Thermal Evolution of Body Size in *Drosophila melanogaster*

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For my Mother

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The Fly

Little Fly,
Thy summer's play
My thoughtless hand
Has brush'd away.

Am not I
A fly like thee?
Or art not thou
A man like me?

For I dance,
And drink, & sing,
Till some blind hand
Shall brush my wing.

If thought is life
And strength & breath,
And the want
Of thought is death;

Then am I
A happy fly,
If I live
Or if I die.

William Blake (Songs of Experience, 1794)

Table of Contents

Abstract

The objective of this research was to investigate the causes and consequences of thermal evolution of body size in *Drosophila melanogaster*. This was done empirically by the integrated study of lines collected along latitudinal gradients and populations that were undergoing long-term evolution at different temperatures in the laboratory. All experiments were conducted in the laboratory using controlled conditions of temperature and larval density.

I examined 20 populations collected along a north-south transect in Australia (17°-43°S) and found that wing size increased with latitude. The genetic cline in wing size was mostly based on variation in cell number (82%). These findings contradicted previous results on thermal selection in the laboratory, where the divergence in wing size was achieved almost entirely by changes in cell size. In another experiment, 6 geographic lines were reared at 5 temperatures spanning the entire viability range for the species. Wing size, cell size and cell number all decreased with increasing development temperature. Cell size determined most of the plasticity in wing size (~75%). Wing size increased with latitude across the reaction norm, mostly due to variation in cell number (87%), which repeated the pattern observed at a single temperature. Although there was significant variation among populations in phenotypic plasticity of the wing traits, a latitudinal trend in this variation was only detected for cell size; variation in plasticity of wing size among populations was attributable to both cellular components. The results of these experiments suggest that thermal evolution acted on body size itself, since cell number is the basis of additive genetic variation for body size within populations at a single temperature.

In the experiments outlined above, it was also found that the size of the wing, relative to the size of the thorax and the relative length of the wing both decreased in response to high selection and developmental temperatures. These results could be explained by thermal selection on flight ability in nature, since high wing-thorax ratios are advantageous for flight at low temperatures. This hypothesis is compatible with the observed cellular basis of the cline in wing size since selection on wing-thorax ratio produces changes in cell number.

In another set of experiments, I investigated whether egg size responded to thermal selection. It was found that egg size increased with latitude among geographic populations from Australia (20) and South America (10). Laboratory populations that had been evolving at 16.5°C laid larger eggs than populations that had evolved at either 25°C or 29°C, suggesting that temperature was an important selective agent in generating the latitudinal clines. Flies from laboratory populations produced larger eggs at 16.5°C

than at 25°C. There was no evidence of gene-environment interaction for the trait. I also studied egg size in populations that had been artificially selected for wing size and found that selection for large wing size within environments increased egg size. However, egg size and body size were not correlated among the Australian populations, so that it is unclear whether body size caused the cline in egg size or egg size was a target of thermal selection.

In further experiments, I studied the impact of egg size on offspring life history characters, by examining genetically equivalent offspring developing from eggs that were different in size as a result of maternal genetic effects (female offspring from crosses among outbred populations that showed genetic differences in egg size). It was discovered that egg size had positive effects on offspring egg viability, hatching rate, hatchling size, larval feeding rate and preadult development rate but no effects on offspring larval competitive ability, adult body size or egg size. These effects could not be predicted from patterns of association between these traits within or among populations.

The results of my research suggest that body size was a principal target of thermal selection in *D. melanogaster*. The mechanisms for this process have not been elucidated. Selection on flight ability could be an important component of thermal evolution of adult body and wing size. Thermal selection on body size could have caused a correlated response in egg size or egg size may have been targeted directly. Maternal genetic effects of egg size can contribute to the thermal evolution of preadult life history traits.

1

General Introduction

"It is not clear what type of selection is involved here."

Jerry A. Coyne and Edward Beecham (1987)

1.1 The Problem

Temperature appears to have general developmental and evolutionary effects on the body size of ectothermic animals, and these seem to act in the same direction. Development at high temperature has been shown to decrease body size in most ectothermic species studied (Section 1.5.1). There is also evidence that temperature can have an evolutionary effect on body size, because genetic differentiation has been shown among populations of several species of ectotherms evolving at different temperatures, with cold adapted populations showing larger body size (Section 1.6.1). The role of temperature in causing these patterns has been established in different species of *Drosophila* by selection experiments where separate populations were kept at different temperatures: those selected at lower temperatures evolved larger body size (Section 1.6.1).

However, despite abundant descriptive evidence for the effects of temperature on body size, little is actually known about their biological causes. Several questions have been raised. Are the developmental and evolutionary effects of temperature caused by a common underlying mechanism? One possibility is that body size is a target of thermal selection (temperature-dependent selection); alternatively, body size could evolve in response to selection on other life history characters, genetically correlated with it (Section 1.4.2). If body size is one of the targets of thermal selection, then the phenotypic plasticity of body size in response to temperature could itself be adaptive. If that is the case, then what is the role of phenotypic plasticity in response to temperature in the thermal evolution of body size in nature? Also, can the phenotypic plasticity of body size itself respond to thermal selection?

My work has tried to clarify these issues by focusing on *Drosophila melanogaster*. This species provides an appropriate model system for the study of thermal evolution for several reasons. Firstly, it displays the best documented set of latitudinal clines in body size among animals (Section 1.6.1). In addition, it can be reared and subjected to experimental manipulations in the laboratory, and it is open to artificial selection experiments that can be used to test evolutionary hypotheses. Finally, a great deal is known about its biology, genetics and developmental biology.

1.2 Development of Body Size in *Drosophila*

Growth in insects occurs by periodic substitutions of the cuticular exoskeleton (moults) during development. These moults are controlled by cyclic changes in levels of the hormone ecdysone, secreted by the prothoracic gland. The production of ecdysone is regulated by the secretion of prothoracicotropic hormone in the brain. The release of prothoracicotropic hormone leading to moulting is initiated early in each larval instar, when a critical weight is reached, after which there is a fixed period of post-critical feeding and growth before moulting occurs. If feeding is prevented before this stage, further development is blocked. Variation in larval critical size and feeding rate during the post-critical period generate differences in size at the following instar. The existence of a critical weight for pupariation and its impact on adult size have been extensively documented in *D. melanogaster* (Beadle et al. 1938; Bakker 1959, 1961; Robertson 1963; Sewell et al. 1975; Burnet et al. 1977).

In *Drosophila*, larval cells do not divide after embryonic development but, instead, increase in ploidy and grow throughout larval life. At metamorphosis larval cells die and imaginal cells differentiate to generate the adult integument and several adult organs. Imaginal cells grow and divide within the larva until puparium formation, when cell proliferation ceases. Although the onset of imaginal morphogenesis is brought about by a decline in the concentration of juvenile hormone, the final size of imaginal discs (sac-like clusters of imaginal cells), which determines adult size, seems to be regulated intrinsically and not by hormonal signals (Bryant and Simpson 1985). In experiments where imaginal discs were allowed to continue growing (e.g. damaging discs in the larva can extend pupariation), the discs did not grow beyond their normal maximum cell number (Simpson et al. 1980; Bryant and Levinson 1985). Furthermore, regeneration and genetic mosaic experiments suggested that the control of cell proliferation in imaginal discs is locally autonomous and controlled by position-dependent cell interactions (Bryant and Simpson 1985; Woods and Bryant 1992; García-Bellido 1994).

1.3 Cellular Basis of Wing Size in *Drosophila*

The wing imaginal disc forms the adult wing and the surrounding thorax and is located beneath the larval thoracic epidermis. At hatching, the wing imaginal disc is constituted by ~40 cells which divide continuously until the end of the third larval instar when it reaches ~50000 cells. Shortly after pupariation, the wing disc cells change dramatically in volume and shape and the disc evaginates. The epidermal cells then secrete a pupal cuticle and an adult cuticle. Between pupal and adult cuticle formation, each cell that will produce a bristle or sensillum divides twice more to produce a cluster of four cells which form the shaft, socket, neuron and neuron sheath. Each non-neural cell secretes an apical cuticular trichome so that trichome density provides a measure of cell surface area (Dobzhansky 1929).

The cells of the future veins have smaller apical surfaces (higher trichome density) and a thicker cuticle than intervein cells. Cell lineage analyses have shown that several vein-mutant phenotypes were associated with changes in the number of cells in the wing blade and the shape of the wing, especially in mutant combinations, suggesting that cell proliferation and differentiation are related: in general, mutations that caused extra veins increased wing size and decreased its relative length, whereas those that removed veins decreased wing size and increased its relative length (Diaz-Benjumea and García-Bellido 1990; García-Bellido and de Celis 1992).

1.4 Quantitative Genetics of *Drosophila* Body Size

1.4.1 Heritability

Laboratory studies in *Drosophila* have discovered high narrow-sense heritabilities (i.e. the ratio of additive genetic variance to total phenotypic variance) for different measures of body size, ranging approximately from 0.2 to 0.5 (e.g. Robertson and Reeve 1952; Tantawy 1961; Misra and Reeve, 1964; Anderson 1973; Coyne and Beecham 1987; Prout and Barker 1989; Wilkinson et al. 1990; Thomas and Barker, 1993). Although field estimates of heritability were generally lower than laboratory estimates, there is evidence for considerable additive genetic variance for body size in natural populations (e.g. Tantawy 1964; Cavicchi et al. 1981; Coyne and Beecham, 1987; Prout and Barker 1989; Riska et al. 1989; Santos et al. 1992a). The size of different structures is genetically correlated in *Drosophila* (Robertson and Reeve 1952; Tantawy and El-Helw 1966; Cowley and Atchley 1990; Wilkinson et al. 1990; Thomas and Barker 1993). However, the relative sizes of the wing and the thorax (Robertson 1962) and the shape of the wing (Cavicchi et al. 1981; Weber 1990, 1992) can also be altered directly by artificial selection.

1.4.2 Genetic correlations with fitness components

In laboratory studies on *Drosophila,* adult life history characteristics such as female fecundity (Robertson 1957b; Tantawy and Rakha 1964), adult longevity (Tantawy and Rakha 1964; Partridge and Fowler 1992) and male mating success (Ewing 1961; Ewing 1964) have generally shown positive genetic correlations with body size (but see Hillesheim and Stearns 1992). In contrast body size seems to be negatively genetically correlated with preadult fitness components: larval (Partridge and Fowler 1993; Nunney 1996) and preadult development rate (Robertson 1957b, 1960a, 1963; Hillesheim and Stearns 1991; Zwaan et al. 1995a), larval competitive ability and larval viability (Santos et al. 1992; Partridge and Fowler 1993). Therefore, body size may display an intermediate optimum and be under stabilising selection, as a result of conflicting selection on the pre-adult and adult periods (Partridge and Fowler 1993).

1.5 Developmental Effects of Temperature

1.5.1 Growth

Development at high temperatures, under apparently non-stressful conditions for growth and development, has been shown to decrease body size in 83.5% of 92 ectothermic species studied (Atkinson 1994). In *Drosophila,* body size has been consistently shown to decrease with increasing developmental temperature (Alpatov and Pearl 1929; Alpatov 1930; Imai 1933; Stalker and Carson 1947, 1948, 1949; Ray 1960; Tantawy 1961; Tantawy and Mallah 1961; Delcour and Lints 1966; Sokoloff 1966; David and Clavel 1967; Levins 1969; Atkinson 1979b; Kuo and Larsen 1987; Thomas 1993; Robertson 1987; Starmer and Wolf 1989; Thomas and Barker 1993; Partridge et al. 1994a). In contrast, rates of development and growth, and growth efficiency, increase with environmental temperature (Partridge et al. 1994b; Neat et al. 1995), i.e. at high temperatures, given a limited amount of food, flies are more efficient at converting "larval weight" into "adult body size".

The environmental effect of temperature on wing size is mostly mediated by changes in cell size in *D. melanogaster* (Alpatov 1930; Robertson 1959a; Delcour and Lints 1966; Masry and Robertson 1979; Kuo and Larsen 1987; Partridge et al. 1994a). Temperature shifts can change adult body size throughout pre-adult development (Masry and Robertson 1979; David et al. 1983), suggesting that temperature must affect both critical weight for pupariation and growth in the post-critical period.

1.5.2 Wing-thorax size ratio and wing shape

Increasing temperature during development has been shown to decrease the wing-thorax size ratio (size of the wing relative to size of the thorax) or increase the wing loading (ratio of body mass to wing area) in different species of *Drosophila* (Pantelouris 1957; Stalker and Carson 1949; Robertson 1987; Starmer and Wolf 1989; Thomas and Barker 1993; David et al. 1994; Barker and Krebs 1995). Relative wing length (ratio of wing length to wing width or aspect ratio) also decreased with environmental temperature in *Drosophila* (Stalker and Carson 1949; Sokoloff 1966).

1.5.3 Egg size

In natural populations of several ectothermic species, egg size has been found to increase in colder areas and at colder times: e.g. in crustaceans (Green 1966; Kerfoot 1974; Brambilla 1982; Clarke et al. 1991), fish (Williams 1967; de Ciechomski 1973; Southward and Demir 1974; Marsh 1984; Daoulas and Economou 1986; Imai and Tanaka 1987; Tanasichuk and Ware 1987) and frogs (Berven 1982; Williamson and Bull 1995). Much of this variation may be attributable to the direct, environmental effects of temperature, because experimental manipulations of temperature can have the same effect in *Daphnia* (Brambilla 1982), *Drosophila* (Imai 1935; David and Clavel 1969; Avelar 1993) and fish (Shrode and Gerking 1977; Marsh 1984; Imai and Tanaka 1987). The reasons for this consistent pattern of phenotypic plasticity are not understood.

1.6 Evolutionary Effects of Temperature

1.6.1 Body size

Genetic clines in fitness-related traits are evolutionarily interesting, because they can reveal how natural selection acts on the traits and help identify mechanisms maintaining genetic variation for them. They also allow study of the roles of genetic and environmental variation, as well as their interactions, in producing phenotypic variation.

Experiments in which individuals from different populations were reared under standard laboratory conditions have revealed genetic clines in body size in *Drosophila melanogaster* from western Europe and Africa (Capy et al. 1993), eastern Europe and central Asia (Imasheva et al. 1994), North America (Coyne and Beecham 1987; Capy et al. 1993), South America (Van't Land et al. 1995) and Australia (James et al. 1995; Chapter 3), with genetically larger flies at higher latitudes (but see Long and Singh 1995). Similar latitudinal size clines with a genetic basis have been found in other *Drosophila* species: *D. robusta* (Stalker and Carson 1947), *D. subobscura* (Prevosti 1955; Misra and Reeve 1964; Pegueroles et al. 1995), *D. obscura* (Pegueroles et al. 1995), *D. simulans*

(David and Bocquet 1975; Capy et al. 1993) but not *D. pseudoobscura* or *D. persimilis* (Sokoloff 1965, 1966). Other ectotherms also show genetic clines of increasing size with latitude: houseflies (Bryant 1977), honey bees (Alpatov 1929), a copepod (Lonsdale and Levinton 1985b), the Atlantic Silverside (Conover and Present 1990), and two species of frogs (Berven et al. 1979; Berven 1982; Riha and Berven 1991). Comparable genetic differentiation in body size also occurs along altitudinal gradients, in *D. melanogaster* (Louis et al. 1982), *D. robusta* (Stalker and Carson 1945) and *D. flavopilosa* (Budnik et al. 1988), and within populations at different seasons, in *D. melanogaster* and *D. simulans* (Tantawy 1964), *D. robusta* (Stalker and Carson 1949) and *D. subobscura* (Prevosti 1955), again with size decreasing at higher temperatures.

The repeatability of these clinal patterns in body size in different species of *Drosophila*, and within *D. melanogaster* in different continents, suggests that they evolve by natural selection, rather than genetic drift (Endler 1986). Recent colonizations by *Drosophila* species (e.g. David and Capy 1982; Pegueroles et al. 1995) to new regions can provide "natural" experiments for testing the response to selection. However, clines cannot unambiguously show temperature dependent selection of body size, since many other factors such as rainfall, day length, number of generations per breeding season, food availability, levels of intra- and interspecific competition and impact of biological enemies are correlated with natural variation in temperature. To establish that temperature *per se* is causal, it must be manipulated independently of other environmental variables.

Experiments on laboratory evolution of *Drosophila* at different temperatures, have established the importance of temperature, or of a causally related variable, as the selective agent. Evolution at low temperatures increased body size in *D. pseudoobscura* in one replicated (Anderson 1966, 1973, but analysed as pseudoreplicated, see Section 2.4.3) and one unreplicated (Matzke and Druger 1977) study, in *D. melanogaster* in two replicated studies (Cavicchi et al. 1985, 1989 but analysed as pseudoreplicated, see Section 2.4.3; Partridge et al. 1994a; Neat et al. 1995) and one unreplicated study (Lint and Bourgois 1987), and in *D. willistoni* in one unreplicated study (Powell 1974). The responses to selection were fast, having been detected after only ~5 years (~300 generations at 25°C) in the replicated studies (Anderson 1966; Partridge et al. 1994a).

In *D. melanogaster*, the evolutionary increase of wing size at low temperatures in the laboratory was achieved mostly by changes in cell size in two studies (Cavicchi et al. 1985, but see Section 2.4.3; Partridge et al. 1994a). This is surprising if body size is the target of thermal selection since, in *Drosophila*, the response to artificial selection for body size within temperatures has been shown to involve mostly responses in cell number (Zarapkin 1935; Robertson 1959b, 1962; L. Partridge, R. E. Langelan, K. Fowler and V.

French, unpublished results). Also, variation in wing size among natural populations of *D. melanogaster* that did not seem to be evolving under thermal selection, was based mostly on cell number (Robertson 1959a). These observations suggest that cell size, rather than wing size, could be the major target of thermal selection. It would be interesting to examine the cellular basis of the thermal evolution of body size in natural populations.

1.6.2 Development time

Larval development time has also shown a latitudinal (James and Partridge 1995) and an altitudinal (Louis et al. 1982) cline in *D. melanogaster*, with faster development in flies collected at higher latitudes and altitudes. Similar trends were found in water striders (Blanckenhorn and Fairbairn 1995), a copepod (Lonsdale and Levinton 1985a, 1985b), the Atlantic Silverside (Conover and Present 1990, Present and Conover 1992) and in two frog species (Berven et al. 1979; Berven 1982; Riha and Berven 1991). Faster pre-adult development also evolved under laboratory selection at lower temperatures in *D. pseudoobscura* (Anderson 1966, but see Section 2.4.3; Matzke and Druger 1977, unreplicated) and in *D. melanogaster* (Kilias and Alahiotis 1985, unreplicated; Lints and Bourgois 1987, unreplicated; Huey et al. 1991; Partridge et al. 1994b; James and Partridge 1995) as a result of faster larval development (Partridge et al. 1994b; James and Partridge 1995). These results suggest that thermal selection during the larval period may be important since, there appears to be strong directional selection for fast larval growth rate at a single temperature (Clarke et al. 1961; Robertson 1963; Sewell et al. 1975; Burnet et al. 1977; Partridge and Fowler 1993).

