Measurement of Male Life-History Traits

In order to assess the fitness of adult male flies from the selection lines, longevity and age-specific fertility were measured. Fertility was estimated as the proportion of wild-type progeny when selection line males competed with *scarlet*-eyed males for matings with *scarlet*-eyed females. Hence, this measurement of fertility incorporates male mating success, sperm competition and fecundity. To determine the influence of environmental temperature, the experiment was carried out at two different temperatures; 18°C and 25°C, with the experimental flies and competitors being reared and tested at the experimental temperature. These measurements were based on those used by Roper *et al.* (1993).

The selection lines and the *scarlet* competitor stock were maintained in standard, low larval density, bottle culture at each experimental temperature for at least two generations prior to the experiment, to avoid the parental and early embryonic effects of temperature and temperature shift (Huey *et al.* 1995; Crill *et al.* 1996). Twenty pairs of flies were taken from each selection line population to be the parents of the experimental flies, and were transferred to laying pots containing

yeasted grape juice medium. After an acclimatory period of 48 hrs at 18°C or 24 hrs at 25°C, the flies were transferred to fresh medium for a 2 hour pre-lay period and then transferred again to fresh medium for egg collection, which lasted for 8 hrs at 18°C and 4 hrs at 25°C. First instar larvae were collected 46 hrs after the midpoint of the lay at 18°C, and 23 hrs after the midpoint at 25°C. Fifty larvae were placed in vials containing 7 ml medium, with four vials per selection line. Collection of emerging virgin male flies from these vials was carried out by anaesthesia over ice within eight hours of eclosion. For the *scarlet* competitor stock, six laying pots were set up as for the selection line populations, each with twenty pairs of flies. Ten vials of fifty larvae were collected from each laying pot. Emerging virgin flies of both sexes were collected from these vials in a similar manner to the selection line flies.

At each temperature, for each selection line, ten virgin *scarlet* females, three selection line males, and seven *scarlet* males were set up in each of thirteen replicate bottles, each containing 70 ml of food medium and active yeast. This design ensured that the selection-line males competed mainly with *scarlet* males, rather than with each other. Flies in each bottle were transferred to fresh bottles every four days at 18°C, and every other day at 25°C, to compensate for the increased rate of living at the higher temperature. The bottles from which the selection line flies were removed were kept at 18°C. Deaths of selection line males were recorded when flies were transferred. All *scarlet* flies were replaced with virgins every four weeks at 18°C, and every two weeks at 25°C. Male fertility was recorded as the proportion of adult progeny that were wild-type emerging from the bottles from which the selection line flies had been removed. A subsample of ten of the thirteen bottles was examined, and the numbers and genotypes of flies in these bottles were maintained by replacing dead flies with individuals from the remaining three bottles. In these three bottles,

dead *scarlet* flies, but not selection line flies, were replaced from a group of virgins. When sufficient death had occurred that fewer than ten bottles could be maintained with a full complement of flies, the number of bottles in the experiment was reduced accordingly.

To provide an estimate of male fitness for each replicate selection line, the total number of wild type flies emerging from all of the bottles from each line was counted. This gave a measure of total lifetime competitive reproductive success for the males of each replicate selection line. This measure was used rather than a measurement of the proportion of progeny sired by the selection line males, because it takes the survival of the selection line males as well as their fertility into account. A measure based on the proportion of wild type progeny produced would reflect only fertility and would also be biased by the declining fertility of the selection line males relative to their younger *scarlet* competitors as the experiment proceeded.

3.3.3 Wing area

To give an indication of the body size of the lines, the wing area of the selection line males used in the experiment was measured. A sample of ten male flies was taken from each of two of the vials of virgin selection line flies collected at the time of initiation of the experiment at each experimental temperature. Wing area was measured as described in the General Materials and Methods (section 2.3.4).

3.3.4 Statistical analysis

The longevity, fertility, fitness and wing size data were subjected to analyses of variance. After transformation of the data, if appropriate, homogeneity of variance was confirmed in all cases with the O'Brien test, which performs an analysis of variance on a new variable, created using group sample variances from the data, to compare spread in the groups. Normality of the error distribution was confirmed with the Shapiro-Wilk test.

The variances among environmental temperatures were significantly heterogeneous for longevity. This among-environment heteroscedasticity was eliminated by transformation following the procedure outlined by Dutilleul and Potvin (1995, equation 6). The transformation eliminates environmental heteroscedasticity without changing the ranking of the trait values across genotypes. Removal of the heteroscedasticity was confirmed using the O'Brien test ($F_{(1,664)}$ = 0.3212, P = 0.5711). The data were subjected to an analysis of variance, treating experimental temperature and selection regime as fixed main effects, and replicate lines as a random effect, nested within selection, and individual fly death times as the unit of replication. The normality of the error distribution of the transformed data set was confirmed using the Shapiro-Wilk test (W = 0.9866, P = 0.6363). Linear contrast analyses were used to compare the longevity of pairs of selection regimes.

The assessment of fertility involved repeated measures on at least some of the same individuals. To minimise pseudoreplication, while examining the temporal patterns of reproduction in lines from different regimes, the data were divided into "early" and "late" phases of adult life. "Early" data consisted of the total number of wild-type flies emerging from each bottle of each line in the first five sampling

intervals, (up to day 20 at 18°C, and day 10 at 25°C), divided by the total number of flies (wild-type and *scarlet*) emerging from the same bottle. "Late" data consisted of the data collected at subsequent sampling intervals, up to the interval at which more than 20% of the flies had died in the replicate line with the highest mortality rate. The homogeneity of variances in the data for the two characters was confirmed using the O'Brien test ($F_{(17,162)} = 1.6387$, $P = 0.2288$ for "early" fertility data $F_{(17,162)} = 1.3555$, $P = 0.1665$ for "late" fertility data). The normality of the error distribution of the "early" and "late" fertility data, were confirmed using the Shapiro-Wilk test ($W = 0.9826$, $P = 0.5412$ and $W = 0.9881$, $P = 0.8779$ respectively). The fertility data were subjected to an analysis of variance, with temperature and selection regime as fixed main effects, and replicate lines as a random effect nested within selection, and with bottles as the unit of replication. Linear contrast analyses were used to compare the fertility of pairs of selection regimes.

Homogeneity of variance of the fitness data was confirmed using the O'Brien test ($F_{(5,12)} = 0.7642$, $P = 0.5927$), and normality of the error terms was confirmed using the Shapiro-Wilk test ($W = 0.9726$, $P = 0.8253$). The fitness data were subjected to a two-way analysis of variance, with temperature and selection as fixed main effects, and replicate lines as the unit of replication.

Homogeneity of variance for the wing size data was confirmed using the O'Brien test ($F_{(17,342)} = 1.2216$, $P = 0.2447$), and normality of the error terms was confirmed using the Shapiro-Wilk test ($W = 0.9790$, $P = 0.1053$). The wing size data were subjected to an analysis of variance, with temperature and selection as fixed main effects, and replicate lines as a random effect nested within selection. There was no significant between-vial variance ($F_{(1,341)} = 0.0007$, $P = 0.9789$), so data from individual body size measurements were used in the analysis.

3.4 Results

3.4.1 Longevity

There were significant effects of both temperature and selection regime upon longevity (Table 3.1, Fig. 3.1). Flies lived significantly longer at the lower experimental temperature. The large-size selection lines lived for significantly longer than flies from the control-size selection lines at both temperatures (18°C: $F_{(1,6)}$ = 63.3563, P = 0.0002; 25°C: $F_{(1,6)}$ = 9.8780, P = 0.0200). The small-size selection lines and the control-size selection lines did not differ significantly from each other in longevity at either temperature (18°C: $F_{(1,6)}$ = 1.7911, P = 0.2293; 25°C: $F_{(1,6)}$ = 0.0026, P = 0.9608). There was a significant interaction between temperature and selection regime, mainly attributable to greater survival of the large-size selection lines relative to the other lines at the lower experimental temperature. There was significant variation between the lines within the selection regimes for survival, but there was no significant interaction between temperature and lines within selection regime.

3.4.2 Male fertility

Both temperature and selection regime had a significant effect on both "early" and the "late" male fertility (Table 3.2, Fig. 3.2). Fertility was higher at the lower experimental temperature. Males from the large-size selection lines sired a greater proportion of progeny than both the small- and control-size selection lines. The small- and control-size selection lines did not differ significantly from one another in

fertility at either experimental temperature, in either phase of adult life. The analysis of variance also revealed a highly significant interaction between temperature and selection regime in both phases, mainly because the large-size selection line males had a greater fertility-advantage at the lower experimental temperature. There was a significant effect on male fertility of lines within selection, but there was no significant interaction between the lines within selection and temperature, for either phase.

Table 3.1 *Two-way nested analysis of variance on longevity of males from the large-, control-, and small-size selected lines reared and tested at 18°C or 25°C. Replicate lines were treated as random effects, nested within selection regime, and selection regime and temperature were analysed as fixed effects.*

effect	MS	d.f.	F ratio	P
Temperature	122095	1	2455.74	<0.0001
Selection regime	3906	2	23.73	<0.02
Lines within selection [A]	165	6	6.68	<0.001
Temperature X selection	652	2	13.11	<0.01
Temperature X lines within selection [B]	50	6	2.02	ns
error [C]	25	648		

ns: $P > 0.05$

[A] Error term for selection regime

[B] Error term for temperature, and temperature X selection regime

[C] Error term for lines within selection and temperature X lines within selection

Fig. 3.1 *Mean lifespan, transformed using the procedure outlined in Dutilleul and Potvin (1995, equation 6), (± 95% CL) of males from the large-, control- and small-size selection lines reared and tested at 18°C or 25°C.*

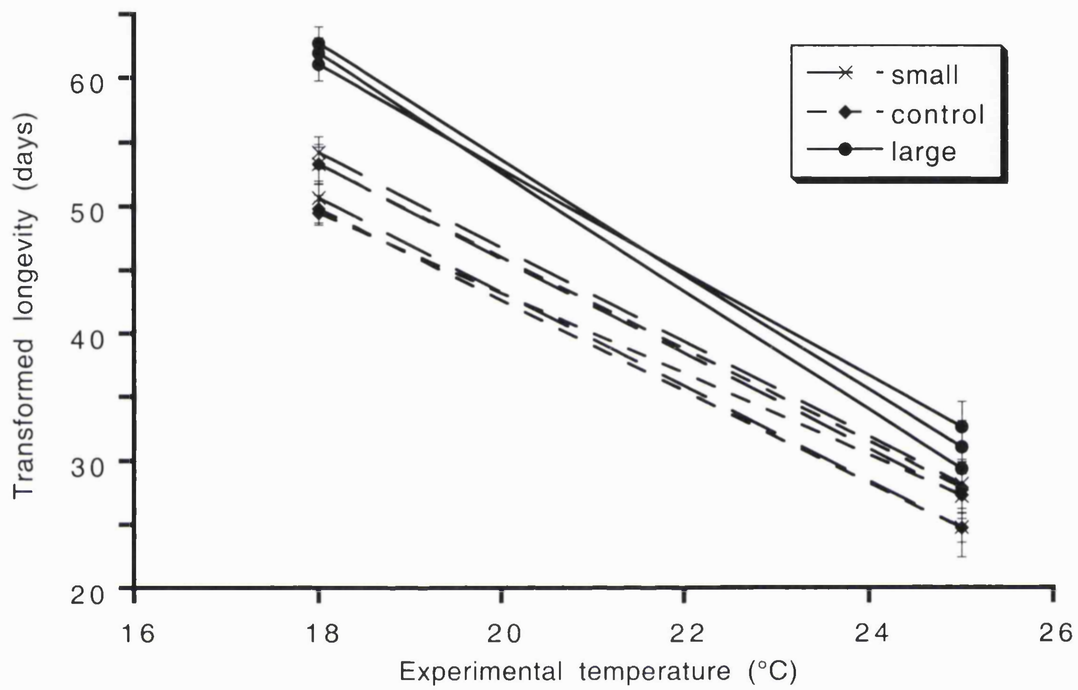


Table 3.2 *Two-way nested analysis of variance on (a) "early" and (b) "late" fertility of males from the large-, control-, and small-size selected lines reared and tested at 18°C or 25°C. Replicate lines were treated as random effects, nested within selection regime, and selection regime and temperature were analysed as fixed effects.*

(a) "early"

effect	MS	d.f.	*F* ratio	*P*
Temperature	0.1426	1	119.50	<0.0001
Selection regime	0.1184	2	17.68	<0.0001
Lines within selection [A]	0.0067	6	2.57	<0.05
Temperature X selection	0.0302	2	25.31	<0.0001
Temperature X lines within selection [B]	0.0012	6	0.40	ns
error [C]	0.0030	162		

(b) “late”

effect	MS	d.f.	*F* ratio	*P*
Temperature	0.0479	1	40.97	<0.001
Selection regime	0.1631	2	26.27	<0.005
Lines within selection [A]	0.0062	6	2.37	<0.05
Temperature X selection	0.0349	2	29.84	<0.001
Temperature X lines within selection [B]	0.0012	6	0.45	ns
error [C]	0.0026	162		

ns: $P > 0.05$

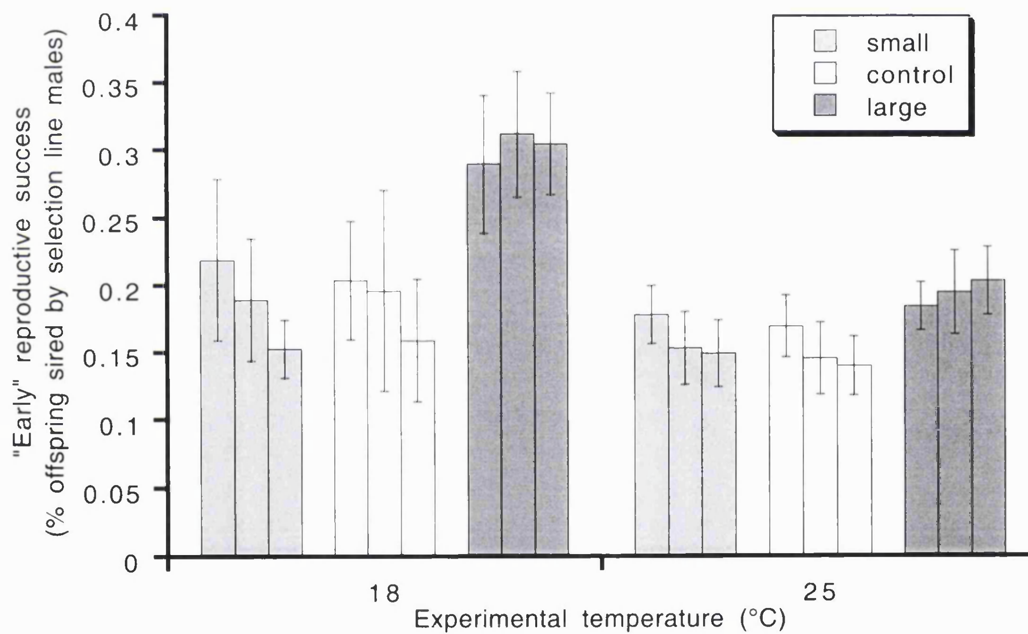
[A] Error term for selection regime

[B] Error term for temperature, and temperature X selection regime

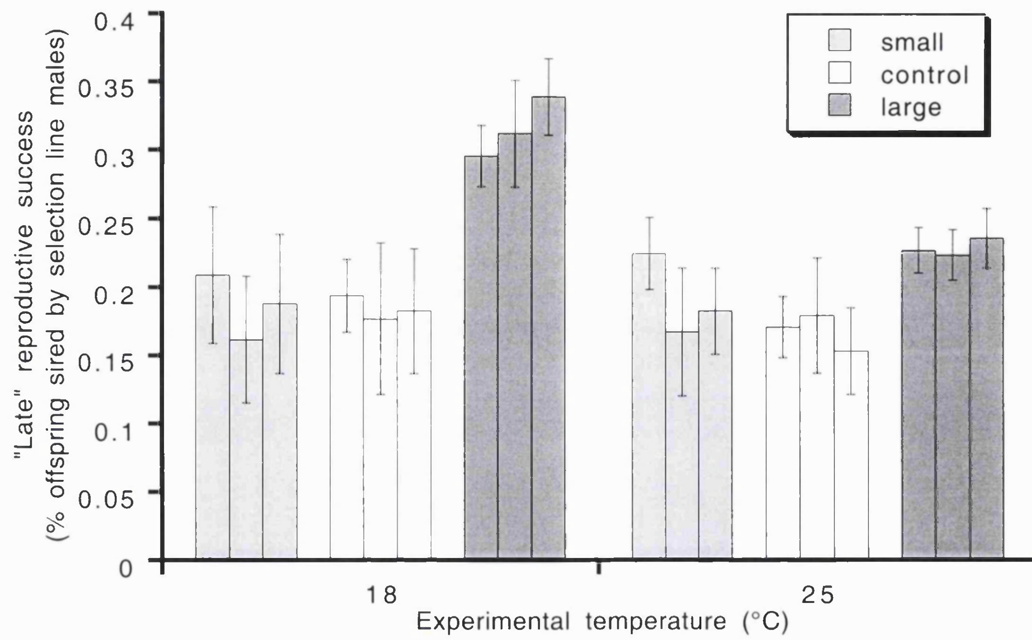
[C] Error term for lines within selection and temperature X lines within selection

