# Latitudinal Variation in *Drosophila melanogaster*

## Ph.D. Thesis

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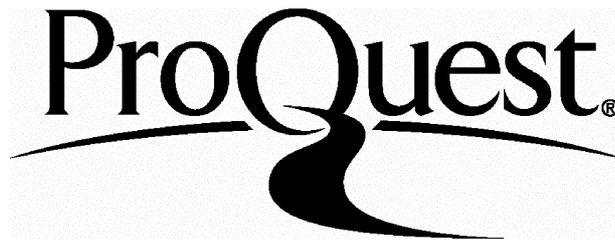
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# Abstract

There is widespread latitudinal variation in ectotherms. This latitudinal variation is often the result of a combination of the evolutionary genetic and developmental effects of the changing environment. *Drosophila melanogaster* is an ideal model organism in which to study this variation. Genetic, latitudinal clines have been found in a number of traits of *Drosophila melanogaster*. Body size, egg size and ovariole number have been found to increase, and development time to decrease with latitude in more than one continent. Parallel latitudinal clines in different continents indicate that natural selection is responsible for this genetic variation. The developmental effects of the environment can act in the same or opposite direction to the evolutionary effects.

To further investigate latitudinal variation in *D. melanogaster*, latitudinal variation in starvation resistance, fat content and larval growth efficiency were studied. Starvation resistance and fat content in South American populations were found not to vary with latitude. Larval growth efficiency was studied in both South American and Australian populations, and larvae originating from high latitude populations were able to convert a fixed amount of food into larger adult body size. In addition, at lower experimental temperatures, larvae originating from both high and low latitudes converted food into larger adult size.

An investigation into the genetic basis of the clines in body size in South America and Australia was performed. Chromosome substitution analysis was done to establish whether any of the chromosomes had large effects on body size, and whether these effects differed between the 2 continents. Chromosome 3 was found to have the largest effect on body size in both continents, with the other chromosomes also having some effect.

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# 1. General Introduction

## 1.1 Spatial Variation

The phenotype of most species varies geographically. This spatial variation occurs as a result of the developmental and evolutionary effects of the environment that the organism inhabits. Organisms respond to the environmental conditions that they occupy and their phenotype adjusts accordingly, either as a short-term or long-term measure. Spatial variation within a species can be the result of phenotypic plasticity or genetic variation. Phenotypic plasticity is purely the effect of the organism's environment on the expression of its phenotype (Scheiner 1993).

An organism's response to the environment can occur on 3 different time scales. The first 2 levels of response occur within the lifetime of an individual. The immediate environment that an organism occupies can elicit an immediate response in a trait. Traits which can respond as quickly as a change in the environment can occur are known as labile (Scheiner 1993). The second level of response occurs over a slightly longer time scale, but still within an organism's lifetime. This response can take from minutes to months to occur (Huey and Berrigan 1996), it may be reversible or non-reversible. For example, developmental temperature has an effect on adult body size in ectotherms. If the temperature is changed after the critical developmental period the size will not change. Thirdly, spatial variation in the environment can persist over a number of generations. This type of spatial variation can lead to a genetic, evolutionary response.

Examples of these 3 types of response can be provided by the ways in which the physiology of an animal can be affected by its thermal environment.

An acute (labile) response occurs when an ectotherm experiences a heat shock. When *Drosophila* are exposed to a high temperature for a short period they produce an immediate response by producing heat shock proteins to counteract the effect of the high temperatures (Lindquist 1986). The second level of response can be illustrated by physiological acclimation caused by changing temperature. When fish are exposed to cold temperatures, the mitochondrial volume density of their muscle increases (Guderley and St Pierre 1996). This process occurs as the fish acclimatise to colder temperatures. The third level of response, at the evolutionary genetic level, can be illustrated by metabolic rates in flies that have evolved at different temperatures. When measured at the same temperature, the metabolic rate of *Drosophila melanogaster* that have evolved at low temperature was higher than that of flies which had evolved at high temperature (Berrigan and Partridge 1997).

Although spatial variation in phenotype may be the result of the response of organisms to spatial environmental variation, this phenotypic variation is not necessarily adaptive. Although adaptive explanations can be found for many of these responses to changing environment, these explanations must be tested thoroughly before it can be assumed that they are correct. Spatial genetic variation may be the result of adaptation due to natural selection but it could also be due to genetic drift or migration and gene flow (Slatkin 1987). If genetic variation is adaptive, it is likely that it will be replicated under similar conditions, either elsewhere in the world, or in the laboratory. It can be more complex to determine whether short-term responses to environmental variation are adaptive (Guderley and St Pierre 1996; Huey and Berrigan 1996; Huey et al. 1999). It must be determined whether these responses result in an increase in fitness that would not otherwise be present.

Spatial variation of the environment can occur on a narrow or a broad scale. An example of narrow scale variation, is variation in local habitat. For example, conditions will vary greatly between an area shaded by a tree and that

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which is not in shade. An example of broad-scale environmental variation is variation with latitude. The environment varies greatly with latitude; this results in a large amount of variation in organisms and the characteristics that they exhibit. Latitudinal variation is a good example of spatial variation and the study of latitudinal variation provides an excellent opportunity to study evolution in action and to try to uncover the mechanisms by which evolution operates.

## 1.2 Genes and the Environment

The spatial variation in phenotype which many species exhibit is determined by both their genotype and their environment. The extent to which this variation is genetic can be determined by common garden experiments where organisms from different populations are reared in a common environment, or by reciprocal transplant experiments where organisms are transplanted into opposite environments (Conover and Schultz 1995).

The association between genetic and environmental variation can follow 3 patterns (Conover and Schultz 1995). Firstly, there may be no covariance between genotype and phenotype. The genetic variation is unlikely to be a product of the environmental conditions. The second type of relationship between genes and environment is termed co-gradient variation. In co-gradient variation, the genetic effect on the phenotype is in the same direction as the environmental effect. An example of co-gradient variation is body size variation with latitude in *Drosophila*, thought to be caused by low temperatures at high latitudes. *Drosophila melanogaster* are larger at high latitudes in the wild, they are genetically larger and a low developmental temperature also acts to make them larger (James et al. 1997). The genetic effect acts to reinforce the effect of the environment on the phenotype. The third pattern of environmental and genetic covariance is counter-gradient variation. In this case the genotype acts to oppose

the effect of the environment on the phenotype. Examples of counter-gradient variation are quite widespread and there may be many more examples as yet undiscovered because the effect of counter-gradient variation can be to produce little phenotypic variation with large amounts of environmental variation. Examples of counter-gradient variation in growth rate occur 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 but the low environmental temperature at high latitudes acts to slow growth rate. Growing seasons are shorter at high latitudes so there is also less time to reach the required size. A large size at the end of the growing season is important to survive the winter (Schultz et al. 1998). So at high latitudes fish are selected to grow faster in a shorter period of time in order to achieve a large size before the winter (Conover and Present 1990; Conover et al. 1997). Wood frog larvae originating from higher altitudes grow faster, they are able to reach metamorphosis faster and at a larger size. This counteracts the developmental effects of temperature at high altitude which slow the rate of growth (Berven 1982b).

## 1.3 Latitudinal Clines

The term cline was first proposed by Huxley in 1938 (Huxley 1938) to mean "a gradation in measurable characters". These gradations in measurable characters have been much studied since the term was first introduced. Endler defines a cline as "a geographic gradient in a measurable character, or gradient in gene, genotype, or phenotype frequency" (Endler 1977). In his book (Endler 1977), Endler concentrates mainly on those clines that occur in the hybrid zones between species. These often occur in quite small geographic areas and may be dependent on small-scale differences in habitat.

However, there are also many clines in characters that are not due to hybrid zones and are instead due to large-scale geographic change and the way that an organism adapts to occupy the environmental conditions that occur throughout its range. Latitude and altitude both have a large effect on the environmental conditions that an organism will encounter. Many species occupy a large geographic range and are affected by the environmental changes that occur across latitudes. The effect of a change in latitude (or altitude) and the accompanying environmental change can be purely a developmental reaction to the environment or a genetic adaptation due to natural selection exerted by climatic conditions. Genetic changes over a geographic range could also be caused by random genetic drift.

Latitudinal clines in various characters have been found in ectotherms from many parts of the world. These clines may be the result of genetic or environmental responses or a combination of the two. In order to demonstrate the genetic basis of a cline the organisms must be removed from their natural environment and reared under standard laboratory conditions, a 'common garden' experiment. If the variation between populations persists even under standard conditions then it can be inferred that there is a genetic component to the cline.

## 1.4 Genetic Clines in Ectotherms

Geographic variation in ectotherms has been widely studied. Clines can be genetic or can be caused by the developmental effect of environmental variation. Here I discuss genetic clines; the genetic nature of the clines in the studies mentioned was confirmed by repeating the measurements after a number of generations of laboratory culture, or by performing reciprocal transplantation experiments. The widespread occurrence of clines related to latitude in ectotherm species suggests that they are the result of natural selection.

## 1.4.1 Body Size

Studies of clinal variation in ectotherms have commonly focused on body size and other morphological traits. Latitudinal clines in body size characters have been found in a number of ectotherm species. Body size variation has been found in *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). In these species larger body size occurs at high latitudes. In addition to clinal variation with latitude, altitudinal clines also occur in ectotherms. The frogs *Rana climitans* and *Rana sylvatica* exhibit larger larval body size at both high latitudes and high altitudes (Berven et al. 1979; Berven 1982b; Berven and Gill 1983; Riha and Berven 1991).

## 1.4.2 Growth Rate

Latitudinal clines in a related trait to body size, growth rate, have also been found in ectotherm species. The rate at which an animal grows can have a significant effect on its ability to survive. Faster growth could be beneficial as it allows the animal to reach maturity quicker. However, in birds and mammals where males grow faster than females, males have higher juvenile mortality rate particularly when food resources are limited (Clutton-Brock et al. 1985). This suggests that high growth rates may be a disadvantage when food is scarce. Species which exhibit clinal variation in growth rate are the ant lion, *Myrmeleon immaculatus* (Arnett and Gotelli 1999), *Scottolana canadensis* (a copepod) (Lonsdale and Levinton 1985) and a number of species of fish, *Morone saxtilis*

(striped bass) (Conover et al. 1997), *Fundulus heteroclitus* (mummichog)

(Schultz et al. 1996), and *Menidia menidia* (Atlantic silverside) (Conover and

Present 1990). Similarly to body size, growth rate is seen to increase with

increasing latitude. The growth rate of the frogs *Rana climitans* and *Rana*

*sylvatica* is higher at both high latitudes and high altitudes (Berven et al. 1979;

Berven 1982b; Berven and Gill 1983; Riha and Berven 1991).

## 1.4.3 Development Time

Development time is also a trait that is highly significant in terms of the

ability of an animal to survive in its surroundings. An animal with a faster

development time will reach maturity and be able to produce its own offspring

sooner than one with a slow development time. However, selection for decreased

development time is correlated with a decrease in pre-adult survival in *Drosophila*

(Chippindale et al. 1997). Development time in the ant lion has been found to

decrease with latitude of origin (Arnett and Gotelli 1999). Ant lions originating

from high latitudes develop quicker to reach a larger adult size than those

originating from low latitudes. Similarly, the frogs *Rana climitans* and *Rana*

*sylvatica* develop faster at both high latitudes and high altitudes (Berven et al.

1979; Berven 1982b; Berven and Gill 1983; Riha and Berven 1991).

## 1.4.4 Growth Efficiency

How efficiently an animal can make use of the food resources available to

it in terms of growth can have a large impact on its fitness. The ability to grow

efficiently where limited resources are available can provide an animal with a

great advantage over its fellows. Efficiency of growth in ectotherms has most

commonly been measured in fish species. Efficiency of growth in the Atlantic

silverside increases with latitude of origin (Present and Conover 1992). When provided with excess food, a fish which has evolved at high latitude can grow more efficiently than one which has evolved at low latitude, (Present and Conover 1992; Billerbeck et al. 2000). However, when the fish were given limited food there was no difference in the growth efficiency of the populations (Billerbeck et al. 2000). The increase in efficiency with latitude can in part explain the difference in growth rate with latitude (Conover and Present 1990), however food consumption also increases with latitude (Present and Conover 1992; Billerbeck et al. 2000).

## 1.4.5 Physiology

All the traits that have been discussed above are affected by the physiology of the organism. A greater understanding of the underlying physiology of these traits would help us to understand the selective forces behind the clinal variation. Unfortunately, although differences in ectotherm physiology with latitude have been observed, only a small number of 'common garden' studies have been performed (see Garland and Adolph 1991 for review). In the Atlantic silverside, routine metabolism ($O_2$ consumption) varies with latitude of origin when measured at high temperature. Respiration is faster in a population originating from high latitude. (Billerbeck et al. 2000). However at 2 lower temperatures, no difference was found between populations originating from high and low latitudes.

## 1.4.6 Egg Size

Egg size variation has also been found in a number of ectotherm species. Individuals originating from higher latitudes produce larger eggs. Species that

exhibit egg size variation include the water strider *Aquarius remigis*,

(Blanckenhorn and Fairbairn 1995) and the wood frog, *Rana sylvatica* (Berven

1982a).


## 1.4.7 Clines in *Drosophila*


The ectotherm in which clinal variation has been most widely studied is

*Drosophila*. *Drosophila* are found throughout the world and exist at a wide-

range of latitudes and altitudes. In *Drosophila*, clinal variation occurs over a very

broad geographic range and in a number of characteristics. The widespread

occurrence of such variation in *Drosophila* species and particularly in

*Drosophila melanogaster* makes this a particularly appropriate model organism

for the study of such a phenomenon. The existence of clines in different

continents allows for comparison between continents and provides evidence for

the adaptive nature of the clines. Latitudinal variation has been found in more

than one continent in *Drosophila* characters such as body size, development time,

egg size and ovariole number and also to some extent in starvation resistance and

fat content. Clinal variation has also been found at the molecular level in a

number of allozyme loci and in a number of chromosomal inversions. Much of

this variation persists in laboratory studies when populations from different

latitudes are kept in a common environment and can therefore be considered to be

genetic. The fact that these genetic clines are repeated in more than one continent

indicates that they are the result of an adaptation due to natural selection rather

than genetic drift.

The suite of traits that are seen to vary with latitude, could be the result of

pleiotropy, with selection acting on one trait and other traits being affected as a

consequence. Alternately, selection could be acting on individual traits

independently. Identification of the genes involved in this variation will enable us

to understand what the target or targets of selection may be. In Chapter 5, I assess the chromosomal contribution to latitudinal variation in body size, this is a first step towards identifying the genes involved.

## 1.4.8 Body Size in *Drosophila*

A large number of geographic clines in body size in a number of *Drosophila* species have been found. Latitudinal variation in 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), and also in *D. melanogaster.* In *D. melanogaster*, latitudinal variation in body size has been found throughout the geographic range of the species. Latitudinal clines in body size have been found in North America (Coyne and Beecham 1987), Eastern Europe, the Caucasus and Central Asia (Imasheva et al. 1993), Australia (James et al. 1995), South America (Van 't Land et al. 1999), France and Africa (David and Bocquet 1975b) and Japan (Watada et al. 1986). In all these case, individuals that evolve at higher latitudes achieve a larger adult body size than those that evolve at lower latitudes. In contrast to these findings a nonmonotonic cline in body size in *D. melanogaster* from North America has also been seen (Long and Singh 1995), with larger flies found at middle latitudes and smaller flies at high and low latitudes.

## 1.4.9 Development Time in *Drosophila*

Development time in *Drosophila* has also been seen to vary with latitude. Development time is a measure of how long it takes to produce an adult fly. If

growth occurred at a constant rate, then it would be expected that it would take longer for a larger adult fly to develop. In laboratory size selection experiments, an increase in body size is correlated with an increase in development time (Partridge and Fowler 1993; Partridge et al. 1999). However, this is not the case in latitudinal clines of *Drosophila*. Development time has been studied in *D. melanogaster* originating from both Australia (James and Partridge 1995) and South America (Van 't Land et al. 1999). Development time varies with latitude, with flies originating from high latitudes developing faster. *Drosophila melanogaster* that evolve at higher latitudes are therefore able to reach a larger adult size in a shorter time.

## 1.4.10 Egg Size in *Drosophila*

Egg size is an important life-history character because it reflects the degree of maternal investment in an individual and is associated with the fitness of the offspring (for review see Azevedo et al. 1997). Egg size has been found to vary with latitude in *D. melanogaster* from both South America and Australia with larger eggs being produced by individuals from higher latitudes (Azevedo et al. 1996).

## 1.4.11 Ovariole Number in *Drosophila*

Ovariole number is an important life-history characteristic related to fitness in *Drosophila*. Ovariole number is related to the maximum daily rate of egg laying in females, although the relationship between rate of egg laying and ovariole number is not linear (David 1970; Bouletreau-Merle et al. 1982). Latitudinal clines in ovariole number in *D. melanogaster* from Australia, Europe, Africa, America, and Japan have been found (David and Bocquet 1975a; David

and Bocquet 1975b; Watada et al. 1986; Azevedo et al. 1996). Clines in 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* from India have also been found (Karan et al. 1998c; Parkash et al. 1998). Larger numbers of ovarioles are found in populations originating from high latitudes.


## 1.4.12 Starvation Resistance and Fat Content in *Drosophila*


In addition to variation in morphological and life history traits, some evidence for latitudinal variation has also been found in traits related to environmental stress such as starvation resistance. Starvation resistance is strongly correlated with fat content in *Drosophila* (David et al. 1975; Zwaan et al. 1991; Zwaan et al. 1995b; Zwaan et al. 1995a), although variation in metabolic rate can affect this correlation (Hoffmann and Parsons 1989).

Little research has been performed on latitudinal variation in starvation and fat content in ectotherm species other than *Drosophila*. Latitudinal clines in starvation resistance have been found in *Drosophila melanogaster, D. ananassae, D. kikkawai* and *Zaprionus indianus* in India, with higher starvation resistance occurring at lower 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). Although some inter-population differences in starvation resistance have been found in other countries (e.g. Da Lage et al. 1990), a cline in starvation resistance has only been found in India (See Chapter 3 for further discussion).

## 1.4.13 Desiccation Resistance in *Drosophila*

Similarly to starvation resistance, desiccation resistance is associated with environmental stress. Desiccation resistance has been studied in many of the same populations and species of *Drosophila* as starvation resistance (Shamina et al. 1993; Parkash et al. 1994; Parkash and Vandna 1994; Karan et al. 1998a; Karan and Parkash 1998). Clines in desiccation resistance are in the opposite direction to those in starvation resistance. While starvation resistance in India decreases with increasing latitude, desiccation resistance increases with increasing latitude. Like starvation resistance, clines in desiccation resistance have so far only been found in India.

## 1.4.14 Physiological Variation in *Drosophila*

Accompanying the latitudinal variation in morphological and fitness-related traits in *Drosophila* some physiological changes would be expected. Studies of physiological variation with latitude in *Drosophila* concentrate on metabolic rate variation. When measured at high temperatures, metabolic rate in *Drosophila melanogaster* has been found to be higher in high latitude populations (Giesel et al. 1991; Berrigan and Partridge 1997). In addition, the metabolic rate of high latitude populations is more sensitive to increasing temperature (Giesel et al. 1991). The higher metabolic rates in high latitude populations could go some way to explaining differences in growth rate and efficiency with latitude, if growth rate and efficiency are a function of metabolic rate. However differences in growth rate between populations have not been found to be related to differences in metabolic rate (De Moed et al. 1998).