The pattern generated by thermal selection on body size and development time is surprising, since the correlation between adult body size and rate of larval development produced by thermal selection is the opposite to that produced by artificial selection at a single temperature on thorax length (Partridge and Fowler 1993), wing size (J. McCabe and L. Partridge, unpublished results), larval period (Nunney 1996) or preadult period (Zwaan et al. 1995a), where large adult size was associated with extended larval or pre-adult development.

1.6.3 Phenotypic plasticity in response to temperature

The fact that both the phenotypic plasticity of wing size in response to temperature and the response of wing size to laboratory thermal selection changed cell size in the same direction, suggests that the former could be adaptive (Gomulkiewicz and Kirkpatrick 1992). However, latitudinal variation (Coyne and Beecham 1987) and laboratory thermal selection (Anderson 1966; Partridge et al. 1994a) have not provided

evidence for thermal evolution in phenotypic plasticity of body size. This is surprising since phenotypic plasticity of body size in response to temperature has been shown to be heritable and to respond to artificial selection in *D. melanogaster* (Scheiner and Lyman 1989, 1991).

In contrast to plasticity of body size, phenotypic plasticity of development time in response to temperature has been shown to change as a result of evolution at constant temperatures, with high temperature populations showing greater plasticity (Huey et al. 1991; Partridge et al. 1994b; James and Partridge 1995), indicating both the presence of genetic variation for plasticity and natural selection for it.

1.6.4 Wing-thorax size ratio and wing shape

Genetically based clines have been shown for wing-thorax size ratio in *Drosophila*: the ratio was found to increase with latitude and altitude in *D. robusta* (Stalker and Carson 1947; Stalker and Carson 1948) and with latitude in *D. subobscura* (Misra and Reeve 1964). Thermal selection experiments on *D. melanogaster* have produced equivocal results on the response in wing-thorax size ratio: one replicated study showed that the wing-thorax size ratio increased in response to selection at cold temperatures (Cavicchi et al. 1989) while another found no evidence for any response in the trait (Partridge et al. 1994a).

Clinal variation in wing shape has not shown a consistent pattern in different species and studies. The length of the wing relative to its width (wing aspect ratio or relative wing length) increased with latitude but remained constant with altitude in *D. robusta* (Stalker and Carson 1947; Stalker and Carson 1948), increased with latitude in European *D. subobscura* in one study (Pegueroles et al. 1995) but did not change in another (Misra and Reeve 1964), increased with latitude and decreased with altitude in *D. pseudoobscura* (Sokoloff 1965), and did not change with latitude in *Drosophila melanogaster* (Long and Singh 1995) (these conclusions are based on reanalyses of indexes estimated from population means of comparable, but not identical, measurements). The only study that has studied wing shape explicitly, detected latitudinal differentiation among populations of *D. melanogaster* in the second principal component of 12 linear distance measurements in the wing, but this measure was not interpretable in terms of general wing shape (Imasheva et al. 1995). Wing shape can apparently respond to thermal selection in the laboratory in *D. melanogaster*, although the use of multivariate descriptors of wing shape, again, does not allow a direct interpretation of the changes (unreplicated short term evolution analysed by discriminant analysis, Cavicchi et al. 1978; replicated long term evolution analysed using discriminant, Fourier and centroid analyses, Cavicchi et al. 1989, 1991, but see Section 2.4.3).

1.6.5 Egg size

Temperature also seems to have an evolutionary effect on the egg size of ectotherms. For instance, insect populations from the colder parts of species' ranges lay larger eggs when kept at constant temperature in the laboratory (Ando 1983; Harvey 1983a; Blackenhorn and Fairbairn 1995). Berven (1982) showed a similar genetic cline in a frog by transplant experiments in the field. A possible interpretation of these data is that lower temperatures promote the evolution of larger eggs, either because larger eggs are more advantageous or because mothers are more able to afford large eggs in colder areas.

1.7 Outline of Thesis

My work has tried to take forward our understanding of some of the issues raised so far. The outcome of thermal selection experiments predicts unambiguously that the cellular basis of latitudinal variation in wing size should be cell size. This pattern was not met in the only study of non-clinal variation among natural populations of *D. melanogaster*, which differed mainly in cell number (Robertson 1959a). An important role of cell number has also been discovered in differentiation of wing, leg and eye sizes among Hawaiian species of *Drosophila* (Stevenson et al. 1995; but see Section 8.4). In Chapter 3 I investigated for the first time the cellular basis of a cline in body size of Australian populations of *D. melanogaster*.

The phenotypic plasticity of body size in response to temperature could respond to thermal selection in nature, since increased plasticity may be advantageous in more variable and more extreme thermal environments. The thermal reaction norms of 6 geographic lines of *D. melanogaster* were examined in Chapter 4. I also compared variation in wing size and its cellular basis between wild-caught and laboratory reared flies from the cline. Since phenotypic plasticity in response to temperature is expected to reinforce the effect of thermal selection in nature, such a comparison can inform us about the importance of temperature in causing phenotypic variation in wing size along the cline. The cellular basis of wing size in field collected flies can give further evidence on this since the effects of temperature and larval density or nutrition have been shown to be qualitatively different in laboratory studies.

Apart from body size, the relative sizes of the wing and thorax, and wing shape have been identified as possible targets of thermal selection (Sections 1.5.2 and 1.6.4). In Chapter 5 I have looked for evidence on this possibility in wild caught and laboratory reared geographic populations. The alternative explanation, that wing-thorax size ratio and wing shape evolved as correlated responses to thermal selection on wing size has also

been examined by looking at the correlated responses to artificial selection on wing size in the laboratory.

In Chapters 6 and 7 I studied the possible role of egg size in the evolution of *Drosophila* life history traits. In Chapter 6 the environmental and evolutionary responses of egg size to temperature were investigated. This was done by examining latitudinal differentiation in two geographic collections from different continents. To establish the role of temperature I also investigated the response of egg size to laboratory thermal selection. In Chapter 7 I studied the maternal effects of egg size on offspring life history traits, controlling for offspring genotype, in order to understand the direct consequences of egg size in causing variation in life history characters.

2

General Materials and Methods

2.1 Populations of *Drosophila melanogaster*

2.1.1 Australian populations

L. Partridge and A. James collected flies at 20 sites on a 2600km transect along the eastern coast of Australia during February of 1993 (Table 2.1; Figure 2.1). Populations were established in cages with 750 males and 750 females from 30 isofemale lines from each population, and kept at 16.5°C for one year, and at 18°C subsequently.

Temperature data from weather stations (mean wet bulb and dry bulb temperatures, at 9:00 and 15:00, and daily minimum and maximum temperatures, yearly or only during the approximate flying season November-March (from Gentili 1971)) were highly correlated with latitude: product-moment correlations $r>0.95$ (Table 2.1).

When reared in standard conditions in the laboratory larvae from high latitude populations developed faster and showed a larger adult thorax length than those from low latitude populations (James and Partridge 1995; James et al. 1995; A. C. James, R. B. R. Azevedo and L. Partridge, unpublished manuscript).

2.1.2 South American populations

One population from Ecuador and nine populations from Chile were collected by J. Van't Land and P. Van Putten (University of Groningen) in 1995, and kept in bottles at 25°C and moderate densities (Table 2.2; Figure 2.2).

Temperature data from weather stations (mean annual temperature, and mean daily minimum and maximum temperatures (J. Van't Land and P. Van Putten, personal communication)) were highly correlated with latitude: product-moment correlations, $r>0.94$ (Table 2.2).

When reared in standard conditions in the laboratory, flies from high latitude populations showed larger wing size than those from low latitude populations, but there was no latitudinal differentiation in preadult development time (Van't Land et al. 1995; J. Van't Land, P. Van Putten, B. Zwaan, H. Villarroel, A. Kamping and W. Van Delden, unpublished results). The cellular basis of the cline in wing size is not known but is currently being investigated (B. Zwaan, personal communication).

Table 2.1 Geographic and climatic characteristics of Australian populations. Annual mean daily maximum and daily minimum temperatures and rainfall are longterm averages for weather stations close to the collection sites (Gentilli 1971).

Lines	Collection Site	Latitude (S)	Longitude (E)	Altitude (m)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)
MO & IN	Innisfail, Queensland	16° 53'	145°45'	< 50	28.8	20.6	2032
BS & EP	Bowen, Queensland	20° 01'	148°15'	< 50	28.5	20.5	1015
KL & LH	Yeppoon, Queensland	23° 08'	150°45'	< 50	25.7	17.7	1342
AG & GL	Hervey Bay, Queensland	25° 33'	152°41'	< 50	26.6	15.0	1185
BH & DG	Northern New South Wales	27° 57'	153°24'	< 50	25.1	15.2	1446
CH & CI	Coffs Harbour, N. S. W.	30° 19'	153°07'	< 50	23.2	13.7	1708
CO	Taree, N. S. W.	31° 54'	152°29'	< 50	24.3	11.9	1178
TY	Hunter Valley, N. S. W.	32° 42'	151°28'	< 50	24.1	10.4	809
CS & PF	Cobram, Victoria	35° 49'	145°34'	110	23.2	9.6	450
ME	Melbourne, Victoria	37° 41'	145°32'	130	19.9	8.2	1020
HS	Melbourne, Victoria	38° 14'	145°02'	< 50	19.3	10.6	735
FT	Forth, Tasmania	41° 11'	146°22'	< 50	16.8	8.0	955
RN	Ranelagh, Tasmania	42° 53'	147°20'	55	16.8	8.3	628

Table 2.2 Geographic and climatic characteristics of South American populations. Annual mean daily maximum and daily minimum temperatures and rainfall are data from 1992 for weather stations adjacent to the collection sites (J. Van't Land and P. Van Putten, personal communication).

Line	Collection Site	Latitude (S)	Longitude (W)	Altitude (m)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)
GU	Guayaquil, Ecuador	2°13'	79°54'	< 50	30.7	20.4	950
AR	Arica, Chile	18°28'	70°19'	< 50	22.7	17.7	0.5
IQ	Iquique, Chile	20°13'	70°10'	< 50	23.1	16.3	0.6
AN	Antofagasta, Chile	23°38'	70°24'	< 50	20.3	17.1	1.7
CO	Copiapó, Chile	27°20'	70°21'	350	?	?	12.0
CQ	Coquimbo, Chile	29°56'	71°24'	< 50	18.0	11.0	78.5
VA	Valparaíso, Chile	33°05'	71°40'	< 50	18.9	10.6	373
LI	Linares, Chile	35°48'	71°36'	140	19.9	6.7	967
VD	Valdivia, Chile	39°48'	73°14'	< 50	16.4	6.5	1871
PM	Puerto Montt, Chile	41°30'	72°50'	< 50	14.5	5.9	1803

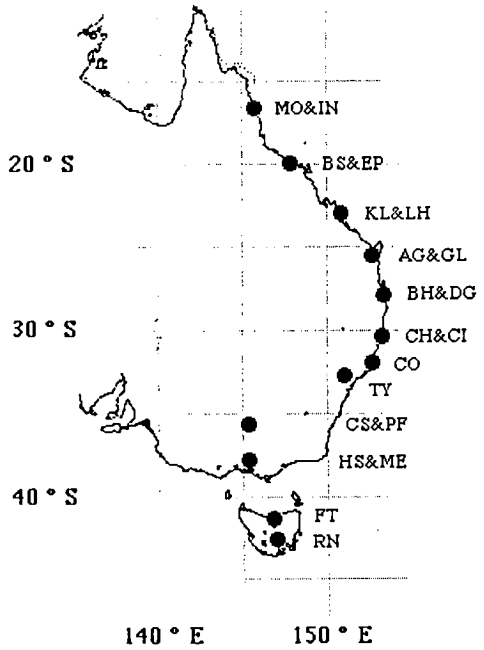

Figure 2.1 Map of eastern Australia and location of sampled populations.

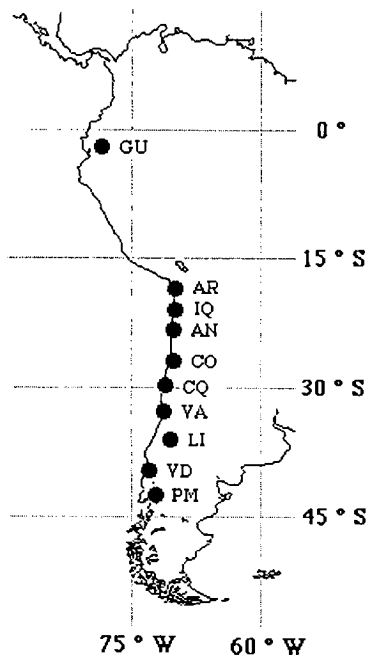

Figure 2.2 Map of western South America with location of sampled populations.

2.1.3 Thermal selection lines

The base stock was established from adults collected in the Brighton (UK) fruit market in 1984, and was maintained as a large random bred stock with overlapping generations in a population cage at 25°C. Six months later, the stock was subdivided to found two temperature groups (16.5°C and 25°C), each with 3 replicate populations. In 1991, each population of the 25°C regime was subdivided in order to found one replicate population at 29°C. The populations kept at 16.5°C evolved faster preadult development and larger adult body size than those kept at 25°C (Huey et al. 1991; Partridge et al. 1994a, 1994b; James and Partridge 1995). The populations evolving at 29°C did not diverge significantly in development time or adult body size after 3 years of selection (James and Partridge 1995; R. B. R. Azevedo, V. French and L. Partridge, unpublished results).

2.1.4 Lines selected for wing area

Unreplicated large, small and control lines, were continuously selected by B. Zwaan for wing area at 25°C. Each selection line was founded with 40 females and 40 males, randomly chosen from 48 isofemale lines collected in North Carolina in 1994. Eggs were then collected from each line in bottles at a moderate density (~400 larvae/bottle) (Section 2.2.4). The adults emerging from each bottle were collected as virgins, and 100 females and 100 males per line were randomly chosen for wing area measurements (Section 2.3.1). In the large line, the 40 females and 40 males showing the highest wing area were chosen to give rise to the following generation, following the procedure just outlined. In the small line the smallest 40 females and 40 males were selected and in the control line a random sample of 40 females and 40 males was used. After generation 9, each generation was produced by collecting eggs from the selected adults and using them to set up a standard density culture of one bottle with 350 larvae per line (Section 2.2.5). The large and small lines have diverged significantly in wing size, as a result of both cell size and cell number (B. Zwaan, personal communication).

2.1.5 Lines selected for cell number

Three replicate large, small and control lines were artificially selected by J. McCabe for wing area keeping cell area constant. Each line was founded with 10 females and 10 males randomly chosen from a random-bred stock collected in Dahomey (Benin) in 1970 and maintained since in population cage culture at 25°C. Eggs were then collected from each line and used to set up standard density cultures of one bottle with 100 larvae per line (see Section 2.2.5). The first 25 adults of each sex to emerge from

each bottle were collected as virgins. Wing area and cell area were measured in the left wing of each fly (Section 2.3). In the large and small regimes, flies were selected for increased and decreased wing size, respectively, while minimizing the selection differential in cell area relative to the control lines. From each line, 10 females and 10 males were selected to produce the following generation, using the procedure just outlined (Section 2.2.5). The lines were selected for 8 generations at 25°C, and on alternate generations at 18°C subsequently. The large and small selection regimes have diverged significantly from the controls, and from each other, in cell number (and wing size) but not in cell size (McCabe et al., in press).

2.2 Rearing Methods

2.2.1 Culture media

Standard food.—Medium used for maintaining populations and rearing experimental animals in bottles or vials. Made by adding 85g sugar, 60g maize meal, 20g dried yeast, 10g agar and 2.5g Nipagin to 1L water.

Agar and yeast.—Sterile agar medium is used as substrate in small vials, a specified amount of yeast in suspension is added. The agar medium is prepared by adding 5g agar to 1L water (Neat et al. 1995).

Grape juice medium.—Substrate used for collecting eggs from flies in laying pots or from cages in petri dishes, to collect first instar larvae from, and to display eggs for measurement. Prepared by adding 50g agar to 1L water and 0.6L grape juice.

2.2.2 Containers

Population cage.—Transparent plastic box (20cm wide x 20cm high x 30cm deep) with a round hole (11cm diameter) in the front covered with a silk stocking with the end cut-open, through which other containers can be moved.

Bottle.—Half-pint glass milk bottle for ~50ml medium, covered with cotton wool.

Vial.—Glass cylindrical vial (7cm high x 2.5cm diameter) for ~8ml medium, covered with cotton wool.

Small vial.—Glass cylindrical vial (5cm high x 1cm diameter) for ~1ml medium, covered with cotton wool.

Laying pot.—Transparent plastic cylinder (6cm high x 4cm diameter), covered with a plastic lid on one side for ~10ml medium, and with a 1cm hole drilled on the other side, covered with cotton wool in order to let air into the pot.

Petri dish.—Plastic dish (1cm high x 9cm diameter) for ~30ml of medium.

2.2.3 Stock maintenance

Cage populations.—Run with continuous generations on a 4-week cycle at 25°C or a 6-week cycle at 16.5°C or 18°C (e.g. Sections 2.1.1 and 2.1.3). Once a week 3 bottles containing fresh standard medium are introduced into the cage, and the 3 bottles which have been in the cage for 4 (or 6) weeks are removed.

Bottle populations.—Kept in discrete generations in bottles (e.g. Sections 2.1.2, 2.1.4 and 2.1.5), where adults of a specified age are allowed to found the next generation (Section 2.2.4).

2.2.4 Egg collections

Samples from population cages.—Eggs can be collected from population cages in bottles with food medium or in petri dishes with grape juice medium. To attract the females to the surface of the food, the medium is supplemented with a dab of live-yeast paste.

Laying pots.—Eggs can also be collected from flies maintained in vials or bottles, outside cages. Flies are transferred to laying pots with grape juice grape juice medium to which a dab of yeast paste was added to encourage egg laying. A period of acclimation is given to the flies. Before egg collection, flies are allowed to lay retained eggs (for ~1 hour at 25°C). Then eggs are collected (for ~2 hours at 25°C).

2.2.5 Experimental designs

Standard design.—The adult flies used in my experiments were usually reared for two generations in controlled conditions outside their source population, in order to obtain individuals of known age, reduced phenotypic variability and not expressing maternal environmental effects. To produce the first generation, a moderate number of eggs (~200) is collected from population cages over several hours (e.g. 6 hours at 25°C or 12 hours at 18°C). The eggs are then incubated at the desired temperature. Flies emerging from bottle or vial cultures are transferred to laying pots. Eggs are collected when the adults reach sexual maturity (Section 2.2.4). Upon hatching, first instar larvae are transferred at a constant density into vials or bottles with food medium using a trimmed paintbrush. These larvae give rise to the second generation.

Reduced design.—In some experiments (e.g. Chapter 7), a simplified version of the standard design was employed whereby the eggs collected from the source population were used to setup the standard density cultures.

2.2.6 Storage

Adult flies to be measured were collected within 36 hours of emergence into centrifuge tubes and frozen at –20°C.

2.2.7 Photoperiod

All flies were reared and kept under a 12h light : 12h dark cycle.

2.3 Measurements

2.3.1 Wing area

Wings to be measured were removed from hardened adult flies, fixed in propanol and mounted in Aquamount on a microscope slide. The areas of the mounted wings were measured (in mm^2) at x50 magnification using a *camera lucida* attached to a dissecting microscope and graphics tablet connected to a computer, by tracing their outlines starting at the humeral-coastal break (~0.4% resolution).