Fig. 3.2 *Mean fertility (± 95% CL) of males from the large-, control- and small-size selection lines reared and tested at 18°C or 25°C. Data is split into (a) "early" and (b) "late" fertility. "Early" fertility is the sum of the proportions of progeny sired by the selection line flies in the first five sampling intervals at each temperature, and "late" fertility is the sum of the proportions of progeny sired by the selection line flies at subsequent sampling intervals.*

(a)



(b)



3.4.3 Male fitness

Temperature did not have a significant effect on fitness (Table 3.3). Selection regime, however, did have a significant effect, with large-size selection lines being significantly fitter than controls. Small-size selection lines were not significantly different in fitness from the control lines. There was also a significant interaction between temperature and selection regime, mainly due to the increased fitness of the large size selection lines when reared and tested at the lower temperature. There was no significant effect of lines within selection.

3.4.4 Wing area

There was a highly significant difference in wing area between flies from the different selection regimes (Table 3.4). Experimental temperature also had a highly significant effect on wing area, which was greater at the lower temperature for all three selection regimes. There was a significant interaction between selection and temperature, mainly due to a reduction in the size-differences between the different selection regimes at 25°C (Fig. 3.3). There was also a significant effect of lines within selection, and of the interaction between temperature and lines within selection.

Table 3.3 *Two-way nested analysis of variance on fitness of males from the large-, control-, and small-size selected lines reared and tested at 18°C or 25°C. Replicate lines were treated as random effects, nested within selection regime, and selection regime and temperature were analysed as fixed effects.*

effect	MS	d.f.	*F* ratio	*P*
Temperature	1233	1	0.01	ns
Selection regime	16550000	2	68.61	<0.0001
Lines within selection [A]	241168	6	1.20	ns
Temperature X selection	1522175	2	7.55	<0.05
error [B]	201687	6		

ns: $P > 0.05$

[A] Error term for selection regime

[B] Error term for temperature, temperature X selection regime, and lines within selection

Table 3.4 *The results of the two-way analysis of variance on wing size of males from the large-, control-, and small-size selected lines reared and tested at 18°C or 25°C. Selection regime and temperature were analysed as fixed effects.*

effect	MS	d.f.	*F* ratio	*P*
Temperature	8.3286	1	374.15	<0.0001
Selection regime	1.8907	2	42.23	<0.0005
Lines within selection [A]	0.0448	6	20.49	<0.0001
Temperature X selection	0.1897	2	8.52	<0.05
Temperature X lines within selection [B]	0.0223	6	10.19	<0.0001
error [C]	0.0022	342		

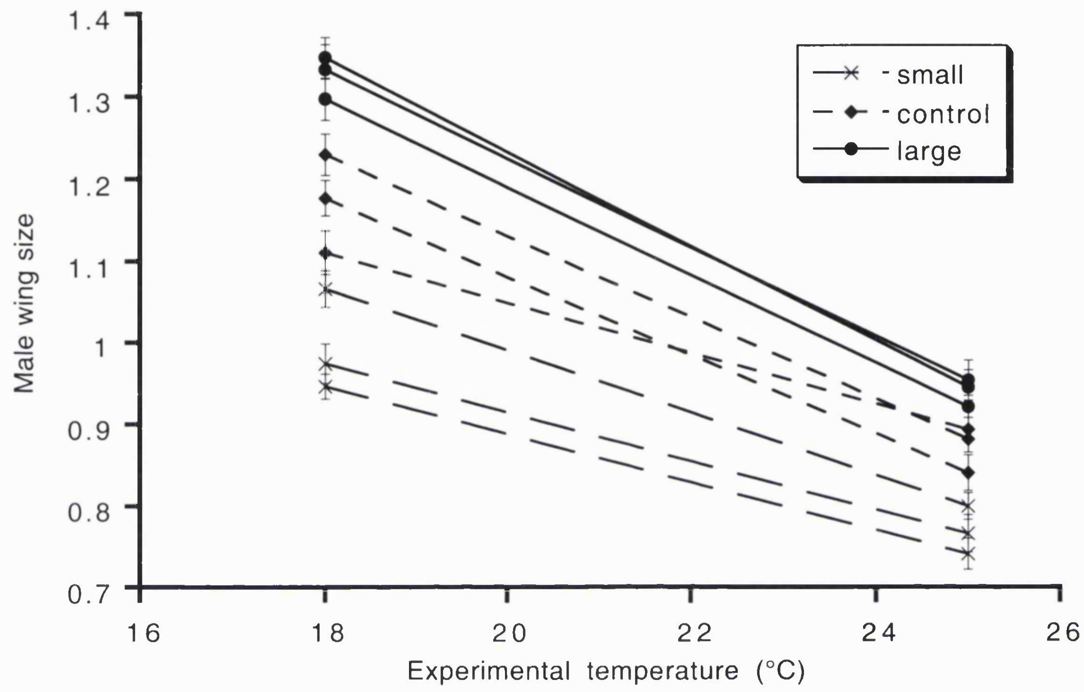
ns: $P > 0.05$

[A] Error term for selection regime

[B] Error term for temperature, and temperature X selection regime

[C] Error term for lines within selection and temperature X lines within selection

Fig. 3.3 *Wing size of the males from the size selected lines which were used in the experiment at* 18°C or 25°C. *Each point corresponds to the mean (± 95% CL) of the twenty samples from each of the three replicate lines within each selection regime.*



3.5 Discussion

The results implicate male body size as a target of thermal selection. Males from the large-size selection lines lived longer than controls and sired relatively more offspring than them at both experimental temperatures, and the extent of their advantage was greater at the lower experimental temperature. The fitness advantage of the large lines was hence greater at the lower experimental temperature. The results implicate body size *per se*, or some trait tightly genetically correlated with it, as the target of thermal selection. It will be important to determine what aspect of body size is important. The size of most external body parts is altered by selection on wing area (Wilkinson *et al.* 1990), and the size of at least some internal structures must also be changed.

The results for male fitness parallel those previously found for females of these selection lines (McCabe and Partridge 1997) and show that, despite the very different behaviour and physiology of females and males, thermal selection acts on their body size in a similar way. Some basic aspect of biological function must therefore be affected. Within a single temperature, increased body size is associated with an increase in lifespan of both males and females, both as a genetic correlation (e.g. Tantawy and Rakha 1964; Tantawy and El-Helw 1966, Partridge and Fowler 1992) and as a phenotypic correlation (e.g. Partridge and Farquhar 1981, 1983; Partridge *et al.* 1986). The mechanisms at work could be similar in the sexes, and might lead to a parallel response to temperature, but it is not clear why temperature affects either relationship. Positive genetic (e.g. male: Ewing 1961; Wilkinson 1987, female: Tantawy 1961; Tantawy and Rakha 1964; Tantawy and El-Helw 1966; Hilleshein and Stearns 1992) and phenotypic (e.g. male: Partridge and Farquhar 1981, 1983; Partridge *et al.* 1987a,b, female: Tantawy and Vetukhiv 1960; Partridge *et al.*

1986) correlations between body size and fertility have also been reported for both sexes. The mechanisms at work in the two sexes are likely to be different, with ability to accrue nutrients and convert them into eggs important for females (Maynard Smith 1958; Lamb 1964) and mating success playing a more important role for males (Cordts and Partridge 1996). Again, the reasons why temperature should affect the relationships between body size and fertility are unclear.

Two possible explanations for these observations are based on body size *per se*. Firstly, the relationship between male fitness and body size may change in response to environmental temperature. Secondly, when reared at the lower experimental temperature, the large-size selection lines were larger than any of the other selection line flies at either experimental temperature, and may have entered a size range in which fitness increases more rapidly with increase in body size. To test which of these explanations is most plausible, it would be necessary to produce lines of flies with a wide range of body sizes, and compare their performance as adults at the two experimental temperatures, so that the effects of phenotypic size and experimental temperature could be separated. Whatever the explanation, the results provided direct evidence for an interaction between genetically determined body size and environmental temperature in the determination of adult male fitness.

Fitness was measured on adult males, but it is possible that the interaction between temperature and genetic variation for size in the determination of adult fitness occurs during the pre-adult period. Lower environmental temperature during growth increases growth efficiency (S. Robinson and L. Partridge, unpubl. observations), and in the present experiment led to larger adult body size at the lower experimental temperature for all lines (Fig. 3.3), as has been observed previously (e.g. Alpatov 1930; Robertson 1959; Partridge *et al.* 1994a). This increased growth

efficiency may be particularly beneficial to the adult fitness of individuals that are, for genetic reasons, capable of achieving larger adult size, and may have contributed to the gene-by-environment interaction for body size observed in the present study. This variation in the pattern of plasticity of body size in response to developmental temperature may have played a role in the determination of adult fitness. The difference in male body size between the large and control selection lines was reduced at the higher experimental temperature (Fig. 3.3). This phenomenon was not observed for female body size one year previously (McCabe and Partridge 1997, Fig. 2). Therefore, this interaction has either evolved in the intervening time, or was always the case for male body size, the plasticity of which has not previously been examined in these lines.

Whatever the mechanism producing the effect of environmental temperature on the fitness advantage of the large line males, it could be important in nature. One study of a latitudinal cline in body size in eastern Australia found that, in flies collected directly from nature, the genetic cline for body size with increasing latitude was steepened by the direct effects of environmental temperature (James *et al.* 1997). Flies from higher latitudes were both genetically and environmentally larger. If the interaction relationship between genetically determined size and environmental temperature in the determination of adult fitness is operative in nature, then selection for genetically increased body size may be more intense in the lower temperatures at higher latitudes, either because the phenotypic plasticity of body size during development is especially beneficial to genetically large individuals, or because it takes genetically large flies into a higher range of body size before selection acts on the adults. A major unanswered question is the identity of the mechanisms

responsible for the phenotypic plasticity of body size of ectotherms generally in response to growth temperature (Atkinson 1994).

4. Costs and benefits of phenotypic plasticity of body size and its cellular components in *Drosophila melanogaster*

4.1 Abstract

Phenotypic plasticity is the ability of a genotype to adopt different phenotypes in response to environmental conditions. The degree of phenotypic plasticity of a trait can be heritable and can evolve in response to selection. In ectotherms, body size evolves in response to thermal environment, with genetically larger individuals found in colder thermal environments. In addition, body size shows phenotypic plasticity, with development at colder temperatures leading to an increase in body size. In this study, we investigated evolution of phenotypic plasticity for body size, following adaptation to constant and cycling thermal environments for 2.5 and 4.5 years. We measured wing area and wing cell area of flies reared at two different temperatures. We found no evidence for evolution of phenotypic plasticity for wing area. However, wing cell area, which was entirely responsible for plasticity of wing area, showed increased phenotypic plasticity in the lines adapted to variable thermal environments. The mechanisms producing plasticity for size in response to temperature, but not plasticity of size itself, therefore increased in variable thermal environments. The results suggest that in variable thermal environments an increased capacity to respond conditionally to thermal environment with altered body size is adaptive, and that it also carries a cost.

4.2 Introduction

Phenotypic plasticity is the ability of a genotype to produce different phenotypes in response to environmental conditions (Bradshaw 1965). The range of phenotypes that can be adopted by a single genotype across a specified set of environments is termed its norm of reaction (Woltereck 1909; Stearns 1989). The phenotypic plasticity of a trait can itself evolve (e.g. in the bacterium *Escherichia coli* (Bennett and Lenski 1997), butterflies (Kingsolver 1995), the mouse *Mus domesticus* (Lynch 1992), and the plant *Impatiens capensis* (Dudley and Schmitt 1996)), and an important issue is the nature of the interplay between evolutionary and plastic responses to a varying environment (e.g. Kingsolver and Huey 1998; de Jong 1999).

The plasticity of body size in *Drosophila* in response to environmental temperature could be adaptive (see Introduction, section 1.10). However, optimal plasticities might not evolve, even if larger adult size were adaptive at lower temperatures, for a variety of reasons. There may be limited variation in temperature within an area, leading to loss of adaptation to those temperatures not usually encountered, as a result of mutation accumulation or trade-offs between fitness at different temperatures. A poor correlation between temperature experienced during larval stages and during adulthood could also impede the evolution of optimal plasticity (Levins 1968; Lively 1986), as could a cost of plasticity (van Tienderen 1991). I have experimentally investigated the effects of thermal regime on the evolution of plasticity of development time and body size to temperature in *D. melanogaster* under laboratory natural selection (Rose and Charlesworth 1981). Two cycling thermal regimes regularly exposed the flies to different temperatures, so that any loss of adaptation to temperatures not usually encountered should have been

prevented or slowed, as assayed by comparison with populations kept at two different constant temperatures. By including one long- and one short-cycle treatment, I was able to test for the importance of a correlation between the temperature encountered during development and that during adulthood.

In *Drosophila melanogaster*, experimental temperature during development has been shown to alter the size of the adult fly predominantly through changes in cell area, with little or no change in cell number (see Introduction, section 1.9.1). Any evolution of plasticity of body size is therefore likely to occur through a change in the plasticity of cell area. Plasticity of cell area alone could evolve in response to thermal selection, if maintaining the cellular machinery of plasticity for body size carries some sort of cost, but is adaptive in variable thermal environments. I therefore also examined how plasticity of cell area in the wing evolved in response to thermal regime.

4.3 Materials and Methods

Wing area, cell density (and thereby cell size and cell number) and larval development time of the thermal selection lines (see General Materials and Methods, section 2.1.3) were investigated in this experiment.