## 1.4.15 Inversion Frequency in *Drosophila*

*Drosophila melanogaster* exhibits a high degree of polymorphism for chromosome inversions (Mourad and Mallah 1960; Watanabe 1967; Singh and Das 1990). A number of these inversions are found in all the main continents, and clines in their frequency have been found throughout the world. The repeatability of these clines in different continents suggests that they may have an adaptive value.

One inversion that has been widely studied is In(2L)t. The frequency of In(2L)t has generally been found to be higher further away from the equator, in studies performed in Japan (Inoue and Watanabe 1979, Inoue, 1984 #37), Australasia (Knibb et al. 1981), India (Singh and Das 1990) and South America (Van 't Land et al. 2000). However, In(2L)t frequency in the USA has been found to be higher in more northerly populations (Stalker 1976) and in southern populations (Mettler et al. 1977). It does appear that there is a world-wide cline in In(2L)t frequency but the results seem less conclusive than for the phenotypic traits. After a relatively short period of time under laboratory conditions populations lose their inversions (Inoue 1979) which suggest that these inversions may not be related to the clinal genetic variation that is maintained even after long periods of laboratory culture.

## 1.4.16 Allozyme Frequency in *Drosophila*

Clines in the frequency of a number of allozymes, in particular Adh and α-Gpdh have been found (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 there is

evidence that the clines in inversion and allozyme frequencies are linked (Voelker et al. 1978; Oakeshott et al. 1982; Knibb 1983), the inversion clines do not always account for the whole of the allozyme cline (Voelker et al. 1978; but see Knibb 1983).

## 1.4.17 Summary of Latitudinal Clines in *Drosophila*

As has been described above, a large number of characters in *Drosophila* are affected by latitude. The most striking factor among this latitudinal variation is that the flies that evolve at higher latitudes appear to be at an unconditional advantage. High latitude flies are larger, develop faster, lay larger eggs and have more ovarioles. All these traits would be advantageous to *Drosophila* living at any latitude, and it would therefore be expected that if selection were not acting to prevent the evolution of these characters then they would evolve in low latitude populations as well. There must therefore be a trade-off occurring. Although clines in *Drosophila* have been studied extensively, not a great deal of evidence for negative fitness components related to high latitude populations has been found. James and Partridge (James and Partridge 1998) studied competitive ability in a latitudinal cline of *Drosophila melanogaster*. They found no differences in competitive ability with latitude, but the body size of the high latitude populations was found to be more sensitive to increases in density and temperature and the combined effects of temperature and density. The high larval densities used in the competition experiments led to relatively high rates of survival. If higher densities were used, latitudinal differences in competitive abilities might be revealed. It would appear that the efficient growth of high latitude populations is adversely affected by stressful conditions of high density and temperature. If *Drosophila* at low latitudes in the wild experience high densities, in addition to high temperatures, this may explain the maintenance of

the cline with latitude. More information about conditions experienced by *Drosophila* in the wild would be helpful in determining whether this is the case. This may not be the sole reason for maintenance of the cline. There may be other factors, including behavioural differences, which are also involved in maintaining this cline. Establishing whether competition levels in the wild vary with latitude and the search for other factors which maintain the cline is a challenge for the future and will increase the understanding of latitudinal variation in *Drosophila* greatly.

## 1.5 Environmental Variation with Latitude

The genetic variation with latitude described above is an adaptation to environmental variation. It is not entirely clear what the main target of selection is or what the selective agent is. There are many environmental factors which vary with latitude, these include, temperature, humidity, rainfall, availability of food resources, levels of competition, day length, number of generations per breeding season and impact of biological enemies.

There is strong evidence that temperature might have a significant role in the evolution of latitudinal variation because in nature, in addition to variation with latitude, similar variation is also seen with altitude and with season (Stalker and Carson 1948; Stalker and Carson 1949; Tantawy 1964; Berven 1982b). Temperature is highly correlated with altitude and season as well as with latitude, and so temperature does appear to be a prime candidate for the selective agent. Temperature is also known to have a large effect on the development of ectotherms (Atkinson 1994).

In order to establish which of these factors are responsible for latitudinal variation, that factor must be manipulated independently of the other factors. This can be done in laboratory selection experiments. Laboratory selection

experiments using temperature as the varying factor have been performed widely and have produced evidence that evolution at different temperatures has a large effect on a number of traits.

# 1.6 The Evolutionary Effect of Temperature on Ectotherms

There is evidence that temperature may be a major selective force in the evolution of latitudinal variation in ectotherms. In order to test this hypothesis, ectotherms must be allowed to evolve at different temperatures with all other factors controlled. Laboratory thermal selection experiments are one way to do this. Animals are kept under controlled temperature conditions for a large number of generations. Laboratory thermal selection experiments on ectotherms have most commonly been performed on *Drosophila* species.

## 1.6.1 Laboratory Thermal Selection in *Drosophila*

The effect of laboratory thermal selection is very similar to that seen in latitudinal clines. *Drosophila melanogaster* and *Drosophila pseudoobscura* increase in size when adapted to low temperature in the laboratory (Anderson 1966; Cavicchi et al. 1985; Partridge et al. 1994a). Replicate selection lines were used in order to confirm that differences between lines were due to natural selection and not genetic drift. In addition to these replicated studies, an increase in size in low temperature evolved *Drosophila willistoni* was found in an unreplicated study (Powell 1974). Accompanying the increase in body size in low temperature evolved populations of *D. melanogaster* and *D. pseudoobscura* is a decrease in development time (Anderson 1966; Partridge et al. 1994b; James

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and Partridge 1995), so flies can grow to a larger size in a shorter time. Egg size has also been seen to increase in low temperature adapted populations of *D. melanogaster* (Azevedo et al. 1996). All the traits mentioned above vary in the same way with laboratory thermal selection as they do in natural geographic variation. This suggests that temperature is likely to have a major involvement in the evolutionary response to latitudinal variation. In addition to these factors, larval growth efficiency is also increased in low temperature selected populations of *D. melanogaster* (Neat et al. 1995). Larvae from populations that have evolved at low temperature can produce a larger fly from a given amount of food than can high-temperature-evolved larvae. Larval growth efficiency in latitudinal clines is discussed in Chapter 4.

# 1.7 Developmental Effect of Temperature in Ectotherms

In addition to the evolutionary effects of temperature, the temperature at which an ectotherm is reared has very significant effects on all aspects of its life cycle. Organisms exhibit a wide range of phenotypes when reared in different environments. This variation in phenotype depending on the environment is called phenotypic plasticity. Developmental temperature has a large effect on the phenotype that an organism exhibits.

## 1.7.1 Body Size

Body size is a character that is much related to many aspects of fitness in ectotherms. The temperature at which an organism develops can have a significant effect on its size and ectotherm size tends to increase at lower temperatures (See Ray 1960 and Atkinson 1994 for reviews). Ray (Ray 1960)

studied the effect of developmental temperature on 17 species, including a number of species of *Drosophila*, the toad *Bufo boreas* and the frog *Rana sylvatica*. It was found that 75% of the species studied increased in body size at lower rearing temperatures. The comprehensive review of Atkinson (Atkinson 1994) studied evidence of temperature effects on body size from 109 studies of ectotherms, plants and protists. Over 80% of the organisms within this study showed a significant reduction in size with increasing temperature.

The body size of species of *Drosophila* is affected by developmental temperature. *Drosophila melanogaster* and *Zaprionus indianus* increase in body size with decreasing temperature (David et al. 1994; Karan et al. 1998b; Karan et al. 1999a; Karan et al. 1999b). In both these species body size reaches a maximum with decreasing temperature and then begins to decrease again as temperature is reduced further. The maximum size of *Drosophila melanogaster* is reached at around 17°C, but varies depending on the specific trait measured as an index of size.

An increase in body size with decreasing temperature appears to be very widespread in ectotherms. This is a similar pattern to that which has been found in endotherms. Bergmann's rule (Bergmann 1847) states that in warm-blooded animals, decreasing temperature leads to a increased body size and therefore a lower relative surface area which will allow less heat loss. Larger warm-blooded animals are therefore better equipped to conserve heat than smaller animals. Bergmann's rule as applied to endotherms is somewhat controversial (Geist 1990; Paterson 1990). Bergmann's explanation for size variation can also be questioned in ectotherms. Small ectotherms such as *Drosophila*, are not able to thermoregulate, they take on the temperature of their surroundings and thus the explanation of heat-loss avoidance cannot be applied to *Drosophila* (Stevenson 1985).

There is evidence that the increase in body size in ectotherms with decreasing rearing temperature is due to an increase in cell size. Van Voorhies

found that cell size increased with increasing rearing temperature in the nematode

*Caenorabditis elegans* and in fish (hybrids of *Oreochromis*) (Van Voorhies

1996; but see Partridge and Coyne 1997). In *Drosophila melanogaster*, the

developmental body size response to temperature is caused by an increase in cell

size (Robertson 1959; Delcour and Lints 1966; Cavicchi et al. 1985; Partridge et

al. 1994b; Partridge et al. 1994a; De Moed et al. 1997). It has yet to be

established what mechanisms underlie this developmental response to

temperature.


## 1.7.2 Development Time and Growth Rate


In ectotherms as a general rule, development time increases and growth

rate decreases as developmental temperature decreases. In Atkinson's review of

body size and temperature, growth rates were studied in a number of species. At

low temperature ectotherms reach a larger adult size but take longer to achieve

that size (Atkinson 1994). Growth rate has been shown to be slowed with

decreasing temperature in a number of ectotherm species including *Fundulus*

*heteroclitus, Morone saxtilis, Menidia menidia* and *Rana sylvatica* (Conover and

Present 1990; Riha and Berven 1991; Schultz et al. 1996; Conover et al. 1997).

Similarly, development time has been shown to increase with decreasing

temperature in a large number of ectotherm species including *Drosophila*

*melanogaster, Sepsis cynipsea* and *Scathophaga stercoraria* (Schultz et al.

1996; James et al. 1997; Blanckenhorn 1999). Development at low temperatures

slows down the rate of growth and also allows a larger body size to be achieved.

### 1.7.3 Growth Efficiency

There has not been a great deal of work into what factors might underlie the developmental response to temperature in ectotherms. Both body size and development time are increased at lower temperatures – the animal becomes larger but takes longer to do it. If colder temperature caused an increase in the efficiency of growth this could allow a larger size to be achieved at low temperatures. In the Atlantic silverside, efficiency of growth increases at low temperatures (Present and Conover 1992). However, larval growth efficiency in *Drosophila* has been seen to decrease at low temperatures (Neat et al. 1995). Larval growth efficiency in *Drosophila* is discussed more fully in Chapter 4.

## 1.8 Summary of the Effects of Temperature

Temperature has a significant effect on many aspects of an ectotherm's life, both in terms of evolution and development. The strong similarities between the evolutionary response to temperature and the evolutionary response to latitude suggest that temperature plays a major role in the evolution of latitudinal variation. The developmental effect of temperature may be one reason why an evolutionary response to temperature occurs. The evolutionary response to temperature has often been seen to be the opposite of the developmental response as in counter-gradient variation (Conover and Schultz 1995). The effect of developmental temperature can also occur in the same direction as the effect of evolutionary temperature. An example of this is *Drosophila* body size which increases with both low developmental temperature and low evolutionary temperature (Partridge et al. 1994a). The plastic response of ectotherm phenotypes to temperature may have an adaptive value. Although phenotypic

plasticity is the result of environmental conditions, the extent and form of the plastic response to the environment shows genetic variation and can evolve, and may or may not be adaptive (Scheiner et al. 1991; Scheiner and Lyman 1991; Scheiner 1993).

Determining whether or not plasticity is an adaptation is not simple (Gotthard and Nylin 1995). In order for plasticity to be demonstrated to be an adaptation it must first be shown to have a genetic basis. It must also be shown to confer an advantage to the organism that has the plastic genotype. This can be done by using transplantation experiments. Individuals adapted to specific environments can be transferred to alternative environments and, if the different phenotypes produced by the same genotypes are shown to have the highest fitness in their own environment, this indicates that the plasticity is adaptive. An example of adaptive plasticity has been seen in Atlantic silverside (Scheiner and Lyman 1989; Conover and Present 1990), where growth rate in high latitude populations is affected more by temperature than it is in low latitude populations. The adaptive nature of this variation was inferred from predictions based on the environmental conditions experienced by the fish in the wild and was confirmed by laboratory experiments. A similar example is found in frogs, where tadpoles from high altitudes grow faster in a cold environment and those from low altitudes grow faster in a warmer environment. The adaptive nature of this variation was demonstrated by reciprocal transplant experiments (Berven et al. 1979; Berven and Gill 1983).

Phenotypic plasticity of body size in response to temperature occurs in a number of species of *Drosophila* (e.g. David et al. 1994; David et al. 1997; Karan et al. 1998b; Karan et al. 1999a). Plasticity in *Drosophila melanogaster* can be selected for in artificial selection experiments, and has been demonstrated to be heritable (Scheiner and Lyman 1989; Scheiner and Lyman 1991). However, it is not clear whether plasticity evolves as an adaptation to environmental conditions. In *Drosophila melanogaster*, laboratory thermal

selection experiments have shown no evolutionary response in levels of plasticity of body size with temperature (Partridge et al. 1994a). Similarly, no latitudinal differences in plasticity were found in a latitudinal cline of *Drosophila melanogaster* (James et al. 1997). Plasticity in *Drosophila* may be adaptive but the level of plasticity does not evolve as a result of temperature, or latitude. Levels of plasticity may respond to fluctuating thermal environment, with higher levels of plasticity occurring in more variable environments. However, laboratory studies have not so far shown any changes in phenotypic plasticity of body size and larval development time in *Drosophila* that had evolved in fluctuating thermal environments (M. Reeve, personal communication).

## 1.9 Latitudinal Variation in *Drosophila*

All ectotherms seem to evolve in response to their environment and particularly in response to temperature. A greater understanding of the mechanisms by which this adaptation occurs will give us further insight into how and why this variation occurs. It is clear that spatial variation in ectotherms is due to a combination of developmental and genetic effects resulting from variation in the environmental conditions that the animals encounter. Developmental and genetic effects can act in the same direction or can oppose each other. In addition, the many different environmental factors the animals encounter may combine or interact to produce differing phenotypes and genotypes. In particular, temperature is known to have a significant effect on many ectotherms, both developmentally and evolutionarily. Understanding the trade-offs at work in adaptation to life in different environments will reveal what physiological factors are of fundamental importance to the lives of these animals.

The existence of world-wide clines in *Drosophila melanogaster* make it an ideal model organism in which to study the evolution of geographic variation.

Studying geographic variation in *Drosophila* will lead to a greater understanding of geographic variation and its similarities and differences between continents. A wider understanding of geographic variation in *Drosophila* will provide indications to the basis of geographic variation in other ectotherm species.

## 1.10 Outline of Thesis

This thesis considers a number of aspects of latitudinal variation in *Drosophila melanogaster* populations originating from South America and Australia. I have tried to increase the understanding of the range of traits that vary with latitude world-wide and the underlying basis of the variation in these traits.

In Chapter 3 I assessed whether latitudinal variation in starvation resistance and fat content exists in South American populations of *Drosophila melanogaster* which have previously been found to exhibit latitudinal variation in a number of other traits. Latitudinal variation in starvation resistance has previously only been found in India, and my results will help to establish whether starvation resistance and fat content can be considered to be among the suite of traits that vary with latitude throughout the world.

In Chapter 4 I assessed larval growth efficiency in populations of *Drosophila melanogaster* originating from both South America and Australia. Latitudinal clines in body size and development time have previously been found in these populations and variation in efficiency of growth could be the reason for this variation. In addition to studying the genetic latitudinal variation of larval growth efficiency, I also studied the developmental effects of temperature on larval growth efficiency in flies from the 2 continents.

In Chapter 5 I assessed the chromosomal contribution to the latitudinal variation in body size in both South America and Australia. No previous study

has attempted to uncover the genetic basis of latitudinal variation in this way by studying both sexes of flies in 2 independent clines from separate continents. I was able to compare the chromosomal basis of genetic latitudinal variation in body size between continents, and assess whether selection and the response to selection might be occurring in the same or in different ways in the separate continents.

# 2. General Materials and Methods

## 2.1 Populations of *Drosophila melanogaster*

### 2.1.1 South American Populations (Chapters 3, 4 & 5)

The 10 South American populations were collected in 1995 by Jan Van 't Land and Pim Van Putten (University of Groningen, The Netherlands) along a latitudinal range from 2.22°S to 41.5 °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 similar distances from the sea. The longitudes of the collection sites varied between 70.17°W and 79.9°W and with the exception of Linares (140m) and Copiapó (350m) the altitude of the collection sites was less than 100m. The populations have been maintained since collection in bottle culture at 25°C. These flies have been found to exhibit latitudinal variation in body size, development time and egg size when reared under standard laboratory conditions (Azevedo et al. 1996; Van 't Land et al. 1999).

### 2.1.2 Australian Populations (Chapters 4 & 5)

The 4 Australian populations were collected in early 1997 from sites in Tasmania, Queensland and the Northern Territory. The Tasmania and Queensland populations were collected by Ary Hoffmann (La Trobe University, Australia) and the Northern Territory population was collected by Linda Partridge (University College London). Latitudes of the collection sites are

shown in Table 2.2. As with the South American populations the collection sites were chosen to be of similar longitude, altitude and distance from the sea, in order to minimise variation caused by factors other than latitude. The longitudes of the populations were between 146°E and 147.1°E, except Darwin, which was at 130.44°E. All the Australian populations were collected from low altitude sites near the coast. These populations have been maintained since collection in bottle culture at 25°C. The Australian populations form part of a cline that has previously been found to exhibit latitudinal variation in body size, development time, egg size and ovariole number (James et al. 1995; James and Partridge 1995; Azevedo et al. 1996).

## 2.1.3 Dahomey Population (Chapter 4)

The Dahomey population was collected in 1970 in Dahomey, West Africa (now Benin) and was maintained from collection in population cage culture with overlapping generations at 25°C.

## 2.1.4 SM5/bw$^{VI}$; TM3/TM6 Balancer Stock (Chapter 5)

The SM5/bw$^{VI}$; TM3/TM6 stock contains balancer chromosomes. These balancer chromosomes contain inversions, which prevent recombination and also contain dominant visible markers that are used to indicate the presence of the chromosome. The balancer chromosomes are homozygous lethal so a fly cannot have 2 copies of the same balancer chromosome. SM5 is a second chromosome balancer chromosome, which carries the curly wing mutation. The other second chromosome contains the bw$^{VI}$ mutation which is a brown variegated eye colour mutation, this mutation is associated with an inversion, and is homozygous lethal (Lindsley and Zimm 1992) but the chromosome is not a balancer because it is

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not completely covered by inversions. The third chromosome balancers are TM3 and TM6, TM3 carries the serrate and stubble mutations and TM6 carries tubby and humeral (Lindsley and Zimm 1992). Crossing schemes involving these balancer stocks were used to perform chromosome substitutions in the South American and Australian populations (Chapter 5). The combination of inversions (to prevent recombination) and visible dominant markers enable the isolation of individual wild type chromosomes which can then be substituted into an alternative genetic background. This stock was maintained in vials at 18°C.

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

| Population | Location | Latitude (°S) |
|:---:|:---:|:---:|
| GU | Guayaquil, Ecuador | 2°13' |
| AR | Arica, Chile | 18°28' |
| IQ | Iquique, Chile | 20°13' |
| AN | Antofagasta, Chile | 23°38' |
| CO | Copiapó, Chile | 27°20' |
| CQ | Coquimbo, Chile | 29°56' |
| VA | Valparaiso, Chile | 33°05' |
| LI | Linares, Chile | 35°48' |
| VD | Valdivia, Chile | 39°48' |
| PM | Puerto Montt, Chile | 41°30' |

**Table 2.2** The locations and latitudes of the collection sites of the Australian
populations

| Population | Location | Latitude (°S) |
|:---:|:---:|:---:|
| DAR | Darwin, Northern Territory | 12.23°S |
| INN | Innisfail, Queensland | 17°30' |
| FLO | Flowerpot, Tasmania | 43°08' |
| CYG | Cygnet, Tasmania | 43°15' |

# 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 is used for maintaining bottle and vial populations and also used
for standard density rearing.