2.3.2 Cell area

The trichomes (Section 1.3) in a standard $0.01mm^2$ area of the same wings (in the posterior medial cell, equidistant from the 4th longitudinal vein, the posterior cross vein and the 5th longitudinal vein) were individually marked on a piece of paper, using a compound microscope at x400 magnification with a *camera lucida* attachment, and counted. The average cell area of a wing was estimated by dividing $0.01mm^2$ by the trichome count (~1.5% resolution).

2.3.3 Cell number

An index of the total number of cells in the wing was calculated by dividing the area of the wing by the average cell area. Although cell size varies throughout the wing, wing area is known to be determined by concordant cell size differences among distinct regions (Delcour and Lints 1966; Partridge et al. 1994a), so using an index of total cell number based on one region is legitimate.

2.3.4 Egg volume

The eggs were chosen at random for measurement, turned on their side on the surface of the grape juice medium and cleaned with a wet trimmed paint brush. In the thermal selection lines (Chapter 6), the length and height of each egg was measured with an eye piece graticule under a dissection microscope at x50 magnification. In all other experiments (Chapters 6 and 7), similar measurements were made using a dissection

microscope at x50 magnification with a *camera lucida* attachment and a graphics tablet connected to a computer. Egg volume (in mm^3) for each egg was approximated, assuming that it is a regular ellipsoid, using the formula (~5% resolution):

$$Volume = \frac{\pi}{6} \cdot (Length) \cdot (Height)^2$$

2.3.5 Body weight

Fresh or dried flies were weighed to the nearest 0.002mg using a Sartorius M500p balance.

2.4 Statistical Analyses

2.4.1 Linear models

All the data were analysed by fitting linear models. For each character (except proportions) I started by fitting a full model (e.g. a factorial model including the effects of Sex, Population and Experimental Temperature and all possible interactions). The heterogeneity of variances among all possible groups determined by the model was then assessed by the tests devised by O'Brien, Brown-Forsythe and Levene. Normality of the standardised residuals from the model was also tested by the Shapiro-Wilk *W* test. If the assumptions of the normal error distribution were generally met by the data, analyses were continued on the raw variable. If the assumptions were violated and were not obviously caused by a few outliers I applied the Box-Cox method to the model in order to find an appropriate transformation for the response variable. If simple transformation were not effective in making the data fit the assumptions of a normal error distribution, other error structures were tried (e.g. Sections 6.4.1 and 7.3.4).

Proportions (Section 7.3.4) were analysed by generalised linear models with a binomial error distribution and logit link (Crawley 1993). If overdispersion was apparent in the minimal adequate model (i.e. if the residual deviance was very different from the residual df) a correction was done by scaling (Crawley 1993).

2.4.2 Latitudinal clines

Latitudinal trends among geographic lines were tested by linear models including latitude as a continuous predictor variable. When variation within lines was considered, latitudinal trends were tested against the term for deviations from the model (e.g. Section 6.4.1). This method does not involve pseudoreplication, since it is statistically equivalent to fitting the model on population means.

In some cases, multiple regression analyses were done, including latitude and main effects such as sex (e.g. Section 4.3.3). In these instances, latitude was centred (i.e. mean latitude was subtracted from the latitude of each population) before analyses; this ensured meaningful comparisons between the intercepts in case of significant higher-order interactions involving latitude.

2.4.3 Selection responses

Divergence among replicated selection regimes was assessed following the method proposed by Lande (1977), i.e. the differences among selection regimes were tested against the variation among lines nested within the selection regime (e.g. Section 6.4.2). Another approach has been used repeatedly in the thermal selection literature whereby divergence among selection regimes is tested against the pooled within replicate variance (e.g. Anderson 1966, 1973; Cavicchi et al. 1985, 1989, 1991). However, this design does not distinguish between the effects of selection and random genetic drift and generates spurious significance tests due to pseudoreplication (Crawley 1993, pp. 56-57). For example, Anderson (1966) studied the differentiation in body size and preadult development time between 2 populations selected at 16°C and 4 populations selected at 25°C and 27°C, after 6 years of selection, but did not use a nested design for the contrasts. As a consequence he erroneously inferred significant divergence in development time and body weight, and grossly overestimated the significance of the actual divergence in wing length (Table 2.3).

Table 2.3 Comparison between pseudoreplicated and nested designs in the analyses of selection responses. Entries are *P*-values listed by Anderson (1963, Table 3) and those obtained applying a nested design to the same data set.

			Statistical Design	
Character	Sex	Temperature	Pseudoreplicated	Nested
Wing length	females	16°C	<0.001	0.02
Wing length	females	19°C	<0.001	0.03
Wing length	males	19°C	<0.001	0.03
Wing length	females	25°C	<0.001	0.11
Body weight	females	25°C	<0.05	0.07
Body weight	males	25°C	<0.05	0.06
Development time	females	19°C	<0.05	0.32
Development time	males	19°C	<0.05	0.19

The patterns observed by Cavicchi and colleagues (1985, 1989, 1991) are more difficult to evaluate since the only the second study listed the means for each replicate line, and the designs of the analyses were never explained. The divergence in wing length, thorax length and head width reported by Cavicchi and colleagues (1989, p. 240) ($P<0.001$, $P<0.01$ and $P<0.05$, respectively) did not differ markedly from that obtained using a nested design ($P=0.011$, $P<0.01$ and $P<0.01$, respectively), even when the only cold-selected replicate was excluded from the analyses (since the other replicate at that temperature was lost) ($P=0.01$ for all three characters).

2.4.4 Phenotypic plasticity

In phenotypic plasticity experiments it is common to observe among-environment heteroscedasticity, i.e. heterogeneity in genotypic variances among environments (references in Dutilleul and Potvin 1995). This situation violates the assumptions of the analysis of variance or regression which are convenient methods to analyse such experiments.

Dutilleul and Potvin (1995) have recently proposed a method for eliminating among-environment heterogeneity of variances. In a standard phenotypic plasticity experiment, a genotype i ($i=1,...,g$) in an environment j ($j=1,...,n$) will have a mean phenotypic value p_{ij}. Let p_j denote the mean of all genotypic means at environment j and r_{ij} the mean residual deviation of genotype i in environment j (i.e. $p_{ij} - p_j$); then the mean phenotypic value can be expressed as:

$$p_{ij} = p_j + r_{ij}$$

If σ_j^2 is the variance among genotypic means at environment j then a suitable transformation of each mean phenotypic value will be:

$$p'_{ij} = p_j + r_{ij} \cdot \sqrt{\frac{\sqrt[n]{\prod_{j=1}^{n} \sigma_j^2}}{\sigma_j^2}}$$

This consists in dividing the residuals r_{ij} by the standard deviation at environment j (Dutilleul and Potvin 1995, equation 6) and then scaling this ratio by the square-root of the geometric mean of the variances of all environments (P. Dutilleul, personal communication). This procedure transforms the data, eliminating the heterogeneity of variances across environments, but retains the relationships between genotypes within environments (the "primary evolutionary information", Dutilleul and Potvin 1995). Comparing the analyses of raw and transformed data will reveal the extent to which variation in phenotypic plasticity (gene-environment interaction) is confounded with among-environment heteroscedasticity (Dutilleul and Potvin 1995) (e.g. Section 4.3.3).

2.4.5 Cellular basis of wing size

To investigate the cellular basis of variation in wing area I followed a regression approach developed by Robertson (1959) and Stevenson and colleagues (1995). If cell area determines all the variation in wing area then the regression of *log (√cell area)* on *log (√wing area)* will have a slope of one; reciprocally, if the wing area variation is completely based on cell number then that slope should be zero. Therefore, the slope of the regression of *log (√cell area)* on *log (√wing area)* estimates the relative contribution of cell area to changes in wing area. This procedure underestimates the contribution of the main cellular component, since log-transformation reduces the variance of large values of the response variable.

2.4.6 Statistical software

Generalised linear models with binomial errors were fitted using GLMStat version 1.6 for the Macintosh written by K. J. Beath. Descriptive statistics and all other analyses were obtained with the JMP package version 3.1 for the Macintosh (SAS 1994).

3

Cellular Basis of a Size Cline in *Drosophila melanogaster*

(Part of A. C. James, R. B. R. Azevedo and L. Partridge. 1995. Genetics 140, 659-666)

3.1 Summary

I examined twenty *Drosophila melanogaster* populations collected from a 2600 km north-south transect in Australia. In laboratory culture at constant temperature (18°C) and standard larval density, a genetic cline in wing area was found, increasing with latitude. The cline was based on clines in both cell size and cell number, but was primarily determined by changes in cell number. These results are discussed in the context of selection processes operating in natural and experimental populations.

3.2 Introduction

Low temperatures during development increase wing size and this effect is mediated mostly by cell size (Section 1.5.1). Low temperatures in the field and in the laboratory also seem to cause an evolutionary increase in wing size. The cellular basis of the response to thermal selection was based on cell size (Section 1.6.1), but this result has not been verified for geographic variation.

In this study I investigated latitudinal differentiation in wing area in Australian populations *D. melanogaster* in standard conditions in the laboratory. I examined the relative importance of cell size and cell number in causing variation in wing area. If the latitudinal variation in life histories results entirely from thermal selection, the cline in wing size is expected to be based on variation in cell size, as was found for laboratory thermal lines (Section 1.6).

3.3 Materials and Methods

I used the 20 Australian geographic lines (Section 2.1.1). This experiment was conducted at 18°C approximately 9 months after the flies were collected. Adults were reared using the standard design (Section 2.2.5) in 30 larvae per vial and 12 vials per population. Wing area, cell area and cell number were measured in 4 flies from each sex per vial (Sections 2.3.1-2.3.3).

The means of all traits for each sex were calculated per vial in both experiments. The vial means were used to calculate the mean and 95% confidence limits for flies from each population.

To investigate the sources of variation in each character, I performed two-way nested analyses of variance with sex and population as crossed fixed effects and vial as a random effect nested within population. Multiple regression analyses with sex as a main effect and latitude as a continuous variable were done in order to test for the existence of significant clines. To compare the strength of the clines for the different wing size traits in each sex, I divided each dependent variable by its mean and estimated the scaled slopes.

To investigate whether the latitudinal variation in wing size was mostly explained by variation in cell size or cell number I did a partial correlation analysis and a regression analysis (Section 2.4.5). The cellular basis of the variation in wing area within populations was investigated by a regression analysis (Section 2.4.5) on the residuals from a one-way analysis of variance among lines on vial means of *log (√cell area)* and *log (√wing area)*, i.e. after removing the differences among populations.

3.4 Results

All characters showed significant variation among sexes, lines and vials within lines ($P<0.001$). A significant sex by line interaction was found for wing area ($F_{[19,220]}=4.39$, $P<0.001$), and significant sex by vial interactions were found for cell area ($F_{[219,1333]}=1.70$, $P<0.001$) and cell number ($F_{[219,1333]}=3.52$, $P<0.001$).

Significant linear regressions with latitude were found for every character (Figure 3.1; Table 3.1). For all characters the intercepts for the regressions with latitude were different between the sexes, but the slopes did not differ significantly between the sexes (Figure 3.1; Table 3.1). However, in cell area, the regression was significant in males ($F_{[1,18]}=5.46$, $P=0.03$) but not in females ($F_{[1,18]}=3.39$, $P=0.08$). Significant deviations from linearity were found in the regressions of wing area on latitude (females: $F_{[11,7]}=7.37$, $P<0.01$; males: $F_{[11,7]}=3.93$, $P=0.04$), with size increasing more rapidly at

higher latitudes. Cell size and number showed non-significant deviations from linearity ($P>0.3$). The addition of a quadratic term to the regression models significantly increased the proportion of residual sum of squares explained by the models for wing area (females: $F_{[11,7]}=24.2$, $P<0.001$; males: $F_{[11,7]}=21.2$, $P<0.001$).

Comparison of the scaled slopes showed that, in both sexes, the clines in wing area and cell number were steeper than that in cell area (Table 3.2). There was a highly significant correlation between wing area and latitude (females: $r=0.801$, $P<0.001$; males: $r=0.794$, $P<0.001$). When cell area was held constant, the partial correlation of wing area on latitude was still high (females: $r=0.759$, $P<0.001$; males: $r=0.720$, $P<0.001$), but when cell number variation was removed it was significant only in males (females: $r=0.364$, $P>0.1$; males: $r=0.482$, $P=0.04$). Comparison of scaled slopes and correlation analysis showed that the latitudinal cline in wing size was determined mainly by variation in cell number. Furthermore, regression analyses (Section 2.4.5) showed that variation in wing size among lines (including the latitudinal cline as well as the deviations from it) was mostly based on cell number: the estimated contribution of cell size to variation in wing size (mean and 95% confidence limits) was 16.3±12.4% in females and 20.7±13.5% in males.

Within populations, both cell size and number contributed significantly to variation in wing size, with a larger contribution of cell number: the estimated contribution of cell size to variation in wing size (mean and 95% confidence limits) was 36.4±8.4% in females and 32.9±8.0% in males.

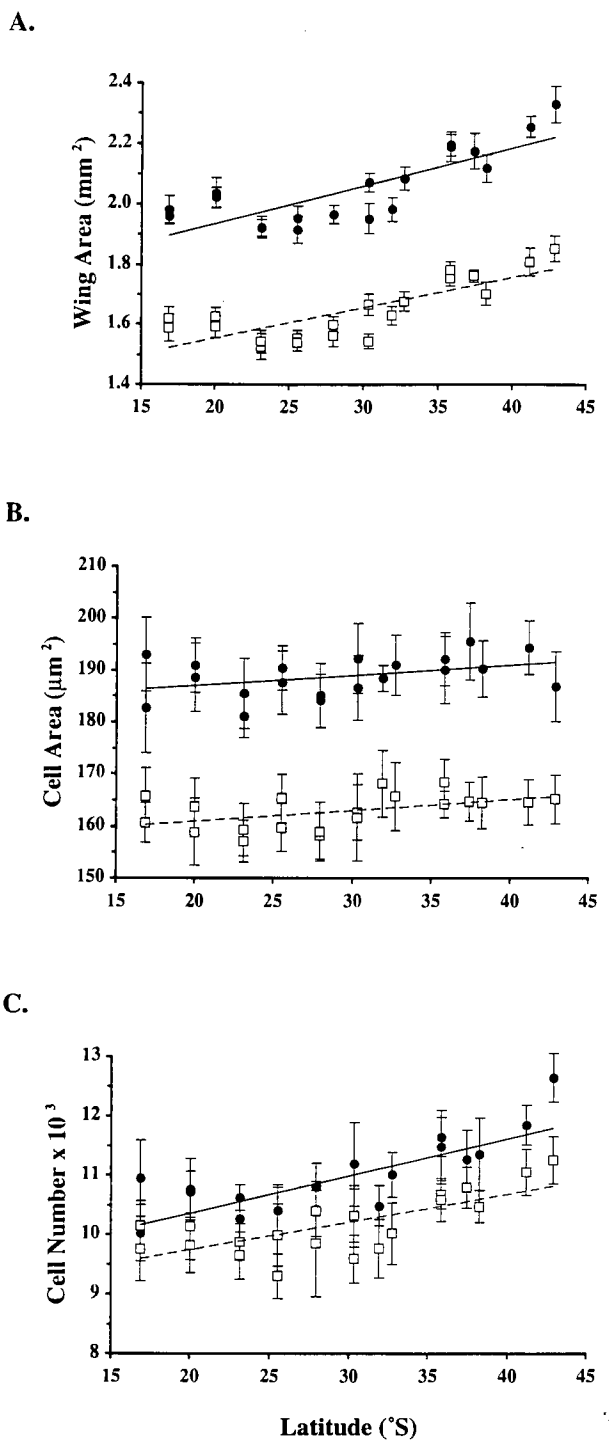

Figure 3.1 Latitudinal clines of wing size characters. Values are line means and 95% confidence intervals. (A) Wing area. (B) Cell area. (C) Cell number. (● females; □ males.)

Table 3.1 Multiple regression analyses on wing characters, with sex as a main effect and latitude as a continuous variable.

Source of variation	df	MS	*F*
Wing area			
Sex	1	1.624	343.07 ***
Regression with latitude†	1	0.300	63.14 ***
Deviations from regression	37	0.005	
Differences between slopes‡	1	0.003	< 1
Deviations within slopes	36	0.005	
Cell area			
Sex	1	6774.33	698.28 ***
Regression with latitude†	1	106.44	10.97 **
Deviations from regression	37	9.70	
Differences between slopes‡	1	0.42	< 1
Deviations within slopes	36	9.96	
Cell number			
Sex	1	5.66	36.69 ***
Regression with latitude†	1	6.98	45.22 ***
Deviations from regression	37	0.15	
Differences between slopes‡	1	0.10	< 1
Deviations within slopes	36	0.16	

† Tested against MS for Deviations from regression. ‡ Tested against MS for Deviations within slopes. ** P<0.01; *** P<0.001.

Table 3.2 Mean response and scaled slope from regression with latitude (and 95% confidence limits) for each character.

Character	Mean	Slope / Mean
Females		
Wing Area (mm^2)	2.05	0.609 ± 0.255
Cell Area (μm^2)	189	0.105 ± 0.117
Cell Number x 10^3	10.9	0.575 ± 0.238
Males		
Wing Area (mm^2)	1.65	0.618 ± 0.234
Cell Area (μm^2)	163	0.127 ± 0.114
Cell Number x 10^3	10.2	0.466 ± 0.222

3.5 Discussion

I have found a genetic cline in wing area of *D. melanogaster*: flies from higher latitudes developed larger wings in experiments where temperature and larval density were controlled. This result is consistent with previous studies of genetic latitudinal clines of body size in ectotherms (Section 1.6.1). The repeated occurrence of these body size clines raises two important issues: what is the selective agent responsible and what is the target of selection?

Temperature decreased with latitude along the transect of collection. The evolution of larger body size in cooler climates was accompanied by an increase in cell size and larval development rate (James and Partridge 1995). Laboratory thermal selection produced a similar pattern in these characters (Section 1.4.2), suggesting that temperature, or a causally associated variable, is indeed the relevant selective agent in nature. Latitudinal variation in temperature is associated with variation in other physical and biological factors and any of these could act as the proximate selective agent. However, the similarity between laboratory and natural populations in effects on body size and development time suggests that the proximate selective agent is the same in both.

The regression of wing size with latitude was not linear. Body size varied little between populations at lower, warmer latitudes, and the slope increased in higher, and cooler latitudes. The shape of the clines may be caused by asymmetrical gene flow between populations along the transect. The productivity of *Drosophila* populations increases with temperature (Birch et al. 1963; Partridge et al. 1995), which could result in higher emigration rates from populations in warmer climates thus reducing the magnitude of genetic differentiation between them.