The lines were assayed twice, first in early 1997, approximately two and a half years after they had been established, and second in early 1999, approximately four and a half years after establishment. The effects of selection temperature and experimental temperature on adult wing area were assessed, by rearing flies from the four selection regimes at both 18 and 25°C. To control for a possible effect of parental thermal environment on offspring performance and size (Huey *et al.* 1995, Crill *et al.* 1996), parents of the experimental flies were also reared at the experimental temperature. Eggs were collected from each population cage by placing yeasted bottles in the cage until a moderate density of eggs had been laid, and these bottles were then placed at the experimental temperature. The eclosing adults were transferred to laying pots containing a yeasted medium of grape juice and agar, and allowed to acclimate to their new environment. After a pre-lay period to oviposit any retained eggs, these adults were then transferred to fresh laying pots to lay the eggs that would hatch to give the experimental flies. Upon hatching, first instar larvae were transferred into yeasted vials of medium, with 30 larvae per vial. The larvae were collected from a single three-hour lay at 25°C, and a single six-hour lay at 18°C. Twenty vials were set up for each replicate selection line at each rearing temperature in the 1997 assay, and 10 vials in the 1999 assay.

4.3.1 Experimental measurements

4.3.1.1 Wing area

Wing area was used as a measure of body size, since a genetic correlation has been shown between the size of different anatomical regions of *Drosophila* adults (Cowley and Atchley 1990; Wilkinson *et al.* 1990). The right wings of four adults of each sex from each vial were removed, fixed with propanol and mounted in Aquamount on microscope slides. Wing area was measured at x50 magnification. In the first assay, a *camera lucida* attached to a dissecting microscope and a Quora graphics tablet connected to a computer was used. In the second, wing area was measured as described in the General Materials and Methods (section 2.3.4).

4.3.1.2 Cell density

Cell density in the wings was measured only in the 1999 experiment, as described in General Materials and Methods (section 2.3.5).

4.3.2 Statistical analysis

4.3.2.1 Wing area analysis

Wing area data from 1997 and 1999 were divided according to sex, and the data for each sex were subjected to an analysis of variance, with experimental temperature and selection regime as fixed main effects, and replicate line as a random effect nested

within selection regime. The unit of replication was the average wing area of flies of one sex from each vial. Linear contrast analyses were used to compare pairs of effects. Homogeneity of variance was confirmed with the O'Brien test (female 1997: $F_{(1,467)} = 0.9791$, $P = 0.0690$; male 1997: $F_{(1,467)} = 0.9809$, $P = 0.1318$; female 1999: $F_{(1,239)} = 2.7349$, $P = 0.0995$; male 1999: $F_{(1,239)} = 3.5524$, $P = 0.0607$), and normality of error was confirmed with the Shapiro-Wilk test (female 1997: $W = 0.9792$, $P = 0.0690$; male 1997: $W = 0.9809$, $P = 0.1318$; female 1999: $W = 0.9831$, $P = 0.4970$; male 1999: $W = 0.9914$, $P = 0.9711$).

4.3.2.2 Cell area and cell number analysis

The cell area and cell number data were subjected to an analysis of variance similar to that used to analyse the wing area data. Homogeneity of variance was confirmed with the O'Brien test (female cell area: $F_{(1,239)} = 2.6778$, $P = 0.1031$; male cell area: $F_{(1,239)} = 1.6639$, $P = 0.1983$; female cell number: $F_{(1,239)} = 2.5070$, $P = 0.1147$; male cell number: $F_{(1,239)} = 1.0329$, $P = 0.3105$), and normal distribution of residuals was confirmed with a Shapiro-Wilk test (female cell area: $W = 0.9879$, $P = 0.8524$; male cell area: $W = 0.9885$, $P = 0.8834$; female cell number: $W = 0.9799$, $P = 0.2627$; male cell number: $W = 0.9835$, $P = 0.5314$).

4.3.2.3 Cell area and cell number plasticity analysis

The interaction term for cell area and cell number was investigated by carrying out analyses of variance of the plasticity of these traits, for the sexes combined. The plasticity variables were generated by subtracting the values at the lower temperature from those at the higher temperature. Homogeneity of variance for the plasticity variables was confirmed with the O'Brien test (cell area: $F_{(3,20)} = 2.6613$, $P = 0.0759$; cell number: $F_{(3,20)} = 0.4738$, $P = 0.7040$). Normal distribution of residuals was confirmed with the Shapiro-Wilk test (cell area: $W = 0.9358$, $P = 0.1346$; cell number: $W = 0.9699$, $P = 0.6642$).

4.4 Results

4.4.1 Wing area

As expected, analysis of both the 1997 and 1999 data revealed that flies of both sexes reared at the lower experimental temperature were significantly larger than those reared at the higher temperature, and females were consistently significantly larger than males (Table 4.1, Fig. 4.1). The 1997 data indicated that there was a significant effect of selection regime on wing area in both sexes. Linear contrast analysis revealed that lines that had evolved at 18°C had significantly larger wings than those that had evolved at 25°C (female: $F_{(1,8)} = 21.6898$, $P < 0.002$; male: $F_{(1,8)} = 14.3952$, $P < 0.01$). The 18°C lines were comparable in size with both the cycling lines, but the 25°C lines were significantly smaller than both the short cycling (female: $F_{(1,8)} = 20.1379$, $P < 0.002$; male: $F_{(1,8)} = 13.5544$, $P < 0.01$) and long cycling lines (female: $F_{(1,8)} =$

21.1313, $P < 0.002$; male: $F_{(1,8)} = 11.8886$, $P < 0.01$). The cycling lines were comparable in size with each other.

The 1999 data indicated that there was a significant effect of selection regime on wing area in the female flies only. These female flies showed similar trends in wing area to the 1997 data; lines that evolved at 18°C had significantly larger wings than those that had evolved at 25°C ($F_{(1,8)} = 10.8514$, $P < 0.02$); 18°C lines were comparable in size with the cycling lines, but 25°C lines were significantly smaller than the both the short cycling ($F_{(1,8)} = 18.8955$, $P < 0.005$) and long cycling lines ($F_{(1,8)} = 8.0240$, $P < 0.05$); and the cycling lines were comparable in size with each other. The male flies in the 1999 data showed no significant effect of selection regime on wing area.

Analysis of both the 1997 and 1999 data for wing area revealed no significant interaction between experimental temperature and selection regime in either sex. There was no significant effect of lines nested within selection regime in either sex in the 1997 data, however this effect was significant in both sexes in the 1999 data. The male data from 1999 also revealed a significant interaction between experimental temperature and lines within selection. However, this interaction was non-significant in the 1997 data for both sexes, and for females in 1999.

Table 4.1. *Analysis of variance on wing area for thermal selection lines reared and tested at 18 and 25°C. (a) 1997 female, (b) 1997 male, (c) 1999 female, and (d) 1999 male. Replicate lines were treated as random effects, nested within selection regime, and selection regime and experimental temperature were analysed as fixed main effects.*

(a)

effect	MS	d.f.	*F* ratio	*P*
Temperature	8.8137	1	2939.07	<0.0001
Selection regime	0.0226	3	10.56	<0.005
Lines within selection [A]	0.0021	8	0.71	ns
Temperature X selection	0.0097	3	3.24	ns
Temperature X lines within selection [B]	0.0030	8	1.15	ns
error [C]	0.0026	445		

(b)

effect	MS	d.f.	*F* ratio	*P*
Temperature	9.3093	1	3423.04	<0.0001
Selection regime	0.0177	3	6.67	<0.02
Lines within selection [A]	0.0027	8	0.98	ns
Temperature X selection	0.0035	3	1.27	ns
Temperature X lines within selection [B]	0.0027	8	1.06	ns
error [C]	0.0026	445		

(c)

effect	MS	d.f.	*F* ratio	*P*
Temperature	3.6132	1	2656.72	<0.0001
Selection regime	0.0244	3	6.88	<0.02
Lines within selection [A]	0.0035	8	2.60	<0.01
Temperature X selection	0.0032	3	2.36	ns
Temperature X lines within selection [B]	0.0014	8	1.88	ns
error [C]	0.0007	217		

(d)

effect	MS	d.f.	*F* ratio	*P*
Temperature	3.4908	1	3006.92	<0.0001
Selection regime	0.0086	3	2.63	ns
Lines within selection [A]	0.0033	8	2.81	<0.01
Temperature X selection	0.0025	3	2.13	ns
Temperature X lines within selection [B]	0.0012	8	2.20	<0.05
error [C]	0.0005	217		

ns: $P > 0.05$

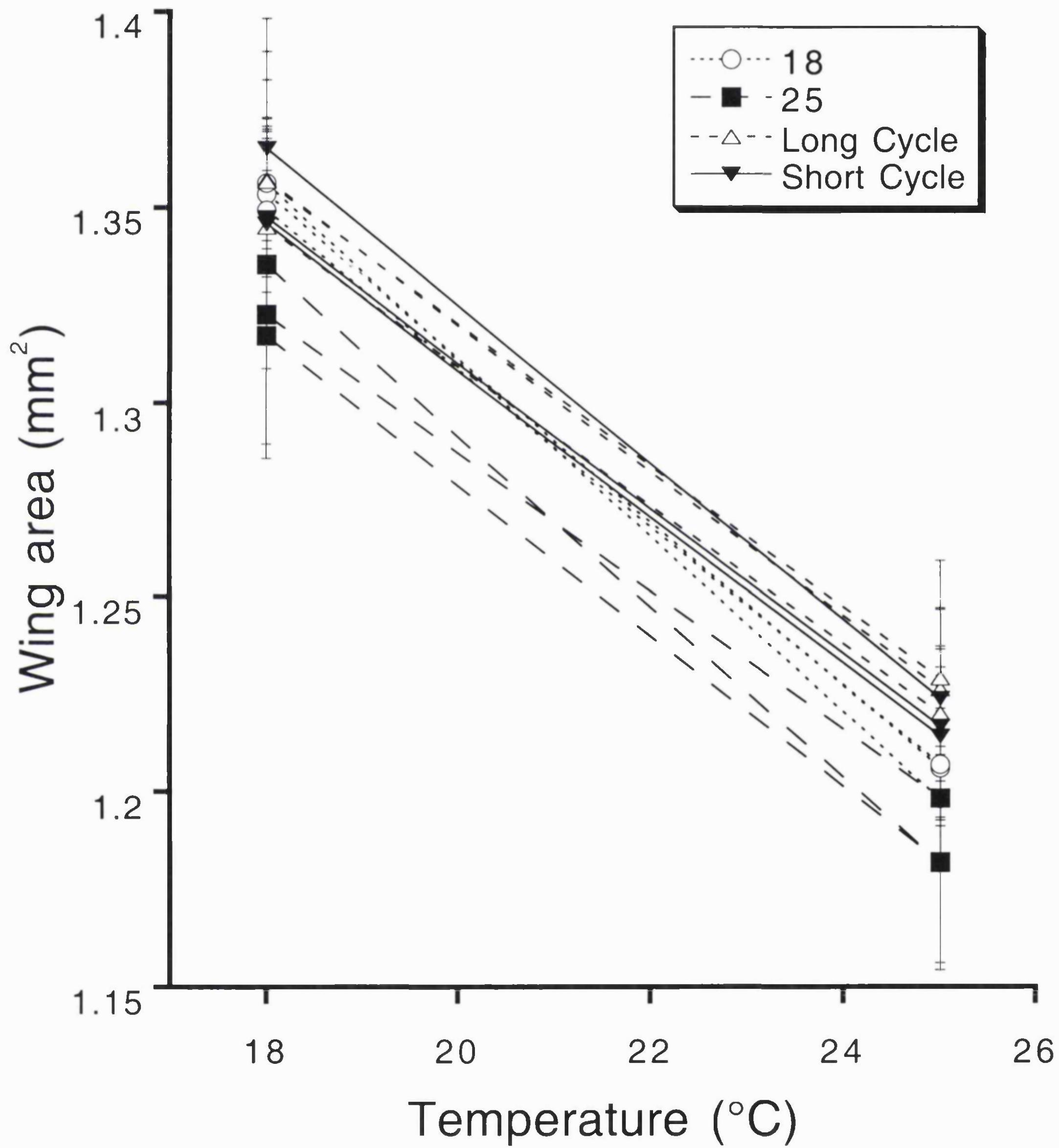
[A] Error term for selection regime

[B] Error term for temperature, and temperature X selection regime

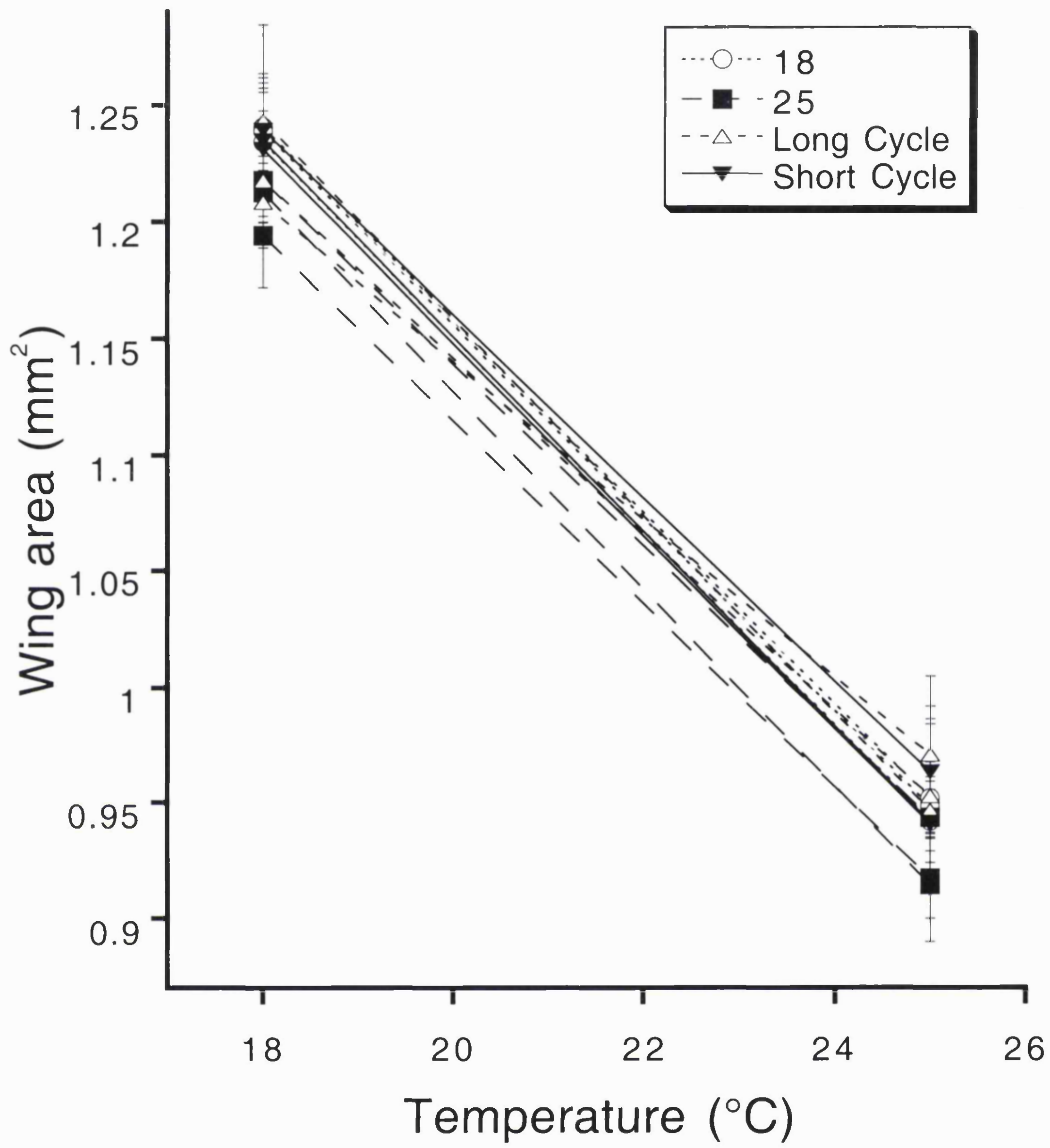
[C] Error term for lines within selection and temperature X lines within selection