### 2.2.1.2 Sugar-yeast food (SY)

100g sugar

100g dried yeast

20g agar

3ml propionic acid

30ml of 10% Nipagin solution in ethanol

1 litre of water

This medium is used for maintaining the Dahomey cage population.

### 2.2.1.3 Grape juice medium

50g agar

600 ml grape juice

1 litre water

42.5ml of 10% Nipagin solution in ethanol

Used for collecting eggs in laying pots or from cages in petri dishes. Eggs or first instar larvae can be easily picked from the surface of the medium.

### 2.2.1.4 Agar and yeast

5mg agar

1 litre water

Yeast in aqueous solution is added to the surface of 1ml of the autoclaved agar medium in small vials (12mm diameter). This medium is used in larval growth efficiency experiments (Chapter 4).

## 2.2.2 Stock Maintenance

### 2.2.2.1    Bottle stocks (South American and Australian populations)

Flies were transferred to a fresh 1/3 pint bottle containing 80ml of ASG medium. The flies were allowed to lay in the bottle for a few hours until a moderate density of eggs had been laid on the surface of the medium, the flies were then removed from the bottle. This process was repeated after 14 days (at 25°C) when the majority of flies had eclosed.

### 2.2.2.2    Vial stocks

These were maintained in the same way as bottle stocks (above), except flies were transferred to vials (22mm diameter, 75mm height) containing 7ml of standard food.

### 2.2.2.3    Cage (Dahomey)

3 bottles containing 80ml of SY food (See General Materials and Methods) were added each week on a 4-week rotation. There were therefore always 12 bottles in the cage, covering a 4-week period. The Dahomey cage is kept at 25°C.

# 2.3 General Methods

## 2.3.1 Egg Collection

### 2.3.1.1    Laying pots

Flies were placed in laying pots containing grape juice medium with a dab of live yeast on the surface. An acclimatisation period of 12-24 hours was allowed followed by a pre-lay period of 1 hour on fresh medium to encourage the laying of any retained eggs. The flies were then transferred to fresh medium for egg collection.

### 2.3.1.2    Cage collection

A petri dish containing grape juice medium was placed into the cage for a short period.

## 2.3.2 Standard Density Culture

Where a standard, uncrowded, larval density was required, 50, first instar larvae were picked from the grape juice medium in a laying pot or petri dish using a mounted needle, and were placed in a vial containing approximately 7ml of standard (ASG) medium. Where crowded larval conditions were required, 200 first instar larvae were picked from the medium and placed into a vial as above.

### 2.3.3 Wing Area Measurement

On emergence, flies were removed from vials under carbon dioxide anaesthesia and were frozen. The left wings of adult flies were then removed, fixed with propanol and mounted on microscope slides using Aquamount. Images of the wings were captured on a PowerMacintosh 8600/200 computer with a video camera attached to a compound microscope. A good approximation to total wing area was achieved by measuring the area between 6 points (including the 4 points where veins intersected the edge of the wing) as illustrated in Figure 2.1. Wing area was measured 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).

**Figure 2.1** The landmarks on the wing that are used to measure wing area
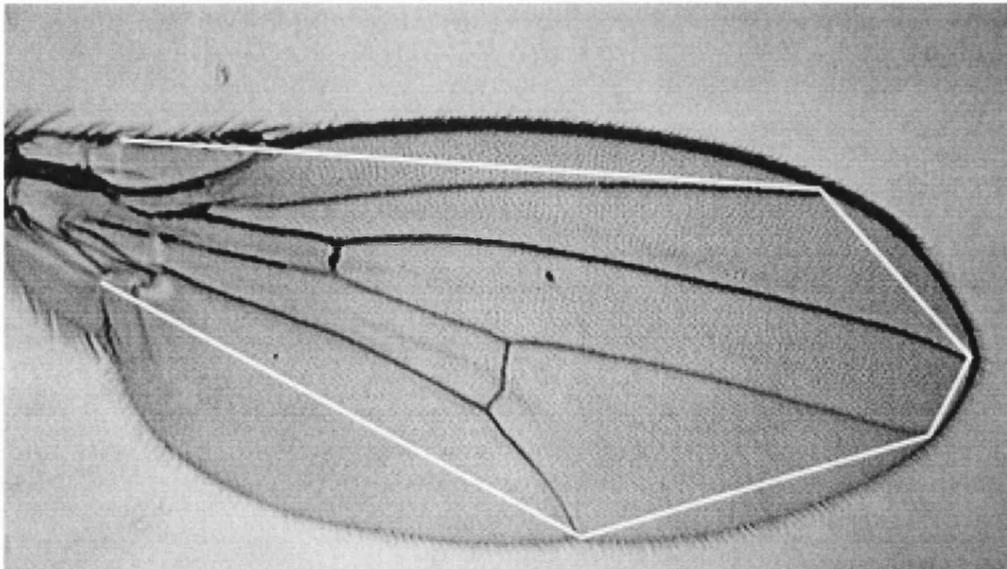
## 2.4 Statistical Analysis

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

# 3. Starvation Resistance and Adult Body Composition in a Latitudinal Cline of *Drosophila melanogaster*

## 3.1 Abstract

Although clines in many traits of *Drosophila melanogaster* have been found throughout the world, a cline in starvation resistance and fat content in *Drosophila melanogaster* has so far been found only in India. Here we investigate starvation resistance and fat content in 10 populations from South America, in which clines in body size, egg size and development time have previously been found (See General Materials and Methods). We find no evidence for a cline in starvation resistance or fat content in South America. We therefore suggest that the cline in starvation resistance in India may have evolved in response to specific climatic variation found only in India.

## 3.2 Introduction

The work described in this chapter has been accepted for publication in Evolution (Robinson et al. in press, see Appendix).

Latitudinal clines in starvation resistance have been found in *Drosophila melanogaster*, *D. ananassae*, *D. kikkawai* and *Zaprionus indianus* in India, with higher starvation resistance occurring at lower latitudes (See General Introduction and 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). In Europe and Africa, differences in starvation resistance have been found between populations of *D. melanogaster*, but these differences were not related to latitude (Da Lage et al. 1990). The latitudinal differences in starvation resistance in India may be explained by ecological and/or climatic factors other than temperature, which also vary with latitude (Karan et al. 1998a). Starvation resistance has often been used as an indirect measure of fat content, because these 2 characters are highly correlated (David et al. 1975; Zwaan et al. 1991; Zwaan et al. 1995b; Zwaan et al. 1995a). However, studying fat content directly would eliminate other factors such as metabolic rate that may affect starvation resistance (Hoffmann and Parsons 1989). It should also be noted that some of the latitudinal ranges studied may not have been wide enough to detect correlations with latitude.

In the present study, starvation resistance and fat content were measured in 10 populations from a transect along a wide latitudinal range in South America. Temperature varies with latitude over this transect, while a number of other climatic factors such as humidity and amount of sun hours do not (Van 't

Land 1997). The collection sites for these populations were low altitude, coastal sites, in order to minimise variation caused by factors other than latitude.

The aims of the present study were: (i) to establish whether starvation resistance and fat content are among the suite of traits that vary with latitude and for which temperature is implicated as the selective agent, and (ii) to establish whether there is clinal variation in body composition in South America. Because these populations are known to increase in body size with latitude, we investigate whether fat content also increases with latitude, which could indicate an ability of high latitude flies to acquire more food or to utilise food more efficiently. Alternatively, increased body size may be achieved by utilising the fat reserves for extra growth. Larval crowding has been shown to influence fat content and starvation resistance in temperate (Zwaan et al. 1991) and tropical (Van 't Land 1997) populations of *D. melanogaster*. Therefore, this experiment was performed using adult flies that had been kept at either high or low density as larvae in order to assess whether differing larval density affects fat content, starvation resistance or the relationship of fat content and starvation resistance with latitude.

## 3.3 Materials and Methods

Starvation resistance and fat content were measured in the South American populations (See General Materials and Methods)

Standard density cultures of 50 and 200 larvae per vial (See General Materials and Methods) were set up for each of the populations (3 vials per density per population). The 2 densities were used to test whether crowding had any effect on starvation resistance. Eclosing adults were collected on ice as virgins and were transferred in single sex groups of 5 to vials containing fresh ASG medium, which were renewed regularly. The flies were kept for 15 days

before measurements were made, because the starvation resistance of female flies changes rapidly in the first 2 weeks of adulthood (Fairbanks and Birch 1970; Service et al. 1985; Zwaan et al. 1991), which could lead to spurious differences when studying younger flies. The flies were kept unmated because there may be a trade off between starvation and egg-laying (Chippindale et al. 1993) and therefore, if mated flies were used, between-line differences in fecundity could confound the results of the study.

## 3.3.1 Experimental Measurements

### 3.3.1.1    Wet weight, dry weight, water content and fat content

Wet weight, dry weight, water content and fat content were determined for 15 flies of each sex from each population and each larval density. The flies used were kept in single sex groups of 5 for weighing. An equal number of flies were taken from each of the original culture vials to equalise any effects caused by between-vial variation. After removal from food, the flies were anaesthetised on ice, and were weighed to the nearest 0.002mg using a Sartorius microbalance to determine wet weight. The flies were dried in an oven at 60°C for 24 hours and were then re-weighed to determine dry weight. To remove fat from the flies, they were placed in sealed tubes with 1ml of diethyl ether for 24 hours with occasional light shaking. They were then removed from the ether and allowed to dry at room temperature for a further 24 hours, and then re-weighed to determine the fat-free dry weight. From the weights obtained it was possible to calculate water content (wet weight - dry weight), fat content (dry weight - fat-free dry weight), relative water content (water content ÷ wet weight) and relative fat content (fat content ÷ dry weight) (David et al. 1975).

### 3.3.1.2    Starvation resistance

Starvation resistance was measured for 15 flies of each sex from each population and each larval density, with an equal number of flies taken from each of the original culture vials. The flies were placed individually in vials containing 1ml of autoclaved agar (5g/l) in order to prevent desiccation. The vials were observed for dead flies after 24 hours had elapsed, and then at intervals of 3 hours. The frequency of observations was decreased as the number of deaths per observation decreased. Death was deemed to have occurred when a fly showed no sign of movement even after the vial was tapped lightly. Time of death was assigned to the midpoint between observations.

## 3.3.2 Statistical Analysis

### 3.3.2.1    Wet weight, dry weight, water content, fat content and starvation resistance

Analysis of covariance was performed on each of the traits, with latitude as the covariate and sex and density as independent variables. A (quasi) minimum adequate model (MAM) was found by a stepwise backward deletion procedure (Crawley 1993). The highest order interaction was removed from the full model if it did not explain a significant proportion of the residual variance ($p > 0.05$), and a reduced model was fitted. The next highest order non-significant interaction with the highest P-value was then removed and a new reduced model was fitted. Non-significant factors or interactions were maintained in the model when any higher-order interactions including the factor or interaction were significant ($p < 0.05$).

### 3.3.2.2 Relationship between fat content and starvation resistance

To determine whether fat content and starvation resistance have a direct relationship, and whether there are factors other than fat content that affect starvation resistance, we performed further tests.

A multiple regression model was fitted using starvation resistance as the dependent variable with sex, crowding, relative fat and latitude as factors. All interactions were included in the model, and a minimum adequate model was then found using a stepwise backward deletion procedure (as above).

# 3.4 Results

## 3.4.1 Wet Weight, Dry Weight, Water Content, Fat Content and Starvation Resistance

Mean values for wet weight, relative water content, relative fat content and starvation resistance are shown in Table 3.1. The results of the MAM analyses of covariance are summarised in Table 3.2. For wet weight and dry weight, latitude, density and sex were all significant main effects with a significant latitude by sex interaction. Wet and dry weight were larger at high latitude, low density and in females. The interaction results from different responses of males and females to increasing latitude. Table 3.1 shows mean values for wet weight for each sex and inspection of this data indicates that increasing latitude had a greater effect on females than it did on males. For water content, latitude, density and sex were all significant with no significant interaction. Water content was greater at high latitudes, low density and in females. For fat content, sex and density were significant main effects. Fat content was higher in females and at

low density. For relative water content, relative fat content and starvation resistance, only sex was significant, with males having a higher relative water content and females having a higher relative fat content and a higher starvation resistance. In summary, only wet weight, dry weight and water content showed clinal variation.

**Table 3.1** Mean values for (a) wet weight, (b) relative water content, (c) relative fat content and (d) starvation time

(a) Mean wet weight (mg)

| Latitude (°S) | Female | | Male | |
| --- | --- | --- | --- | --- |
| | Low density | High density | Low density | High density |
| 2.22 | 5.373 | 4.701 | 3.669 | 3.080 |
| 18.47 | 6.023 | 6.263 | 4.209 | 3.641 |
| 20.22 | 6.325 | 5.804 | 4.617 | 3.651 |
| 23.63 | 6.748 | 5.678 | 4.398 | 3.620 |
| 27.33 | 6.982 | 5.950 | 4.658 | 3.620 |
| 29.93 | 6.734 | 5.842 | 4.526 | 3.816 |
| 33.08 | 6.392 | 5.319 | 4.210 | 3.505 |
| 35.8 | 7.596 | 6.477 | 4.489 | 3.769 |
| 39.8 | 6.857 | 6.238 | 4.578 | 3.663 |
| 41.5 | 7.373 | 6.290 | 4.671 | 3.968 |

Table continued overleaf

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(b) Mean relative water content

| Latitude (°S) | Female | | Male | |
|---|---|---|---|---|
| | Low density | High density | Low density | High density |
| 2.22 | 0.667 | 0.656 | 0.674 | 0.673 |
| 18.47 | 0.599 | 0.658 | 0.658 | 0.671 |
| 20.22 | 0.650 | 0.636 | 0.686 | 0.683 |
| 23.63 | 0.649 | 0.665 | 0.673 | 0.680 |
| 27.33 | 0.645 | 0.653 | 0.683 | 0.679 |
| 29.93 | 0.632 | 0.632 | 0.670 | 0.665 |
| 33.08 | 0.603 | 0.650 | 0.667 | 0.687 |
| 35.8 | 0.687 | 0.671 | 0.687 | 0.689 |
| 39.8 | 0.653 | 0.637 | 0.682 | 0.678 |
| 41.5 | 0.621 | 0.632 | 0.668 | 0.669 |

(c) Mean relative fat content

| Latitude (°S) | Female | | Male | |
|---|---|---|---|---|
| | Low density | High density | Low density | High density |
| 2.22 | 0.057 | 0.070 | 0.043 | 0.036 |
| 18.47 | 0.136 | 0.062 | 0.059 | 0.044 |
| 20.22 | 0.090 | 0.092 | 0.041 | 0.039 |
| 23.63 | 0.068 | 0.056 | 0.041 | 0.032 |
| 27.33 | 0.077 | 0.074 | 0.026 | 0.033 |
| 29.93 | 0.092 | 0.087 | 0.036 | 0.044 |
| 33.08 | 0.130 | 0.082 | 0.058 | 0.029 |
| 35.8 | 0.038 | 0.058 | 0.034 | 0.027 |
| 39.8 | 0.073 | 0.087 | 0.036 | 0.031 |
| 41.5 | 0.092 | 0.085 | 0.045 | 0.042 |

Table continued overleaf

Table 3.1 continued

(d) Mean starvation time (hours)

| Latitude (°S) | Female | | Male | |
|---|---|---|---|---|
| | Low density | High density | Low density | High density |
| 2.22 | 60.100 | 62.283 | 28.892 | 32.608 |
| 18.47 | 104.383 | 68.083 | 33.617 | 32.783 |
| 20.22 | 80.107 | 48.768 | 31.920 | 26.821 |
| 23.63 | 77.033 | 65.767 | 30.575 | 32.217 |
| 27.33 | 75.267 | 61.367 | 25.533 | 26.767 |
| 29.93 | 55.667 | 70.258 | 30.642 | 29.408 |
| 33.08 | 102.383 | 70.600 | 33.917 | 33.483 |
| 35.8 | 48.308 | 39.750 | 23.933 | 26.442 |
| 39.8 | 68.217 | 66.083 | 28.717 | 27.917 |
| 41.5 | 79.817 | 79.550 | 36.017 | 36.417 |

Table 3.2 Minimum adequate model analysis of covariance with (a) Wet weight,
(b) dry weight, (c) water content, (d) relative water content, (e) fat content, (f)
relative fat content and (g) starvation resistance as dependent variables, latitude as
covariate and sex and density as independent variables

| Source | d.f. | Mean Square | F Ratio | $p$ |
|---|---|---|---|---|
| (a) Wet weight | | | | |
| Latitude | 1 | 4.1156 | 48.5867 | <0.0001 |
| Density | 1 | 6.0321 | 71.2119 | <0.0001 |
| Sex | 1 | 3.8403 | 45.3363 | <0.0001 |
| Latitude*Sex | 1 | 0.5944 | 7.0173 | 0.0120 |
| Error | 35 | 0.0847 | | |
| (b) Dry weight | | | | |
| Latitude | 1 | 0.5555 | 43.7765 | <0.0001 |
| Density | 1 | 0.8550 | 67.3765 | <0.0001 |
| Sex | 1 | 0.5842 | 46.0365 | <0.0001 |
| Latitude*Sex | 1 | 0.1322 | 10.4216 | 0.0027 |
| Error | 35 | 0.0127 | | |
| (c) Water content | | | | |
| Latitude | 1 | 1.6471 | 24.9101 | <0.0001 |
| Density | 1 | 2.3451 | 35.468 | <0.0001 |
| Sex | 1 | 17.2082 | 260.2572 | <0.0001 |
| Error | 36 | 0.0661 | | |

| Source | d.f. | Mean Square | F Ratio | $p$ |
|---|---|---|---|---|
| (d) Relative water content | | | | |
| Sex | 1 | 0.0098 | 36.2976 | <0.0001 |
| Error | 38 | 0.0003 | | |
| (e) Fat content | | | | |
| Sex | 1 | 1.1753 | 105.1813 | <0.0001 |
| Density | 1 | 0.0754 | 6.7475 | 0.0134 |
| Error | 37 | 0.0112 | | |
| (f) Relative fat content | | | | |
| Sex | 1 | 0.0172 | 55.7984 | <0.0001 |
| Error | 38 | 0.0003 | | |
| (g) Starvation resistance | | | | |
| Sex | 1 | 15022.1070 | 111.1708 | <0.0001 |
| Error | 38 | 135.1000 | | |

## 3.4.2 Relationship Between Fat Content and Starvation Resistance

The MAM multiple regression analysis is shown in Table 3.3. A number of significant interactions were found. The interaction between relative fat and latitude was significant (parameter estimate = 0.41312). This interaction indicates that the relationship between starvation resistance and relative fat varied with increasing latitude. The sign of the parameter estimate is positive, indicating that starvation resistance and relative fat were more closely correlated with increasing latitude. The interaction between relative fat and density was also significant (parameter estimate = 13.18336). This interaction indicates that larval density has an effect on the relationship between fat content and starvation resistance. The sign of the parameter estimate indicates that at high density, fat explained more of the variation in starvation resistance than it did at low density.