Latitudinal variation in wing area was explained by clines in both cell size and cell number, with the latter having the predominant effect. This result could imply that natural selection in the field acts directly on adult body size since, in artificial selection experiments at a single temperature, cell number is the basis of additive genetic variation for body size within populations (Section 1.6.1). However, my observations on natural populations were not concordant with the results of laboratory thermal selection, in which divergence in wing area was exclusively a consequence of changes in cell size

(Cavicchi et al. 1985; Partridge et al. 1994a). Another difference between the results from laboratory and natural populations was that in thermally selected lines the wing size variation within populations was predominantly based on cell number (L. Partridge, B. Barrie, K. Fowler and V. French, unpublished results), while my results showed both cell size and number were involved. The ecological and genetic conditions in the laboratory and in the field are not strictly comparable. In particular, constant temperatures, absence of gene flow and limited time of evolution in laboratory thermal selection could account for the differences in the results.

4

Phenotypic Plasticity of Wing Size in Response to Temperature in Geographic Populations of *Drosophila melanogaster*

(Part of A. C. James, R. B. R. Azevedo and L. Partridge. Genetics, in press)

4.1 Summary

Phenotypic plasticity in response to temperature for wing size, cell size and cell number in geographic lines of *Drosophila melanogaster* was examined, in order to understand the role of phenotypic plasticity in the expression of clinal variation in nature. All characters were plastic in response to temperature but did not show geographic variation in plasticity. The developmental effect of temperature on wing size was due to changes in cell size, whereas latitudinal variation in wing size was mostly based on cell number. Wild caught flies were smaller and latitudinal trends of size were stronger than when tested under standard rearing conditions. The reduction in wing size was caused by fewer cells while the steeper cline was produced by greater latitudinal variation in cell area. My results suggest that flies in nature experienced reduced nutrition, possibly caused by larval crowding, and a latitudinal gradient of decreasing developmental temperatures.

4.2 Introduction

I have examined phenotypic plasticity in response to temperature in a latitudinal cline for wing size in *Drosophila melanogaster*. My aim was to understand the evolution of plasticity in the cline, and the role of plasticity in body size in the phenotypic expression of the cline in nature.

It has not been established whether plasticity of body size in response to temperature is adaptive (Section 1.6.3). Phenotypic plasticity of body size in response to temperature in *D. melanogaster* did not respond to thermal selection in the laboratory (Partridge et al. 1994a) or diverge along a latitudinal cline (Coyne and Beecham 1987).

In the present study I took 6 populations collected at high, intermediate and low latitudes in eastern Australia, and examined their phenotypic plasticity in response to temperature for wing area. I also investigated the role of cell size and cell number in producing genetic and environmental and gene-environment interaction variation in wing size.

The relative contributions of genetic variation, environmental variation and gene-environment interaction to the expression of phenotypic clines in nature has rarely been examined. A study of latitudinal variation in wing length in Coyne and Beecham (1987) found a steeper clines for wing length in *D. melanogaster* reared in the laboratory at three different temperatures than in the flies collected from nature. This finding was interpreted as meaning that environmental and genetic effects of latitudinal variation in temperature on body size were severely confounded by other sources of environmental variation. However, those populations were collected in different years and seasons, which could inflate the environmental heterogeneity that was present in the cline at a single time. The lines used in my study were all collected in the same month of a single year (Section 2.1.1). I compared the steepness of the clines wing size characters for field-collected adults from the cline in eastern Australia and measured in the present study with that of flies reared under standard conditions in the laboratory (Chapter 3).

Phenotypic plasticity of wing size in response to different environmental factors can involve different cellular mechanisms. In particular, the response of wing size to temperature has been shown to involve mostly changes in cell size (Section 1.5.1) in contrast to the response to variation in larval crowding or nutrition, which affected both cellular components but mostly cell number (Robertson 1959b). In the present study I compared the patterns of clinal variation in cell size and cell number in wild caught flies with those of flies from the same populations reared under standard conditions. My aim was to determine the role of environmental variation in general and of variation in temperature in particular in the phenotypic expression of the size cline in nature.

4.3 Materials and Methods

4.3.1 Phenotypic plasticity in response to temperature

This experiment was conducted approximately 2 years after the flies were collected on a subset of two replicate Australian lines from each of three latitudinal regions: (1) tropical Queensland (MO and IN), (2) intermediate latitudes (GL and BH) and (3) temperate Tasmania (FT and RN) (Section 2.1.1; Table 2.1). These sites were chosen since they covered the full latitudinal range and were representative of the geographic differentiation for larval development time and body size (James and Partridge 1995; James et al. 1995; Chapter 3).

Flies from each line were reared for two generations at 14°C, 18°C, 21°C, 25°C, and 29°C, temperatures which span the range of viability and fertility of this species (David et al. 1983). Following the standard experimental design (Section 2.2.5), I produced 20 replicate vial cultures with 30 larvae in each, for each line at each temperature. I measured wing and cell area in 4 randomly selected pairs from each replicate vial (Sections 2.3.1-2.3.3).

4.3.2 Geographic variation in field-collected flies

Flies collected at 19 of the field sites (all except FT) were preserved in alcohol (Section 2.1.1). Field collected males from one site (IN) were lost. Wing area, cell area, and cell number were measured on 10 individuals of each sex (Sections 2.3.1-2.3.3). These data are compared to the data for flies reared under standard laboratory conditions 9 months after collection (Chapter 3).

4.3.3 Statistical analyses

Wing area, cell area and cell number were linearised by taking their square-root. The patterns of geographic variation in phenotypic plasticity in response to temperature were investigated separately for each sex by repeated measures analyses of variance on line means at each environment. Temperature and latitude were considered as crossed fixed effects and site as a random effect nested within latitude (Section 2.4.3). Variances among experimental temperatures were significantly heterogeneous for cell size in males (Levene's test: $P<0.025$). The among environment heteroscedasticity was eliminated for all traits by transformation following Dutilleul and Potvin (1995) (Section 2.4.4). The analyses on transformed and untransformed produced qualitatively different results for cell size in males but not in the other (homoscedastic) cases. The residuals from the analyses of variance for all transformed traits were normally distributed ($P>0.05$).

I investigated the cellular basis of plasticity in wing size with temperature and of among line variation in wing area, by analyses of covariance on $log\ (\sqrt{cell\ area})$ with main effects of line and temperature, respectively, and $log\ (\sqrt{wing\ area})$ as the covariate, using the line means at each temperature (Section 2.4.5).

To compare latitudinal variation wing size characters between field caught and laboratory reared flies, I did multiple regressions with centred latitude as a continuous variable and rearing environment (field collected vs. laboratory raised) and sex as fixed main effects (Section 2.4.2). Laboratory data corresponding to those samples for which there were no field data were excluded from the analysis. To test for the possibility of laboratory evolution in the lines, I did a repeated measures analysis of variance for each trait on the line means reared in the laboratory at 18°C after 9 months and 2 years,

separately for each sex. Time and latitude were considered as crossed fixed effects and line as a random effect nested within latitude.

4.4 Results

4.4.1 Phenotypic plasticity in response to temperature

All wing size characters decreased with temperature during development (Figure 4.1; Table 4.1). There were non-linear effects of latitude on wing area in both sexes, and concordant effects on cell number, but not on cell area (Table 4.1). Flies from high latitudes had larger wings with more cells than those from the other four sites ($P<0.05$; Figure 4.1). No clinal variation in degree of plasticity was found in wing size traits (Table 4.1). Cell area was the principal determinant of the plasticity of wing area in response to temperature (Table 4.2). Variation between sites in wing area in the phenotypic plasticity experiment was mainly due to cell number, and consistent between experimental temperatures (Figure 4.1; Table 4.2).

4.4.2 Geographic variation in field-collected flies

Flies collected in the field had wings with fewer and smaller cells than the flies reared in the laboratory at 18°C (Chapter 3). All traits increased significantly with latitude (Figure 4.2; Table 4.3). The slopes of the regression lines were significantly steeper for the field collected flies for all traits, although the effect was on the borderline of significance for cell number (Figure 4.2; Table 4.3). Cell size contributed significantly more than did cell number to geographic variation in wing size in the field (~40%) relative to geographic variation in the laboratory (~13%) (Table 4.2).

4.4.3 Laboratory adaptation

I found no evidence for differentiation in the laboratory, between 9 months and 2 years of capture, in wing size traits (effects of the Latitude by Time interaction: $P>0.1$).

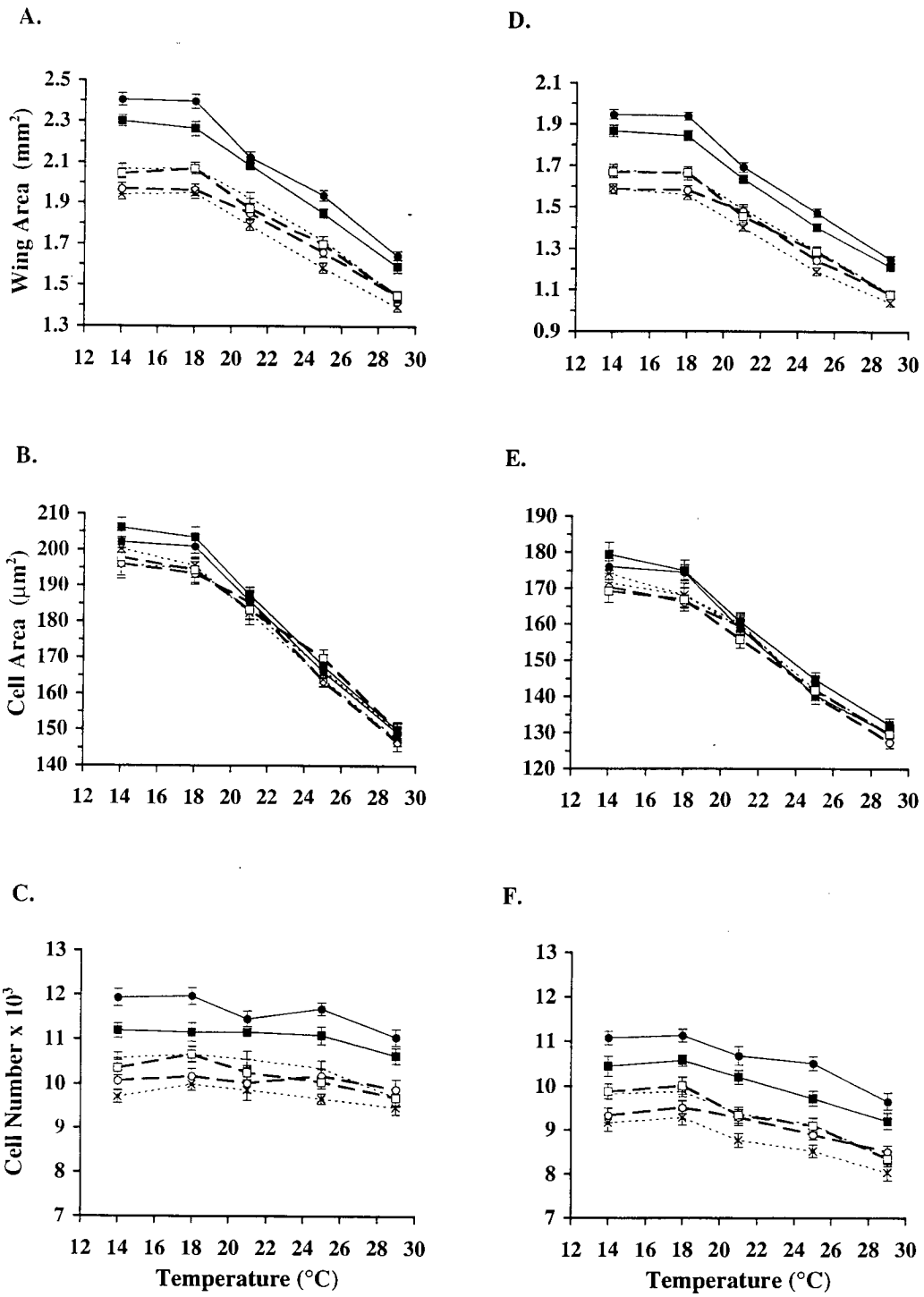

Figure 4.1 Reaction norms of wing size characters for 6 geographic lines reared at 5 experimental temperatures. Values are line means and 95% confidence limits. (A) Wing area in females. (B) Cell area in females. (C) Cell number in females. (D) Wing area ratio in males. (E) Cell area in males. (F) Cell number in males. (o IN, □ MO, × GL, + BH, ■ FT, ● RN.)

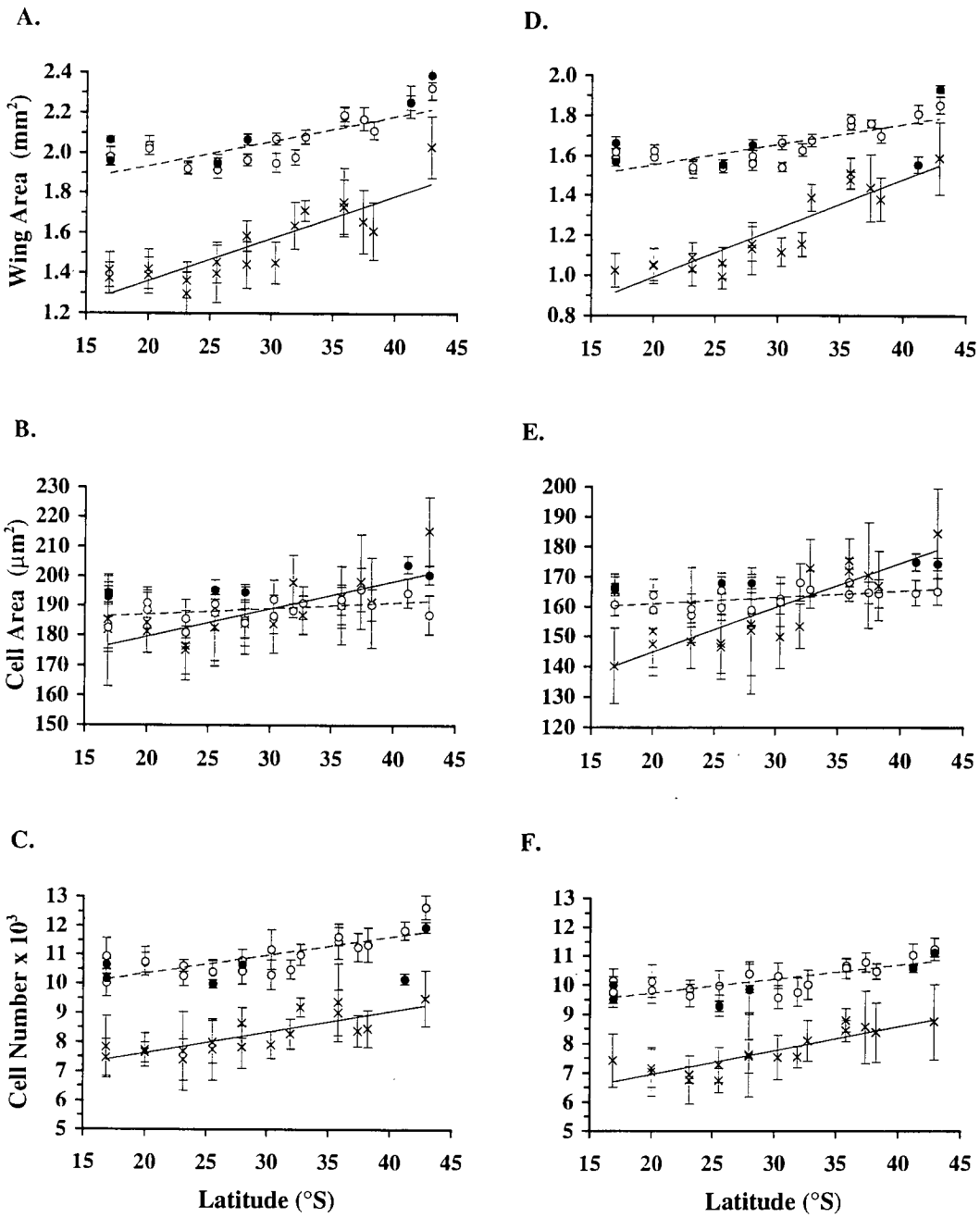

Figure 4.2 Latitudinal clines of wing size characters in field collections and laboratory rearing. Values are line means and 95% confidence intervals. (A) Wing area in females. (B) Cell area in females. (C) Cell number in females. (D) Wing area in males. (E) Cell area in males. (F) Cell number in males. (× wild caught flies, o reared in the laboratory after 9 months captivity, ● reared in the laboratory after 2 years captivity.)

Table 4.1 Phenotypic plasticity in response to temperature of wing size in geographical populations.

	Temperature		Latitude		Site within Latitude		Latitude x Temperature	
Character	R^2	F	R^2	F	R^2	F	R^2	F
√ Wing Area (females)	0.780	1029.35 ***	0.194	12.58 *	0.023	40.61 ***	<0.001	0.43
√ Wing Area (males)	0.826	992.37 ***	0.156	15.24 *	0.015	24.54 ***	<0.001	0.27
√ Cell Area (females)	0.986	732.51 ***	0.006	4.23	0.002	2.07	0.002	0.66
√ Cell Area (males)	0.984	749.60 ***	0.008	5.12	0.002	2.38	0.001	0.78
√ Cell Number (females)	0.120	15.28 **	0.725	8.53 †	0.127	21.62 ***	0.004	0.27
√ Cell Number (males)	0.395	99.17 ***	0.501	8.26 †	0.091	30.50 ***	0.001	0.13

Repeated measures analyses of variance on transformed data, with Temperature and Latitude as crossed fixed effects and Site as a random effect nested within Latitude. The MS for Latitude were tested against the MS for Site within Latitude (df=3). All other MS were tested against Residual MS (Site x Temperature, df=12). † P =0.05; * P <0.05; ** P <0.01; *** P <0.001.

Table 4.2 Cellular basis of variation in wing area.

Source of variation	Females	Males
Phenotypic plasticity experiment		
Temperatures	83.4 ± 4.2	67.6 ± 3.0
Sites	13.4 ± 6.5	13.1 ± 6.9
Flies collected in the field		
Sites	35.3 ± 9.9	46.5 ± 9.0

Percentage of variation in wing area attributable to cell area (means and 95% confidence limits).

Table 4.3 Multiple regression analyses of wing size characters in field collected and laboratory reared flies.

Character	√Wing Area	√Cell Area	√Cell Number
Latitude	171.851 ***	75.74 ***	112.505 ***
Rearing Environment (RE)	588.81 ***	5.16 *	824.226 ***
Sex	369.05 ***	526.67 ***	56.59 ***
Latitude x RE	27.65 ***	35.83 ***	6.55 *
Latitude x Sex	1.47	6.01	<0.01
RE x Sex	0.02	3.10	0.17
Latitude x RE x Sex	1.51	2.25	1.14

Values are F ratios with the Residual MS (df=66) as denominator. Latitude was centered before analysis.

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

4.5 Discussion

Wing size was highly plastic in response to developmental temperature: flies raised in high temperatures acquired smaller wings containing smaller and fewer cells. High latitude flies had larger wings with more and larger cells at all experimental temperatures.