Fig. 4.1 *Wing area (±95% CL) from the (a,b) 1997 and (c,d) 1999 data for females and males respectively from the four selection regimes, reared and tested at 18°C and 25°C. Replicate lines are all shown on the graph.*

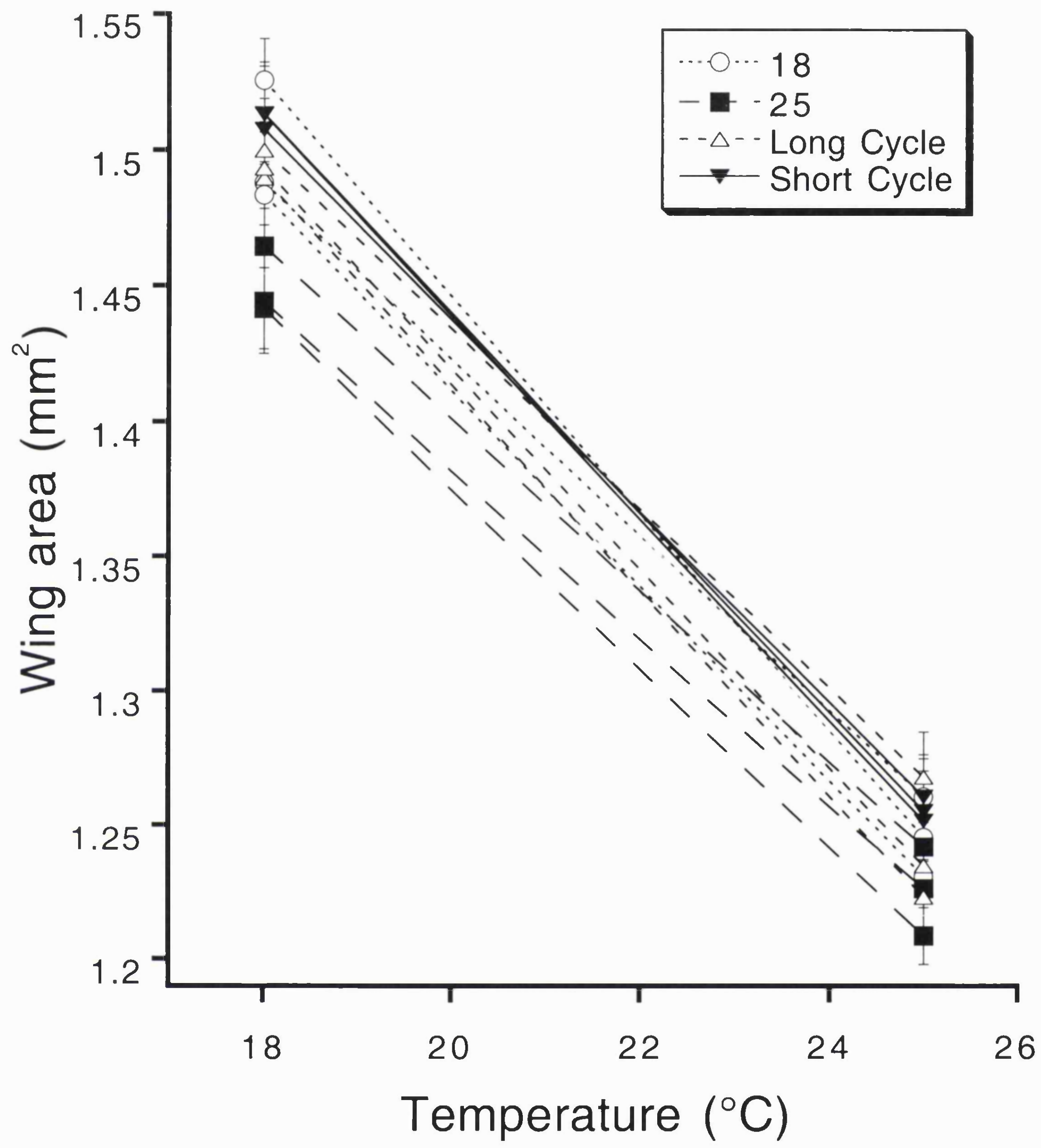
(a)



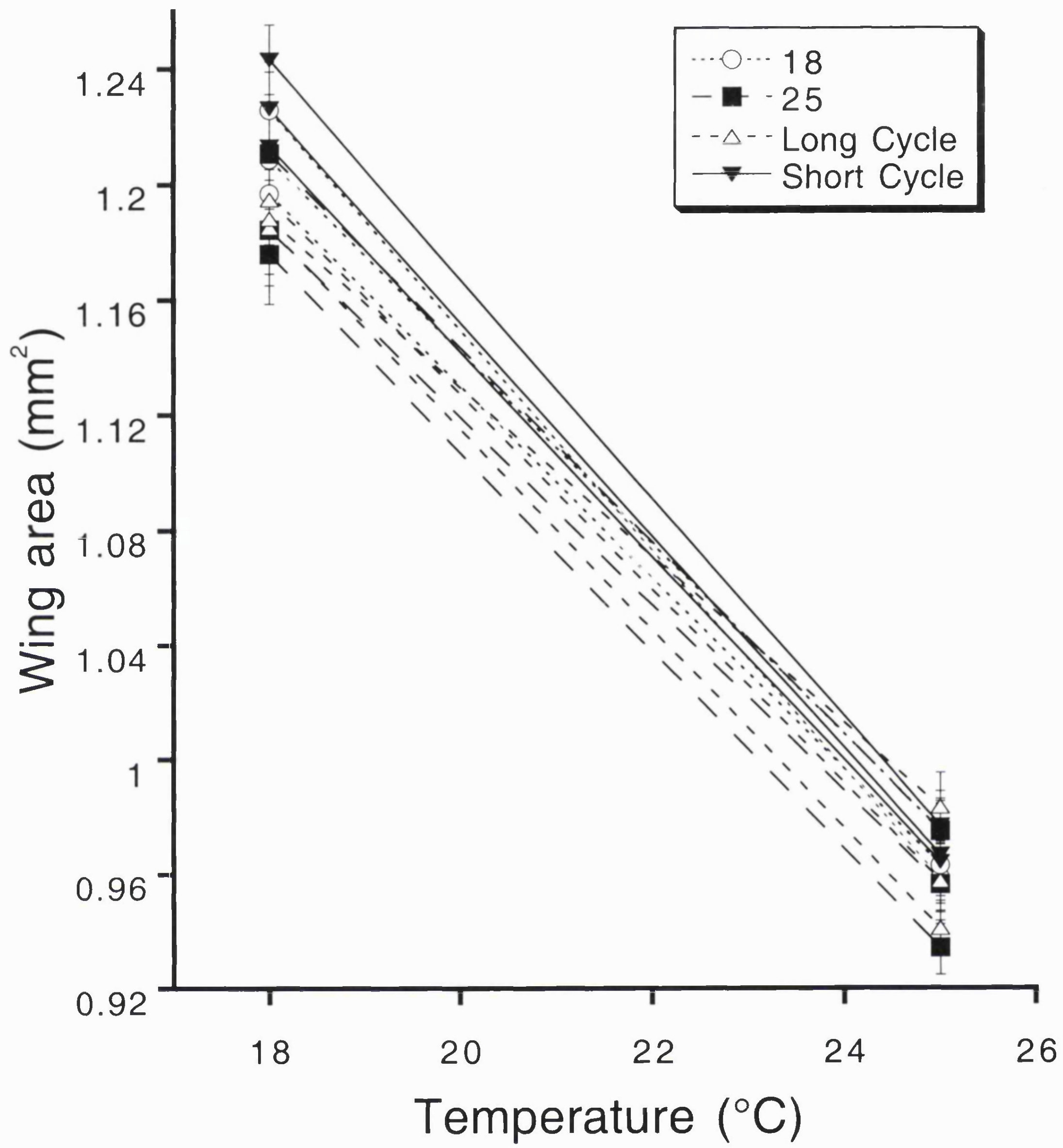
(b)



(c)



(d)



4.4.2 Cell area

The cells from wings of flies of both sexes reared at the higher temperature were significantly smaller than from those reared at the lower temperature (Table 4.2, Fig. 4.2). Analyses of variance revealed a significant effect of selection regime on cell area in both sexes. Linear contrast analyses revealed that cells from lines that had evolved at 18°C were significantly larger than those from lines that had evolved at 25°C in female flies only ($F_{(1,8)} = 35.1741$, $P < 0.0005$). The cell sizes in the cycling lines were comparable in both sexes (female: $F_{(1,8)} = 0.0930$, $P = 0.7681$; male: $F_{(1,8)} = 0.4171$, $P = 0.5365$). The short cycling lines had significantly larger cells than the 25°C lines in both sexes (female: $F_{(1,8)} = 47.9771$, $P < 0.0001$, male: $F_{(1,8)} = 9.0695$, $P < 0.02$), as did the long cycling lines (female: $F_{(1,8)} = 43.8632$, $P < 0.0002$, male: $F_{(1,8)} = 13.3546$, $P < 0.01$). Cell area was not significantly different, however, between the cycling lines, nor between the cycling lines and the 18°C lines, in either sex.

There was a significant effect of replicate lines within selection for cell area in the male flies only, and a significant interaction between experimental temperature and selection regime in both sexes. This interaction was analysed by examining plasticity directly (see below). There was also a significant interaction between experimental temperature and lines within selection in the female flies only.

Table 4.2 Analysis of variance on wing cell area in the of the thermal selection lines reared and tested at 18 and 25°C. (a) female, (b) male. Replicate lines were treated as random effects, nested within selection regime, and selection regime and experimental temperature were analysed as fixed main effects.

(a)

effect	MS	d.f.	*F* ratio	*P*
Temperature	2.6×10^{-7}	1	4234.73	<0.0001
Selection regime	1.5×10^{-9}	3	21.34	<0.0005
Lines within selection [A]	6.9×10^{-11}	8	1.12	ns
Temperature X selection	1.3×10^{-9}	3	20.44	<0.0005
Temperature X lines within selection [B]	6.1×10^{-11}	8	2.22	<0.05
error [C]	2.8×10^{-11}	217		

(b)

effect	MS	d.f.	*F* ratio	*P*
Temperature	1.8×10^{-7}	1	4973.84	<0.0001
Selection regime	9.8×10^{-10}	3	5.06	<0.05
Lines within selection [A]	1.9×10^{-10}	8	5.27	<0.0001
Temperature X selection	1.6×10^{-9}	3	42.08	<0.0001
Temperature X lines within selection [B]	3.7×10^{-11}	8	1.80	ns
error [C]	2.1×10^{-11}	217		