**Table 3.3** Minimum adequate multiple regression model with starvation resistance as the dependent variable, latitude and relative fat content as continuous factors and sex and density as discrete factors

| Source | d.f. | Mean Square | F Ratio | $p$ |
|---|---|---|---|---|
| Sex | 1 | 620.8157 | 14.8234 | 0.0007 |
| Density | 1 | 747.5490 | 17.8494 | 0.0002 |
| Latitude | 1 | 650.7022 | 15.537 | 0.0005 |
| Relative fat | 1 | 347.1335 | 8.2886 | 0.0077 |
| Sex*Density | 1 | 490.9332 | 11.7221 | 0.0020 |
| Latitude*Sex | 1 | 611.9400 | 14.6114 | 0.0007 |
| Sex*Relative fat | 1 | 65.0617 | 1.5535 | n.s. |
| Latitude*Density | 1 | 655.9321 | 15.6618 | 0.0005 |
| Density*Relative fat | 1 | 814.1069 | 19.4386 | 0.0001 |
| Latitude*Relative fat | 1 | 685.7342 | 16.3734 | 0.0004 |
| Latitude*Sex*Density | 1 | 495.9863 | 11.8428 | 0.0019 |
| Latitude*Density*Relative fat | 1 | 696.0447 | 16.6196 | 0.0004 |
| Error | 27 | 41.8800 | | |

# 3.5 Discussion

The results of our analysis showed that relative water content, fat content, relative fat content and starvation resistance did not vary clinally. Overall water content increased with increasing latitude, but this was paralleled by the increase in dry body weight; flies from each latitude contained the same amount of water per unit body weight. The multiple regression model for starvation resistance found a significant latitude by relative fat interaction, indicating that latitude affected the relationship between relative fat content and starvation resistance. Similarly, the crowding by relative fat interaction indicated that the relationship between fat and starvation resistance was affected by larval density. Factors other than fat content must therefore have partly determined starvation resistance. Previous studies have shown that starvation resistance is directly correlated with fat content (See Introduction). However, other factors such as metabolic rate may also play a role, as has been suggested by our study. Previous studies of clines have concentrated on starvation resistance alone and concluded that it reflected differences in fat content (Da Lage et al. 1990; Shamina et al. 1993; Parkash et al. 1994; Parkash and Vandna 1994). However, not measuring fat content may miss effects due to other factors. In studying both fat content and starvation resistance directly, we have been able to establish that there is no clinal variation in either in the South American populations studied and that other factors such as metabolic rate differences may explain the apparent variation in starvation resistance, independent of fat content.

Other studies of starvation resistance in *D. melanogaster* have found clinal variation in the Indian subcontinent (Karan et al. 1998a) but not in Europe and Africa (Da Lage et al. 1990 and see General Introduction). The South American populations that we studied showed no evidence of clinal variation in starvation resistance.

The lack of a cline in starvation resistance of the South American populations could be a consequence of adaptation to laboratory conditions. However, this is unlikely because other geographically varying traits such as body size have not undergone laboratory adaptation since collection (Azevedo et al. 1996; Van 't Land et al. 1999). Another possible reason for the difference in results between our study and that of Karan et al. (1998) is the difference in methods used. Karan et al. (1998) studied only males and tested starvation resistance at 3 to 4 days of age. Our study examined starvation resistance of both males and females. Incorporating females gives a more reliable estimate because they live longer. The females we used were kept as virgins, because there is a trade off between egg production and starvation resistance (Chippindale et al. 1993). The flies used were also kept for 15 days before starvation resistance was measured because female starvation resistance increases steeply early in life, before reaching a plateau. Starvation resistance was measured at 15 days to avoid any confounding effects of between population differences in the time taken to reach this plateau. The flies used in our study remained healthy until tested for starvation resistance and the differences between the methods would not be likely to explain the difference in results. It should also be noted that the study of Da Lage et al. (1990) used very similar methods to that of Karan et al. (1998) and also failed to find a latitudinal cline in starvation resistance. However they did study only 3 populations.

In addition to differences in methods, our study covered a broader range of latitudes than that of Karan et al. (1998). The broader latitudinal range means that we also covered a wider range of temperature than the study of Karan et al. (1998) and this is further evidence to suggest that temperature variation is not solely responsible for the variation in starvation resistance found in Indian populations.

The apparent inconsistency of the results of Karan et al. (1998) and the ones we report here can be accounted for if differences in starvation resistance

are the result of adaptation to climatic factors other than temperature, which vary with latitude and differ between continents. Karan et al. (1998) suggested that the clinal variation in starvation resistance found in India was due to either average yearly temperature or average winter temperature. However, average yearly temperature in India is not well correlated with latitude. It is therefore unlikely that average yearly temperature is responsible for clinal variation of a trait. Similarly, although winter temperature in India is negatively correlated with latitude, summer temperature is positively correlated with latitude, and it is not clear why only winter temperature should affect this trait. In our study, average yearly temperature was highly correlated with latitude and we found no significant clinal variation in starvation resistance. It therefore seems likely that the variation in starvation resistance in India is actually due to climatic factors other than temperature, or a combination of factors (which may include temperature) that differ between South America and India. Sampling populations along latitudinal clines with varying climatic factors could pinpoint the key parameters in the evolution of these and other traits. In addition, specific hypotheses can be tested using laboratory evolution experiments.

# 4. Temperature and Clinal Variation in Larval

# Growth Efficiency in *Drosophila melanogaster*

## 4.1 Abstract

This study investigated the effects of geographic origin and experimental temperature on larval growth efficiency in *D. melanogaster*. Larvae from populations that had evolved at high latitudes were found to use limited food more efficiently, so that the overall adult body size achieved was larger. Larvae reared at a lower experimental temperature (18°C) used food more efficiently than those reared at a higher temperature (25°C). The increases in growth efficiency found in populations from high latitudes could explain their increased body size and more rapid development (See General Introduction).

## 4.2 Introduction

The work described in this chapter has been submitted for publication in Journal of Evolutionary Biology (Robinson and Partridge submitted-a).

The underlying physiological basis of latitudinal and temperature variation that is seen in *Drosophila* is at present not understood. Some research has been performed on geographical differences in vertebrate physiology. However, very few of the studies were designed to establish whether differences found were genetic or purely environmental (See Garland and Adolph 1991 for review). In invertebrates, the majority of the research on latitudinal variation in physiology has focused on *Drosophila*. The main focus of these physiological studies in *Drosophila* is metabolic rate, which has been seen to increase with latitude (See General Introduction; Giesel et al. 1991; Berrigan and Partridge 1997). But so far no studies have been performed that relate the variation in *Drosophila* body size to physiological factors.

*D. melanogaster* larvae that are adapted to low temperature in the laboratory require less food resources to produce a given overall adult size (See General Introduction; Neat et al. 1995). Growth efficiency may be the main target of selection in thermally adapted populations. Changes in growth efficiency could also account for the changes in other characters. A larva with a higher efficiency of conversion of food resources to adult body size may be able to achieve a larger overall adult size more rapidly, if metabolic efficiency is limiting for growth rate. An increase in metabolic efficiency may also cause a change in reproductive efficiency, thus increasing egg size and ovariole number. It is therefore important to know if this evolution of higher growth efficiency also occurs in natural populations living at higher latitudes. The first aim of this study was to investigate whether larval growth efficiency is affected by latitude of origin

in *Drosophila melanogaster* originating from a wide latitudinal range in 2 continents.

A previous study documented that larval growth efficiency was greater at an experimental temperature of 25°C than at 16.5°C (Neat et al. 1995). This contrasted with the evolutionary effects found in the same study, where larvae from low temperature selection lines were found to use food more efficiently than those from high temperature selection lines. However, the reduction in growth efficiency at the lower temperature could have been due to the stressful combination of a very low temperature and a very low food level. Although it has been demonstrated repeatedly that the adult body size of a number of species of *Drosophila* that are fully fed as larvae, increases with decreasing developmental temperature (See General Introduction and e.g. Ray 1960; Powell 1974; Coyne and Beecham 1987; Partridge et al. 1994a), this trend is reversed at extremely low temperatures (David et al. 1994; David et al. 1997; Karan et al. 1998b; Morin et al. 1999), suggesting that very low temperatures limit growth. Neat et al. (1995) found no difference in adult body size between adults reared at the 2 experimental temperatures (16.5°C and 25°C) when the larvae had been fully fed, suggesting that the lower temperature used here was too low. Furthermore, the levels of food that the larvae consumed in this experiment were extremely low, which may have further compromised growth, particularly at the low temperature. The second aim of the present study was to determine the effect of developmental temperature on larval growth efficiency of *D. melanogaster*. A low temperature of 18°C was used, rather than 16.5°C, because this temperature is sufficiently different from 25°C for any temperature related differences to be apparent, and size under full feeding is maximal at 18°C (David et al. 1994; James et al. 1997; Karan et al. 1998b; Morin et al. 1999).

## 4.3 Materials and Methods

### 4.3.1 Fly Populations

The South American, Australian and Dahomey populations were used in this experiment (See General Materials and Methods)

### 4.3.2 Measurement of Larval Growth Efficiency

Eggs were collected using laying pots as described in the General Materials and Methods. Eggs were picked from the surface of the medium using a needle, and placed individually in vials (12mm x 50mm, 110 vials per population) containing 1ml of agar (5g/l) and1.5mg of yeast in solution (See General Materials and Methods). This quantity of yeast was provided because previous studies have shown that this is a sufficient amount of food for the majority of the larvae to reach adulthood, but it produces a substantial reduction in body size compared to ad lib feeding (See Neat et al. 1995 for response curves). For example, mean female wing area for Innisfail (Australia) with 1.5mg of yeast = 1.06 mm$^2$, with 10mg of yeast (excess food) = 1.28 mm$^2$, for Cygnet with 1.5mg of yeast = 1.21 mm$^2$, with 10mg of yeast = 1.49 mm$^2$. The vials were checked regularly for eclosing adults, and all flies were removed and frozen soon after emergence. The left wings of all of the emerged flies were then removed, mounted on microscope slides and measured (See General Materials and Methods).

To ensure that any differences in size between populations were not due to differences in the amount of food ingested by the larvae rather than differences in growth efficiency, a further test was performed. The addition of a further egg to vials already utilised by experimental larvae allowed us to determine the

amount of yeast remaining that would be available for larval consumption.
Individual eggs from the 2 South American cline end populations (Guayaquil and
Puerto Montt) were placed in vials containing agar and 1.5ml yeast solution as in
the previous experiment. When pupariation had occurred, the pupae were
removed from the experimental vials. Any vial in which no pupa was found was
discarded. A freshly laid egg from a standard laboratory stock (Dahomey) was
then added to each of the vials. The Dahomey eggs had been collected on a petri
dish (See General Materials and Methods). The vials containing the eggs were
then left for 3 days (72 hours). On the third day, the Dahomey larvae were
removed from the vials and were weighed to the nearest 0.002mg using a
Sartorius microbalance. It was not possible to retrieve larvae from all the vials.
In some cases it was apparent that very young larvae had crawled up the side of
the vial, presumably in search of food, and had died. These larvae were not
weighed. There was no significant difference in the number of larvae retrieved
from the vials that had been previously occupied by the 2 populations. This
method provided us with a measure of the amount of yeast remaining that was
available for larval consumption, and whether this differed between the 2
populations.

## 4.3.3 Experimental Groups and Treatments

Larval growth efficiency was measured in all 10 South American and all 4
Australian populations at 25°C; it was also measured at 2 temperatures, 18°C and
25°C, for 2 cline end populations from each continent (Australia: Innisfail and
Cygnet, South America: Guayaquil and Puerto Montt. See Table 2.1 and Table
2.2 for latitudes). In order to control for parental effects, the parents of the
experimental individuals were also reared at the experimental temperature.

## 4.3.4 Statistical Analysis

### 4.3.4.1    Larval growth efficiency method

A Wilcoxon rank sum test was performed to establish if there were differences in size between the larvae raised in the vials that had previously been vacated by larvae of the 2 South American populations.

### 4.3.4.2    Larval growth efficiency in clines from Australia and South America

The variances of the wing area measurements for different sites within each sex were found to be homogeneous. The Australian populations can be considered to be 2 replicate populations each from high and low latitudes, and they were therefore analysed using an analysis of variance, with replicate sites nested within latitude (high or low). The South American populations form a continuous latitudinal cline, and they were therefore analysed using least squares regression analysis of variance, to look for a relationship between wing area and latitude.

### 4.3.4.3    Larval growth efficiency at two temperatures

In order to determine if there were differences between lines and between temperatures, 2 way analyses of variance were performed on each sex with line and temperature as main effects.

# 4.4 Results

## 4.4.1 Larval Growth Efficiency Method

There was no significant difference in weight between the Dahomey larvae reared in the vials previously occupied by Guayaquil or Puerto Montt larvae (Wilcoxon statistic Z=-0.722, $p$=0.470, Guayaquil mean = 0.0890 ± 0.021mg (95%), N=45, Puerto Montt mean = 0.090 ± 0.015mg (95%) N=48), indicating that there was no significant difference in the amount of food available for larval consumption in the vials previously occupied by the larvae from the 2 populations.

## 4.4.2 Larval Growth Efficiency in Clines from Australia and South America

In both continents, wing area, and therefore larval growth efficiency, increased with latitude. In the South American populations, for both males and females, there were highly significant ($p$<0.001) differences between populations, and standard least squares regression showed a significant increase in wing area with latitude (female $p$<0.05, male $p$<0.01) (Figure 4.1, Table 4.1). Analysis of variance of the data from both sexes in the Australian populations showed a significant effect of latitude ($p$<0.001), but no significant difference between replicate populations (Figure 4.1, Table 4.2).

## 4.4.3 Larval Growth Efficiency at Two Temperatures

The wing areas of each of the lines at the 2 temperatures are shown in Figure 4.2.

For both South America and Australia, wing area (and larval growth efficiency) was higher at the lower temperature and in the high latitude lines. For both males and females of Australian and South American origin there were highly significant effects of both temperature and line ($p<0.001$) (Table 4.3). For Australian males there was also a significant interaction effect between line and temperature ($p<0.005$).

**Figure 4.1** Mean wing area and 95% confidence intervals for adult flies

produced from food-restricted larvae: (a) Female, South America, (b) Male, South

America, (c) Female, Australia, (d) Male, Australia


(a) Female, South America

(b) Male, South America



**(b)**

Y-axis: **Mean Wing Area (mm$^2$)**

X-axis: **Latitude ( $^o$S)**
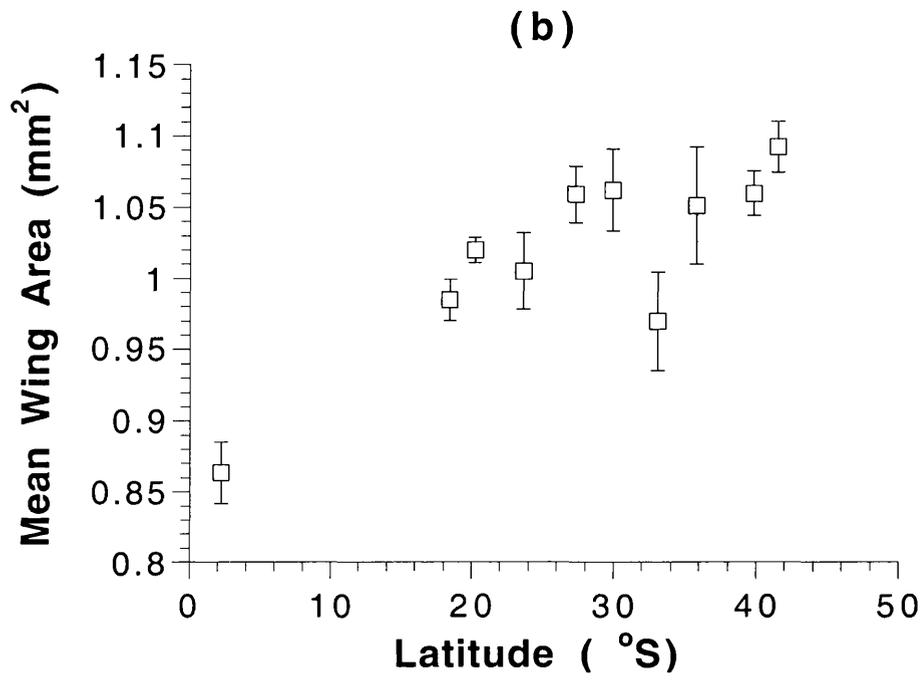
(c) Female, Australia

**( c )**

(d) Male, Australia



**(d)**

Y-axis: **Mean Wing Area (mm$^2$)** — 1.1, 1.05, 1, 0.95, 0.9, 0.85

X-axis: **Latitude ($^o$S)** — 10, 15, 20, 25, 30, 35, 40, 45
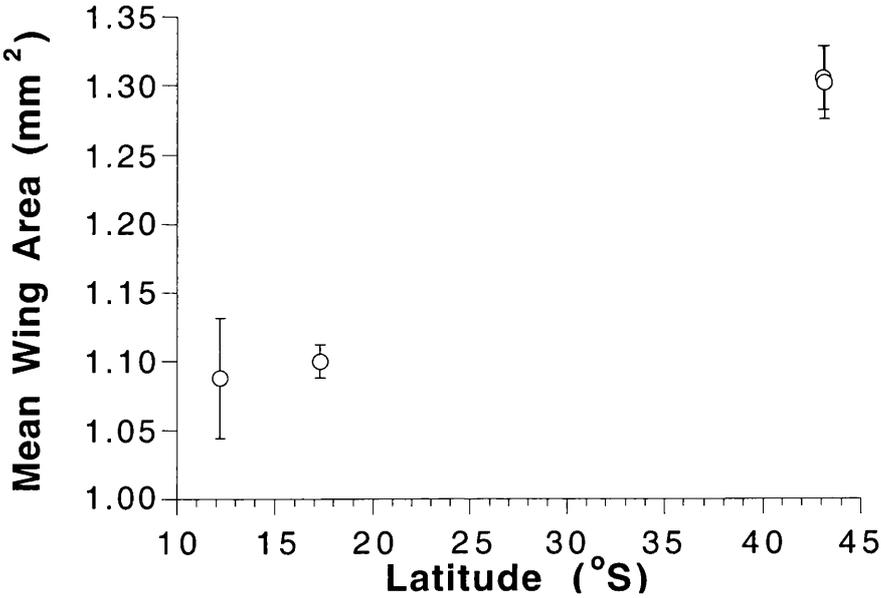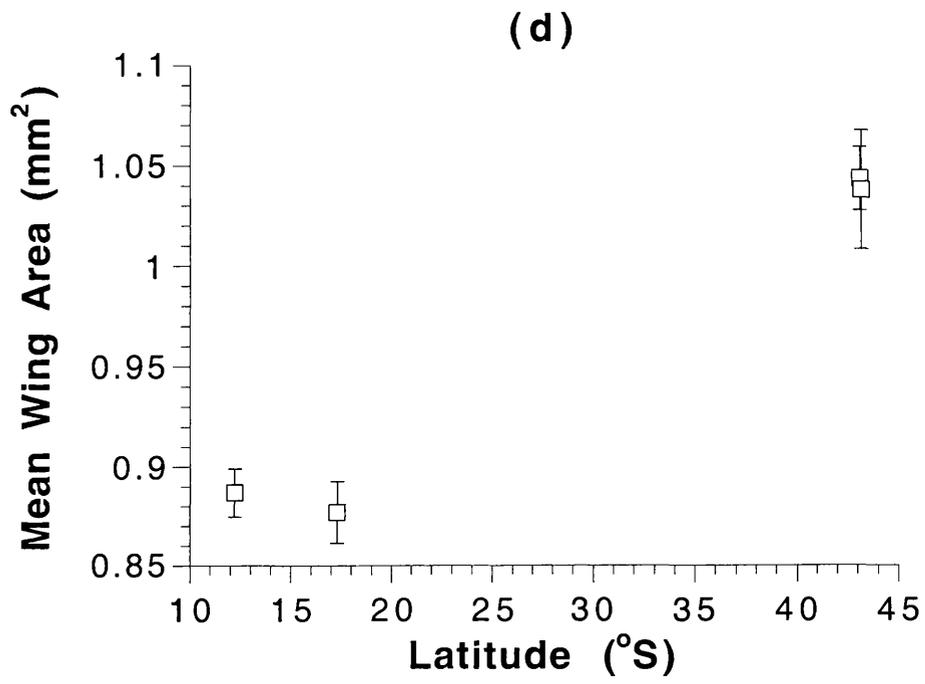
**Table 4.1** Least squares regression analysis of variance of wing area of food restricted flies among South American populations. (a) Female, (b) Male

| Source | d.f. | Sum of Squares | F | $p$ |
|---|---|---|---|---|
| **(a) Female** | | | | |
| Line | 9 | 0.874 | 16.75 | <0.0001 |
| Linear regression with latitude | 1 | 0.415 | 7.24 | <0.05 |
| Deviations | 8 | 0.459 | 9.89 | <0.0001 |
| Pure error | 275 | 1.594 | | |
| **(b) Male** | | | | |
| Line | 9 | 1.052 | 30.58 | <0.0001 |
| Linear regression with latitude | 1 | 0.731 | 18.27 | <0.01 |
| Deviations | 8 | 0.32 | 10.47 | <0.0001 |
| Pure error | 274 | 1.047 | | |