Wing area showed geographic variation in plasticity, but there was no clinal trend, coinciding with Coyne and Beecham's (1987) results for North American *D. melanogaster*. The lack of a latitudinal trend in plasticity was unexpected. If the developmental and evolutionary responses of body size to temperature are both adaptive, and for the same reasons, then one would expect to see higher levels of plasticity in environments with more variable thermal regimes, such as those found at higher latitudes, since a more plastic response might be able to track more accurately the seasonal changes in temperature. The lack of increase in plasticity with latitude could be a consequence of gene flow from lower latitudes. Alternatively, the temperatures experienced during growth and breeding could be more similar at different latitudes than suggested by the annual range of temperature variation, if the activity season is shorter at higher latitudes (Conover and Present 1990). Another possibility would be if increased plasticity implied a fitness cost, preventing the genetic increase in plasticity at higher latitudes (Van Tienderen 1991; Gomulkiewicz and Kirkpatrick 1992). Finally, the developmental mechanisms controlling the plastic response may have a range that is difficult to increase by selection. However, this is unlikely since artificial selection for increased plasticity of thorax length in response to temperature has been successful (Scheiner and Lyman 1991).

Plasticity of wing area in response to temperature was found to be mostly caused by changes in cell area, in accordance with previous studies (Section 1.5.1). Latitudinal variation in wing area was produced mainly by changes in cell number at all temperatures, supporting previous observations at a single temperature (Chapter 3). Flies captured in nature had smaller wings than those reared under standard laboratory conditions. This reduction in wing area was accounted for mostly by a reduction in cell number rather than cell area. This finding suggests that the wild flies were smaller because they experienced poor nutrition and/or larval crowding, which have been found to reduce cell number (Robertson 1959b), unlike environmental temperature. An alternative explanation would be the evolution of larger body size under laboratory conditions. However, the marked similarity of the repeated measurements on these populations at 9 months and 2 years after collection argue against this possibility. In addition, only after intense divergent artificial selection on body size for 30 generations

(Partridge and Fowler 1993) did size differences approach the observed magnitude of the difference between field-collected and laboratory-reared flies, and these collections had been in captivity for fewer generations when first measured.

The latitudinal cline in wing size was significantly steeper for flies collected in nature, mostly due to an increase in the steepness of the cline for cell area. This suggests that latitudinal variation in environmental temperature in nature increased the steepness of the phenotypic cline. This is also suggested by the reaction norms of the lines from which there was field data (Figure 3.1). Flies collected at RN differed from the other populations (MO, GL and BH) by ~19% which is much greater than the difference predicted across the measured thermal reaction norm (7-9%) if all populations experienced the same temperature; i.e. flies in RN probably developed at a lower temperature in the field than flies from the other sites. This finding supports the idea that the evolutionary and developmental responses of body size to temperature share a common function, since they show co-gradient variation in nature, and that temperature is an important selective agent for body size in nature, since its environmental impact on size varies in the predicted way along the cline.

5

Latitudinal Variation of Body Shape in *Drosophila melanogaster*

(with A. C. James, J. McCabe and L. Partridge)

5.1 Summary

The environmental and evolutionary effects of temperature on wing-thorax size ratio and relative wing length and width were investigated using geographic populations of *Drosophila melanogaster*. I found that wing-thorax size ratio and relative wing length and width increased with latitude in field collected flies. When the same populations were reared in the laboratory, wing-thorax size ratio and relative wing width showed genetically based latitudinal clines in the same direction as the field clines, but there was no significant genetic latitudinal differentiation in relative wing length. All three characters were found to decrease in response to temperature during development. My observations are compatible with an adaptive association between low temperature and large wing-thorax size ratio, possibly in relation to flight. Lines selected for large wing size were found to evolve increased wing-thorax size ratio and relative wing width and decreased relative wing length. This suggests that direct selection on wing size could account for the observed latitudinal differentiation in wing-thorax size ratio and relative wing width but not in relative wing length.

5.2 Introduction

There is some evidence that thermal selection can change wing size relative to thorax length (wing-thorax size ratio, inversely related to wing loading, the ratio of body mass to wing area) and wing shape in *Drosophila*. The wing-thorax size ratio, was found to increase genetically with latitude in *D. robusta* and *D. subobscura* and to increase in response to selection at cold temperatures in the laboratory in one study of *D. melanogaster* (Section 1.6.4). The situation is less clear with respect to wing shape. Genetically based clines do not agree in different species or in different studies on the same species (Section 1.6.4). Also, although it was shown that changes in wing shape could evolve during thermal selection in the laboratory, the use of multivariate analyses has obscured the interpretation of the changes (Section 1.6.4). In this study I have

investigated a latitudinal cline in wing-thorax size ratio, relative wing length and relative wing width, in field collected and laboratory reared Australian *D. melanogaster.*

Given that temperature can affect the evolution of wing-thorax size ratio and, possibly, wing shape, is there evidence that those effects reflect an adaptive association between particular shapes and different temperatures? In *D. melanogaster,* it was shown that flies that were capable of flying between 13°C and 15°C in the field had higher values of an index proportional to wing-thorax size ratio and relative wing length, compared to flies that could only fly above 16°C, although they did not differ significantly in thorax size (Stalker 1980). Therefore a high wing-thorax size ratio and/or relative wing length could be advantageous at low temperatures if selection acted on flight ability (Ennos 1989; Wooton 1992). This is possible since flight has high energetic costs in insects (e.g. Chadwick and Gilmour 1940; Sacktor 1975; Yuval et al. 1994) which can affect major fitness components such as female fecundity (Roff 1977; Inglesfield and Begon 1983; Gunn et al. 1988), male fertility (Benjamin and Bradshaw 1994) and starvation and desiccation resistance (Graves et al. 1992).

Temperature also seems to have developmental effects on wing-thorax size ratio and the wing aspect ratio (length/width): in general, both characters decrease with increasing temperature (Section 1.5.2). I have studied the reaction norms in response to temperature of wing-thorax size ratio, relative wing length and relative wing width, in geographic populations to assess the possible role of phenotypic plasticity in establishing the clines in nature.

The phenotypic plasticity in response to temperature of body size and for wing-thorax size ratio and wing shape could be adaptive. Flies that developed at 15°C showed a higher total power output when flying at that temperature but a lower one when flying at 22°C and 30°C, relative to flies that developed at higher temperatures (Barnes and Laurie-Ahlberg 1986). Therefore, if the plasticity in morphological characters and in flight performance are causally related, then large body size, wing-thorax size ratio or relative wing length could be advantageous for flight at low temperatures.

Temperature could have an important effect on the evolution of the wing–flight muscle system in insects. In small insects, like dipterans, that do not generate enough metabolic heat to regulate body temperature (Stevenson 1985), the wing-beat frequency increases linearly with temperature (Chadwick 1939; Reed et al. 1942; Yurkiewicz and Smyth 1966a; Hargrove 1980; Unwin and Corbet 1984). Ambient temperature influences the efficiency of insect flight since this depends on the match between the current wing-beat frequency and the mechanical resonance frequency of the wing–flight muscle system (Machin et al. 1962; Yurkiewicz and Smyth 1966b; Yurkiewicz 1968; Unwin and Corbet 1984). Therefore, selection on flight performance at different

temperatures is expected to favour different optima for characters that influence the physical parameters of the flight system, such as body weight, wing-thorax size ratio and wing shape (Reed et al. 1942; Weis-Fogh 1973; Ellington 1984a, 1984b, 1984c).

Comparison among 3 geographic populations of *D. melanogaster* (Barnes and Laurie-Ahlberg 1986) and among 2 closely related groups of *Drosophila* species (Reed et al. 1942) indicated that cold adapted flies had low wing-beat frequency. Since the wing-beat frequency also decreases environmentallly with temperature, an increase in wing-thorax size ratio and relative wing length could be advantageous at lower temperatures in order to increase the lift generated for the same weight.

If body and wing shape were not themselves targets of thermal selection, they might have evolved as correlated responses to selection on body size. In particular, selection on wing size could be involved, since it can alter the wing-thorax size ratio (Robertson and Reeve 1952) and wing shape (Cavicchi et al. 1981). In contrast, artificial selection on wing-thorax size ratio did not change either thorax size or wing shape (Robertson 1962). To investigate the possible role of direct selection on wing size in causing the latitudinal trends in wing-thorax size ratio and wing shape I have examined the responses of wing-thorax size ratio, relative wing length and relative wing width to artificial selection on wing size.

5.3 Materials and Methods

5.3.1 Geographic lines

Geographic variation in flies collected in the field.—See Section 4.3.2. Thorax length was measured on 25 individuals of each sex and wing traits were measured on 10 individuals of each sex.

Geographic variation in flies reared in the laboratory in standard conditions.—See Section 3.3. Thorax length and wing traits were measured on 5 and 4 flies from each sex per vial, respectively.

Phenotypic plasticity in response to temperature.—See Section 3.3.2. Thorax length and wing traits were measured on 4 flies from each sex in 5 and 8 vials per line and temperature, respectively.

5.3.2 Lines selected for cell number

Three replicate large, small and control lines, artificially selected for wing area keeping cell size constant were used in this experiment (Section 2.1.5). Flies from each line were reared at 18°C and 25°C for two generations following the standard experimental design (Section 2.2.5), in 5 replicate vial cultures with 30 larvae in each, for each line at each temperature. Thorax length and wing traits were measured in 4 randomly selected pairs from each replicate vial.

5.3.3 Measurements

Thorax length was defined as the length from the most anterior humeral bristle to the posterior tip of the scutellum. In the geographic variation study thorax length was measured using a *camera lucida* attached to a dissecting microscope and a graphics tablet connected to a Macintosh computer. In the other studies, thorax length was measured to the closest 0.02mm using an eyepiece graticule (~2% resolution).

Wing area was measured as described in Section 2.3.1. Four landmarks in the wing were then marked and their coordinates recorded (Figure 5.1). The shape of individual wings was described by two indices: relative wing length was defined as *(distance AC)^2/(wing area)* and relative wing width was defined as *(distance BD)^2/(wing area)*. The definitions of wing length and width follow Long and Singh (1995). Relative wing length is directly related to the wing aspect ratio since *Length/Width* $\approx$ *Length^2/Area*). Since AC and BD are non-orthogonal and the angle BD between the segments (~63°) may be variable, relative wing length and width are not strongly correlated. The functional significance of relative wing width is not clear. In order to complement the interpretation of variation in wing shape, the variation in the BC angle between the length and width measurements was discussed. The two measures of wing shape provided a simple representation of the overall shape of the wing. This was demonstrated by multivariate analyses of the 6 linear measurements among the landmarks used (Figure 5.1). Principal components were extracted separately in each sex from a covariance matrix calculated on the vial means in the experiment where the geographic populations were reared at 18°C in the laboratory. Among the line means in each sex, relative wing length was significantly correlated with the second and fourth (P<0.01) principal components, whereas relative wing width was significantly correlated with the first (P<0.01) and third (P<0.001 in males and P=0.12 in females) principal components.

Wing-thorax size ratio was calculated as *($\sqrt{}$wing area)/(thorax length)*, which has been shown to be inversely related to wing loading since *(body weight)/(wing area)* $\approx$ *(thorax length)^3/(wing area)* (Stalker 1980; Starmer and Wolf 1989). This character was only measured on individual flies in the cell number selection lines. In the laboratory experiments with the geographic lines the wing-thorax size ratios were estimated from vial means of wing and thorax size and in the field flies estimates were made based on line means.

5.3.4 Statistical analyses

Latitudinal clines.—I investigated the variation in the shape characters by a two-way analysis of variance with population and sex as crossed fixed effects, separately on individual values for field collected flies and vial means for laboratory reared flies. Latitudinal trends in the different traits were tested by linear regression of line means for each rearing environment (field or laboratory) and sex. The latitudinal trends were then compared among sexes and rearing environments by multiple regression on line means, including these main effects with centred latitude as a continuous variable. The laboratory data corresponding to those samples for which there were no field data (sites FT and IN, both sexes) were excluded from the analyses. Residuals from regressions were normally distributed for relative wing length and width (Shapiro-Wilk W test, $P>0.15$). The residuals were not normally distributed for wing-thorax size ratio ($P<0.001$) but since this was caused by two outliers ($P>0.15$ when outliers removed) I did not transform the variable.

Phenotypic plasticity.—The patterns of phenotypic plasticity in response to temperature were investigated separately for each sex by repeated measures analyses of variance on line means in each environment. Temperature and latitude (or selection regime) were considered as crossed fixed effects and line as a random effect nested within latitude (or selection regime). Variances among lines means were not significantly heterogeneous across experimental temperatures for any trait ($P>0.05$), and the analyses of data transformed so as to eliminate among environment heteroscedasticity (Section 2.4.4), produced qualitatively similar results to the analyses on untransformed data. Residuals from the analyses of variance of all traits were normally distributed ($P>0.05$).

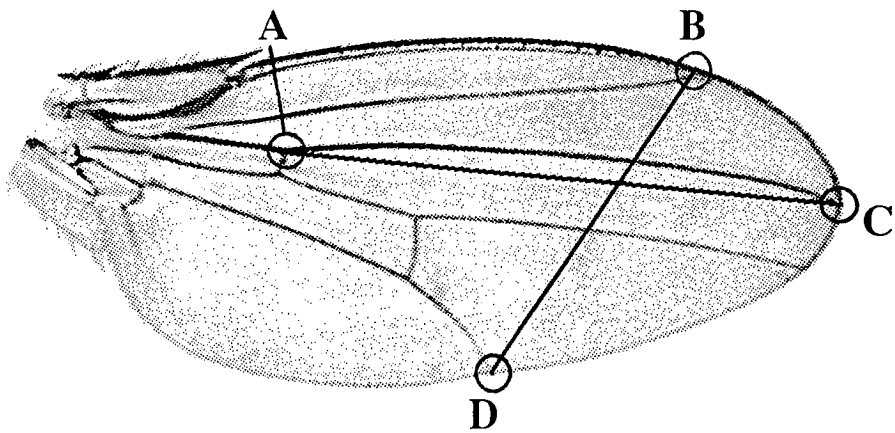

Figure 5.1 Picture of *Drosophila* wing with landmarks used in this study.

5.4 Results

5.4.1 Geographic lines

Latitudinal variation in field and laboratory reared flies.—Wing-thorax size ratio, relative wing length and relative wing width varied significantly among lines, both in the field and laboratory reared flies ($P<0.001$). All traits showed significant sexual dimorphism ($P<0.001$) except relative wing length in the field. There were no cases of significant population by sex interaction.

The three characters showed significant linear latitudinal trends in field collected flies (Figure 5.2): wing-thorax size ratio (females $P<0.1$, males $P<0.001$), relative wing length (females $P<0.01$, males $P<0.05$) and relative wing width (both sexes $P<0.01$) increased with latitude. Flies reared in standard conditions in the laboratory showed significant positive latitudinal trends in wing-thorax size ratio (both sexes $P<0.001$) and in relative wing width, in females ($P<0.01$). Relative wing width in males and relative length in both sexes did not change significantly with latitude ($P>0.05$).

The observation that both relative wing length and width increased with latitude was caused by a correlated cline in the angle BC between the length and width segments. This angle decreased linearly with latitude in both field and laboratory reared flies (field $P<0.05$ for both sexes; laboratory $P<0.01$ in females and $P<0.05$ in males) so that both relative wing length and width increased with latitude.

Multiple regression analyses showed that the slopes of the regressions with latitude were constant between the two rearing environments for wing-thorax size ratio and relative wing width but were significantly steeper in the field collections for relative wing length (Table 5.1). Wing-thorax size ratio was lower and relative wing length was higher in field caught flies than in laboratory reared flies, whereas relative wing width did not differ in the two rearing environments. The difference between environments was significantly larger in females than in males for wing-thorax size ratio, and this confounded the sexual dimorphism in the trait (Table 5.1).

Phenotypic plasticity in response to temperature.—Wing-thorax size ratio, relative wing length and relative wing width showed significant plasticity in response to temperature (Table 5.2). All traits showed a significant linear decrease with temperature (linear contrast: $P<0.001$ in both sexes of each character) (Figure 5.3). Wing-thorax size ratio was the only trait that showed a significant latitudinal trend in overall level of response, with a significantly higher ratio in high latitude populations. Wing relative length and width were larger in the high latitude lines, but the difference was not significant. Relative wing width in females was the only trait exhibiting

significant latitudinal differentiation in phenotypic plasticity, with flies from intermediate latitudes being less plastic.

As with geographic variation discussed above, the positive covariation in relative wing length and width at different temperatures was explained by significant phenotypic plasticity in the angle BC. This angle increased non-linearly at higher temperatures (with the greatest change between 25°C and 29°C) so that both relative wing length and width decreased with temperature.

5.4.2 Lines selected for cell number

Wing-thorax size ratio, relative wing length and relative wing width showed a significant decrease in response to temperature (Figure 5.4; Table 5.3). Wing-thorax size ratio did not show a significant response to selection when both temperatures were considered. However, significant differentiation occurred at 25°C with lines selected for large wing size showing higher wing-thorax size ratios (females $F_{[2,8]}$=10.34, P=0.01; males $F_{[2,8]}$=6.77, P=0.03). Wing shape responded to selection: lines selected for large wing size showed reduced relative wing length and increased relative wing width in both sexes. The degree of plasticity in any trait did not respond to selection.

The opposite responses to selection of relative wing length and relative wing width were caused by the fact that the angle BC did not respond to selection in either sex (P>0.15). The positive covariation in relative wing length and width in response to temperature was explained by the same pattern as in the Australian reaction norms.

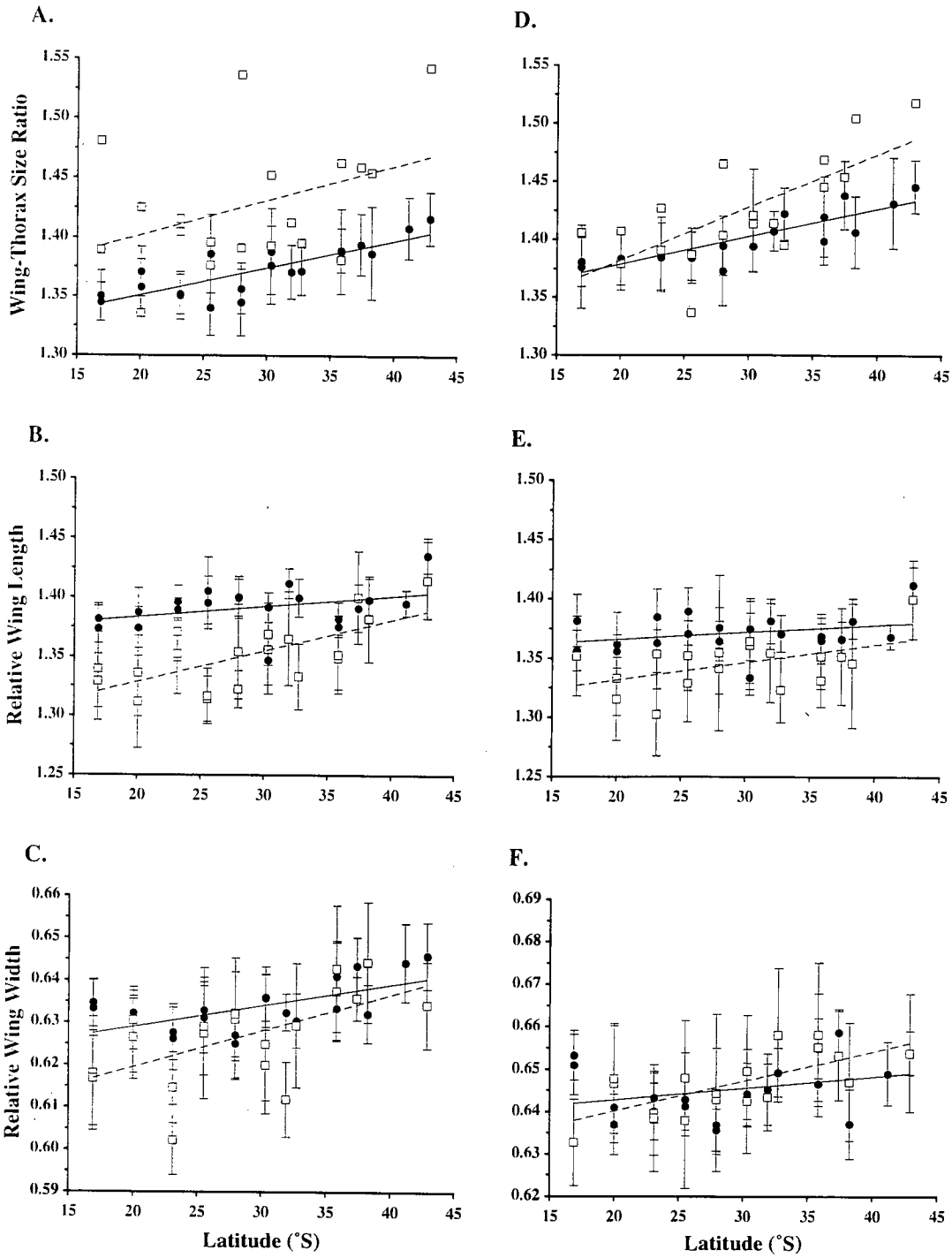

Figure 5.2 Latitudinal clines of body and wing shape in field collections and laboratory rearing. Values are line means and 95% confidence intervals. (A) Wing-thorax size ratio in females. (B) Relative wing length in females. (C) Relative wing width in females. (D) Wing-thorax size ratio in males. (E) Relative wing length in males. (F) Relative wing width in males.
(□ wild caught flies; ● flies reared in the laboratory at 18°C.)