ns: $P > 0.05$

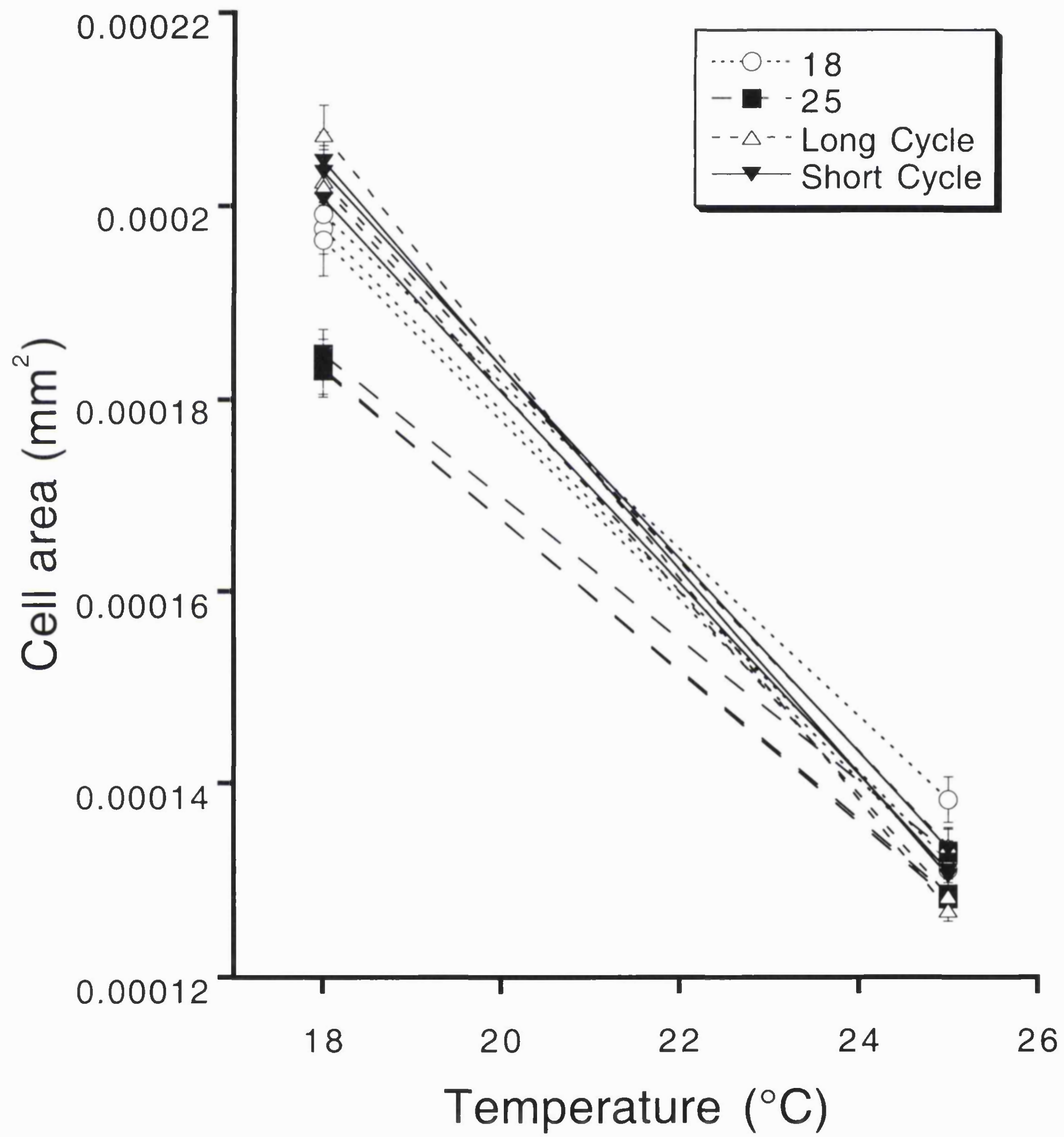
[A] Error term for selection regime

[B] Error term for temperature, and temperature X selection regime

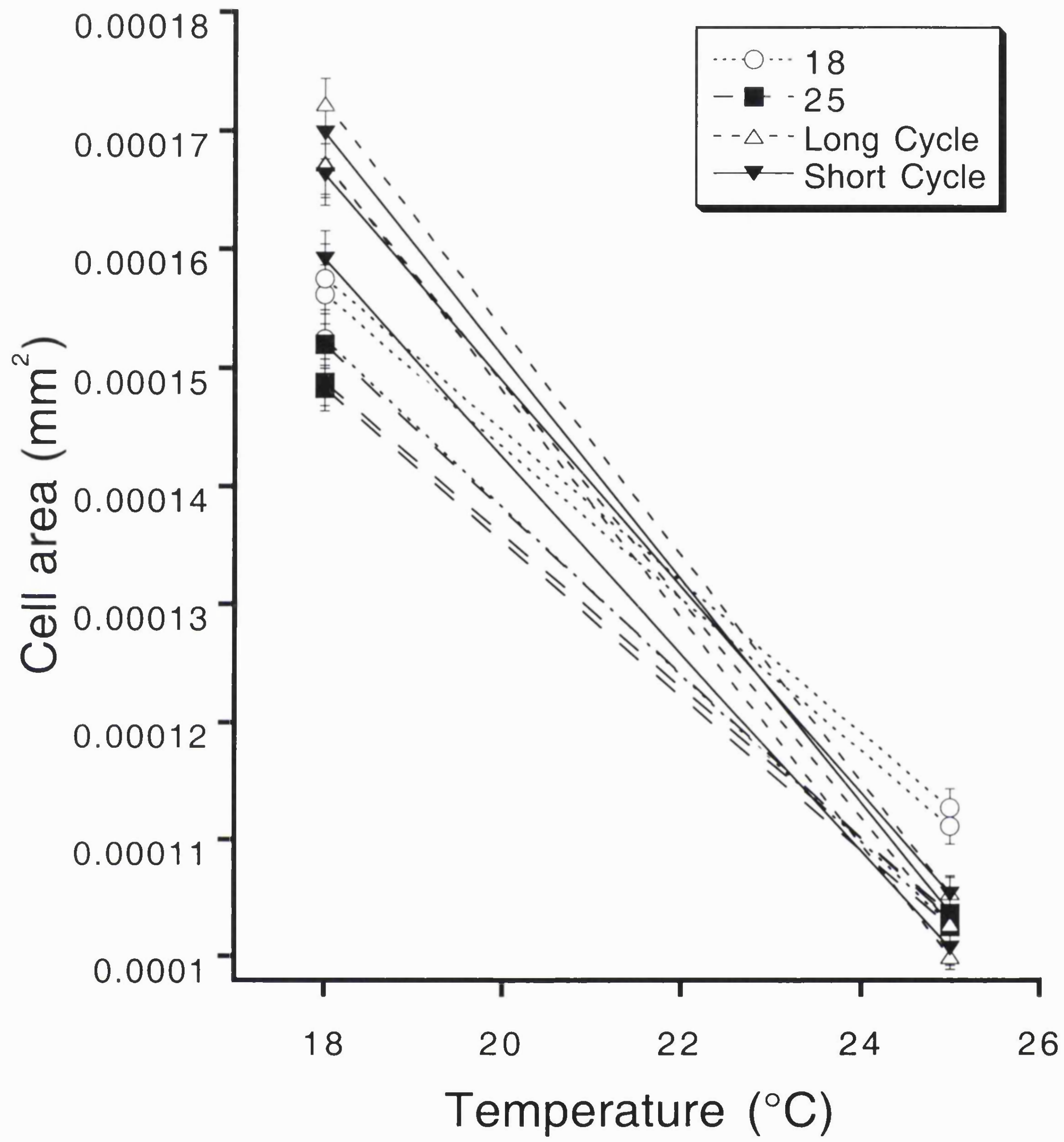
[C] Error term for lines within selection and temperature X lines within selection

Fig. 4.2 *Cell area (±95% CL) of (a) females and (b) males from the four selection regimes, reared and tested at 18°C and 25°C. Replicate lines are all shown on the graph.*

(a)



(b)



4.4.3 Cell number

There was a significant effect of experimental temperature on cell number in both sexes (Table 4.3, Fig. 4.3). Wings of flies reared at the higher temperature had a significantly greater number of cells, in opposition to the observed trend in wing area. The increased wing area at lower temperatures was attributable entirely to increased cell size.

There was no significant effect of selection regime upon cell number in either sex, but there was a significant effect of replicate lines within selection in both sexes. There was a significant interaction between experimental temperature and lines within selection in both sexes, and a significant interaction between experimental temperature and selection regime. This variation in plasticity of cell number was analysed directly (see below).

Table 4.3 *Analysis of variance on cell number in the wings of replicate lines reared and tested at 18 and 25°C. (a) female, (b) male. Replicate lines were treated as random effects, nested within selection regime, and selection regime and experimental temperature were analysed as fixed main effects.*

(a)

effect	MS	d.f.	*F* ratio	*P*
Temperature	219300000	1	825.57	<0.0001
Selection regime	559900	3	0.98	ns
Lines within selection [A]	573000	8	2.16	<0.05
Temperature X selection	1641000	3	6.18	<0.02
Temperature X lines within selection [B]	265600	8	2.96	<0.005
error [C]	89690	217		

(b)

effect	MS	d.f.	*F* ratio	*P*
Temperature	166400000	1	402.68	<0.0001
Selection regime	1679000	3	1.81	ns
Lines within selection [A]	927700	8	2.25	<0.05
Temperature X selection	4720000	3	11.42	<0.005
Temperature X lines within selection [B]	413200	8	4.33	<0.0001
error [C]	95470	217		

ns: $P > 0.05$

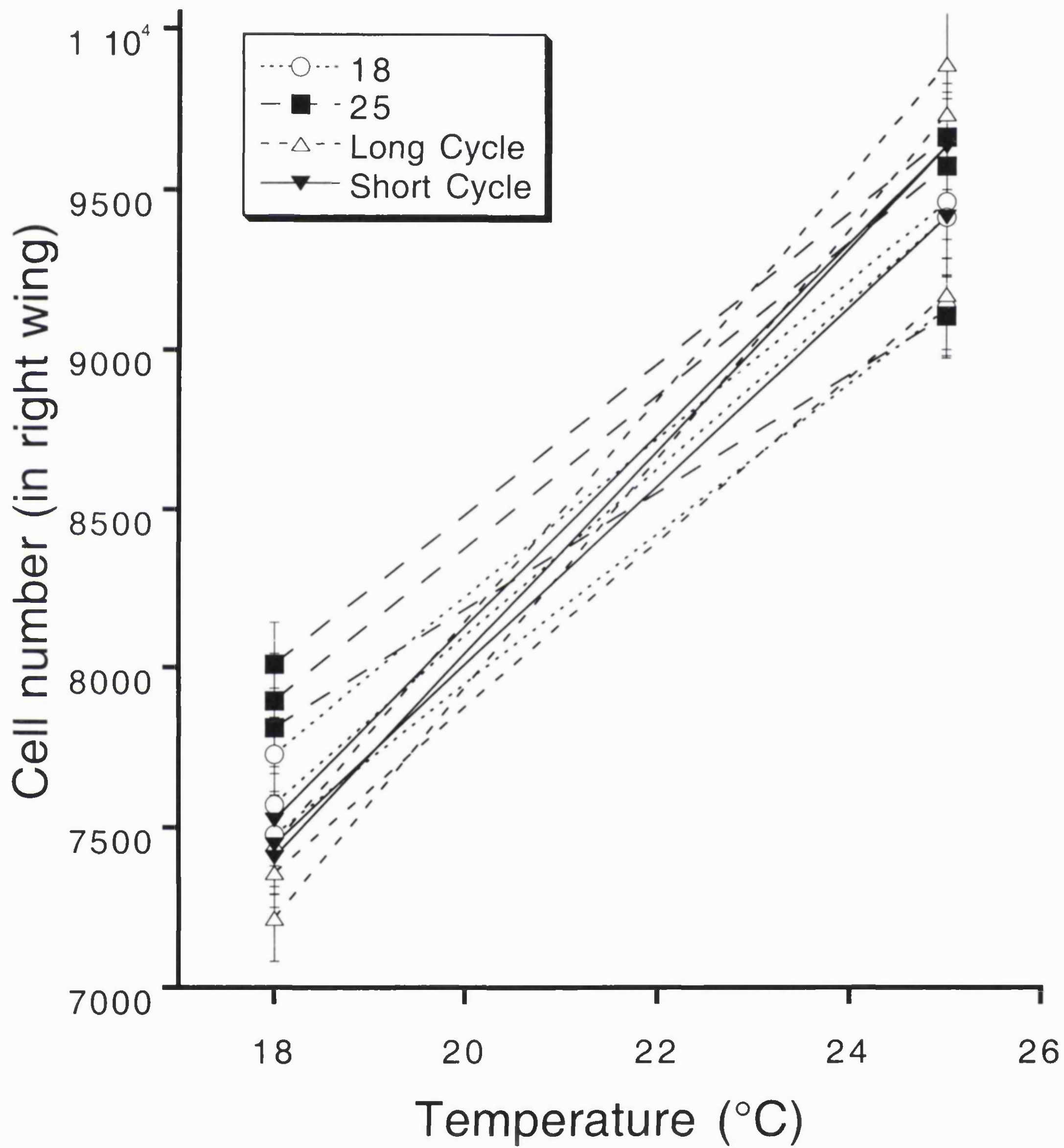
[A] Error term for selection regime

[B] Error term for temperature, and temperature X selection regime

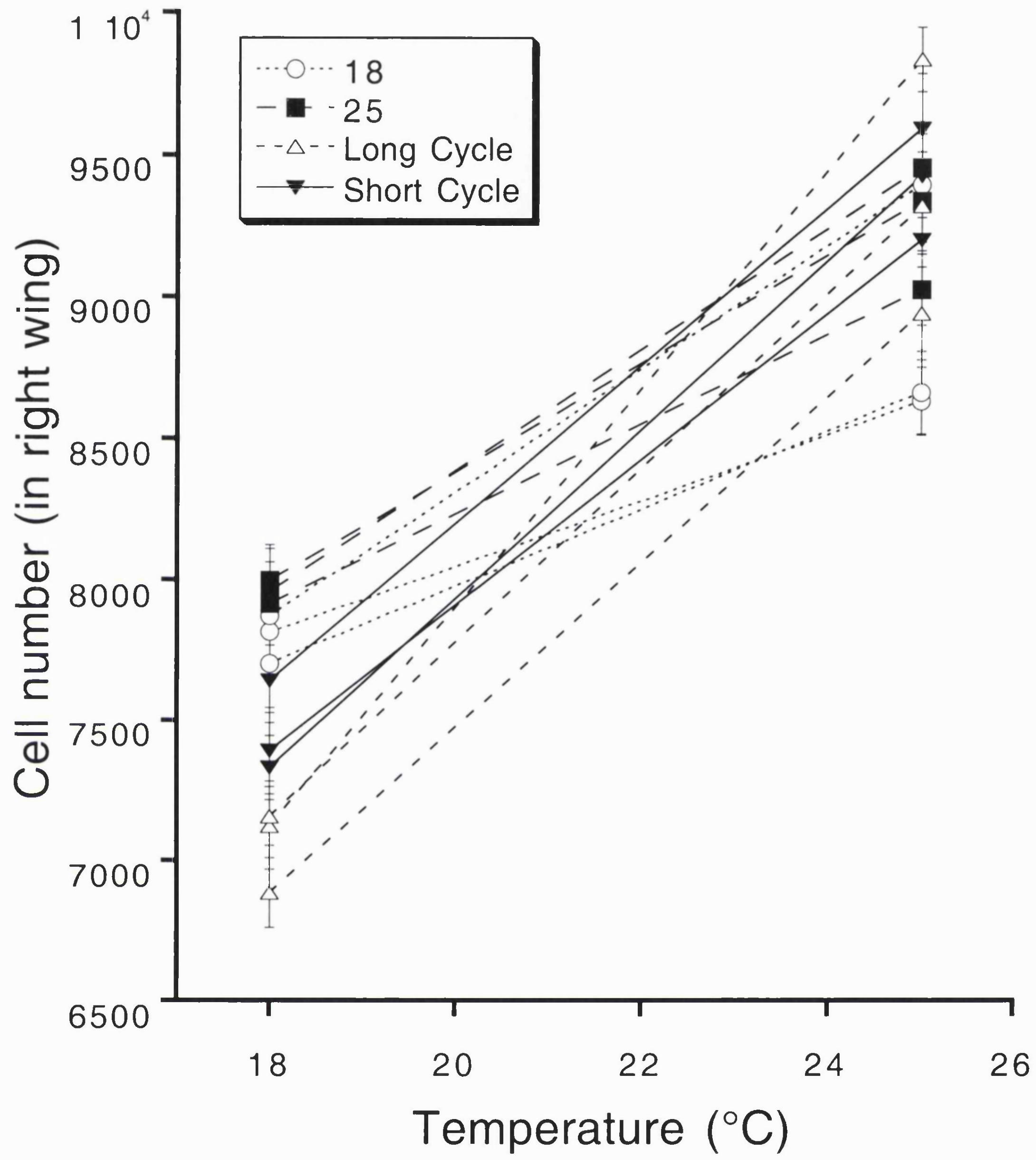
[C] Error term for lines within selection and temperature X lines within selection

Fig. 4.3 *Cell number (±95% CL) of (a) females and (b) males from the four selection regimes, reared and tested at 18°C and 25°C. Replicate lines are all shown on the graph.*

(a)



(b)



4.4.4 Plasticity of cell area and cell number

Analysis of variance of the 1999 cell area plasticity revealed that there was a significant effect of selection regime ($F_{(3,8)} = 10.7403$, $P < 0.005$), and sex ($F_{(1,11)} = 6.5967$, $P < 0.05$). Linear contrast analysis of the cell area plasticity revealed that the constant temperature lines did not differ significantly from each other. The long and short cycling lines also did not differ significantly in cell area plasticity from each other. However, the cycling lines showed significantly increased plasticity relative to the constant temperature lines (constant temperature and cycling lines: $F_{(1,8)} = 30.2798$, $P < 0.001$). Cell area plasticity was significantly greater in females. There was no significant effect of lines within selection on cell area plasticity ($F_{(8,11)} = 1.9283$, $P = 0.1547$).

Analysis of variance of cell number plasticity revealed that there was a significant effect of selection regime ($F_{(3,8)} = 45.6175$, $P < 0.0001$), and sex ($F_{(1,11)} = 45.9927$, $P < 0.0001$). Linear contrast analysis of the cell number plasticity revealed that the constant temperature selection regimes did not differ significantly from each other. The long and short cycling lines also did not differ significantly in cell number plasticity from each other. However, the cycling lines showed significantly increased plasticity relative to the constant temperature lines (constant temperature and cycling lines: $F_{(1,8)} = 126.7508$, $P < 0.0001$). Cell number plasticity was significantly greater in females. There was no significant effect of lines within selection on plasticity of wing area ($F_{(8,11)} = 0.7929$, $P = 0.6204$).

4.5 Discussion

In line with previous studies (Anderson 1966; Cavicchi *et al.* 1985; Partridge *et al.* 1994a), wing area showed a significant evolutionary increase at the lower experimental temperature. The direction of evolution can be deduced because the Dahomey base stock had a history of culture at 25°C. In addition, both sets of cycling lines showed an increase in body size, as great as that seen in the 18°C constant temperature lines. The period of the thermal cycle appeared to be of no consequence, with the short-cycle lines showing as great an increase in wing area as that seen in the long-cycle lines. The physiological time spent at the higher experimental temperature was greater in both cases, assuming a Q_{10} of 2. These results therefore suggest that, in the cycling thermal environments, selection for increased scope for growth, or for larger size *per se,* at the lower experimental temperature was more intense than for smaller size at the higher experimental temperature.