**Table 4.2** Analysis of variance of wing area of food restricted Australian populations with replicate populations nested within latitude. (a) Female, (b) Male

| Source | d.f. | Sum of Squares | F | $p$ |
|---|---|---|---|---|
| **(a) Female** | | | | |
| Latitude | 1 | 1.126 | 277.76 | <0.0001 |
| Latitude (replicate) | 2 | 0.002 | 0.27 | n.s. |
| Error | 106 | 0.43 | | |
| **(b) Male** | | | | |
| Latitude | 1 | 0.659 | 315.91 | <0.0001 |
| Latitude (replicate) | 2 | 0.002 | 0.42 | n.s. |
| Error | 110 | 0.23 | | |

**Figure 4.2** Mean wing area and 95% confidence intervals of food restricted flies against temperature. (a) Female, (b) Male

(a) Female
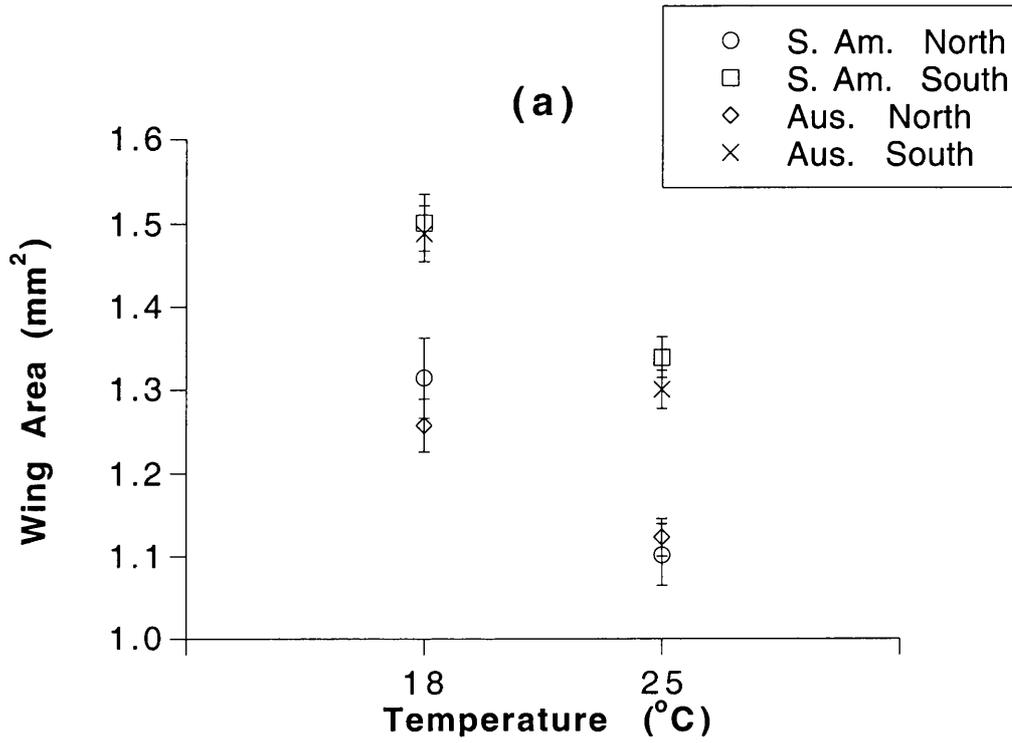
(b) Male
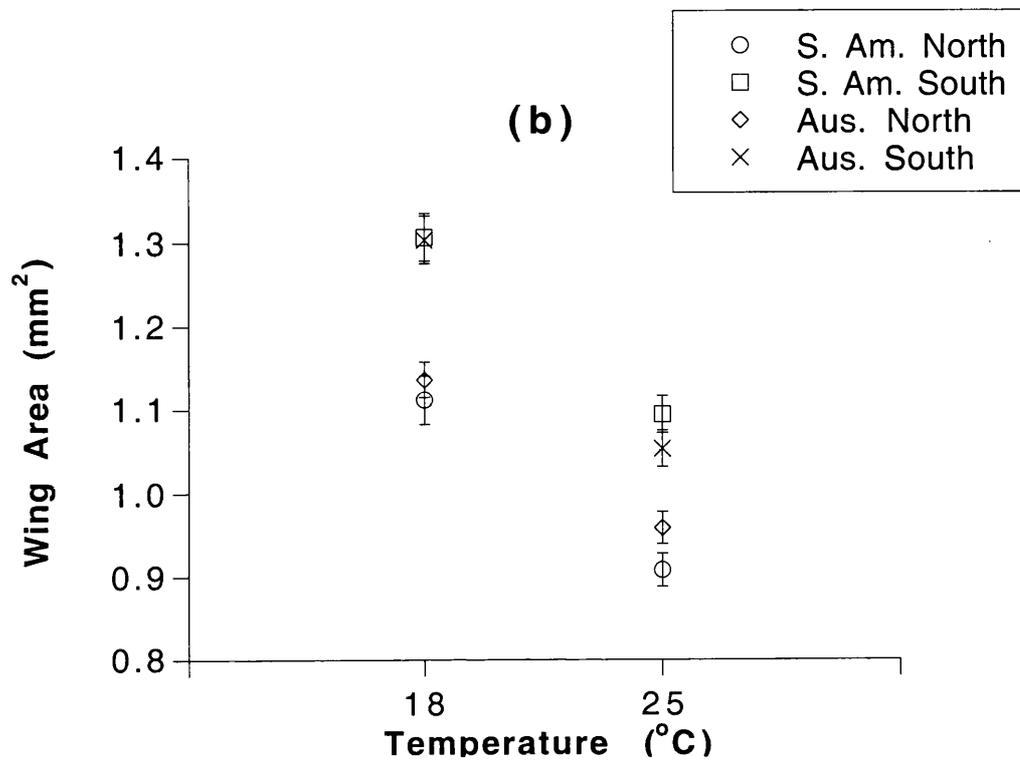
**Table 4.3** Analysis of variance of wing area of food restricted Australian and South American populations at 18°C and 25°C. (a) South American Female, (b) South American Male, (c) Australian Female, (d) Australian Male

| Source | d.f. | SS | F | $p$ |
|---|---|---|---|---|
| **(a) South American Female** | | | | |
| Line | 1 | 0.8605328 | 187.3781 | <0.0001 |
| Temperature | 1 | 0.56450527 | 122.9191 | <0.0001 |
| Line*Temperature | 1 | 0.01627461 | 3.5437 | n.s. |
| Error | 75 | 0.3444371 | | |
| **(b) South American Male** | | | | |
| Line | 1 | 0.8797934 | 330.8312 | <0.0001 |
| Temperature | 1 | 1.1238787 | 422.6153 | <0.0001 |
| Line*Temperature | 1 | 0.0012159 | 0.4572 | n.s. |
| Error | 90 | 0.2393408 | | |

Table continued overleaf

Table 4.3 continued

| Source | d.f. | SS | F | *p* |
|--------|------|-----|---|-----|
| **(c) Australian Female** | | | | |
| Line | 1 | 1.179817 | 240.3504 | <0.0001 |
| Temperature | 1 | 1.0419202 | 212.2583 | <0.0001 |
| Line*Temperature | 1 | 0.0060074 | 1.2238 | n.s. |
| Error | 131 | 0.6430446 | | |
| **(d) Australian Male** | | | | |
| Line | 1 | 0.6500434 | 190.016 | <0.0001 |
| Temperature | 1 | 1.5764231 | 460.8086 | <0.0001 |
| Line*Temperature | 1 | 0.0382497 | 11.1809 | 0.0011 |
| Error | 139 | 0.4755181 | | |

# 4.5 Discussion

These experiments were designed to measure larval growth efficiency, the ability of a larva to turn a fixed amount of food into adult body size. The accuracy of the measurement of larval growth efficiency is dependent on their being no differences in the amount of food consumed by larvae from different populations. Our study showed that there was no difference in the amount of food consumed by high and low latitude larvae. Mean weights of Dahomey larvae when removed from the vials 72 hours after laying, were higher than would be expected of freshly hatched larvae (Guayaquil = 0.089 ±0.021mg, Puerto Montt =0.090±0.015 compared to freshly hatched weight of 0.02mg, data from Partridge et al. 1994b) indicating that there was a small amount of food remaining in the vials. However, the amount of growth achieved by the Dahomey larvae was not affected by the geographic origin of the previous occupant of the vial, indicating that the amount of food consumed by the larvae from the 2 populations did not differ. These results therefore indicate that our study provides a true measure of larval growth efficiency.

The effects found in our study parallel those of laboratory thermal selection (See General Introduction) and imply that the clines found are the result of natural selection. Our results further implicate temperature as a selective force in the cline (See General Introduction). Larval growth efficiency was found to be greater in high latitude populations as a result of natural selection. The increase in efficiency may occur because it is more beneficial for a larva to grow efficiently at low temperatures (high latitudes) or because low temperatures increase the potential for growth and hence make it easier to achieve higher efficiency.

Temperature has been shown to have developmental effects on the size of *Drosophila* as well as on that of numerous other ectotherms (See General

Introduction). In the present study, temperature was demonstrated to have an environmental effect on growth efficiency, with larvae raised at 18°C being more able to convert food into larger adult size than those raised at 25°C. This result may underlie the evolutionary response of growth efficiency to temperature, with patterns of allocation of nutrients possibly becoming genetically altered. The mechanisms underlying the response of growth efficiency to experimental temperature are not understood, and warrant further investigation.

In laboratory thermal selection, as in latitudinal clines, development time has been seen to be slower in populations from higher selection temperatures (See General Introduction; Anderson 1966; James and Partridge 1995). The increase in body size and accompanying faster development of flies that have evolved at lower temperatures (or higher latitudes) could be explained by the increase in growth efficiency. Greater efficiency could enable a fly to reach a larger size in shorter time. The increase in efficiency of food use may also explain the variation of reproductive characters (ovariole number and egg size) with latitude. An increase in larval growth efficiency could lead to the formation of more ovarioles and the maintenance of this increased efficiency in the adult could lead to the production of larger eggs. If both growth efficiency and reproductive efficiency are increased, then the amount of resources available for reproductive function will be increased.

# 5. Chromosome Substitution Analysis of Body Size in Two Parallel Latitudinal Clines of *Drosophila melanogaster*

## 5.1 Abstract

This study investigated the contribution of individual chromosomes to body size variation in *D. melanogaster* from 2 continents (Australia and South America) and the differences in chromosomal contribution between those 2 continents. Chromosome 3 had the largest effect on body size in both continents but chromosomes 1 and 2 also had a significant effect. Significant differences between the 2 continents were also found, with chromosome 3 having a larger effect in South America. There were also indications that epistasis varied between continents. These results suggest that, although the clines in body size in the 2 continents did not have an identical genetic basis, there were strong similarities between them.

## 5.2 Introduction

The work described in this chapter has been submitted for publication in Genetical Research (Robinson and Partridge submitted-b).

Latitudinal clines in body size have been found in a number of ectotherm species (See General Introduction). Latitudinal clines in *Drosophila melanogaster* have been found throughout the world with body size increasing with increasing latitude (See General Introduction). The existence of the parallel clines throughout the world indicates that these clines are likely to be due to natural selection. The selective agent thought to cause these clines is temperature, because body size is affected by laboratory thermal adaptation in the same way as in geographic clines (See General Introduction).

In ectotherms, evolution at higher latitudes (or lower temperatures) tends to lead to increases in body size, but the mechanisms are not fully understood. Obtaining a greater understanding of the genetic basis of this phenomenon in *Drosophila* could provide insights into the mechanisms involved in the thermal evolution of body size in ectotherms in general as well as in *Drosophila*. Identification of the genes involved is one way of approaching this complex physiological problem. Gene identification can be approached by using a quantitative trait locus mapping approach. A first step towards this is to identify which (if any) of the chromosomes has a predominant effect on body size. This can be done using a chromosome substitution procedure. A number of previous studies have analysed body size in *Drosophila* using chromosome substitution procedures. One study (Chakir et al. 1995), which looked at wing length of *D. melanogaster* males only, showed that all 3 major chromosomes play a role in size differences between African and French populations of *D. melanogaster*, with a significant interaction found between chromosomes 2 and 3. A similar

study performed on laboratory thermal selection lines (Cavicchi et al. 1989) showed that both second and third chromosomes have an effect on body size. However, the magnitude of the chromosomal effects differed when body dimensions were measured at different environmental temperatures. Line cross analysis can also provide valuable insights into the genetic architecture of body size. A study of the genetic basis of wing area in 3 parallel clines of *D. melanogaster* (Australia, South America and Africa) estimated additive, dominance, epistatic and maternal effects for each cline and found marked differences between them (Gilchrist and Partridge 1999). These results suggest that the phenotypic variation seen in the parallel clines has, at least in part, a different genetic basis.

In the present study we used a chromosome substitution procedure to assess the contribution of the 3 main chromosomes to latitudinal variation in body size in 2 continents (South America and Australia). Chromosome substitution is relatively simple to perform in *Drosophila melanogaster* and can be very informative (e.g. Robertson 1954; Caligari and Mather 1975; Sokolowski 1980; Caligari and Mather 1988). Chromosome substitutions can give some indication of whether a trait might be controlled by one or a few genes and whether there are interactions between genes. The aim of this study was: (a) to investigate chromosomal contribution to latitudinal variation in body size in *D. melanogaster* from 2 continents, South America and Australia, and (b) to determine whether the chromosomal basis of these 2 clines is the same. Similarities in the chromosomal basis of the 2 clines would indicate that the clinal variation in body size in different clines may be the result of a response to selection at the same loci in each cline. Differences in the chromosomal basis of the 2 clines would indicate that the response to selection is produced by different genetic variants in different clines, and that adaptation of life history traits to latitude can therefore be achieved by different genetic routes.

## 5.3 Materials and Methods

### 5.3.1 Fly Populations

One population from each end of the clines from South America and Australia were used in this experiment. The populations used were; Guayaquil and Puerto Montt (South America) and Innisfail and Cygnet (Australia). Although Innisfail was not the lowest latitude Australian population, it was used because it deviated less in longitude from Cygnet and the size of the flies in the population were not significantly larger than those from Darwin. See General Materials and Methods for details of these populations.

### 5.3.2 Chromosome Substitution

The contribution of each chromosome to body size was assessed by using a chromosome substitution procedure in which one chromosome at a time from the low latitude (Small) line was inserted in the high latitude (Big) background and vice-versa to produce all 6 possible chromosome substitution lines. To obtain the chromosome substitution lines, 5 generations of crosses were required (Figure 5.1). An SM5/bw$^{VI}$; TM3/TM6 balancer stock (See General Materials and Methods) was used in this chromosome substitution procedure, and all crosses were performed at 25°C. The South American and Australian populations used had been typed as P cytotype using a PCR technique (Ballinger and Benzer 1989) and therefore the balancer stock used was also P cytotype in order to avoid problems of hybrid dysgenesis. Efforts were made to make the chromosome substitution lines as outbred as possible so that a large number of parental chromosomes could be assessed. As can be seen from

Figure 5.1, all the wild type flies introduced to the crosses were female, and equal numbers of males and females were used in the crosses. The first cross was performed using 35 females from each of the South American populations and 60 from each of the Australian populations while, in the second cross, a further 45 South American and a further 30 Australian females from each population were introduced. Throughout the crossing procedure the maximum possible number of flies was recovered at every generation to use for the subsequent cross. The data therefore came from a random, moderate sized sample of the chromosomes from each population.

**Figure 5.1** The scheme of crosses required to insert one big (B) chromosome into the small (S) background for each of the three chromosomes. (a) chromosome 1, (b) chromosome 2, (c) chromosome 3. Substitutions of one small chromosome into the big background were performed following the same scheme of crosses

(a) Chromosome 1

$\female\female B_1/B_1$; $B_2/B_2$; $B_3/B_3$     x     $X/Y$; $SM5/bw^{Vl}$; $TM3/TM6\ \male\male$

$\female\female B_1/B_1$; $B_2/B_2$; $B_3/B_3$     x     $B_1/Y$; $B_2/bw^{Vl}$; $B_3/TM6\ \male\male$

$\female\female B_1/B_1$; $B_2/B_2$; $B_3/B_3$   x   $B_1/Y$; $SM5/B_2$; $TM3/B_3\ \male\male$

$\female\female B_1/B_1$; $B_2/bw^{Vl}$; $B_3/TM6$   x   $S_1/Y$; $S_2/S_2$; $S_3/S_3\ \male\male$

$\female\female B_1/B_1$; $SM5/B_2$; $TM3/B_3$     x     $B_1/Y$; $S_2/bw^{Vl}$; $S_3/TM6\ \male\male$

$\female\female B_1/B_1$; $SM5/S_2$; $TM3/S_3$     x     $B_1/Y$; $SM5/S_2$; $TM3/S_3\ \male\male$

$B_1/B_1$ or $B_1/Y$; $S_2/S_2$; $S_3/S_3$

$♀♀ S_1/S_1; S_2/S_2; S_3/S_3$    x    $X/Y; SM5/bw^{VI}; TM3/TM6 ♂♂$

$♀♀ S_1/S_1; S_2/S_2; S_3/S_3$    x    $S_1/Y; S_2/bw^{VI}; TM3/S_3 ♂♂$

$♀♀ S_1/S_1; S_2/S_2; S_3/S_3$    x    $S_1/Y; SM5/S_2; S_3/TM6 ♂♂$

$♀♀ S_1/S_1; S_2/bw^{VI}; TM3/S_3$    x    $B_1/Y; B_2/B_2; B_3/B_3 ♂♂$

$♀♀ S_1/S_1; SM5/S_2; S_3/S_3$    x    $S_1/Y; B_2/bw^{VI}; TM3/B_3 ♂♂$

$♀♀ S_1/S_1; SM5/B_2; TM3/S_3$    x    $S_1/Y; SM5/B_2; TM3/S_3 ♂♂$

$S_1/S_1 \text{ or } S_1/Y; B_2/B_2; S_3/S_3$

(c) Chromosome 3

♀♀ $S_1/S_1$; $S_2/S_2$; $S_3/S_3$    x    X/Y; SM5/bw$^{VI}$; TM3/TM6 ♂♂

♀♀ $S_1/S_1$; $S_2/S_2$; $S_3/S_3$    x    $S_1$/Y; SM5/$S_2$; $S_3$/TM6 ♂♂

♀♀ $S_1/S_1$; $S_2/S_2$; $S_3/S_3$    x    $S_1$/Y; $S_2$/bw$^{VI}$; TM3/$S_3$ ♂♂

♀♀ $S_1/S_1$; SM5/$S_2$; $S_3$/TM6    x    $B_1$/Y; $B_2/B_2$; $B_3/B_3$ ♂♂

♀♀ $S_1/S_1$; $S_2/S_2$; TM3/$S_3$    x    $S_1$/Y; SM5/$B_2$; $B_3$/TM6 ♂♂

♀♀ $S_1/S_1$; SM5/$S_2$; TM3/$B_3$    x    $S_1$/Y; SM5/$S_2$; TM3/$B_3$ ♂♂

$S_1/S_1$ or $S_1$/Y; $S_2/S_2$; $B_3/B_3$

### 5.3.3 Wing Area Measurement

Flies from each line were reared at a standard density of 50 larvae per vial (See General Materials and Methods) in 5 vials per line and frozen on emergence. The left wings of 12 flies of each sex from each vial were then removed and mounted on microscope slides. Wing area was measured following the protocol in General Materials and Methods.