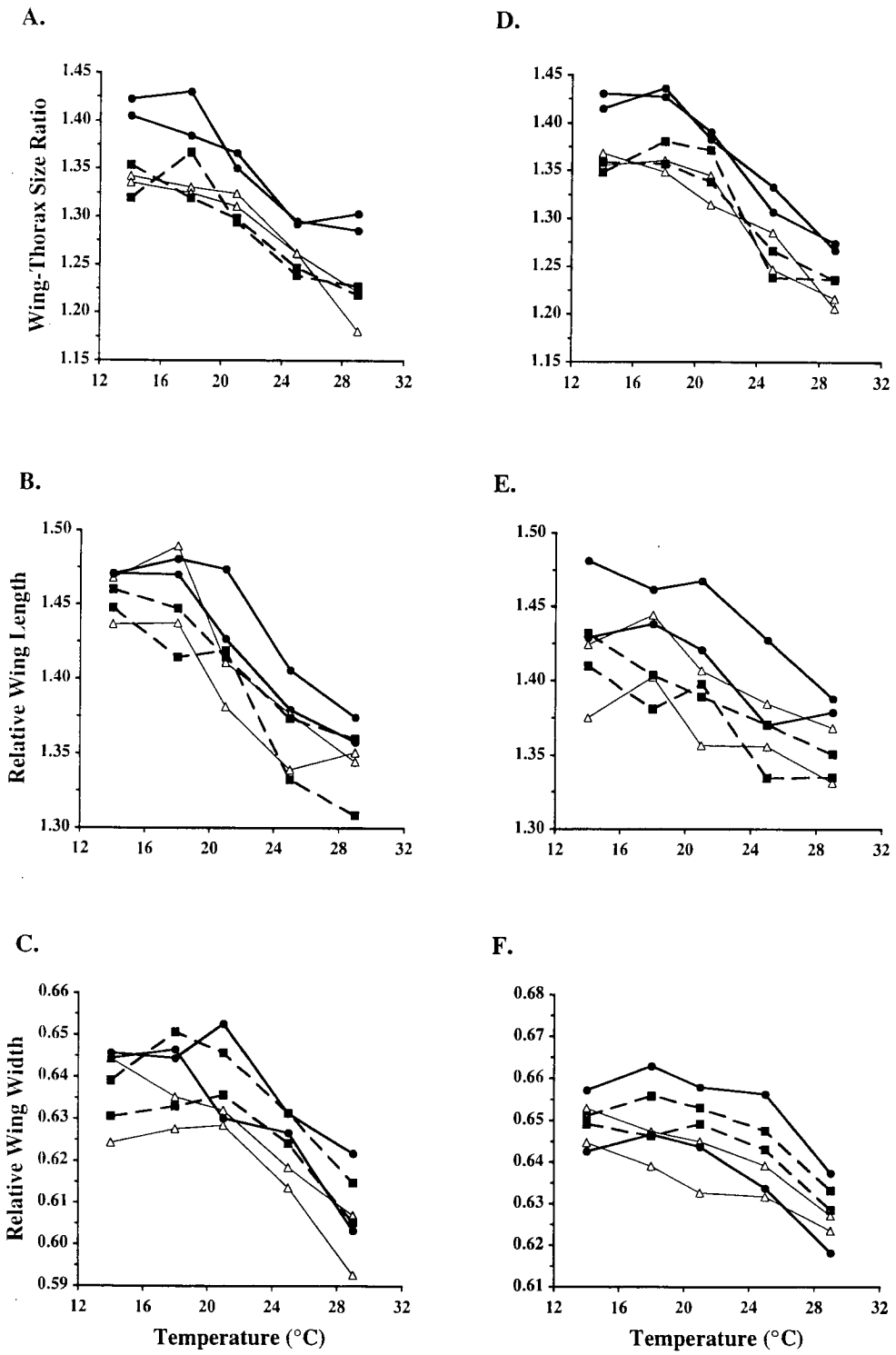

Figure 5.3 Reaction norms of body and wing shape for 6 geographic lines reared at 5 experimental temperatures. Values are line means. (A) Wing-thorax size ratio in females. (B) Relative wing length in females. (C) Relative wing width in females. (D) Wing-thorax size ratio in males. (E) Relative wing length in males. (F) Relative wing width in males. (● high, Δ intermediate and ■ low latitude lines.)

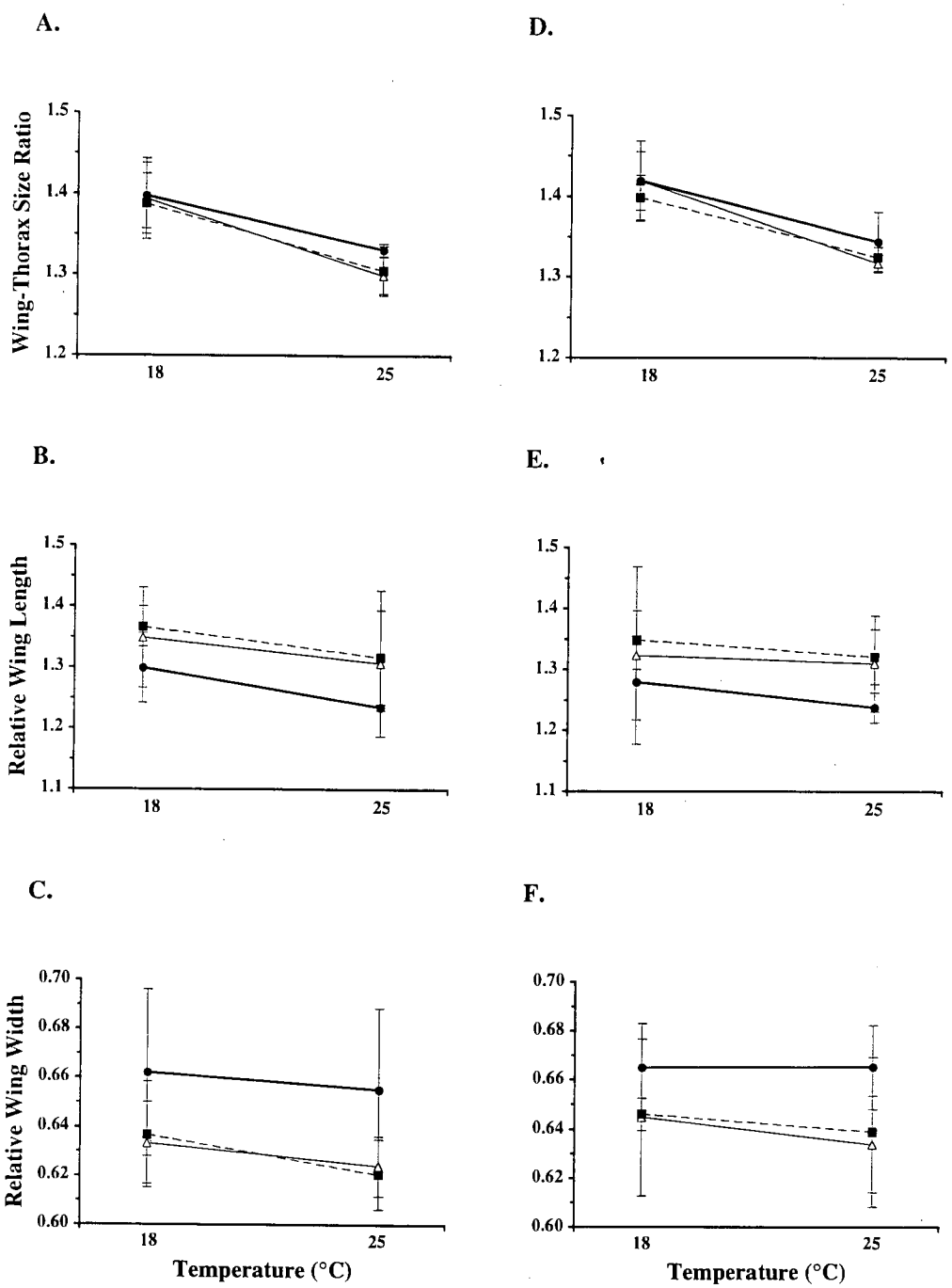

Figure 5.4 Reaction norms of body and wing shape for lines artificially selected for wing size reared at 2 experimental temperatures. Values are line means and 95% confidence limits for each selection regime. (A) Wing-thorax size ratio in females. (B) Relative wing length in females. (C) Relative wing width in females. (D) Wing-thorax size ratio in males. (E) Relative wing length in males. (F) Relative wing width in males. (● up, Δ control and ■ down selection.)

Table 5.1 Multiple regression analyses of body and wing shape in field collected and laboratory reared flies.

Character	Wing-thorax size ratio	Relative Wing Length	Relative Wing Width
Latitude	34.26 ***	23.54 ***	27.37 ***
Rearing Environment (RE)	29.61 ***	60.72 ***	2.54
Sex	2.60	9.11 **	101.79 ***
Latitude x RE	1.92	4.27 *	3.79
Latitude x Sex	0.79	1.44	0.640
RE x Sex	5.30 *	2.48	5.79 *
Latitude x RE x Sex	0.57	0.68	<0.01

Values are F ratios with the Residual MS (df=66) as denominator. Latitude was centered before analysis.

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

Table 5.2 Phenotypic plasticity in response to temperature of body and wing shape in geographical populations.

	Temperature		Latitude		Population		Latitude x Temperature	
Character	R^2	F	R^2	F	R^2	F	R^2	F
Wing-Thorax Size Ratio (females)	0.675	53.22 ***	0.257	297.30 ***	0.001	0.14	0.029	1.13
Wing-Thorax Size Ratio (males)	0.786	95.09 ***	0.173	842.93 ***	<0.001	0.05	0.016	0.95
Relative Wing Length (females)	0.794	71.54 ***	0.083	1.98	0.063	7.53 **	0.027	1.24
Relative Wing Length (males)	0.457	40.30 ***	0.276	2.22	0.186	21.90 ***	0.046	2.05
Relative Wing Width (females)	0.693	37.14 ***	0.123	1.62	0.113	8.10 **	0.015	0.41
Relative Wing Width (males)	0.565	102.36 ***	0.103	0.56	0.277	66.91 ***	0.038	3.43 *

The MS for Latitude were tested against the MS for Population within Latitude (df=3). All other MS were tested against Residual MS (Population x Temperature, df=12). * P <0.05; ** P <0.01; *** P <0.001.

Table 5.3 Phenotypic plasticity in response to temperature of body and wing shape in populations selected for wing size.

	Temperature		Selection regime		Replicate line		Selection x Temperature	
Character	R^2	F	R^2	F	R^2	F	R^2	F
Wing-Thorax Size Ratio (females)	0.879	250.99 ***	0.037	2.38	0.046	2.20	0.017	2.46
Wing-Thorax Size Ratio (males)	0.885	289.17 ***	0.036	2.84	0.038	2.09	0.022	3.64
Relative Wing Length (females)	0.293	39.20 ***	0.452	6.77 *	0.200	4.47 *	0.001	0.62
Relative Wing Length (males)	0.097	8.76 *	0.548	6.07 *	0.271	4.06	0.017	0.77
Relative Wing Width (females)	0.099	26.72 **	0.680	10.99 **	0.186	8.36 *	0.013	1.8
Relative Wing Width (males)	0.045	3.15	0.662	10.95 **	0.181	2.12	0.026	0.91

The MS for Selection regime were tested against the MS for Line within Selection (df=3). All other MS were tested against Residual MS (Line x Temperature, df=12). * P <0.05; ** P <0.01; *** P <0.001.

5.5 Discussion

I found that wing-thorax size ratio, relative wing length and relative wing width increased with latitude in field collected flies. However, when the same populations were reared in the laboratory, I only found evidence for a significant genetic component to the latitudinal clines in wing-thorax size ratio and relative wing width, but not in relative wing length. In two experiments, all characters were found to decrease in response to increasing temperature during development. The similarity between the developmental and evolutionary responses to temperature provides possible evidence of an adaptive association of large wing-thorax size ratio and relative wing width at low temperature. The patterns of latitudinal variation and phenotypic plasticity in wing-thorax size ratio are in general agreement with previous *Drosophila* studies (Section 1.6.4).

Since latitudinal differentiation in wing-thorax size ratio and relative wing width was positively correlated with variation in wing size, those traits could have evolved as correlated responses to direct selection on wing size. In order to test this possibility, I investigated the correlated responses of wing-thorax size ratio and relative wing width in lines selected for large and small wing size. We used lines selected keeping cell size constant (Section 2.1.5) to approximate the cellular basis of latitudinal differentiation in wing size (Chapter 3). In this experiment we found that lines selected for large wing size evolved increased wing-thorax size ratio and relative wing width but that they also evolved decreased relative wing length. These results provide evidence that, although direct selection on wing size could account for the observed latitudinal differentiation in wing-thorax size ratio, it could not entirely explain the evolution in wing shape. These results provide further evidence that high relative wing width is adaptive at low temperatures.

Thermal selection on the wing-thorax size ratio independent of body size, as predicted by the observed advantage of low wing loading (high wing-thorax size ratio) for flight at low temperatures (Stalker 1980), could account for the latitudinal clines in wing size and cell number, as shown by artificial selection on wing-thorax size ratio (Robertson 1962). However, Robertson (1962) could not detect correlated responses in thorax size or wing aspect ratio (~relative wing length) so that selection on wing-thorax size ratio might not be an important target of thermal selection in nature.

The differentiation in relative wing length, caused by artificial selection on cell number in the wing, may have involved the evolution of genes involved in cell differentiation. Cell lineage analyses suggest that cell proliferation and differentiation are related: in general, mutations that caused extra veins increased wing size and

decreased its relative length, whereas those that removed veins decreased wing size and increased its relative length (Section 1.3).

My study suggests that wing shape could be a target of thermal selection. A high relative wing width seems to be favoured at cold temperatures, possibly in relation to flight ability. This could be tested experimentally by selecting on relative wing width keeping the size of the wing and thorax constant, and then studying the correlated responses in flight performance at different temperatures. Wing shape has also been shown to change during short and long term laboratory thermal selection (Section 1.6.4), although the precise nature of the changes in terms of relative wing width was not clear. The response of wing-thorax size ratio and relative wing width to thermal selection in the laboratory require further investigation. The precise ways in which temperature and morphology interact to determine flight performance within species and populations also deserve further study.

Wild caught flies showed higher wing-thorax size ratio but lower relative wing length than those reared in laboratory conditions. This discrepancy pattern cannot be explained simply by absolute differences between the temperatures experienced by flies in the field and those used in the laboratory, since both traits have been shown to change consistently across temperatures; rather, it suggests that other differences in the field preadult environment (e.g., larval densities or daily thermal range) could be involved. The impact of fluctuating temperatures on body shape has not been investigated in *Drosophila*. Both thorax length and wing area were smaller in field collected flies (A. C. James, R. B. R. Azevedo and L. Partridge, unpublished manuscript; Chapter 4) but thorax length showed a greater reduction under field conditions relative to wing area: the ratio of size in nature to size in the laboratory, averaged across populations, was significantly lower for thorax length (83%) than for $\sqrt{}$wing area (86%) (paired t-test in both sexes, $P<0.005$). If larval density was higher in the field, then these observations would mean that larval density has a stronger effect on thorax length than on wing area, whereas temperature has a stronger effect on wing area than on thorax length; although this has not yet been investigated in *D. melanogaster*, it has been shown to be the case in *D. mulleri* (Starmer and Wolf 1989), The interaction between temperature and larval densities in the field in determining morphological and life history traits constitutes a major challenge for the future, since larval density could be an important proximal agent of thermal selection (Partridge and French 1996).

6

Thermal Evolution of Egg Size in *Drosophila melanogaster*

(R. B. R. Azevedo, V. French and L. Partridge. 1996. Evolution 50, 2338-2345)

6.1 Summary

I measured the size of eggs produced by populations of *Drosophila melanogaster* that had been collected along latitudinal gradients in different continents or that had undergone several years of culture at different temperatures in the laboratory. Australian and South American populations from higher latitudes produced larger eggs when all were compared at a standard temperature. Laboratory populations that had been evolving at 16.5°C produced larger eggs than populations that had evolved at 25°C or 29°C, suggesting that temperature may be an important selective agent in producing the latitudinal clines. Flies from laboratory populations produced larger eggs at an experimental temperature of 16.5°C than at 25°C, and there was no indication of genotype-environment interaction for egg size. Evolution of egg size in response to temperature cannot be accounted for by differences in adult body size between populations. It is not clear which life history traits are direct targets of thermal selection and which are showing correlated responses, and disentangling these is a task for the future.

6.2 Introduction

Egg size is an important life history character since it is positively associated with offspring fitness (Chapter 7). Environmental variables associated with changes in egg size are of interest because they could yield insights into the mechanisms by which greater egg size improves offspring fitness and by which females' ability to invest in eggs is controlled. There are indications that one such variable, at least for ectotherms, may be environmental temperature (Section 1.5.3).

In addition to environmental effects, patterns of geographic variation indicate that temperature may have evolutionary effects on the egg size of ectotherms (Section 1.6.5). This could occur if lower temperatures promoted the evolution of larger eggs, either by an advantage provided by larger eggs or by a reduced cost to mother laying large eggs in colder areas. However, to establish that temperature *per se* is causal, it must be manipulated independently of other environmental variables.