Rearing at lower experimental temperature led to a significant increase in body size in all the lines examined, consistent with many previous studies (e.g. Ray 1960; David *et al.* 1983; Partridge *et al.* 1994a). This developmental effect of temperature operated almost exclusively through increased cell area, as previously reported (e.g. Alpatov 1930; Robertson 1959; Delcour and Lints 1966; Masry and Robertson 1979; Cavicchi *et al.* 1985; Kuo and Larsen 1987; Partridge *et al.* 1994a; James *et al.* 1997). However, cell number was reduced when lines were reared at the lower temperature. Some previous studies showed no alteration of cell number with changes in developmental temperature (e.g. Robertson 1959; Partridge *et al.* 1994a), and some have shown a reduction in cell number with increasing developmental temperature

(e.g. Alpatov 1930; Cavicchi *et al.* 1985). The pattern seen in the present study could be indicative of responses in populations exposed to narrow thermal ranges, and in the early stages of adaptation to variable regimes.

Plasticity of wing area showed no evidence of evolutionary response to thermal environment. If exposure to only a narrow thermal range leads to a loss of adaptation of body size to thermal environments not encountered, I would have expected to observe significantly reduced plasticity in the constant temperature lines relative to the cycling lines. These results do not provide any support for this hypothesis. We did find evidence for evolution of plasticity in the trait that produces plasticity in wing area, namely cell area, but it did not lead to reduced plasticity of wing area in the constant temperature lines. Furthermore, wing area plasticity was comparable between the two sets of cycling lines. If a strong correlation between larval and adult thermal environment promoted the evolution of increased wing area plasticity, we might have observed increased plasticity of wing area in the long-cycle lines.

The failure of plasticity of wing area to respond to thermal selection regime, together with the evidence for very limited evolution of this trait in nature, suggest that temperature itself has a direct effect on scope for growth. It has previously been shown that opportunity for growth during the pre-adult stages increases with rearing at lower experimental temperatures (Robinson and Partridge 2001). The invariance of plasticity of body size with evolution in different thermal regimes would then occur because temperature determines the opportunity for growth during the pre-adult period. The extent of opportunity for growth is then exploited by alterations in cell size.

Although wing area plasticity did not evolve in the lines examined in this experiment, the plasticity of the cellular traits underlying wing area plasticity did. Plasticity of both wing area components; cell area and cell number, increased in the cycling lines relative to the constant temperature lines. The increase in plasticity of cell area did not lead to an increase in plasticity of total wing area, because it was compensated by the increase in plasticity of cell number in the opposite direction.

5. Longevity and fecundity of populations along a latitudinal cline from Eastern Australia

5.1 Abstract

Latitudinal clines of a large number of characteristics have been observed worldwide in *Drosophila*, however, traits which are closely related to fitness, such as longevity and fecundity appear to have received relatively little investigation along geographic clines. In this study, I investigated latitudinal trends in longevity and fecundity in eight replicated populations from along a latitudinal cline of *Drosophila melanogaster* from Eastern Australia, when reared and tested in the laboratory at two experimental temperatures. I found no evidence for a latitudinal cline of either longevity or lifetime fecundity, however, there was significant evidence for a change in the pattern of fecundity along the cline. Populations from higher latitudes tended to lay more eggs later in life than populations from lower latitudes, when reared at the higher experimental temperature.

5.2 Introduction

Latitudinal clines of numerous characteristics have been observed worldwide both in ectotherms in general (see Introduction, section 1.5) and in *Drosophila* in particular (see Introduction, section 1.6). However traits which are very closely related to fitness, such as longevity and fecundity (Charlesworth 1980; Stearns 1992; Lessells 1991), seem to have received relatively little investigation along geographic clines.

No studies have previously investigated clinal trends in longevity, though there are two lines of evidence that suggest that we might expect to observe a clinal trend in longevity with latitude (see Introduction, section 1.6.7). First, since body size is generally observed to vary with latitude (see Introduction, sections 1.5.1 and 1.6.1), and body size has been observed to be associated with increased longevity, (McCabe and Partridge 1997; Chapter 3 of this thesis), we might expect to find a latitudinal trend of increased longevity with increasing latitude. However, a laboratory thermal experiment, in which *Drosophila* were adapted to different thermal environments for several years, found that longevity was significantly greater when flies were reared and tested at the temperature to which they had adapted, at least in females (Partridge *et al.* 1995), despite the fact that the lines adapted to lower temperatures were larger when reared at either environmental temperature (Partridge *et al.* 1994a). This evidence suggests that differences in body size alone are unlikely to account for differences in longevity between populations of flies that have evolved at different temperatures.

Second, starvation resistance has been shown to vary latitudinally in several clines, with increased starvation resistance found in flies from lower latitudes (see Introduction, section 1.6.5), and there is some evidence suggesting a correlation

between increased starvation resistance and increased longevity (see Introduction, section 1.6.7). Consequently, starvation resistance differences along a cline might be expected to be associated with a latitudinal trend of decreased longevity with increasing latitude.

There is little experimental evidence for clinal variation in fecundity, (see Introduction, section 1.6.8). *Drosophila* from tropical populations have been observed to have decreased fecundity in laboratory conditions relative to temperate populations (Bouletreau-Merle *et al.* 1982). However, that study only examined two unreplicated populations of *Drosophila*, from France and North Africa. Other studies, such as that carried out on three replicated populations of the water strider *Aquarius remigis* (Blanckenhorn and Fairbairn 1995), and that carried out on nine populations of the pitcher-plant mosquito *Wyeomyia smithii* (Bradshaw *et al.* 2000), found no evidence for a latitudinal cline of fecundity upon rearing at common temperatures.

A laboratory thermal selection experiment, in which flies were adapted to different thermal environments for several years, found that that fecundity is greater when flies are reared and tested at the temperature to which they had adapted (Partridge *et al.* 1995), again independent of body size (Partridge *et al.* 1994a).

In this study, I examined the longevity and fecundity of eight populations of *Drosophila melanogaster* collected from a cline along the Eastern coast of Australia, and analysed them to see if there was latitudinal variation in these traits along the cline. The cline shows significant latitudinal variation in body size (W. J. Kennington, pers. comm.), and shows evidence of weak latitudinal variation in starvation resistance, with increased starvation resistance at lower latitudes, but in one sex only (Hoffmann *et al.* 2001).

5.3 Materials and Methods

5.3.1 Populations

The Australian clinal populations were used in this experiment (see General Materials and Methods, section 2.1.4). The eight populations were produced by outbreeding isofemale lines from each location along the cline, that had been collected in the wild a year before the experiment was carried out, and maintained in vials at 25°C. After two generations of random outbreeding between the isofemale lines, in standard density culture using bottles, as described in General Methods and Methods (section 2.3.3), the offspring were collected to be the experimental flies.

5.3.2 Measurement of Longevity

Longevity was measured for two replicates of each of the eight Australian populations, at two temperatures; 18°C and 25°C. Three hundred male and three hundred female flies were collected for each replicate from the standard density culture bottles on the day of eclosion, and transferred to cages. Care was taken not to anaesthetise flies within three hours of eclosion. Instead, flies were transferred into empty bottles, and only anaesthetised and sexed after three hours.

Cages were maintained with fresh plates of grape juice medium covered with excess yeast, which were replaced three times per week. A bottle of water bunged with damp cotton wool was placed in each cage to maintain humidity, and replaced once per week. Deaths in each cage were tallied six days per week at 25°C, and three days per week at 18°C.

5.3.3 Measurement of Fecundity

To measure fecundity of the flies, an unyeasted plate of grape juice medium was placed in each cage once per week at 25°C, and once per fortnight at 18°C. These plates were left in the cages for six hours, at both temperatures, after which they were removed, and plates of yeasted grape juice medium were replaced. The eggs laid on the plates were counted and recorded.

5.3.4 Statistical Analysis

5.3.4.1 Longevity Analysis

The median longevity of flies of each sex in each cage was analysed. To investigate the effects of sex and temperature on longevity, and to test for the existence of significant latitudinal clines for longevity, an analysis of covariance test was carried out on the median longevities. Sex and temperature were analysed as fixed main effects, and latitude was used as the covariate. Normality of the error distribution was confirmed using the Shapiro-Wilk test ($W = 0.9580$, $P = 0.0703$). Homogeneity of variance between the two temperatures and the two sexes was compared using the O'Brien test (temperature: $F_{(1,62)} = 0.8660$, $P = 0.3557$; sex: $F_{(1,62)} = 1.1306$, $P = 0.2918$).

5.3.4.2 Fecundity Analysis

In order to compare the total lifetime fecundity of the populations, the numbers of eggs collected from all plates taken from each cage were summed to produce an estimate of the fecundity of the flies in each cage. An analysis of covariance test was then performed on these fecundity data, with experimental temperature as a fixed main effect and latitude as the covariate. Normality of the error distribution was confirmed using the Shapiro-Wilk test ($W = 0.9647$, $P = 0.5623$) and homogeneity of variance between the two experimental temperatures was compared using the O'Brien test ($F_{(1,30)} = 4.1545$, $P = 0.0504$).

5.3.4.3 Pattern of Fecundity Analysis

In order to compare the pattern of fecundity of the populations, the number of eggs collected in the first two samples from each cage were divided by the total number of eggs collected from all samples taken from that cage. This gives an index of the early fecundity of the population.

An analysis of covariance test was then performed on these indices of early fecundity, with experimental temperature as a fixed main effect and latitude as the covariate. Normality of the error distribution was confirmed using the Shapiro-Wilk test ($W = 0.9709$, $P = 0.5840$) and homogeneity of variance between the two experimental temperatures was compared using the O'Brien test ($F_{(1,30)} = 1.1094$, $P = 0.3006$).

5.3.4.4 Age-specific fecundity Analysis

To investigate the age-specific fecundity of the populations, the number of eggs collected at each sample for each cage was divided by the number of female flies alive in that cage at that time. Linear regressions with latitude were then carried out on these age-specific fecundities, for each sampling interval at each temperature, to investigate whether there was any latitudinal clinal variation for age-specific fecundity.

These regressions were carried out for the first four sampling intervals at each temperature, after which all of the flies had died in some of the cages.

The Bonferroni method was applied to correct for multiple comparisons, using a significance level (α') of 0.01274. [$(1-\alpha')^4 = 0.95$].

5.4 Results

Median longevities, for each sex of each population at each temperature, and egg counts, for each population at each temperature, are presented in Table 5.1.

Table 5.1 *(a) Median longevities, for each sex of each population at each temperature, and (b) fecundities, with the percentage of fecundity accounted for in the first two samples given in parentheses, for each population and temperature.*

(a)

	18°C		25°C	
Population	Female	Male	Female	Male
MEG	58 58	74 72	33 34	42 42
TOW	70 70	79 80	29 33	43 41
ROC	64 67	77 74	24 30	26 38
COO	70 67	79 77	29 38	41 49
BEL	67 61	79 79	34 33	45 41
MIL	61 70	67 74	27 30	37 38
YY	64 56	81 72	29 34	36 41
HUO	70 67	79 79	33 32	47 41

(b)

Population	18°C		25°C	
MEG	1553 (93.1%)	1383 (79.5%)	11206 (97.4%)	8641 (90.0%)
TOW	1133 (66.5%)	1129 (83.8%)	11681 (92.4%)	12278 (89.6%)
ROC	867 (84.7%)	1453 (91.7%)	11595 (99.5%)	10041 (85.5%)
COO	834 (97.4%)	786 (78.7%)	10836 (95.6%)	7759 (96.9%)
BEL	824 (95.4%)	1293 (94.6%)	16670 (72.7%)	14593 (70.1%)
MIL	947 (90.8%)	1226 (92.4%)	5106 (84.1%)	7661 (82.9%)
YY	906 (88.0%)	549 (84.0%)	8920 (89.3%)	9906 (95.4%)
HUO	1118 (93.5%)	984 (87.2%)	13224 (62.1%)	10932 (79.5%)

5.4.1 Longevity Analysis

The analysis of covariance on longevity revealed a significant effect of both temperature and sex on longevity (Table 5.2, Fig. 5.1). The males lived significantly longer than females, and populations reared and tested at the lower experimental temperature of 18°C lived significantly longer than those reared and tested at the higher experimental temperature of 25°C. There was no significant evidence for a clinal relationship between longevity and latitude, nor was there any significant evidence for differences in slope between the sexes or experimental temperatures.

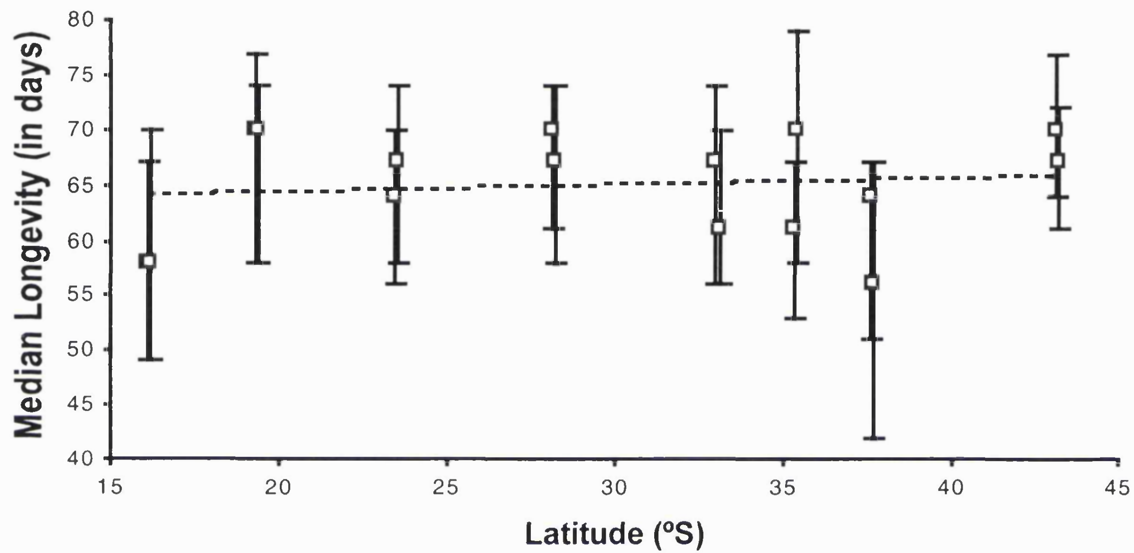
Table 5.2 *Analysis of covariance on median longevity of replicated populations reared at 18°C and 25°C. Sex and temperature were analysed as fixed main effects, and latitude was the covariate.*

effect	MS	d.f.	*F* ratio	*P*
Temperature	19321.00	1	954.43	<0.0001
Regression with Latitude	9.64	1	0.48	ns
Sex	1681.00	1	83.04	<0.0001
Temperature X Regression with Latitude	0.76	1	0.04	ns
Sex X Regression with Latitude	0.16	1	0.01	ns
Temperature X Sex	20.25	1	1.00	ns
Temperature X Sex X Regression with Latitude	1.32	1	0.07	ns
error	20.24	56		