### 5.3.4 Statistical Analysis

The contributions of the individual chromosomes and the effect of continent on body size were analysed using a 5 way analysis of variance with chromosomes 1, 2 and 3, sex, and continent as main effects. Before performing the analysis, wing areas were standardised within each sex (mean = 0, standard deviation = 1) to ensure that comparisons between sexes were not affected by the sexual dimorphism in size. Similarly, wing areas were standardised between continents to ensure that comparisons between continents were not affected by the difference in mean size of the corresponding cline end populations.

## 5.4 Results

Wing areas of the chromosome substitution lines from each continent are shown in Figure 5.2. On average, the substitution of a big chromosome 3 in a small background accounts for 77% of the size difference between big and small.

Substituting big chromosomes 1 or 2 into a small background each account for 13% of the overall difference in size between big and small.

The results of the analysis of variance are shown in Table 5.1. All 3 chromosomes had a significant effect on body size. Chromosome 3 had the largest effect ($p<0.0001$, $F= 2851$, Table 5.1), with chromosomes 1 and 2 having significant effects of much smaller magnitude (for chromosome 1, $p<0.001$, $F=4.9$; for chromosome 2, $p<0.0001$, $F=13.5$).

Significant interaction effects between chromosomes 2 and 3 ($p<0.001$) and between chromosomes 1 and 2 ($p<0.05$), indicate some degree of epistasis. There was a significant chromosome 3 by continent interaction ($p<0.0001$) and inspection of the data indicated that chromosome 3 had a larger effect on the body size of South American flies. There was a significant chromosome 1 by sex interaction ($p<0.05$), with chromosome 1 having a larger effect on female body size. Significant interactions were also found between chromosomes 2, 3 and continent ($p<0.005$), between chromosomes 1, 2, 3 and continent ($p<0.0001$) and between chromosomes 1, 2, 3, sex and continent ($p<0.05$), indicating that the effects on body size of interchromosomal interactions differed between the 2 continents. These multiway interactions indicate that the effects of interactions between the chromosomes differ between the 2 continents and between the sexes.

**Figure 5.2** Mean wing areas and 95% confidence intervals of chromosome

substitution lines. B represents big (high latitude) chromosome, S represents small

(low latitude) chromosome. Position of letter represents chromosome number, e.g.

BBS = big chromosomes 1 and 2, small chromosome 3. (a) South American

females, (b) South American males, (c) Australian females, (d) Australian males

(a) South American females



**(a)**

South American females wing area plot. Y-axis: Wing area ($mm^2$) from 1.15 to 1.50. X-axis: Line (B, S, BSS, SBS, SSB, SBB, BSB, BBS).
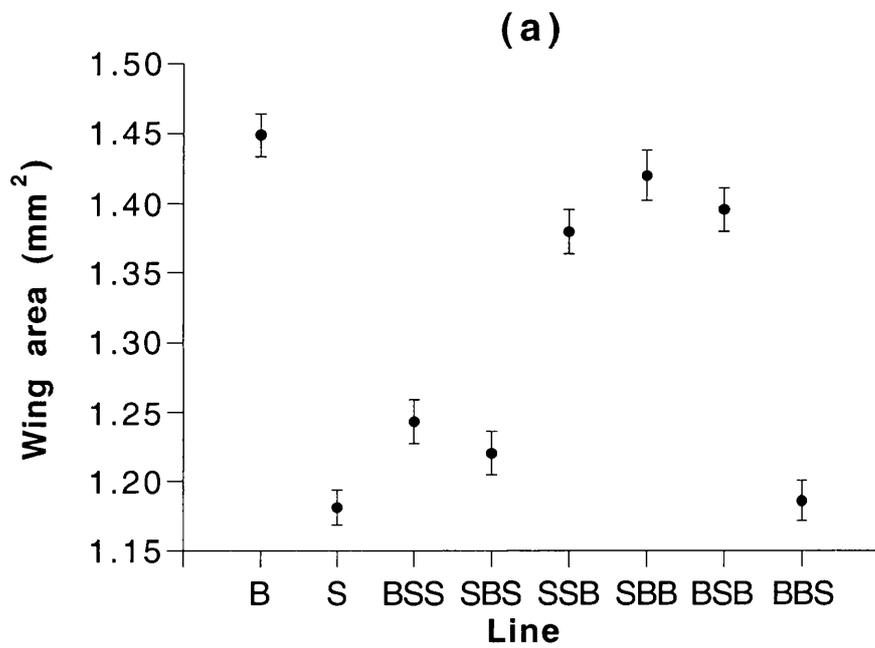
(b) South American males
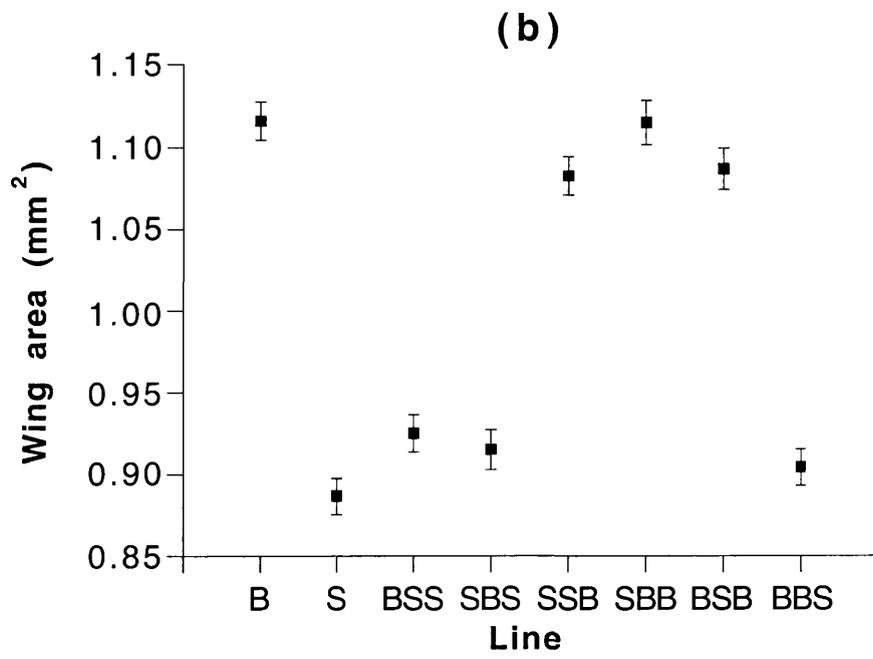
(c) Australian females
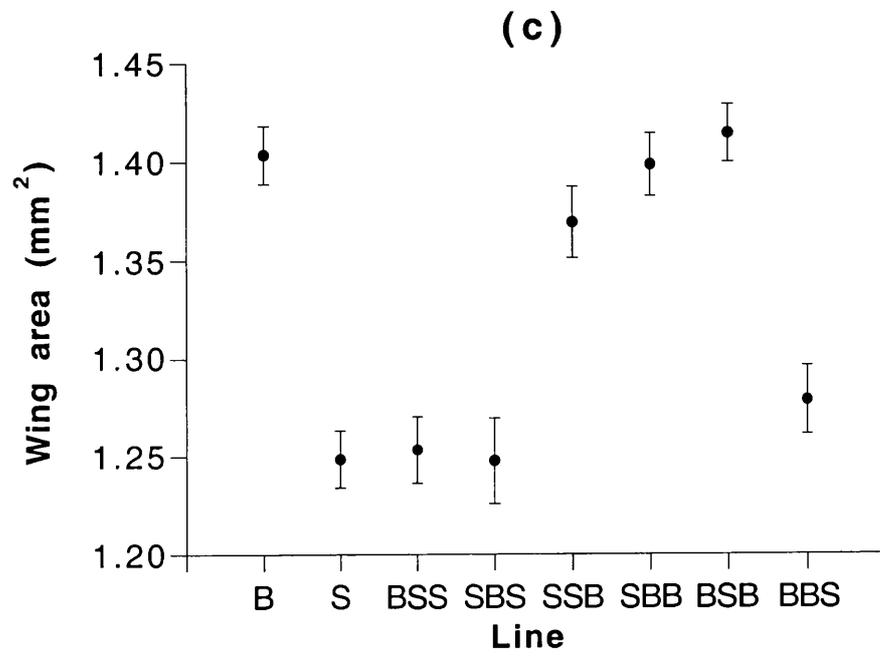
(d) Australian males
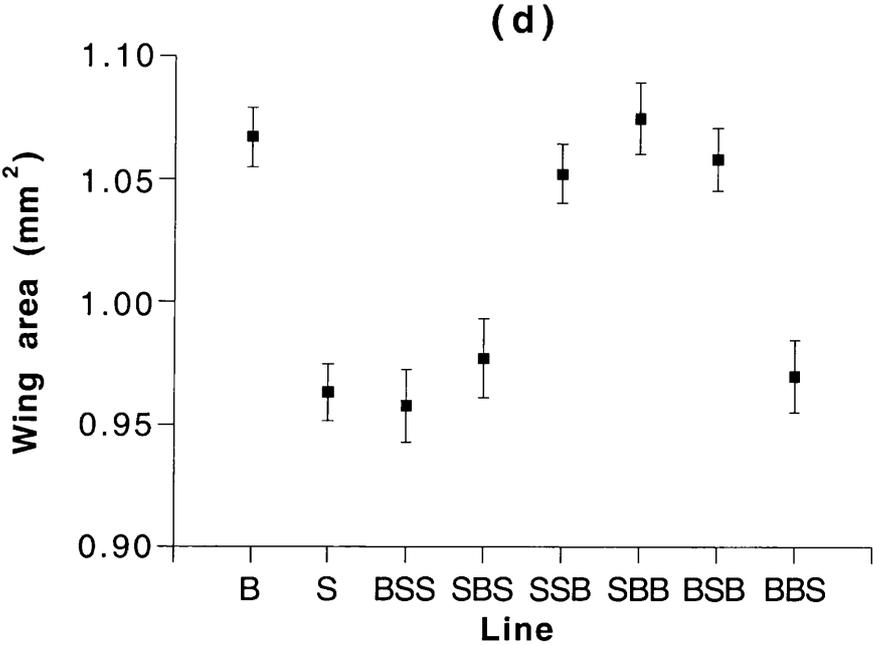
**Table 5.1** Five-way analysis of variance of wing area with chromosomes 1, 2, and 3 and sex and continent as main effects

| Source | d.f. | Sum of Squares | Mean square | F Ratio | $p$ |
|---|---|---|---|---|---|
| Chromosome 1 | 1 | 4.8889 | 4.8889 | 12.092 | 0.0005 |
| Chromosome 2 | 1 | 13.4813 | 13.4813 | 33.3444 | <0.0001 |
| Chromosome 3 | 1 | 1153.0115 | 1153.0115 | 2851.832 | <0.0001 |
| Sex | 1 | 0.2892 | 0.2892 | 0.7153 | n.s. |
| Continent | 1 | 0.3723 | 0.3723 | 0.921 | n.s. |
| Chr1*Chr2 | 1 | 5.1838 | 5.1838 | 12.8216 | 0.0004 |
| Chr1*Chr3 | 1 | 0.4294 | 0.4294 | 1.062 | n.s. |
| Chr2*Chr3 | 1 | 4.2381 | 4.2381 | 10.4825 | 0.0012 |
| Chr1*Sex | 1 | 4.0918 | 4.0918 | 10.1206 | 0.0015 |
| Chr2*Sex | 1 | 0.1236 | 0.1236 | 0.3057 | n.s. |
| Chr3*Sex | 1 | 0.023 | 0.023 | 0.0569 | n.s. |
| Chr1*Continent | 1 | 0.1339 | 0.1339 | 0.3313 | n.s. |
| Chr2*Continent | 1 | 0.0064 | 0.0064 | 0.0157 | n.s. |
| Chr3*Continent | 1 | 12.6698 | 12.6698 | 31.3371 | <0.0001 |
| Sex*Continent | 1 | 0.5883 | 0.5883 | 1.4552 | n.s. |

Table continued overleaf

Table 5.1 continued

| Source | d.f. | Sum of Squares | Mean square | F Ratio | *p* |
|---|---|---|---|---|---|
| Chr1*Chr2*Chr3 | 1 | 0.4675 | 0.4675 | 1.1563 | n.s. |
| Chr1*Chr2*Sex | 1 | 0.0005 | 0.0005 | 0.0013 | n.s. |
| Chr1*Chr3*Sex | 1 | 0.6088 | 0.6088 | 1.5058 | n.s. |
| Chr2*Chr3*Sex | 1 | 0.1865 | 0.1865 | 0.4613 | n.s. |
| Chr1*Chr2*Continent | 1 | 0.6774 | 0.6774 | 1.6754 | n.s. |
| Chr1*Chr3*Continent | 1 | 0.6056 | 0.6056 | 1.4978 | n.s. |
| Chr2*Chr3*Continent | 1 | 3.2758 | 3.2758 | 8.1022 | 0.0045 |
| Chr1*Sex*Continent | 1 | 0.8257 | 0.8257 | 2.0422 | n.s. |
| Chr2*Sex*Continent | 1 | 0.4267 | 0.4267 | 1.0554 | n.s. |
| Chr3*Sex*Continent | 1 | 0.8418 | 0.8418 | 2.0821 | n.s. |
| Chr1*Chr2*Chr3*Sex | 1 | 0.0017 | 0.0017 | 0.0041 | n.s. |
| Chr1*Chr2*Chr3*Continent | 1 | 9.7066 | 9.7066 | 24.008 | <0.0001 |
| Chr1*Chr2*Sex*Continent | 1 | 0.0496 | 0.0496 | 0.1228 | n.s. |
| Chr1*Chr3*Sex*Continent | 1 | 0.4103 | 0.4103 | 1.0148 | n.s. |
| Chr2*Chr3*Sex*Continent | 1 | 0.4286 | 0.4286 | 1.0601 | n.s. |
| Chr1*Chr2*Chr3*Sex*Cont | 1 | 2.427 | 2.427 | 6.003 | 0.0144 |
| Model | 31 | 1215.8367 | 39.2205 | 97.0072 | <0.0001 |
| Error | 1809 | 731.3888 | 0.4043 | | |

# 5.5 Discussion

Chromosome 3 had the largest effect on body size in both clines, accounting for the majority of the body size differences between cline ends (approximately 77%). The large effect of chromosome 3 indicates that the majority of clinal variation in body size in both continents may be determined by a small number of genes (or even a single gene) with major effects, located on chromosome 3. Chromosomes 1 and 2 also had significant but much smaller effects on body size in both continents.

Although chromosome 3 accounted for most of the difference in body size between cline ends in both continents, we also found significant differences between the 2 continents. Chromosome 3 had a larger effect in South America than in Australia. These results support previous work where differences in the genetic architecture of 3 parallel clines were found (Gilchrist and Partridge 1999). However, the similarities in the chromosomal basis of size variation in the 2 clines suggest that a large part of the response to selection may be achieved in a similar way in the 2 clines examined.

There are a number of explanations why mechanisms causing clinal variation in the same traits may differ between continents. Firstly, the selective agents may differ between the 2 continents. Although there is evidence that temperature plays a major role in latitudinal variation in body size, a number of other environmental factors vary with latitude. These include factors such as food availability, humidity and rainfall. These factors may differ between continents and could lead to differences in the genetics of the size clines between continents. Even if the clines in different continents are the result of the same selective agents, the genetic basis of the response to that selection may not be the same. Selection may be acting on body

size (or on some other related trait) but there could be a variety of genetic routes to the same response to selection. Mutations occur by chance, and those producing a fitter phenotype will be selected for, but there may be effects of mutation order and of interaction between mutations in determining the phenotype. In a computer simulation of a polygenic character under selection, it was found that the order in which mutations occur can have a significant effect on genotypic evolution while producing the same final phenotype, (Clarke et al. 1988). Epistasis may also play a role in differences between continents, as the genetic background in the 2 continents may differ, so may the possibility of epistasis and its effect on body size.

In addition to the effects of individual chromosomes on body size, interactions between chromosomes also had an effect on size in the clines studied. Interactions occurred between chromosomes 1 and 2 and between chromosomes 2 and 3. These interchromosomal interactions are an indication that epistasis plays a role in the size differentiation in the clines. While chromosome interactions indicate epistasis, this study was not designed specifically to investigate epistatic differences and therefore cannot estimate the total epistasis in the 2 clines. Epistatic effects within chromosomes may also occur but these could not be assessed by the method used in the present study. Some epistatic differences between the 2 clines were also found. Significant interactions were found between chromosomes 2 and 3 and continent and chromosome 1 and 2 and 3 and continent. The data indicated that interactions between the chromosomes were greater in South America than Australia. A previous study has shown that there are differences in the epistatic component of body size between South America and Australia (Gilchrist and Partridge 1999), and our results support this.

Sex differences in chromosomal contribution to body size were also found. A significant interaction between chromosome 1 and sex indicated that chromosome 1 played a larger role in producing latitudinal variation in female body size than it did

in male body size in both continents.  Previous studies of chromosomal contribution to body size studied the size of only one sex and so were unable to detect any genotype by sex interaction (Cavicchi et al. 1989; Chakir et al. 1995).

In addition to the clinal variation in body size of *Drosophila* with latitude, shorter development time, larger egg size and higher ovariole number have been reported at higher latitudes in more than one continent  (See General Introduction). Further investigations are required to assess whether the variation of these traits within a continent has the same genetic basis as that for body size.  A previous study of ovariole number, wing length and sternopleural bristle number in *D. melanogaster* from France and Africa found effects on all 3 traits on all 3 chromosomes. Significant interactions between chromosomes differed between the traits, indicating that the traits did not necessarily have the same genetic basis (Chakir et al. 1995). Further work to localise QTLs responsible for body size will enable us to more fully understand the genetic basis of latitudinal variation and the differences between clines.

# 6. Discussion

## 6.1 Latitudinal Variation in Ectotherms

Clines in a number of characteristics of ectotherms occur throughout the world. Latitudinal variation has been seen in species of fish and frogs as well as in many insect species (See General Introduction). These clines are the result of a combination of the developmental and evolutionary genetic effects of the environment that the animals occupy. A number of environmental factors vary with latitude and ectotherms adapt to the environmental conditions that they experience. A number of traits of *Drosophila* vary clinally with latitude and the response of *Drosophila* to changing latitude is similar to that of other ectotherms. *Drosophila* provides a good model for studying latitudinal variation. In this thesis I have studied a number of aspects of latitudinal variation in *Drosophila melanogaster*.