I have investigated the thermal evolution of egg size in *Drosophila melanogaster* by two approaches. Firstly I compared the patterns of geographical variation in egg size among natural populations collected along two latitudinal transects in different continents, and correlated egg size with other morphological and life-history traits. Secondly, I investigated the role of temperature in the evolution of egg size directly, using laboratory natural selection of replicated populations at three different temperatures. I have also investigated the importance of gene-environment interaction for egg size in relation to temperature, by making measurements on laboratory populations at two different temperatures.

6.3 Materials and Methods

6.3.1 Experimental methods

The eggs to be measured were laid by flies reared at constant temperature and low density, since the rearing conditions of the females can affect egg size (Gause 1931; Imai 1935; Eigenbrodt and Zahl 1939; Crill et al. 1996). Unless otherwise stated, the parents of the experimental flies were also reared at the experimental temperature to control for parental effects of temperature. Crill and colleagues (1996) found no evidence for maternal effects of temperature on egg size but significant paternal effects. In all experiments, females from different populations were mated to males from their own population. Paternal genotype has been previously shown to have no significant effect on egg size (R. B. R. Azevedo, unpublished results).

In each experiment, the eggs to be compared among populations were laid synchronously over a few hours in constant temperature by females of standard age (± 1 day), since female fecundity and egg size are very sensitive to environmental temperature during egg laying (Imai 1935; David and Clavel 1969; Avelar 1993; Huey et al. 1995; Crill et al. 1996). These estimates are valid since, although there are daily fluctuations in egg size, these are consistent across populations (Parsons 1962; David 1963; Avelar 1993; R. B. R. Azevedo, unpublished results).

Australian populations.—See section 2.1.1. The experiment described here was done 18 months after the populations were collected, at a constant temperature of 18°C.

Adults were reared using the reduced design in 10 vials of standard medium per population each with 30 first instar larvae (Section 2.2.5). Eggs were collected from 2 laying pots per population, each with 25 pairs aged ~13 days, over 3 consecutive 4-hour laying periods (Section 2.2.4). I measured 50 eggs per population chosen at random from the samples (Section 2.3.4). In order to determine if egg size was related to ovary size, I measured the size of the reproductive organs of the females from each population. I chose 12 females aged 15-18 days from each line, dissected them in saline solution, and counted the number of ovarioles in each ovary.

South American populations.—See section 2.1.2. This experiment was done 13 months after the populations were established in the laboratory, at a constant temperature of 25°C. Adults were obtained from eggs collected directly from each population into two bottles with food medium (~200 eggs per bottle; Section 2.2.4). Eggs were collected from 2 laying pots per population, each with 20 pairs aged ~7 days, over 3 consecutive 3-hour laying periods (Section 2.2.4). I measured 50 eggs per population chosen at random from the samples (Section 2.3.4).

Thermal selection lines.—See section 2.1.3. These experiments were carried out in 1993, when laboratory natural selection had been in operation for 9 years at 16.5°C and 25°C, and for 4 years at 29°C.

In the first experiment, I investigated whether evolution in different thermal environments (16.5°C, 25°C and 29°C) led to a divergence in egg size when measured at 25°C. Flies from each population were reared by the standard design in 15 vials of standard medium per population each with 50 first instar larvae over 5 consecutive days (3 vials per day; Section 2.2.5). Virgins were collected from these cultures and transferred to fresh vials. For each population and experimental day, 6 couples were chosen and kept separately in vials containing 7ml of Lewis medium with charcoal. After 5 days (age 7 days) with daily transfers to fresh vials, I measured 4 eggs per female.

In a second experiment I investigated whether selection in the 16.5°C and 25°C environments produced a significant gene-environment interaction for egg size at these two temperatures. In this experiment the parents of the experimental flies did not develop at the experimental temperature: adults from each of the 6 lines were obtained from eggs collected directly from the cages and reared at each experimental temperature (Section 2.2.4). Eggs were collected for measurement once daily on 5 consecutive days, over periods of 6 hours at 16.5°C and 3 hours at 25°C, from 5 laying pots per population, each with 50 pairs aged 11-16 days at 16.5°C and 7-11 days at 25°C (Section 2.2.4). I measured 5 eggs per laying pot (250 eggs per line per temperature).

6.3.2 Statistical analyses

The data on the geographical populations were analysed by linear models to test for latitudinal trends (Section 2.4.2). The data on the thermal selection lines were treated by nested analyses of variance (Section 2.4.3). Unless otherwise stated, the variances were homogeneous among groups ($P>0.05$). In all cases the standardised residuals were normally distributed (Shapiro-Wilk test, $P>0.05$) (Section 2.4.1).

6.4 Results

6.4.1 Geographic lines

The variances of egg volume were significantly heterogeneous among populations for both latitudinal collections (O'Brien, Brown-Forsythe and Levene tests: Australian populations $P<0.001$, South American populations $P<0.05$), and increased with mean egg size (Spearman rank correlation: Australian populations $r_S=0.770$, $P=0.0001$; South American populations $r_S=0.527$, $P=0.11$). In the Australian populations, the heteroscedasticity was partly caused by two extreme values but their deletion did not make the variances homogeneous ($F_{[19,978]}>2.12$, $P<0.005$). However, the coefficients of variation were homogeneous among populations (Australian populations after removal of outliers $F_{[19,978]}<1.55$, $P>0.06$; South American populations $F_{[9,490]}<1.47$, $P>0.15$), which suggested that the errors followed a gamma instead of a normal distribution. To analyse the variation in egg size among geographic populations, I fitted generalised linear models with gamma error distribution and identity link to each transect (Crawley 1993). One outlier was excluded from the analysis of the Australian gradient since it was significant by Grubbs' test ($T=3.49$, two-tailed $P<0.01$) (Sokal and Rohlf 1995). Egg volume increased significantly with latitude (Figure 6.1; Table 6.1). Deviations from regression were highly significant, and populations collected at the same latitude frequently produced eggs that differed significantly in size. There was no indication of curvilinearity in the relationships between egg size and latitude. The slopes of the regression models were extremely similar. The differences in intercept are explained by the different temperatures in the two experiments.

The mean number of ovarioles per ovary of each female in the Australian populations was analysed by standard least-squares regression since it followed the assumptions of a normal error distribution. Ovariole number was also variable among populations and increased significantly with latitude although non-linearly (Figure 6.2; Table 6.2).

Among the Australian populations, egg size was not significantly correlated with ovariole number among populations, although both traits were positively related to

female thorax length and larval development rate (Table 6.3). When latitude or thorax length was held constant there were no significant partial correlations between the different traits ($P>0.05$, corrected by sequential Bonferroni procedure on each set of partial correlations).

6.4.2 Thermal selection lines

In the first experiment at 25°C I calculated the mean egg volumes for each female and then calculated the mean egg volume for the females of each experimental day. The day means were analysed in randomised block analyses of variance using experimental day as blocking factor and selection temperature as a fixed effect. In the comparison between the 16.5° and 25°C thermal selection lines, replicate line was considered a random effect nested within selection regime. I found that females from the lines selected at 16.5°C laid significantly larger eggs than those from the 25°C selection regime ($F_{[1,4]}=7.82$, $P=0.049$; Figure 6.3). There were no significant differences among lines within selection temperatures ($F_{[4,20]}=1.61$, $P>0.2$). In the comparison between the 25° and 29°C selected lines, because of the way the 29°C lines were derived (see Section 2.1.3), I considered replicate line as a random effect crossed with selection regime. There were no significant effects ($P>0.3$; Figure 6.3) which means that the lines selected at 25°C and 29°C did not diverge in egg size.

In the second experiment, at 16.5°C and 25°C, I calculated the mean egg volume for each thermal selection line on each day. Comparisons among lines from different selection regimes were done in factorial randomised block analysis of variance with experimental and selection temperatures as fixed effects, line replication as a random effect nested within selection regime and day nested within experimental temperature as a blocking factor (see Table 6.4). Egg size showed significant plasticity with temperature, with an average 5.2% increase at the lower experimental temperature (Fig. 6.4). The eggs from the thermal lines selected at 16.5°C were larger than those from the 25°C selection regime. There were no significant gene-environment interactions among lines or selection regimes.

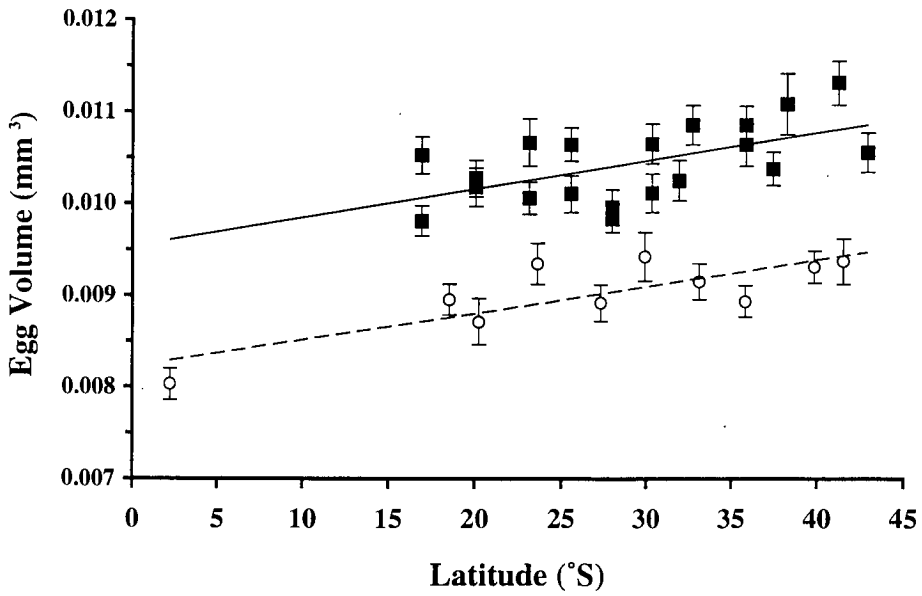

Figure 6.1 Latitudinal clines of egg size. Line means and 95% confidence intervals and linear regressions with latitude for Australian (■, $y = 0.009552 + 0.000030x$) and South American (o, $y = 0.009552 + 0.000030x$) populations.

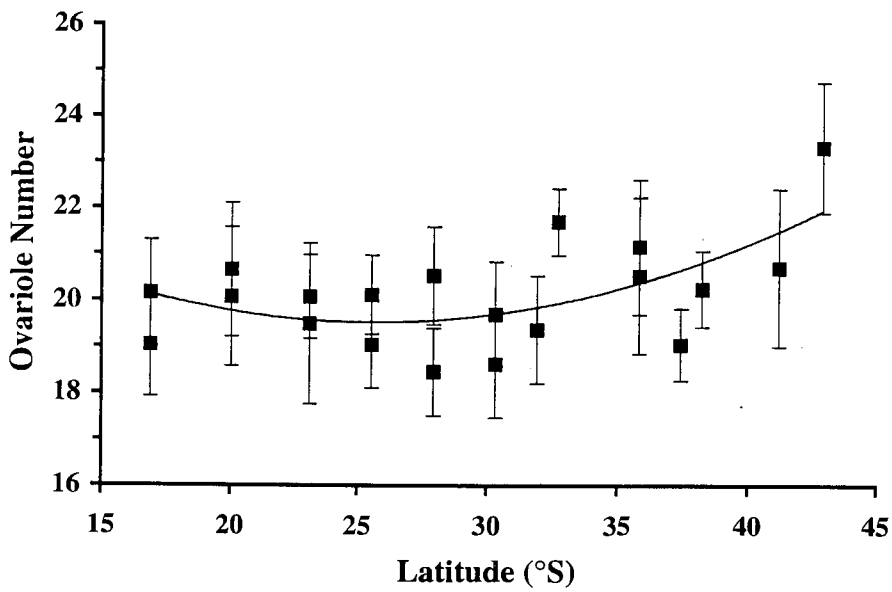

Figure 6.2 Latitudinal variation in ovariole number in the Australian populations. Line means and 95% confidence intervals and quadtratic regression with latitude ($y = 24.7984 - 0.4123x + 0.0081x^2$).

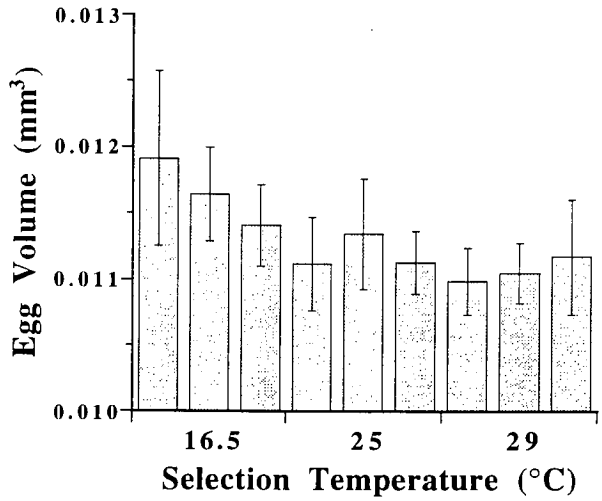

Figure 6.3 Laboratory thermal selection lines at 25°C: mean egg volume for each of the 3 replicate selection lines and 95% confidence limits based on corrected day means.

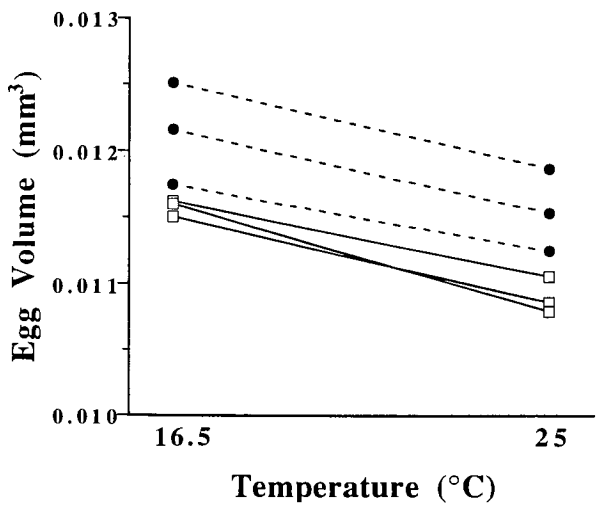

Figure 6.4 Laboratory thermal selection lines at 16.5°C and 25°C: mean egg volume for each replicate selection line plotted against experimental temperature. (● 16.5°C and □ 25°C selected lines.)

Table 6.1 Geographical populations. Generalised linear model analyses of the variation in egg volume among lines.

Source of variation	df	Deviance †	*F*	
Australian populations				
Line	19	1.460	15.38	* * *
Linear regression with latitude ‡	1	0.514	9.88	* *
Quadratic regression with latitude ‡	1	0.060	1.16	
Deviations	17	0.885	10.42	* * *
Pure error	979	4.893		
South American populations				
Line	9	0.992	12.56	* * *
Linear regression with latitude ‡	1	0.715	29.38	* * *
Quadratic regression with latitude ‡	1	0.106	4.36	
Deviations	7	0.170	3.52	* *
Pure error	490	3.388		

Generalised linear models with gamma errors and identity link. † Given by $2 \cdot \sum\left[-\log\left(\frac{y}{\mu}\right)+\frac{y-\mu}{\mu}\right]$ where y is the data and μ is the fitted value under the model (Crawley 1993). The tabled values are the changes in deviance associated with the different sources of variation and are analogous to SS in least-squares. ‡ The changes in deviance due to regression were estimated sequentially and tested against the mean deviance for deviations from regression. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 6.2 Australian populations. Polynomial regression analysis of variation in ovariole number among lines.

Source of variation	df	SS	*F*	
Line	19	294.68	4.15	***
Linear regression with latitude †	1	53.31	4.78	*
Quadratic regression with latitude †	1	51.77	4.64	*
Deviations	17	189.60	2.98	***
Pure error	220	822.00		

† Regression SS were estimated sequentially and tested against the MS for deviations from regression. * $P<0.05$; *** $P<0.001$.

TABLE 6.3 Australian populations. Correlation analysis of life history characters among lines. Pearson product-moment correlations (above diagonal) and partial correlations with latitude held constant (below diagonal).

Character	E	LDR	O	T
E		0.599 *	0.306	0.466
LDR	0.403		0.518 †	0.662 **
O	0.078	0.373		0.390
T	0.187	0.564 †	0.092	

(E) egg volume and (O) ovariole number data from this study, (LDR) larval development rate data from James and Partridge (1995), and (T) thorax length data from James et al. (1995). Significance tests for correlations and partial correlations were corrected by a sequential Bonferroni procedure (Rice 1989).

† $P<0.10$; * $P<0.05$; ** $P<0.01$

Table 6.4 Laboratory thermal selection lines: randomised block factorial analysis of variance on egg volume for each experimental temperature, with selection regime and experimental temperature as fixed effects, population replication as a random effect and day of measurement nested within experimental temperature as a blocking factor.

Source of variation	*df*	SS x 10^8	*F*	
Selection †	1	552	8.74	*
Population within selection ‡	4	253	24.07	* *
Temperature §	1	601	84.27	* * *
Selection x Temperature ‡	1	3	1.12	
Population x Temperature	4	10	0.53	
Day within temperature	8	76		
Error	40	198		

† Tested against Population MS; ‡ Tested against Population x Temperature MS; § Tested against synthetic MS = Population x Temperature MS + Day MS – Error MS with 3.77 *df.* * $P<0.05$; ** $P<0.01$; *** $P<0.001$

6.5 Discussion

I have detected two geographical clines in egg size, in populations from different continents, with a genetic increase with latitude when all populations were kept at constant temperature. This finding raises two questions: what selective forces were responsible for the clines in egg size, and was egg size itself the target of natural selection?

The operation of natural selection, as opposed to drift and dispersal, in producing clines is in general deduced from repeatability in different places (Endler 1986). Although geographical variation in egg size of *D. melanogaster* has been found previously (Oksengorn-Proust 1954; Cals-Usciati 1964; David and Legay 1977), extensive latitudinal trends had not been studied. The demonstration in this study of independent latitudinal clines in egg size in two continents suggests that natural selection is responsible. An indication that geographic variation in temperature is at least one of the agents of selection comes from the results of laboratory thermal selection. Populations that underwent laboratory evolution at 16.5°C for 9 years had larger eggs than those from populations maintained at the two higher temperatures (25°C and 29°C). Since there were replicate lines at each selection temperature to control for any effects of genetic drift, these data indicate that the lower temperature did select for larger egg size. Four years of evolution at 29°C did not result in a significant change in egg size when measured at 25°C, presumably indicating no selection for reduced egg size at this temperature. The lack of any significant curvilinearity in the latitudinal cline could therefore indicate that the lower latitude populations do not frequently encounter temperatures as high as 29°C. It is not possible to deduce the temperatures encountered by this species in nature from standard meteorological measurements, partly because of habitat selection (Jones et al. 1987), and partly because the proportion of the year when the flies are active declines with increasing latitude. It is also possible that the mechanisms of thermal natural selection are different between laboratory and the field. The temperature encountered by the laboratory strains was constant whereas field temperatures are subject to daily and seasonal variations. In addition, the shapes of latitudinal clines may be affected by geographic variation in levels of gene flow (Chapter 3).