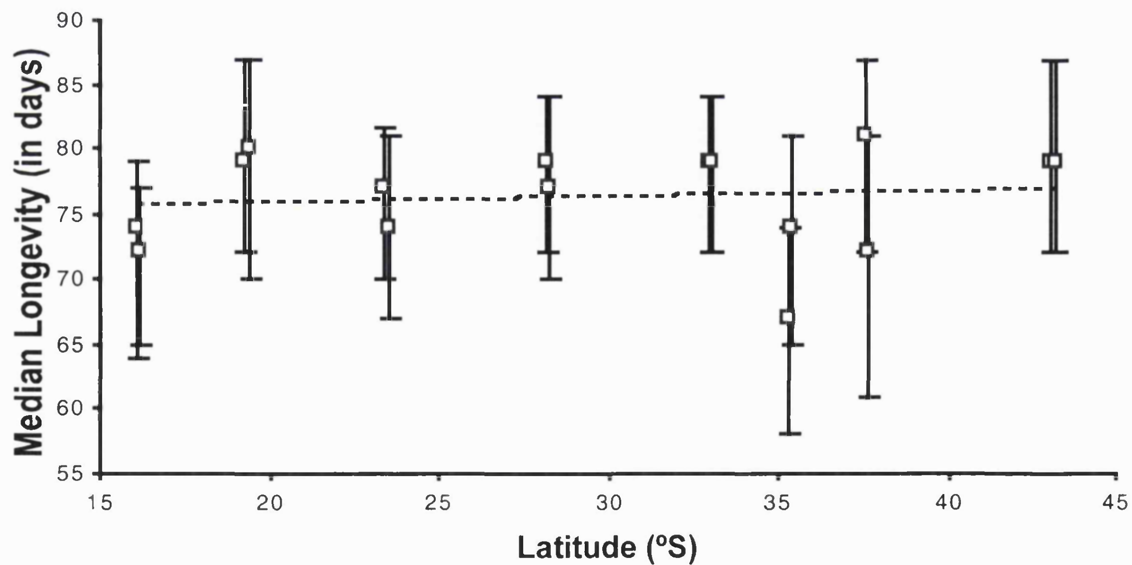
ns: $P > 0.05$

Fig. 5.1 *Graphs to show median longevity of each population, by latitude. Data has been split into (a) 18°C female, (b) 18°C male, (c) 25°C female, (d) 25°C male. Error bars indicate upper and lower quartiles. Dashed lines indicate regression lines through the data.*

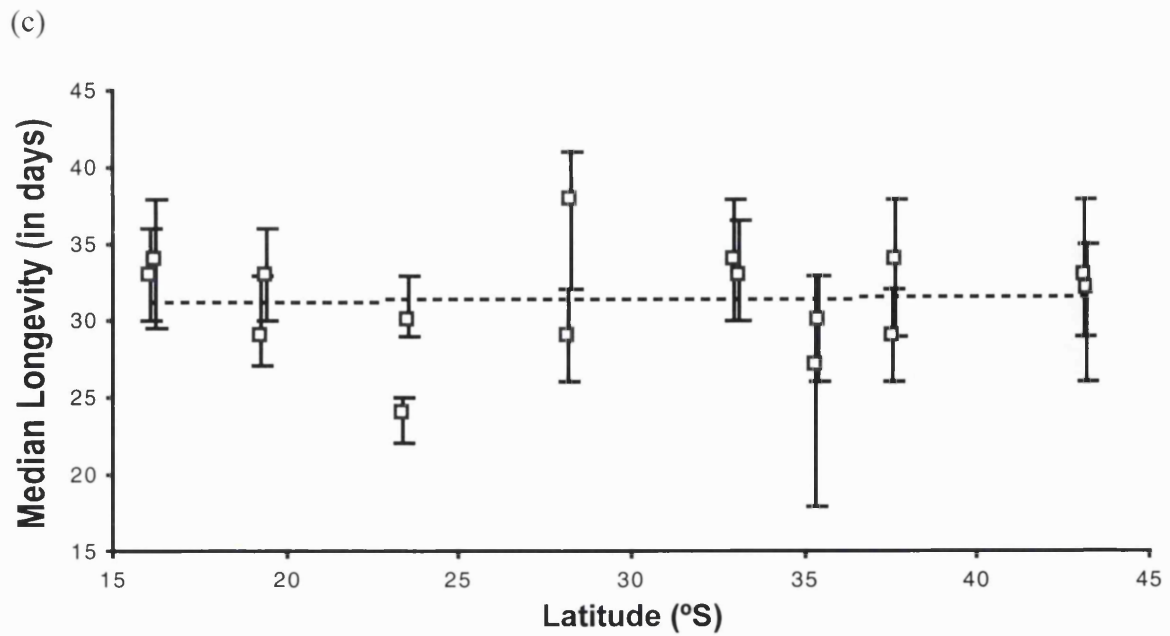
(a)



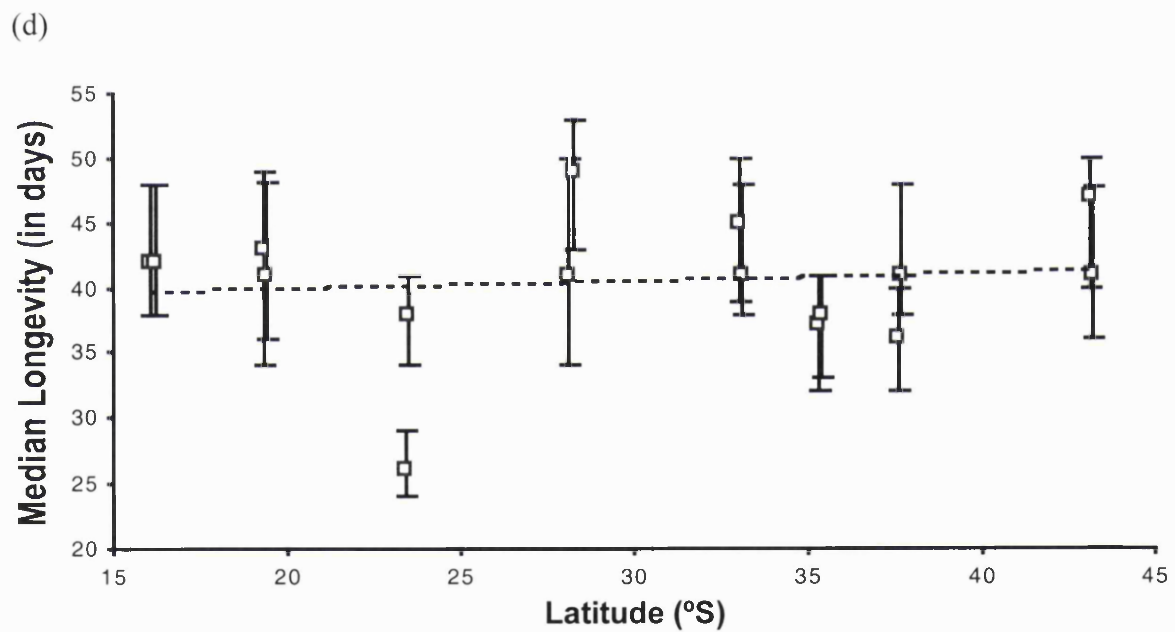
(b)



(c)



(d)



5.4.2 Fecundity Analysis

The analysis of covariance on fecundity revealed a significant effect of temperature (Table 5.3). Flies reared and tested at 25°C produced significantly more eggs than those reared and tested at 18°C. There was no significant evidence for a clinal relationship between fecundity and latitude, nor was there any significant evidence for differences in slope between the experimental temperatures.

Table 5.3 *Analysis of covariance on fecundity of populations reared at 18°C and 25°C. Temperature was treated as a fixed main effect, and latitude was the covariate.*

effect	MS	d.f.	*F* ratio	*P*
Temperature	741741128	1	172.72	<0.0001
Regression with Latitude	162704	1	0.04	ns
Temperature X Regression with Latitude	108687	1	0.03	ns
error	4294497	28		

ns: $P > 0.05$

5.4.3 Pattern of Fecundity Analysis

The analysis of covariance on the indices of early fecundity revealed no significant effect of temperature, nor any evidence of a clinal relationship between the pattern of fecundity and latitude (Table 5.4). However, the analysis of covariance did find a significant difference in slope between the experimental temperatures, as suggested by the significant interaction term between temperature and regression with latitude (Table 5.4, Fig. 5.2). At 18°C, there was no significant clinal relationship between the pattern of fecundity and latitude ($F_{(1,14)} = 2.5690$, $P = 0.1313$). However, at 25°C, there was a significant clinal decrease in the proportion of eggs laid early with increasing latitude ($F_{(1,14)} = 7.2589$, $P = 0.0175$).

Table 5.4 *Analysis of covariance on the index of early fecundity of populations reared at 18°C and 25°C. Temperature was treated as a fixed main effect, and latitude was the covariate.*

effect	MS	d.f.	*F* ratio	*P*
Temperature	0.001028	1	0.15	ns
Regression with Latitude	0.007623	1	1.09	ns
Temperature X Regression with Latitude	0.006675	1	9.59	<0.005
error	0.006962	28		

ns: $P > 0.05$

Fig. 5.2 *Proportion of total fecundity sampled in the first two sampling intervals for each cage in the experiment. Open circles represent data collected at 18°C, filled circles represent data collected at 25°C. The dashed line shows a regression with latitude through the data collected at 18°C, the solid line is a regression with latitude through the data collected at 25°C.*

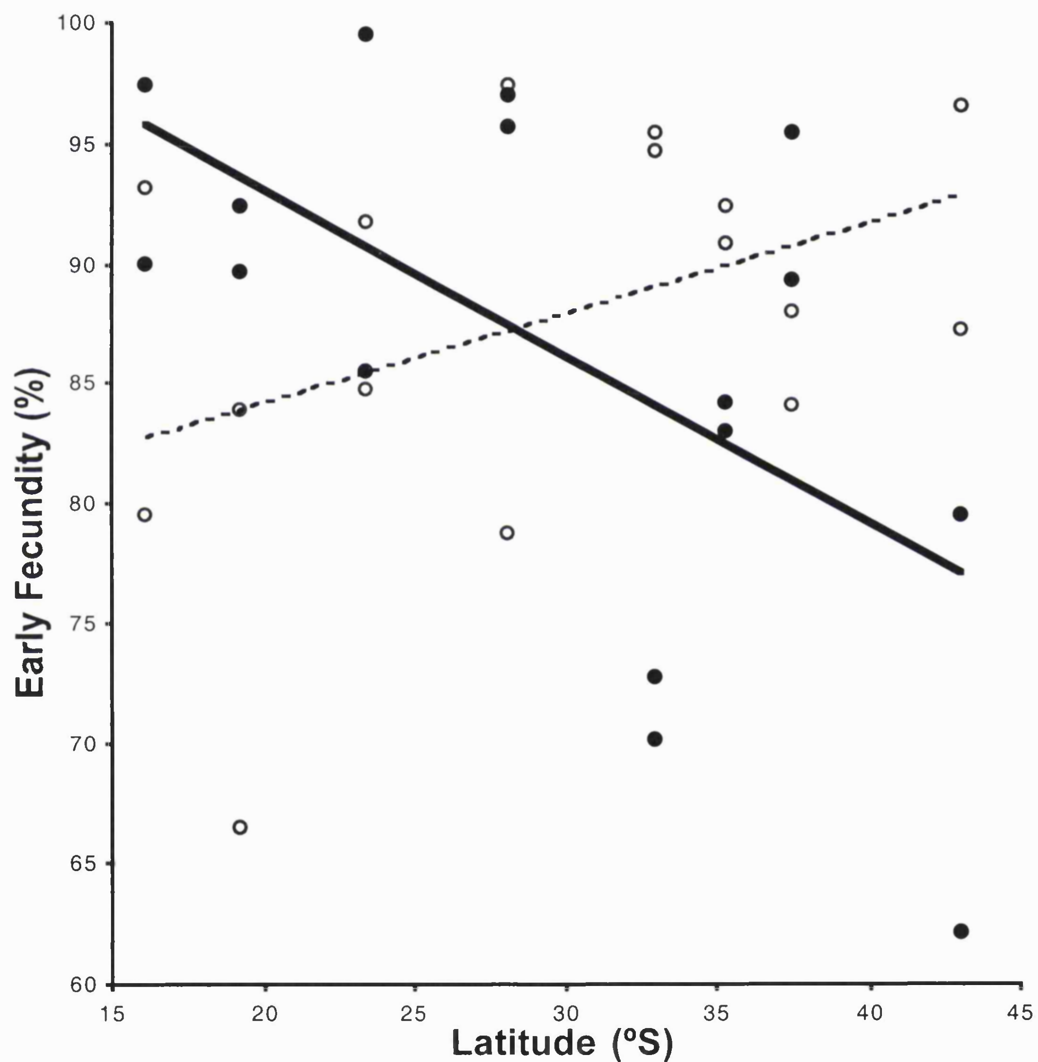


FIG 5.2 to go here

5.4.4 Age-specific fecundity Analysis

The linear regressions revealed no significant clinal variation in age-specific fecundity for any of the samples at either temperature, after applying the Bonferroni method.

5.5 Discussion

Males and females from all of the populations were much longer lived when reared and tested at the lower experimental temperature, as has previously been observed by many studies with *Drosophila* and other ectotherms (e.g. Loeb and Northrop 1917; Alpatov and Pearl 1929; MacArthur and Baillie 1929; Maynard Smith 1958; Klass 1977; Parsons 1978; Partridge *et al.* 1995; Reeve *et al.* 2001).

This study found no evidence for a latitudinal cline for longevity at either experimental temperature, in either sex. This observation contradicted the only previous study of longevity along a latitudinal cline, which found a significant difference in longevity between the extreme ends of a North American cline (Matzkin and Eanes, unpublished data, reported in Schmidt *et al.* 1999).

A latitudinal cline for longevity might also have been expected, since a correlation has been suggested between increased longevity and stress resistance, and a significant latitudinal variation for starvation resistance had previously been observed along the cline, at least in one sex. This evidence suggests that the relationship between longevity and stress resistance is not as significant as had been previously thought.

There was also no evidence for latitudinal variation in fecundity *per se* along the cline, nor any interaction between latitude and experimental temperature. This finding is contrary to a previous study that found that low latitude populations expressed a lower fecundity than high latitude populations when reared at a common temperature (Bouletreau-Merle *et al.* 1982). However, that study only examined two unreplicated populations, and consequently may not reveal the true picture of latitudinal variation along the cline. Other studies, on different species of ectotherm, the water strider and the pitcher-plant mosquito, however, revealed no such clinal trend in lifetime fecundity. The study on the water strider examined three replicated populations along a latitudinal cline in North America, and the pitcher-plant mosquito study examined nine unreplicated populations along a latitudinal cline in Japan.

A laboratory selection experiment that looked at longevity and fecundity of lines of *Drosophila melanogaster* that had evolved in different thermal environments found no evidence of an effect of selection temperature on fecundity overall. However, it did find a significant interaction between selection temperature and experimental temperature, such that populations which had evolved at a higher temperature had a greater fecundity when reared at a higher temperature (Partridge *et al.* 1995). A similar observation had previously been made comparing fecundity schedules in wild-caught lines of *Drosophila pseudoobscura* from two different thermal environments, in which females from lines originating in areas with cooler summers showed higher fecundity at lower temperatures, and lower fecundity at higher temperatures (Dobzhansky 1935).

There was some evidence in this study for differences in patterns of fecundity along the cline. The proportion of eggs laid early decreased with increasing latitude

at the higher experimental temperature, although there was no significant trend at the lower experimental temperature. This suggests that at the higher experimental temperature, flies from higher latitudes (i.e. those from colder climates) laid more eggs later in life, than those from lower latitudes. At the lower experimental temperature, flies from populations along the cline laid similar proportions of eggs early.