Understanding latitudinal variation in *Drosophila melanogaster* could help us to understand latitudinal variation in other ectotherms. The similar responses to latitude in ectotherms may have similar genetic basis and similar physiological processes may be affected by latitude in similar ways across the ectotherms. Effects in *Drosophila* may even have some bearing on latitudinal variation in endotherms, since they too, show increased size with latitude (Bergmann 1847).

## 6.2 Latitudinal Variation in *Drosophila melanogaster*

A number of traits have previously been found to vary with latitude in *D. melanogaster*. Body size, egg size and ovariole number have all been shown to

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increase with increasing latitude, and development time decreases with increasing latitude. These results have been replicated in different continents, indicating that this genetic variation is due to natural selection. A latitudinal cline in starvation resistance has also been found in populations originating from India with higher starvation resistance occurring at lower latitudes (See General Introduction). I studied latitudinal variation in 3 traits, starvation resistance, fat content and larval growth efficiency.

Starvation resistance and fat content were studied in populations of *D. melanogaster* from South America. These populations had previously been shown to exhibit clines in other traits (See General Materials and Methods). Although variation in starvation resistance and fat content between sites was found, this variation was not related to changes in latitude (Chapter 3). Therefore, starvation resistance and fat cannot be considered to be among the suite of traits that vary with latitude throughout the world. The lack of a cline in starvation resistance in South America may indicate that the latitudinal cline found in India is the result of genetic drift, and is not adaptive. Alternately, it could indicate that the selective agent responsible for the majority of the latitudinal variation so far observed is not responsible for variation in starvation resistance. A number of factors vary with latitude and some of these factors differ in their relationship to latitude between continents. The evolution of latitudinal variation in starvation resistance in India may be the result of a pattern of variation in climatic factors that occurs specifically in India.

Latitudinal variation in larval growth efficiency was studied in populations of *Drosophila melanogaster* originating from South America and Australia, in which latitudinal variation in a number of traits had previously been found (See General Materials and Methods). Latitudinal variation in larval growth efficiency was found in both South American and Australian populations (Chapter 4). The replication of

the latitudinal variation indicates that it is the result of natural selection rather than genetic drift. Furthermore, the existence of clines in larval growth efficiency complement previous findings of clines in body size and development time. The efficiency with which an organism can use its food is highly significant in terms of that organism's fitness. Being able to make a larger adult from a given amount of food is advantageous, because larger adult body size is known to be advantageous in terms of fitness, and because the fly is able to make good use of what may be limited food resources. Variation in larval growth efficiency may be responsible for the latitudinal variation in both body size and development time.

In general, it appears that the traits exhibited by *Drosophila* originating from high latitudes would be advantageous at all latitudes. However, if this would the case we would expect these traits to have evolved at all latitudes. There must therefore be some aspects of the traits exhibited at high latitudes that would confer negative fitness effects on flies experiencing low latitude conditions. A study of larval competitive ability in a cline of *D. melanogaster* found no variation in competitive ability with latitude (James and Partridge 1998). However the study did show that the ability of high latitude populations to grow efficiently is compromised to some extent at high temperature and density. High temperatures are characteristic of low latitude areas and there is some evidence that larvae at low latitudes are present at higher densities (Lemeunier et al. 1986; Davis et al. 1993). Low latitude populations may have evolved to deal with the stresses of high temperature and high density at the expense of more efficient growth. There may be other trade-offs that have yet to be revealed. Other trade-offs may involve the other environmental factors that vary with latitude. Although when given a limited amount of food, *D. melanogaster* larvae originating from high latitude populations are able to produce a larger body size, they may have to allocate the majority of their resources to growth in order to do this. If the majority of resources are allocated to growth when food is limited, then

this would have a negative effect on reproductive characters. However, fully fed high latitude *Drosophila* have a larger number of ovarioles so there may in fact be an overall increase in the efficiency of food use in terms of both growth and reproduction. This could be tested by comparing the fecundity of flies from low and high latitudes that have been provided with low food levels during development. In laboratory thermal selected lines, *D. melanogaster* which evolved at low temperature were found to have higher fecundity when measured at low temperature and flies that evolved at high temperature were found to have higher fecundity when measured at high temperature (Partridge et al. 1995).

Although much is now known about latitudinal variation in *Drosophila* there is much more still to learn. It is important to discover what the trade-offs are that maintain these clines.

## 6.3 The Target of Selection

Although a number of traits vary with latitude it is not clear which, if any, of the traits so far identified is the target of selection. The latitudinal variation in these traits is clearly an adaptation due to natural selection but selection may be acting separately on the individual traits or variation in one trait may be the result of selection on another. The variation of a whole suite of traits with latitude could be the result of pleiotropy or the result of selection acting individually on a number of traits.

Body size is a possible target of selection as increased body size has often been associated with increased fitness (Robertson 1957; Tantawy and Vethukiv 1960; Tantawy and Rahkha 1964; Partridge and Farquhar 1981; Partridge and Farquhar 1983; Partridge et al. 1987). Body size consists of 2 components, the

number of cells and the size of those cells. Variation in body size can be caused by either a variation in the number, or the size of cells, or a combination of both (Zwaan et al. 2000). The contribution of variation in cell area and cell number, to overall wing area in *D. melanogaster* has previously been studied (Zwaan et al. 2000). It was found that although body size increased with latitude in both South America and Australia, the contribution of cell size and cell number to overall body size varied between the 2 continents. The contribution of cell size to the Australian cline was much smaller than that of the South American cline. It appears that selection is not acting on either cell size or cell number directly, but it could be acting on body size as a whole.

The combination of large body size and fast development time at high latitudes could implicate larval growth efficiency as a target of selection. The high larval growth efficiency in high latitudes enables the larvae to convert a fixed amount of food into a larger sized adult, and may also enable this process to occur more quickly.

Other factors may also be involved. It is clear that we do not yet know all there is to know about latitudinal variation in *Drosophila* and the trade-offs that may be involved. Establishing what the target of selection is, is a challenge for the future.

## 6.4 Selective Agents

It is known that the majority of latitudinal variation seen in *Drosophila* is the result of genetic variation due to natural selection. However, it is not clear what the selective agent is that causes this variation. A large number of environmental factors vary with latitude and these may all have some effect on the latitudinal variation in these traits.

A primary candidate for selective agent in these clines is temperature. Temperature is correlated with latitude throughout the world. Temperature has large effects on the development and evolution of ectotherms (See General Introduction). The effects of latitudinal variation in *Drosophila* have often been paralleled in laboratory studies of thermal evolution. Body size and egg size increase in populations that have evolved at low temperatures and in populations that have evolved at high latitudes. Similarly, development time decreases in populations that have evolved at low temperatures and high latitudes. Larval growth efficiency has previously been found to increase in low temperature evolved populations (Neat et al. 1995). In Chapter 4, it was confirmed that larval growth efficiency also increases in high latitude evolved populations. This result provides further evidence that temperature is the selective agent involved in the suite of traits that vary with latitude.

Laboratory thermal selection experiments have shown that temperature is involved in the evolution of latitudinal variation in body size, egg size, development time and larval growth efficiency. Clines in these traits also occur in more than one continent. The combination of evidence from laboratory thermal selection experiments and the existence of parallel clines in these traits throughout the world strongly implicates temperature as the selective agent responsible for latitudinal variation.

Although clines in many traits occur in more than one continent, clines in starvation resistance and desiccation resistance have only been found to occur in India (Chapter 5). If temperature was the selective agent responsible for clines in starvation and desiccation resistance then it would be expected that parallel clines would occur throughout the world. The fact that these clines are not paralleled in other continents suggests that they are not the result of thermal selection. Since latitudinal clines in starvation and desiccation resistance in *Drosophila* have so far only been found in India, it is possible that they are the result of genetic drift.

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However, the clinal variation has been observed in more than one species of *Drosophila*, which strongly suggests that it is adaptive. In order to confirm that temperature is not involved in the evolution of starvation and desiccation resistance, laboratory thermal selection lines could be tested for variation in these traits. If temperature is not responsible for latitudinal variation in these traits, they must be the result of a particular environmental factor or factors that vary with latitude in India but not in other continents.

In India, desiccation and starvation resistance exhibit opposite latitudinal clines (Karan et al. 1998a; Karan and Parkash 1998). Desiccation resistance is higher at high latitudes and starvation resistance is higher at low latitudes. These opposite clines contrast to the results of selection experiments where selection on increased desiccation resistance leads to a correlated increase in starvation resistance (Hoffmann and Parsons 1989; Hoffmann and Parsons 1993; Harshman et al. 1999; Hoffmann and Harshman 1999). This is an interesting contrast and suggests that the pressures in the natural environment differ from those applied in laboratory selection. The opposite clines in the wild indicate a possible trade-off between the 2 traits, due perhaps to opposing selection pressures. It is not clear what pressures may be acting on flies in the wild and further information on the environmental conditions which flies encounter in the wild would be useful in interpreting this variation.

One possible candidate for the selective agent is humidity, which does not vary with latitude in South America (Van 't Land 1997). High levels of desiccation resistance are found in *Drosophila* from arid areas (David et al. 1983; Hoffmann and Parsons 1991). This suggests that humidity could be responsible for the variation in desiccation resistance but it unclear whether this would lead to the correlated but opposite changes in starvation resistance. Laboratory humidity selection experiments could help to clarify this. Discovering what lies behind the

response of starvation resistance and desiccation resistance of *Drosophila* to latitude is a challenge for the future.

In other ectotherm species, other selective agents have been proposed as responsible for latitudinal variation. In the Atlantic silverside, *Menidia menidia*, growth rate increases with latitude. It has been suggested that this increase in growth rate is a result of the shorter growing season at high latitudes (Conover and Present 1990).

It is clear that temperature plays a major role in the latitudinal variation of many traits, which are seen to vary in multiple continents. However, some latitudinal variation such as that found in starvation resistance in India does not appear to be related to temperature and may be the result of other environmental factors, which so far remain unidentified.

## 6.5 Developmental Effect of Temperature

The temperature at which an organism develops has a large effect on its phenotype. This developmental effect may underlie the evolutionary response to temperature. The developmental effect of temperature can either act in the same (co-gradient variation) or opposite direction (counter-gradient variation) as the evolutionary effect of temperature (See General Introduction). Body size in *Drosophila* has been shown to increase with both developmental and evolutionary temperature. In this thesis I have shown that this is also true for larval growth efficiency (Chapter 4). Larval growth efficiency is high in populations that have evolved at high latitudes or low temperatures (Chapter 4). Larval growth efficiency is also high at low developmental temperatures. The response of larval growth efficiency to temperature (both evolutionary and developmental) must, at least in part,

be responsible for the response of body size. Low temperatures slow the speed of growth but must somehow permit that growth to be more efficient. It would be most interesting to discover how this occurs.

## 6.6 The Genetic Basis of Latitudinal Variation

Latitudinal variation is widespread in ectotherm species. Understanding the genetic basis of this variation may help us to understand how natural selection acts to produce this variation. In Chapter 5, I assessed the chromosomal contribution to latitudinal variation in body size in populations originating from South America and Australia. The majority of latitudinal body size variation in *Drosophila melanogaster* originating from both South America and Australia has been localised to chromosome 3. This result differs from that found for laboratory size-selected lines where all 3 chromosomes have an effect (B. Zwaan, personal communication), which suggests that the target of selection in latitudinal variation is not simply size. Laboratory thermal selected lines have shown major effects on both chromosomes 2 and 3 (Cavicchi et al. 1989). The differences between the results in Chapter 5 and those for laboratory thermal selection suggest that the latitudinal response may be more complex than simply a response to environmental temperature. The largest effect on body size in both South America and Australia has been found to be on chromosome 3. The similarities between the 2 continents suggest that the genetic response to latitude may be the same in both clines. Selection may be acting on the same gene or genes in both continents to produce the same response

Chromosome substitution analysis will only give a broad indication of whether the genetic basis of the traits is similar. In order to get a more detailed picture we must look at what genes are involved in this variation. Further

information on the genetic basis of the clines could be obtained by performing quantitative trait locus (QTL) mapping studies.

Quantitative trait locus (QTL) mapping is a technique that is used to locate regions of chromosomes that have a large effect on quantitative traits. This technique has been used in a number of animal and plant species in particularly maize, mice and *Drosophila* (Tanksley 1993; Doebley et al. 1995; Mitchell-Olds 1995; Keightley et al. 1996; Mackay 1996). QTL mapping is performed using 2 populations which differ for a particular trait, this can either be natural variation or variation due to artificial selection. These populations must also contain markers spaced throughout their genomes which are easily identifiable and which diverge between the 2 populations. Crosses between the 2 populations are performed to produce a range of phenotypes that differ from the 2 extremes of the parental populations. There are a number of methods for this and which method is used depends to some extent on the organism being studied and the nature of the variation (Tanksley 1993; Falconer and Mackay 1996). The markers present in the 2 populations make it possible to determine which parts of the offspring's genome originate from which parental population. Statistical analysis can then be applied to the results of both phenotype and genotype to infer which areas of the chromosome are responsible for the largest changes in phenotype.

In *Drosophila*, QTL mapping has been used to look at a number of traits. Bristle number has been particularly well studied using lines selected for low and high bristle numbers (Long et al. 1995; Mackay 1995; Mackay 1996; Gurganus et al. 1998; Lyman and Mackay 1998). There are number of different types of marker which can be used for QTL mapping in *Drosophila*. Very early studies used visible mutants (Thoday 1961; Wolstenhume and Thoday 1963), but the disadvantage of these is that they can affect the fitness of the flies, these mutants also had to be crossed into the populations to be studied. The ideal markers would already be

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present in the genomes of the populations to be studied. More recent studies, including many of those performed on bristle number have used transposable element insertion sites as markers. These are present throughout the genome and tend to vary between populations. They can be relatively easily visualised by in situ hybridisation of the polytene chromosomes of the salivary gland. Recent advances in knowledge and technology have made the use of molecular markers a possibility. Microsatellites are very variable between populations and are present throughout the *Drosophila* genome at relatively small intervals. Modern technology makes identifying the alleles present relatively quick and easy. The latest QTL studies in *Drosophila* are now using microsatellites as markers.

Preliminary QTL analysis of recently collected Australian populations has indicated that major QTLs exist on chromosome 3, and that there are also QTLs present on chromosome 2 (J. Gockel and W. J. Kennington, personal communication). This result is in accordance with my findings in Australia. It would be interesting to perform QTL mapping on other traits. This will allow us to discover whether the different traits have the same genetic basis and thus see how natural selection is acting to cause latitudinal variation.

Localisation of QTLs allows us to get one step nearer to discovering the actual genes that result in latitudinal variation. The complete genome of *Drosophila melanogaster* has now been sequenced (Adams et al. 2000) and this will help greatly in the search for the genes responsible for latitudinal variation. Combining the information from QTL mapping studies with information on the genes present in those specific regions of the genome will enable the identification of candidate genes that may be responsible for the variation in these traits.

Discovering what genes are responding to latitudinal selection in *Drosophila* may help us to understand the result of latitudinal selection in other species.

# 6.7 Comparing Latitudinal Variation Across Continents

Parallel latitudinal clines have been found in a number of continents. Although it is known that these clines are the result of natural selection it is not known whether the genetic basis of the response is the same throughout the world. Selection may always act on the same gene or genes to provide the response to latitudinal change or, selection may act to alter phenotype by a number of different genetic routes.

In Chapter 5 I performed chromosome substitution analysis of body size on flies from both South America and Australia. My study showed that chromosome 3 was responsible for the majority of body size variation in both continents. This could indicate that selection is acting on the same genes on chromosome 3 in both continents to produce the size variation. However, I found that the chromosome 3 had a larger effect on body size in South America. There were also differences in the effects of interactions between the 2 continents. My results indicated that there were major similarities and minor differences between the 2 continents.

In addition to chromosome substitutions, information on the genetic basis of latitudinal clines can also be provided by line cross analysis. A line cross analysis study of latitudinal variation in body size has been performed on flies from clines in 3 continents, South America, Australia and Africa (Gilchrist and Partridge 1999). This study showed that there were differences in the genetic architecture of the clines from the 3 continents.

As mentioned above, a preliminary QTL study of an Australian cline has shown large effects on body size are located on chromosome 3 (J. Gockel and W.J. Kennington, personal communication). It would be most interesting to compare the location of the QTLs found in the Australian populations with the South American

populations. This will provide further information on whether or not the genetic basis of the 2 clines is similar.

From the results of my chromosome study it appears that there may be major similarities in the genetic basis of the 2 clines in body size. These major similarities are accompanied by minor differences that are perhaps the result of differences in environmental conditions between the 2 continents.

## 6.8 Further Work

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

It appears that temperature variation is not responsible for latitudinal variation in starvation resistance and fat content (Chapter 3). This could be tested by assessing laboratory thermal selection lines for differences in starvation resistance. One possibility for the selective agent responsible for variation in starvation resistance is humidity and laboratory humidity selection experiments could be performed to discover whether humidity variation is responsible. Similarly, food level selection experiments could be performed to see whether starvation resistance evolves in response to food availability.

Chromosome substitution analysis of body size in South America and Australia (Chapter 5) revealed similarities between the 2 continents. Studying other traits in the same chromosome substitution lines would provide an indication of whether the traits have a similar genetic basis. Large body size and short development time in high latitude populations may be the result of the greater larval growth efficiency shown in high latitude populations. Chromosome substitution analysis would indicate whether the genetic basis of the traits is similar or different.

Similarities would indicate that the latitudinal variation in the traits might be the result of pleiotropy. Differences would indicate that the different traits are selected for separately.

QTL mapping studies of latitudinal variation in body size and other traits would provide a further insight into the genetic basis of these traits. Comparing QTLs between continents would indicate whether the variation does have a common genetic basis or not.

Latitudinal variation in *Drosophila* is a clear example of variation induced by the response of natural selection to changes in the environment, particularly to temperature. The lessons we can learn from the study of latitudinal variation in *Drosophila* will enable us to have a greater understanding of natural selection and the way that organisms can evolve to meet the challenges of their environment.

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# Appendix.

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## STARVATION RESISTANCE AND ADULT BODY COMPOSITION IN A LATITUDINAL CLINE OF *DROSOPHILA MELANOGASTER*

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*Abstract.*—Latitudinal geographic variation in *Drosophila melanogaster* is pervasive. Parallel clines in traits such as body size, egg size, ovariole number, and development time have been found on several continents throughout the world. However, a cline in starvation resistance and fat content in *D. melanogaster* has so far been found only in India. Here we investigate starvation resistance and fat content in 10 populations from South America, in which clines in body size, egg size, and development time have previously been found. We find no evidence for a cline in starvation resistance or fat content in South America. We therefore suggest that the cline in starvation resistance in India may have evolved in response to specific climatic variation found only in India.

*Key words.*—*Drosophila melanogaster*, fat content, latitudinal cline, starvation resistance.

Many traits of *Drosophila melanogaster* vary clinally with latitude on multiple continents. Flies from relatively high latitudes have larger body size (Stalker and Carson 1947; David and Bocquet 1975; Watada et al. 1986; Coyne and Beecham 1987; Imasheva et al. 1993; James et al. 1995; van 't Land et al. 1999), shorter development time (James and Partridge 1995; van 't Land et al. 1999), larger egg size (Azevedo et al. 1996), and higher ovariole number (Watada et al. 1986; Capy et al. 1993; Azevedo et al. 1996). The repeatability of the clines in different continents suggests that they are the result of natural selection (Endler 1977). However, it is unclear what selective agent is responsible for these clines or which traits are the targets of selection. Laboratory studies have indicated that temperature plays a significant role in the evolution of these characters. Flies that evolve at lower temperatures in the laboratory are larger (Anderson 1966; Cavicchi et al. 1985; Partridge et al. 1994), have a shorter development time (Anderson 1966; James and Partridge 1995), and have larger eggs (Azevedo et al. 1996). As well as the evolutionary effect, temperature has a direct environmental effect on these traits; at lower temperature, body size and egg size are greater and development time longer (Azevedo et al. 1996; James et al. 1997).

Latitudinal clines in starvation resistance have been found in *D. melanogaster*, *D. ananassae*, *D. kikkawai*, and *Zaprionus indianus* in India, with higher starvation resistance occurring at lower latitudes (Karan et al. 1998; Karan and Parkash 1998). Other studies in India have also found latitudinal trends in starvation resistance in smaller numbers of populations of *D. melanogaster* (Shamina et al. 1993), *D. kikkawai* (Parkash and Vandna 1994) *D. bipectinata*, and *D. malerkotliana* (Parkash et al. 1994). In Europe and Africa, differences in starvation resistance have been found between populations of *D. melanogaster*, but these differences were not related to latitude (Da Lage et al. 1990). The latitudinal differences in starvation resistance in India may be explained by ecological and/or climatic factors other than temperature (Karan et al. 1998). Starvation resistance has often been used

as an indirect measure of fat content, because these two characters are highly correlated (David et al. 1975; Zwaan et al. 1991, 1995a, b). However, studying fat content directly would eliminate other factors, such as metabolic rate, that may affect starvation resistance (Hoffmann and Parsons 1989). It should also be noted that some of the latitudinal ranges studied may not have been wide enough to detect correlations with latitude.

In the present study, starvation resistance and fat content were measured in 10 populations from a transect along a wide latitudinal range in South America. Temperature varies with latitude over this transect, whereas a number of other climatic factors, such as humidity and amount of sun hours do not (van 't Land 1997). The collection sites for these populations were low-elevation, coastal sites to minimize variation caused by factors other than latitude. These populations have been maintained at 25°C since collection in 1995 and the latitudinal variation in body size, egg size, and development time is still present in the populations (Azevedo et al. 1996; van 't Land et al. 1999).

The aims of the present study were to establish whether starvation resistance and fat content are among the suite of traits that vary with latitude and for which temperature is implicated as the selective agent and to establish whether there is clinal variation in body composition in South America. Because these populations are known to increase in body size with latitude, we investigate whether fat content also increases with latitude, which could indicate an ability of high-latitude flies to acquire more food or to utilize food more efficiently. Alternatively, increased body size may be achieved by using the fat reserves for extra growth. Larval crowding has been shown to influence fat content and starvation resistance in temperate (Zwaan et al. 1991) and tropical (van 't Land 1997) populations of *D. melanogaster*. Therefore, this experiment was performed using adult flies that had been kept at either high or low density as larvae to assess whether differing larval density affects fat content, starvation resistance, or the relationship of fat content and starvation resistance with latitude.

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## MATERIALS AND METHODS

### Fly Populations

The 10 South American populations have been described elsewhere (Azevedo et al. 1996; van 't Land et al. 1999). They were collected in 1995 along a latitudinal range from 2.22°S to 41.5°S and have been maintained since collection in bottle culture at 25°C. The South American populations have previously been found to exhibit clinal variation in body size, development time, and egg size (Azevedo et al. 1996; van 't Land et al. 1999). Starvation resistance, wet weight, dry weight, water content, and fat content were measured in these populations. All experiments were carried out at 25°C with constant day length (12 h light, 12 h dark).

### Production of Experimental Flies

To collect eggs, flies from each of the populations were transferred to laying pots and allowed an acclimatisation period of 12 h and a 4-h period on fresh food to encourage the laying of retained eggs. They were then allowed to lay on fresh medium for two consecutive periods of 4-h. First-instar larvae were picked from the surface of the medium and transferred to vials containing 7 ml of standard medium (composition: 1.0% [w/v] agar, 8.5% sugar, 6.0% maize meal, 2.0% dried yeast extract, 2.5% [v/v] of a 10% Nipagin solution in ethanol). To test whether crowding conditions had any effect on the characters measured or on their relationship to latitude, two larval densities, 50 larvae per vial and 200 larvae per vial, were used. For each population, three vials were set up at each larval density. Eclosing adults were collected as virgins and were transferred in single-sex groups of five to vials containing fresh medium, which were renewed regularly. The flies were kept for 15 days before measurements were made, because the starvation resistance of female flies changes rapidly in the first two weeks of adulthood (Fairbanks and Birch 1970; Service et al. 1985; Zwaan et al. 1991), which could lead to spurious differences when studying younger flies. The flies were kept unmated because there may be a trade-off between starvation and egg-laying (Chippindale et al. 1993), thus, any between-line differences in fecundity could confound the effects of our study if mated flies were used. The flies were then anaesthetized using carbon dioxide and removed from the food.

### Experimental Measurements

#### Wet weight, dry weight, water content, and fat content

Wet weight, dry weight, water content, and fat content were determined for 15 flies of each sex from each population and each larval density. The flies used were kept in single-sex groups of five for weighing. An equal number of flies was taken from each of the original culture vials to equalize any effects caused by between-vial variation. After removal from food, the flies were anaesthetized on ice and were weighed to the nearest 0.002 mg using a Sartorius (Gottingen, Germany) microbalance to determine wet weight. The flies were dried in an oven at 60°C for 24 h and were then reweighed to determine dry weight. To remove fat from the flies, they were placed in sealed tubes with 1 ml of diethyl ether for 24

h with occasional light shaking. They were then removed from the ether and allowed to dry at room temperature for another 24 h, and then reweighed to determine the fat-free dry weight. From the weights obtained, it was possible to calculate water content (wet weight—dry weight), fat content (dry weight—fat-free dry weight), relative water content (water content/wet weight) and relative fat content (fat content/dry weight; David et al. 1975).

### Starvation resistance

Starvation resistance was measured for 15 flies of each sex from each population and each larval density, with an equal number of flies taken from each of the original culture vials. The flies were placed individually in vials containing 1 ml of autoclaved agar (5 g/L) to prevent desiccation. The vials were observed for dead flies after 24 h had elapsed and then at 3-h intervals. The frequency of observations was decreased as the number of deaths per observation decreased. Death was deemed to have occurred when a fly showed no sign of movement even after the vial was tapped lightly. Time of death was assigned to the midpoint between observations.

### Statistical Analysis

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

#### Wet weight, dry weight, water content, fat content, and starvation resistance

Analysis of covariance was performed on each of the traits, with latitude as the covariate and sex and density as independent variables. A (quasi) minimum adequate model (MAM) was found by a stepwise backward-deletion procedure (Crawley 1993). The highest-order interaction was removed from the full model if it did not explain a significant proportion of the residual variance ($P > 0.05$), and a reduced model was fitted. The next-highest-order nonsignificant interaction with the highest $P$-value was then removed and a new reduced model was fitted. Nonsignificant factors or interactions were maintained in the model when any higher-order interactions including the factor or interaction were significant ($P < 0.05$).

### Relationship between fat content and starvation resistance

To determine whether fat content and starvation resistance have a direct relationship and whether there are factors other than fat content that affect starvation resistance, we performed additional tests. To test whether the relationship between fat content and starvation resistance differed linearly with latitude, regression analyses were performed on population means of male and female starvation resistance and on population means of male and female relative fat content at each density. A multiple regression model was fitted using starvation resistance as the dependent variable with sex, crowding, relative fat, and latitude as factors. All interactions were included in the model, and a minimum adequate model was then found using a stepwise backward-deletion procedure (as above).

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TABLE 1. Mean values for (a) wet weight, (b) relative water content, (c) relative fat content, and (d) starvation time.

| Latitude (°S) | Female Low density | Female High density | Male Low density | Male High density |
|---|---|---|---|---|
| **(a) Mean wet weight (mg)** | | | | |
| 2.22 | 5.373 | 4.701 | 3.669 | 3.080 |
| 18.47 | 6.023 | 6.263 | 4.209 | 3.641 |
| 20.22 | 6.325 | 5.804 | 4.617 | 3.651 |
| 23.63 | 6.748 | 5.678 | 4.398 | 3.620 |
| 27.33 | 6.982 | 5.950 | 4.658 | 3.620 |
| 29.93 | 6.734 | 5.842 | 4.526 | 3.816 |
| 33.08 | 6.392 | 5.319 | 4.210 | 3.505 |
| 35.8 | 7.596 | 6.477 | 4.489 | 3.769 |
| 39.8 | 6.857 | 6.238 | 4.578 | 3.663 |
| 41.5 | 7.373 | 6.290 | 4.671 | 3.968 |
| **(b) Mean relative water content** | | | | |
| 2.22 | 0.667 | 0.656 | 0.674 | 0.673 |
| 18.47 | 0.599 | 0.658 | 0.658 | 0.671 |
| 20.22 | 0.650 | 0.636 | 0.686 | 0.683 |
| 23.63 | 0.649 | 0.665 | 0.673 | 0.680 |
| 27.33 | 0.645 | 0.653 | 0.683 | 0.679 |
| 29.93 | 0.632 | 0.632 | 0.670 | 0.665 |
| 33.08 | 0.603 | 0.650 | 0.667 | 0.687 |
| 35.8 | 0.687 | 0.671 | 0.687 | 0.689 |
| 39.8 | 0.653 | 0.637 | 0.682 | 0.678 |
| 41.5 | 0.621 | 0.632 | 0.668 | 0.669 |
| **(c) Mean relative fat content** | | | | |
| 2.22 | 0.057 | 0.070 | 0.043 | 0.036 |
| 18.47 | 0.136 | 0.062 | 0.059 | 0.044 |
| 20.22 | 0.090 | 0.092 | 0.041 | 0.039 |
| 23.63 | 0.068 | 0.056 | 0.041 | 0.032 |
| 27.33 | 0.077 | 0.074 | 0.026 | 0.033 |
| 29.93 | 0.092 | 0.087 | 0.036 | 0.044 |
| 33.08 | 0.130 | 0.082 | 0.058 | 0.029 |
| 35.8 | 0.038 | 0.058 | 0.034 | 0.027 |
| 39.8 | 0.073 | 0.087 | 0.036 | 0.031 |
| 41.5 | 0.092 | 0.085 | 0.045 | 0.042 |
| **(d) Mean starvation time (h)** | | | | |
| 2.22 | 60.100 | 62.283 | 28.892 | 32.608 |
| 18.47 | 104.383 | 68.083 | 33.617 | 32.783 |
| 20.22 | 80.107 | 48.768 | 31.920 | 26.821 |
| 23.63 | 77.033 | 65.767 | 30.575 | 32.217 |
| 27.33 | 75.267 | 61.367 | 25.533 | 26.767 |
| 29.93 | 55.667 | 70.258 | 30.642 | 29.408 |
| 33.08 | 102.383 | 70.600 | 33.917 | 33.483 |
| 35.8 | 48.308 | 39.750 | 23.933 | 26.442 |
| 39.8 | 68.217 | 66.083 | 28.717 | 27.917 |
| 41.5 | 79.817 | 79.550 | 36.017 | 36.417 |

TABLE 2. Minimum adequate model analysis of covariance with (a) wet weight, (b) dry weight, (c) water content, (d) relative water content, (e) fat content, (f) relative fat content, and (g) starvation resistance as dependent variables, latitude as covariate, and sex and density as independent variables.

| Source | df | Mean square | F-ratio |
|---|---|---|---|
| **(a) Wet weight** | | | |
| Latitude | 1 | 4.1156 | 48.5867*** |
| Density | 1 | 6.0321 | 71.2119*** |
| Sex | 1 | 3.8403 | 45.3363*** |
| Latitude × sex | 1 | 0.5944 | 7.0173* |
| Error | 35 | 0.0847 | |
| **(b) Dry weight** | | | |
| Latitude | 1 | 0.5555 | 43.7765*** |
| Density | 1 | 0.8550 | 67.3765*** |
| Sex | 1 | 0.5842 | 46.0365*** |
| Latitude × sex | 1 | 0.1322 | 10.4216** |
| Error | 35 | 0.0127 | |
| **(c) Water content** | | | |
| Latitude | 1 | 1.6471 | 24.9101*** |
| Density | 1 | 2.3451 | 35.468*** |
| Sex | 1 | 17.2082 | 260.2572*** |
| Error | 36 | 0.0661 | |
| **(d) Relative water content** | | | |
| Sex | 1 | 0.0098 | 36.2976*** |
| Error | 38 | 0.0003 | |
| **(e) Fat content** | | | |
| Sex | 1 | 1.1753 | 105.1813*** |
| Density | 1 | 0.0754 | 6.7475* |
| Error | 37 | 0.0112 | |
| **(f) Relative fat content** | | | |
| Sex | 1 | 0.0172 | 55.7984*** |
| Error | 38 | 0.0003 | |
| **(g) Starvation resistance** | | | |
| Sex | 1 | 15022.1070 | 111.1708*** |
| Error | 38 | 135.1000 | |

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

all significant with no significant interaction. Water content was greater at high latitudes, low density, and in females. For fat content, sex and density were significant main effects. Fat content was higher in females and at low density. For relative water content, relative fat content, and starvation resistance, only sex was significant, with males having a higher relative water content and females having a higher relative fat content and a higher starvation resistance. In summary, only wet weight, dry weight, and water content showed clinal variation.

## RESULTS

*Wet weight, dry weight, water content, fat content, and starvation resistance*

Mean values for wet weight, relative water content, relative fat content, and starvation resistance are shown in Table 1. The results of the MAM analyses of covariance are summarized in Table 2. For wet weight and dry weight, latitude, density, and sex were all significant main effects with a significant latitude-by-sex interaction. Wet and dry weight were larger at high latitude, low density, and in females. The interaction results from different responses of males and females to increasing latitude. Table 1 shows mean values for wet weight for each sex; inspection of this data indicates that increasing latitude had a greater effect on females than it did on males. For water content, latitude, density, and sex were

*Relationship between fat content and starvation resistance*

Regression analyses of male starvation resistance against female starvation resistance at low and high larval density were significant (both $P < 0.05$). Regression analysis of male relative fat against female relative fat was significant only at low larval density ($P < 0.05$). These results suggest differences between fat content and starvation resistance. The MAM multiple regression analysis is shown in Table 3. A number of significant interactions were found. The interaction between relative fat and latitude was significant (parameter estimate = 0.41312). This interaction indicates that the relationship between starvation resistance and relative fat varies with increasing latitude. The sign of the parameter estimate is positive, indicating that starvation resistance and

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TABLE 3. Minimum adequate multiple regression model with starvation resistance as the dependent variable, latitude and relative fat content as continuous factors, and sex and density as discrete factors.

| Source | df | Mean square | F-ratio |
|---|---|---|---|
| Sex | 1 | 620.8157 | 14.8234*** |
| Density | 1 | 747.5490 | 17.8494*** |
| Latitude | 1 | 650.7022 | 15.537*** |
| Relative fat | 1 | 347.1335 | 8.2886** |
| Sex × density | 1 | 490.9332 | 11.7221** |
| Latitude × sex | 1 | 611.9400 | 14.6114*** |
| Sex × relative fat | 1 | 65.0617 | 1.5535 |
| Latitude × density | 1 | 655.9321 | 15.6618*** |
| Density × relative fat | 1 | 814.1069 | 19.4386*** |
| Latitude × relative fat | 1 | 685.7342 | 16.3734*** |
| Latitude × sex × density | 1 | 495.9863 | 11.8428** |
| Latitude × density × relative fat | 1 | 696.0447 | 16.6196*** |
| Error | 27 | 41.8800 | |

*** $P < 0.001$; ** $P < 0.01$.

relative fat are more closely correlated with increasing latitude. The interaction between relative fat and density was also significant (parameter estimate $= 13.18336$). This interaction indicates that larval density has an effect on the relationship between fat content, and starvation resistance. The sign of the parameter estimate indicates that at high density, fat explains more of the variation in starvation resistance than it does at low density.

## DISCUSSION

The results of our analysis showed that relative water content, fat content, relative fat content, and starvation resistance did not vary clinally. Overall, water content increased with increasing latitude, but this was paralleled by the increase in dry body weight; flies from each latitude contained the same amount of water per unit body weight. The multiple regression model for starvation resistance found a significant latitude-by-relative fat interaction, indicating that latitude affected the relationship between relative fat content and starvation resistance. Similarly, the density-by-relative fat interaction indicated that the relationship between fat and starvation resistance was affected by larval density. Factors other than fat content must therefore have partly determined starvation resistance. Previous studies have shown that starvation resistance is directly correlated with fat content (David et al. 1975; Service 1987; Zwaan et al. 1991). However, other factors (e.g., metabolic rate; Hoffmann and Parsons 1989) may also play a role, as has been suggested by our study. Previous studies of clines have concentrated on starvation resistance alone and concluded that it reflected differences in fat content (Da Lage et al. 1990; Shamina et al. 1993; Parkash et al. 1994; Parkash and Vandna 1994). However, studies that do not measure fat content may miss effects due to other factors. In studying both fat content and starvation resistance directly, we have been able to establish that there is no clinal variation in either and that other factors such as metabolic rate differences may explain the apparent variation in starvation resistance, independent of fat content.

Other studies of starvation resistance in *D. melanogaster* have found clinal variation in the Indian subcontinent (Karan

et al. 1998), but not in Europe and Africa (Da Lage et al. 1990). The South American populations that we studied showed no evidence of clinal variation in starvation resistance. The starvation resistance of the South American populations may be a consequence of adaptation to laboratory conditions. However, this is unlikely because other geographically varying traits such as body size have not undergone laboratory adaptation since collection (Azevedo et al. 1996; van 't Land et al. 1999). Another possible reason for the difference in results between our study and that of Karan et al. (1998) is the differences in methods used. Karan et al. (1998) studied only males and tested starvation resistance at 3–4 days of age. Our study examined starvation resistance of both males and females. Incorporating females gives a more reliable estimate because they live longer. The females we used were kept as virgins, because there is a trade-off between egg production and starvation resistance (Chippindale et al. 1993). The flies used were also kept for 15 days before starvation resistance was measured because female starvation resistance increases steeply early in life, before reaching a plateau. Starvation resistance was measured at 15 days to avoid any confounding effects of between-population differences in the time taken to reach this plateau. The flies used in our study remained healthy until tested for starvation resistance and the differences between the methods would not be likely to explain the difference in results. It should also be noted that the study of Da Lage et al. (1990) used very similar methods to that of Karan et al. (1998) and also failed to find a latitudinal cline in starvation resistance. However, they only studied three populations.

In addition to differences in methods, our study covered a broader range of latitudes than that of Karan et al. (1998). The broader latitudinal range means that we also covered a wider range of temperature than the study of Karan et al. (1998) and this is further evidence to suggest that temperature variation is not solely responsible for the variation in starvation resistance found in Indian populations.

The apparent inconsistency of the results of Karan et al. (1998) and the ones we report here can be accounted for if differences in starvation resistance are a result of adaptation to climatic factors other than temperature, which differ between continents. Karan et al. (1998) suggested that the clinal variation in starvation resistance found in India was due to either average yearly temperature or average winter temperature. However, average yearly temperature in India is not well correlated with latitude. It is therefore unlikely that average yearly temperature is responsible for clinal variation of a trait. Similarly, although winter temperature in India is negatively correlated with latitude, summer temperature is positively correlated with latitude, and it is not clear why only winter temperature should affect this trait. In our study, average yearly temperature was highly correlated with latitude and we found no significant clinal variation in starvation resistance. Thus, it seems likely that the variation in starvation resistance in India is actually due to climatic factors other than temperature or a combination of factors (which may include temperature) that differ between South America and India. Sampling populations along latitudinal clines with varying climatic factors could pinpoint the key parameters in the evolution of these and other traits. In addition, specific

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hypotheses can be tested using laboratory evolution experiments.

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