This again differs from a previous study of wild populations, which found an increased proportion of eggs laid early in life in higher latitude populations when reared at a constant temperature in the laboratory (Bouletreau-Merle *et al.* 1982). However, as mentioned earlier in this discussion, that study was not replicated and only examined flies from two populations along a latitudinal cline. Nonetheless, the difference between the observations of that study and this could reflect differences between individual clines. The results of Bouletreau-Merle *et al.* investigated fecundity of populations from Europe and North Africa, and concluded that the observed differences in patterns of fecundity could have evolved in response to the more stable environment in the North African area, and the less predictable temperature European climate. This variable European environment can result in variable resource availability, and consequently European populations have a higher early fecundity (and lifetime fecundity) in order to take advantage of resources when they are available. If the environments along the Australian cline differ from those of the European and North African environments, this could explain why such different observations were made in their fecundities.

6. Discussion

6.1 Latitudinal Variation in Ectotherms

Spatial variation in numerous characteristics of ectotherms has been observed throughout the world, and latitudinal clines have been observed in frogs, fish, and a wide variety of insect species (see Introduction, section 1.5). These clines occur in response to the evolutionary and developmental environments that the species encounter. Numerous environmental factors vary along the latitudinal clines, and these environmental conditions can act as selective forces.

A number of traits of *Drosophila melanogaster* vary clinally along latitudinal clines, and there is significant evidence to implicate temperature as the main factor in the evolution of this latitudinal variation (see Introduction, section 1.7). One such trait observed to vary along latitudinal clines of *Drosophila*, and other ectotherms, is body size. In this thesis, I have studied the effects of body size on fitness, as well as examining the effects of thermal adaptation on body size plasticity and its components, in *Drosophila melanogaster*. I have also looked for latitudinal variation in life history traits in the species.

These experiments assist in the understanding of latitudinal variation both in *Drosophila melanogaster* and, by implication, in other ectotherms. Similar responses to latitude in other ectothermic species may have similar genetic bases and physiology. Additionally, effects observed in *Drosophila* could provide information about similar latitudinal variation in endotherms, since they also show latitudinal variation, most notably increased body size with latitude (Bergmann 1847).

6.2 Selective Agents

The majority of the observed latitudinal variation in *Drosophila* is the result of genetic variation, established by natural selection. There is strong evidence to suggest that the selective agent for this variation is temperature. However, a large number of environmental factors vary with latitude, and may also contribute to the observed latitudinal variation.

Temperature is correlated with latitude worldwide, with higher temperatures consistently found at lower latitudes. Additionally, temperature is consistently observed to affect both development and evolution of ectotherms (see Introduction, sections 1.7 and 1.9). Latitudinal variation has also been paralleled in laboratory studies of the effects of thermal variation (see Introduction, section 1.8), with characteristics including body size, egg size, development time and larval growth efficiency showing the same trends in laboratory stocks adapted to different thermal environments as in wild populations adapted to different latitudes. Consistent with these observations are the results of Chapter 4, which found that a genetic increase in body size evolved in the replicated lines adapted to the lower constant thermal environment relative to the lines adapted to the higher constant thermal environment.

Evidence for the contribution of another factor to the establishment of latitudinal variation along clines of *Drosophila* comes from the observation of clinal variation in desiccation resistance and starvation resistance in India (see Introduction, sections 1.6.5 and 1.6.6). The uniqueness of these clines to the Indian subcontinent originally suggested that they could have arisen through genetic drift, however, similar resistance clines have been observed in several species of *Drosophila* in the continent, strongly suggesting that the trend is adaptive. Since these trends are not

observed elsewhere in the world, this suggests that temperature alone is not responsible for the latitudinal variation of these traits. One possible candidate for the selective agent of these clines is humidity, which has been shown not to vary with latitude in South America (van't Land 1997). Desiccation resistance is greater in *Drosophila* from arid areas (David *et al.* 1993; Hoffmann and Parsons 1991). Hence, humidity differences along the cline could explain the desiccation resistance variation, but it is unclear how this selection would lead to the observed opposite changes in starvation resistance along the cline. Laboratory humidity selection experiments could resolve the extent to which humidity differences result in variation in desiccation resistance, and whether starvation resistance is affected by such selection.

Evidence for a latitudinal cline for starvation resistance has since been observed in female *Drosophila* from Eastern Australia, although there was no evidence for a cline in desiccation resistance in those flies (Hoffmann *et al.* 2001). It is possible that the selective agent responsible for the starvation resistance cline in Eastern Australia could be the same as that responsible for the clines in India.

In other species of ectotherms, other selective agents have been proposed as explanations for observed latitudinal variation. In the Atlantic silverside (*Menidia menidia*) growth rate is observed to increase with latitude. It has been suggested that this growth rate variation could be due to the shorter growing season at higher latitudes (Conover and Present 1990).

It seems clear that temperature plays a major role in the latitudinal variation of many traits, which are observed in different continents. However, latitudinal variation such as that found in starvation resistance in India and Eastern Australia are

unlikely to be related to temperature, and the selective agent for this variation has yet to be identified.

6.3 The Target of Thermal Selection

Although a large number of traits vary with latitude, it remains unclear which, if any, of the traits identified is the target of thermal selection. Latitudinal variation of these traits is clearly an adaptation to the thermal environment, but selection might either be acting on individual traits or variation in one trait may be the result of selection on another. The consistent observed variation of a suite of traits in different latitudinal clines might either be due to pleiotropy, or the result of selection acting individually on a number of traits.

Body size is a possible target for selection, as increased body size has often been associated with increased fitness in several *Drosophila* species (Robertson 1957; Tantawy and Vethukhiv 1960; Tantawy and Rahkha 1964; Partridge and Farquhar 1981,1983; Partridge *et al.* 1987b). In order to investigate whether body size *per se* has direct effects on fitness, body size was manipulated, ensuring that other correlated characteristics did not alter with this artificial manipulation of body size. These lines showed increased female longevity and female fecundity with increased body size, when the lines were reared and tested at two different temperatures (McCabe and Partridge 1997). In Chapter 3 of this thesis, I found that increased body size was associated with both increased male longevity and male mating success at both experimental temperatures investigated. In both sexes, increased body size led to increased fitness, and this increase in fitness was proportionally greater when flies were reared and tested at lower experimental

temperatures, strongly implicating body size as a target for thermal selection. It is unclear whether the relationship between fitness and body size changes at different environmental temperatures, or whether the increased size of the genetically-large flies when reared at lower experimental temperatures allows them to enter a size range where fitness increases more rapidly with increases in body size.

The combination of large body size and fast development time at higher latitudes could implicate larval growth efficiency as a target of selection. High larval growth efficiency at higher latitudes enables these larvae to convert a fixed amount of food into a larger adult size, and could allow this process to occur more rapidly.

Other factors could also be involved in latitudinal variation. It is clear that latitudinal variation in *Drosophila* in particular, and in ectotherms in general, merits further research. Establishing the target of the observed thermal selection remains a challenge for future research.

6.4 Body Size

The body size of an organism has major consequences for its life history and ecology (Bonner 1965; Peters 1983; Calder 1984; Schmidt-Neilsen 1984; Damuth 1987). While many studies have investigated the radiation of such phenotypic properties as body shape and coloration, relatively little work has been carried out on the study of body size. This inherently seems odd, since body size dictates with far greater priority than factors of colour and shape what an organism's requirements for food, water and oxygen will be, and therefore which environments the organism would be able to successfully exploit (Calder 1984). An organism's size has implications too for the structure of its population, with studies indicating that population size is

negatively correlated with individual body size (Damuth 1987). These consequences of body size make it an important subject meriting research to reveal its implications for ecological structure and the life history of species.

6.5 Body Size and Temperature

As mentioned above, there is considerable evidence for body size as a target for thermal selection, and in Chapter 3, I found significant evidence for an increase in fitness due to increased body size at both experimental temperatures examined.

Further evidence for increased fitness with increased body size is provided in Chapter 4 of this thesis. In this chapter, I examined body size of lines of *Drosophila melanogaster*, originally maintained at 25°C, that had adapted to two constant temperatures (18°C and 25°C), and two cycling thermal environments (in both of which the lines spent time at both of the two temperatures, see General Materials and Methods, section 2.1.3, for more details). Lines adapted to the lower thermal temperature exhibited an increased body size relative to those adapted to the higher thermal temperature, as had previously been observed in numerous studies (see Introduction, section 1.8), suggesting that increased body size is associated with increased fitness at lower temperatures.

The cycling lines both spent more physiological time at the higher experimental temperature, however, despite this, they expressed an increase in body size comparable to that observed in the lines adapted to a constant temperature of 18°C. These results suggest that in the cycling temperature lines, selection for larger body size at the lower experimental temperature was more intense than selection for smaller size at the higher experimental temperature. This could be due to a more than

linear increase in fitness with body size as suggested to explain the observations of Chapter 3. Additionally, the cost of growing large in terms of pre-adult mortality (Fowler and Partridge 1993; Santos *et al.* 1994; Chippindale *et al.* 1997) may be increased at higher growth temperatures.

However, greater body size is not always associated with greater fitness upon rearing and testing at all temperatures. In Chapter 5, I investigated longevity and fecundity of populations along a latitudinal cline of *Drosophila melanogaster* from Eastern Australia, and found no evidence for a latitudinal cline of either longevity or total fecundity, when the lines were reared at either of two common temperatures, even though the higher latitude populations were genetically larger at both temperatures.

Similarly, replicated lines of flies laboratory thermally adapted to two different temperatures had greater longevity and fecundity when reared and tested at the temperature to which they were adapted, despite the lines adapted to the lower environmental temperature being larger at both rearing temperatures (Partridge *et al.* 1995). These results suggest that different genes can determine body size changes, or that genetic changes in body size can be associated with changes in different traits that impact on fitness, since increased body size is not always found to be associated with increased fitness upon rearing and testing at different temperatures.

The absence of a latitudinal cline of longevity in Chapter 5 was also somewhat surprising, as a significant cline for starvation resistance has been reported in the populations examined, at least in one sex (Hoffmann *et al.* 2001), and longevity and stress resistance have previously been believed to be correlated. The evidence of this study suggests that the relationship between longevity and starvation resistance may not be as significant as previously thought.

Despite finding no evidence for a latitudinal cline in total fecundity, there were significant differences in the patterns of fecundity along the cline. At the higher experimental temperature, there was an increased tendency to lay eggs later with increasing latitude. No such trend was generally observable at the lower experimental temperature.

6.6 Cellular Traits Influencing Body Size

Body size changes can either be manifested through changes in cell area or cell number. In *Drosophila*, as in most ectotherms, increases in body size have been shown to alter the size of the adult predominantly through changes in cell area, with little or no change in cell number (see Introduction, section 1.9.1).

In Chapter 4, the developmental increase in body size with development at lower temperature, observed in all the lines examined, occurred through increased cell area, as previously observed (Alpatov 1930; Robertson 1959; Delcour and Lints 1966; Masry and Robertson 1979; Cavicchi *et al.* 1985; Partridge *et al.* 1994a,b; de Moed *et al.* 1997; James *et al.* 1997).

However, this increased body size in the lines in Chapter 4 with development at lower temperatures was associated with a decrease in cell number, in all the lines. Previous studies had observed either no alteration of cell number with changes in developmental temperature (e.g. Robertson 1959; Partridge *et al.* 1994a), or an increase in cell number with decreasing developmental temperatures (e.g. Alpatov 1930; Cavicchi *et al.* 1985). The observed decrease in cell number with development at lower temperatures in Chapter 4 could be a response to the narrow thermal ranges

to which the constant temperature lines were exposed, and represent a stage in the adaptation of variable temperature lines to their thermal environment.

6.7 Plasticity of Body Size and its Cellular Components

In Chapter 4, I found no evidence for adaptation of phenotypic plasticity of body size. Wing area plasticity was comparable in lines adapted to both constant and cycling environments, suggesting that there is a cost to increased phenotypic plasticity (e.g. van Tienderen 1991).

However, although wing area plasticity did not evolve in the lines examined in Chapter 4, the plasticity of the cellular traits underlying wing area plasticity did. Plasticity of both wing area components; cell area and cell number, increased in the cycling lines relative to the constant temperature lines. The increase in plasticity of cell area did not lead to an increase in plasticity of total wing area, since it was compensated by the increase in plasticity of cell number in the opposite direction in the cycling lines.

Thus the observed increase in cell area plasticity cannot be explained by a positive contribution to plasticity of total wing area. Instead, the result suggests that the capacity to alter growth rate in response to temperature, a factor exclusively achieved through changes in cell area, is selectively favoured in variable thermal environments. Unfortunately, the design of this experiment means that we cannot reveal the response of wing area to temperature when temperature changes during development. A selective advantage of an increased capacity to alter growth rate through cell area plasticity in response to temperature would be most likely manifested during temperature transitions, which the cycling lines would have been

more frequently exposed to. The loss of plasticity in cellular traits in the constant temperature lines suggests that the capacity to change cell growth rate carries a cost (e.g. van Tienderen 1991).

The opposition between the cell number and cell area plasticities is also an interesting observation, suggesting that the two are compensating for each other, and suggesting that there might be overall stabilising selection on plasticity of body size. This seems reasonable since, were plasticity of body size not under some sort of stabilising selection, it would have been difficult to explain why body size plasticity is not observed to produce an optimal body size at each temperature in wild populations.

6.8 Further Work

Although much is known about latitudinal variation in *Drosophila*, there is still a great deal left to discover.

In Chapter 3, I found that lines selected for increased body size had a greater fitness at all temperatures, and a proportionally much greater fitness when reared and tested at a lower experimental temperature. It was unclear whether the relationship between body size and fitness is different at different environmental temperatures, or whether the increased size of the large flies upon rearing at a lower experimental temperature allows them to enter a size range where fitness increases more rapidly with increases in body size. To investigate which of these explanations is the most plausible, it would be necessary to produce lines of flies with a wide range of sizes, and compare their fitnesses at the two experimental temperatures, so that the effects of phenotypic size and experimental temperature can be more clearly elucidated.

Chapter 4 suggested that there is a cost to increased plasticity, such as the increased plasticity of cell area observed in cycling lines relative to constant temperature lines. It would be interesting to investigate whether the increased cell area plasticity of the cycling lines provides a fitness advantage over the constant temperature lines, by rearing and testing the lines at different environmental temperatures. Since increased cell area plasticity is thought to give the cycling lines an increased ability to alter growth rate, this suggests that it allows them to respond more optimally to a wide range of environments.

Chapter 5 found no evidence for latitudinal variation of either longevity or female lifetime fecundity along a cline in Eastern Australia, even though there was some evidence for variation in the pattern of fecundity along the cline. It would be interesting to see if these results can be observed in other latitudinal clines. In particular, it would be interesting to see whether the differences observed between two populations for longevity in North America (Matzkin and Eanes, unpublished data, reported in Schmidt *et al.* 1999), or the differences between two populations for fecundity in France and North Africa (Bouletreau-Merle *et al.* 1982), are representative of clinal trends in these continents.

The observed latitudinal variation in numerous traits of *Drosophila*, and body size in particular, provides a clear example of variation induced by the response of natural selection to environmental cues, particularly to temperature. The lessons we can learn from the study of latitudinal variation in *Drosophila* can provide a greater understanding of the mechanism of natural selection, and the study of body size in particular can provide information about life history and ecology.

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