Lower temperature could select for increased egg size because of a direct physiological effect on mothers or offspring, or through its consequences for population dynamics. My data do not throw any light on this issue, and it requires further investigation. In the laboratory thermal lines, long-term culture at 16.5°C has been shown to be associated with the evolution of more rapid larval development and growth,

larger adult body size and higher efficiency of food use during larval development than in the 25°C populations. It will be important to determine if egg size is directly related to these other traits, either as a cause or as a consequence. For instance, by experimental manipulation, I have shown that larger egg size is causal of more rapid larval development (Chapter 7). Furthermore, artificial selection for development rate has been shown to produce a positive correlated response in egg size (Bakker 1969). A significant correlation between egg size and rate of larval development was apparent among geographic stocks (Table 6.3). The differences between the Australian populations (and between thermal selection lines) in development rate may therefore be, at least in part, a consequence of the differences between them in egg size. However, the residuals from the regressions of these two traits against latitude were not significantly correlated and the populations from the South American transect did not differ significantly from one another in development time (J. Van't Land, P. Van Putten, B. Zwaan, H. Villarroel, A. Kamping and W. Van Delden, unpublished results). These findings suggest that development rate can also be altered in other ways.

The data from the geographic populations suggest that the differences between them in adult body size are not causal in producing the differences in egg size, because the two traits were not significantly correlated, whether latitude was or was not held constant. This is surprising because selection for increased body size at a single temperature resulted in increased egg size (R. B. R. Azevedo, J. McCabe, B. Zwaan and L. Partridge, unpublished results).

The 16.5°C thermal selection lines use food more efficiently in larval growth than do the 25°C lines (Neat et al. 1995). The thermal evolution of egg size might be explained if females with a low-temperature evolutionary history use food more efficiently in the production of eggs. A trade-off between egg size and egg number does not seem to be important. Among the Australian populations, variation in egg size was not associated with variation in ovariole number, when latitude was or was not held constant, although both traits increased in value with latitude. If anything, ovariole number has been shown to be positively but weakly associated with daily fecundity (Robertson 1957a; David 1970; Boulétreau-Merle et al. 1982). In the thermal selection lines there was gene-environment interaction for fecundity, with each set of lines showing higher fecundity than the other at their own evolutionary temperature (Partridge et al. 1995). Again, these data do not support the importance of a trade-off between egg size and egg number in the thermal evolution of egg size, since all the lines showed an increase in egg size when rearing temperature was decreased (Figure 6.4), and so the correlation between egg size and fecundity changed sign between the two measurement temperatures.

My experiments showed a direct effect of experimental temperature on egg size. This could be explained by temperature during the development of the female or during oogenesis in the adult female, or both. In *Drosophila*, egg size and fecundity are very sensitive to laying temperature (David and Clavel 1969; Avelar and Rocha Pité 1989; Avelar 1993; Huey et al. 1995) but not to rearing temperature (Imai 1935; Huey et al. 1995; but see Crill et al. 1996). The physiological basis of this pattern of phenotypic plasticity is not understood, nor is it clear if it is adaptive.

The extent of the difference in egg size between the 16.5°C and 25°C selection lines did not differ when measurements were made at the two selection temperatures (Figure 6.4), so there was no indication of genotype-environment interaction. This result should be treated with some caution, because the parents of the experimental flies were not raised at the experimental temperature and there may be parental effects on egg size (Crill et al. 1996). My data suggests that the thermal evolution of egg size was not accompanied by a change in plasticity for the trait, and that genotype-by-environment interaction may, therefore, not be important in the expression or maintenance of differences in egg size between the laboratory thermal lines or the latitudinal populations. Direct measurement of the pattern of plasticity of egg size with temperature in the geographic populations would be needed to confirm this.

Egg size could evolve in response to temperature because of an effect of temperature on population dynamics. For instance, large eggs could be especially beneficial for offspring if levels of larval competition are high, or small eggs could be favoured if high levels of competition between adult females mean that they are in low nutritional status. I do not have sufficient information about the effect of temperature on population dynamics to evaluate its role in thermal evolution of egg size.

7

Life History Consequences of Egg Size in *Drosophila melanogaster*

(R. B. R. Azevedo, V. French and L. Partridge. American Naturalist, in press)

7.1 Summary

I used a novel approach to study the effects of egg size on offspring fitness components in *Drosophila melanogaster*. Populations that differed genetically in egg size were crossed and the female offspring from these reciprocal crosses were examined for life history traits. These flies expressed effects of egg size because they developed from eggs of different sizes as a result of maternal genetic effects but displayed an equivalent range of nuclear genetic variation. The crosses used four independent pairs of outbred populations, that differed in the pattern of covariation between egg size and life history traits, so that the maternal effects of egg size on offspring characters could be contrasted to the associations present among the parental populations. Egg size showed positive maternal genetic effects on embryonic viability and development rate, hatchling weight and feeding rate, and egg-pupa and egg-adult development rate, but no consistent effects on larval competitive ability, adult weight or egg size in the offspring. My method revealed a pattern of causality that could not be deduced from intra- or inter-population comparisons and, therefore, provides a good way of disentangling the causes and consequences of variation in egg size while controlling for zygotic genetic effects.

7.2 Introduction

The role of maternal effects in general, and of egg size in particular, in *Drosophila* life history evolution has been largely neglected. The phenotype of an individual can be affected not only by its genotype and the environment it experiences during development, but also by the phenotype of its parents. This type of influence is called maternal inheritance (since mothers generally have the strongest effects on the offspring) and can involve a variety of mechanisms that are independent from the

nuclear genes of the offspring, such as the cytoplasmic transmission of mitochondria, chloroplasts, microorganisms or RNA transcripts, the provisioning of the egg with yolk, parental care or cultural transmission (reviews: Labeyrie 1967; Mousseau and Dingle 1987; Roach and Wulff 1987; Kirkpatrick and Lande 1989; Bernardo 1996a). Maternal effects can influence evolution and themselves evolve. The direction and rate of response to selection on a particular character depends on the maternal traits that influence it, as well as on its additive genetic variation (Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990). Maternal inheritance can also produce time lags in the selection response, because individuals may show the effects of a gene without possessing it (Kirkpatrick and Lande 1989).

Egg size will be an important agent of maternal inheritance if it reflects reproductive investment in offspring. The relationship between egg size and offspring fitness characters has been empirically investigated in animals, using a variety of methods (reviews of studies in plants: McGinley et al. 1987; Roach and Wulff 1987; Roff 1992). In table 7.1 I summarise the results of 136 studies of the relationship between egg size and offspring fitness components in animals. A total of 124 species from 91 genera were examined, mostly insects, fish and birds (including 24%, 16% and 30% of the species, respectively). Most studies reported phenotypic correlations within populations where variation in egg size was either not manipulated (101 studies on 102 species), or was caused by differences in maternal size and/or age (12 studies on 10 species) or resulted from manipulations of maternal diet (9 studies on 9 species). In these phenotypic studies, egg size consistently showed a positive relationship with preadult size (loosely defined as size at any stage before adulthood, e.g. the size of an insect larva; positive correlations in 93% of species; no negative correlations) and, although the effect frequently disappeared before adulthood, positive effects on adult size were common (positive correlations in 46% of species; no negative correlations). Positive correlations were also widespread with components of survival, such as egg hatchability, juvenile survival or starvation resistance (59% of species showed positive correlations; 34% no correlation). The association between egg size and development rate was unclear (no correlation in 50% of species). However, invertebrates, which lay relatively small eggs, usually developed faster with increasing egg size (62% of species showed positive correlations; 7% negative correlations). In conclusion, these phenotypic studies have tended to show positive associations between egg size and some component of offspring fitness: in 63% of the species studied, or 60% when correlations with preadult size are disregarded. This pattern does not seem to be associated with any particular taxon.

However, the measurement of phenotypic correlations within populations (or among populations or species) is not appropriate for establishing causal relationships

between egg size and other traits, because it does not allow discrimination between the effects of egg size and other maternal effects or the effects of offspring genotype and environment (David 1961; Sinervo 1990; Bernardo 1996b). For example, physiologically stressed females may lay pathologically small eggs that subsequently lack particular vital resources or develop in a poor environment, thus generating a positive phenotypic correlation. Furthermore, the simultaneous study of other types of maternal effects, such as age and diet, could be inappropriate since these may generate variation in reproductive investment in offspring independently of egg size (David 1961; Parsons 1962; Rossiter 1991b; Rossiter et al. 1993; Bridges and Heppel 1996). In order to ensure that egg size is causal in influencing offspring traits, it is necessary to manipulate egg size genetically or experimentally (Sinervo 1993).

Artificial selection has generally revealed positive correlations between egg size and life history characters. Selection on egg size produced positive correlated responses in adult weight in *D. melanogaster* (Parsons 1964), and in larval weight and overwintering survival in spruce budworm (Harvey 1983b, 1985). Selection for desiccation resistance in a mosquito produced a positive response in egg size (Sota 1993). In *D. melanogaster*, egg size was increased in response to selection for fast development (Bakker 1969) and for large wing size (R. Azevedo, J. McCabe, B. Zwaan and L. Partridge, unpublished results). The only negative result was that, in *D. funebris*, selection for wing length produced a negative response in egg length (Zarapkin 1934, 1935). However, selection experiments confound maternal effects of egg size on offspring traits and additive genetic correlations between the trait expressed in the mother and the size of its eggs.

The effects of egg size have only rarely been studied by quantitative genetic methods, presumably because these methods can only be applied to organisms with short generation times, large family sizes and that can be bred in controlled conditions. Two major types of maternal effects on offspring traits can be distinguished (Falconer 1989). Firstly, mothers that differ in the average investment in eggs can provide different environments for their offspring. For example, in the pied flycatcher, the variation in egg size among broods affected offspring adult tarsus-length (Potti and Merino 1994). Secondly, each mother can cause environmental variation in the offspring by varying egg provisioning among siblings. For example, in the gypsy moth, within clutch variation in egg size had positive effects on embryonic and larval development rate and pupal weight (Rossiter 1991a). Although both types of maternal effects influence the environment of the offspring, they may have an detectable genetic basis (Willham 1963; Thompson 1976). The genetic component of egg size effects has never been examined by quantitative genetic methods.

Direct experimental manipulation of egg size can establish causality because it uncouples egg size from other aspects of maternal genotype and phenotype. In western fence lizards, egg size was found to covary within and among populations with offspring fitness traits such as hatchling size, sprint speed and stamina (Sinervo 1990). However, the precise allometric relationship between egg size and hatchling size were significantly different within and among populations, suggesting that they could not both accurately reflect the causal relationship between the traits. Sinervo (1990) obtained artificially small eggs by extracting variable amounts of yolk and showed that these small eggs developed faster into smaller hatchlings (Sinervo 1990). The allometric relationship between egg size and hatchling size matched the pattern of covariation among, not within, populations (Sinervo 1990). Egg size was also shown to affect hatchling sprint speed but not their stamina (Sinervo 1990; Sinervo and Huey 1990). Sinervo and Licht (1991) achieved a sophisticated method for altering egg size in side blotched lizards, whereby yolk allocation in the female before oviposition was manipulated. Larger clutches of smaller eggs were obtained by injecting follicle-stimulating hormone into females and larger eggs were obtained by removing the yolk from some follicles (Sinervo and Licht 1991). Applying this approach to field studies of natural selection on offspring size, it was shown that larger eggs produced larger hatchlings which experienced a juvenile and adult survival advantage late in the reproductive season, but a disadvantage earlier in the season (Sinervo et al. 1992; Sinervo and Doughty 1996). In locusts, within populations, egg size was found to be positively correlated with larval size and negatively correlated with larval ovariole number (Albrecht et al. 1959). Verdier (1957 cited in Albrecht et al. (1959)) reduced egg size in a locust by ligaturing and showed that egg size determined larval size but did not have an effect on the number of ovarioles in the larva, therefore refuting the causal relationship suggested by the phenotypic correlation.

In *D. melanogaster*, quantitative genetic methods within populations, although potentially very informative, are difficult to implement since the error involved in measuring egg size is high and close to the coefficient of variation of egg size within populations (~5%). In addition, it is not possible to manipulate egg size without disrupting positional information in the cytoplasm of the developing embryo (Illmensee 1972; Schubinger 1976, Vogel 1977).

I developed a new approach in *D. melanogaster* for disentangling the causes and consequences of variation in egg size while controlling for zygotic genetic effects. Maternal effects can be detected by comparing the offspring of the homogametic sex from reciprocal crosses between populations: if the two groups of offspring are significantly different from each other, then maternal effects are a likely explanation,

since the offspring possess an equivalent range of nuclear genetic variation (Figure 7.1). The offspring of the heterogametic sex are not comparable in the same way, since they may differ in nuclear genetic constitution (Figure 7.1). This approach has revealed maternal effects of unknown origin on a variety of *Drosophila* characters (Table 7.2). The maternal effects of egg size on offspring life history characters can be studied if the populations used in the reciprocal crosses lay eggs of different sizes and if egg size is maternally determined. If populations that differ genetically in egg size are crossed, then the offspring of the homogametic sex from the reciprocal crosses will express maternal effects, since they develop from eggs of different size but have a common range of zygotic genetic variation (Figure 7.1). If, moreover, the crossed populations are reared under similar, controlled, environmental conditions, then the maternal effects of egg size will have a genetic basis; referred in this chapter as maternal genetic effects.

This approach has never been used explicitly to investigate effects of egg size, but some earlier studies contain data from which the effects of egg size can be deduced (Table 7.3). For example, Bakker (1969) selected on larval development rate in *D. melanogaster*, and found a positive correlated response in egg size and a negative response in adult weight. When he did reciprocal crosses between the fast and slow lines, he found that larvae emerging from large eggs grew faster up to 72 hours (III instar). Since the larvae were not sexed, X-chromosome effects could be causing the difference (a possibility which is not considered in the other studies listed in Table 7.3). In another experiment, larvae from the reciprocal crosses were allowed to feed for 72 hours, and then reared to adulthood without any more food, and it was found that the adult females developing from larger eggs became heavier adults (Bakker 1969). This experiment showed a positive maternal genetic effect on larval growth rate (Bakker 1969), caused by egg size.

I did reciprocal crosses of four pairs of independently derived outbred populations of *D. melanogaster* that displayed large genetic differences in egg size: two populations from different latitudes in Australia (AU), two populations from different latitudes in South America (SA), two lines selected for large and small wing size (WS) and two lines selected for high and low cell number in the wing (CN). In all pairs of populations, the one with bigger eggs also had larger adult body size. The WS pair of populations differed in cell size, but not the AU or CN. The populations in each pair did not differ in preadult developmental rate, except the CN lines, where the larger flies laid bigger eggs but developed more slowly. The comparisons among the female offspring from these reciprocal crosses tested whether population differences in body size were caused by differences in egg size and if egg size had effects on other life history traits.

Table 7.1 Review of studies into the relatinship of egg size and offspring fitness components within animal populations.

	Fitness Component § (+ O −)				
Taxon (No. species)	Survival	Development Rate	Preadult Size	Adult Size	Sources
Phenotypic correlations:					
Mollusca (3)	2 0 0	0 1 0	1 0 0	. . .	Kraeuter et al. 1982; Orton and Sibly 1990
Annelida (1)	1 0 0	1 0 0	1 0 0	. . .	Levin et al. 1991
Arthropoda (28)	11 6 1	9 2 1	6 0 0	8 6 0	Green 1954†; Leonard 1970; Curtsinger 1976a; Lawlor 1976†; Richards and Myers 1980; Wittman 1981; Bell 1983; Perelle et al. 1983; Karlsson 1984; Steinwascher 1984; Palmer 1985; Honek 1987; Larsson 1989; Tessier and Consolatti 1989†; Marshall 1990; Carlberg 1991; Ebert 1991†; McLain and Mallard 1991; Wallin et al. 1992; Fox 1993, 1994; Lampert 1993; Carrière and Roff 1995; Toda et al. 1995; Avelar unpubl.
Pisces (15)	8 1 1	1 4 0	8 1 0	5 2 0	de Ciechomski 1966; Bagenal 1969; Fowler 1972; Kazakov 1981; Knutsen and Tilseth 1985; Mann and Mills 1985; Rana 1985; Marsh 1986; Lagomarsino et al. 1988; Henrich 1988†; Hutchings 1991; Marteinsdottir and Able 1992; Silverstein and Hershberger 1992,1994; Jonasson 1993; Mire and Millett 1994
Tetrapoda (52)	15 17 0	1 9 5	34 2 0	7 15 0	Nisbet 1973, 1978; Schifferli 1973; Murton et al. 1974; Brooke 1978; Lloyd 1979; O'Connor 1979; Kaplan 1980, 1985, 1992; Moss et al. 1981; Congdon et al. 1983; Furness 1983; Thomas 1993; Bancroft 1984; Birkhead and Nettleship 1984; Crump 1984; Ferguson and Fox 1984; Richter 1984; Gutzke

					and Packard 1985; Lessells 1986; Quinn and Morris 1986; Petranka et al. 1987; Berven and Chadra 1988; Galbraith 1988; Greig-Smith et al. 1988; Newman 1988†; Stockland and Amundsen 1988; Amundsen and Stockland 1990; Pinckney 1990; Reid and Boersma 1990; Whitehead and Tschirner 1990; Whithead et al. 1990; Bolton 1991; Brooks et al. 1991; Grant 1991; Martin and Arnold 1991; Croxall et al. 1992; Magrath 1992; Sydeman and Emslie 1992; Tejedo and Reques 1992; Arnold 1993; Janzen 1993; Meathrel et al. 1993a, b; Robertson and Cooke 1993; Williams et al. 1993; Semlitsch and Schmiedehausen 1994†; Thessing and Ekman 1994; Campos and Magnusson 1995; Parichy and Kaplan 1995; Rowe 1995; Smith et al. 1995
Maternal age/size effects:					
Insecta (4)	1 2 1	2 2 0	0 1 0	1 2 0	Harvey 1977; Wiklund and Persson 1983; Ruohomäki et al. 1993; Fox 1993; Fox and Dingle 1994
Pisces (5)	2 0 1	0 2 0	5 0 0	2 0 0	Gall 1974; Pitman 1979; Beacham and Murray 1985; Beacham et al. 1985; Zastrow 1989; Ahnesjö 1992
Amphibia (1)	1 0 0	. . .	1 0 0	0 1 0	Semlitsch and Gibbons 1990
Manipulation of maternal diet:					
Arthropoda (7)	4 0 0	1 0 0	4 1 0	0 2 0	Klomp and Teerink 1967; Solbreck 1989; Gliwicz and Guisande 1992†; Guisande and Gliwicz 1992; Fox and Dingle 1994; Boersma 1995; Guisande and Harris 1995
Echinodermata (1)	0 0 1	. . .	1 0 0	. . .	George et al. 1991
Pisces (1)	1 0 0	1 0 0	1 0 0	. . .	Iguchi and Yamaguchi 1994

Experimental manipulation of egg size:					
Insecta (1)	. . .	. . .	1 0 0	. . .	Verdier 1957
Echinodermata (1)	. . .	1 0 0	1 0 0	1 0 0	Sinervo and McEdward 1988; Hart 1995
Tetrapoda (3)	1 0 0	0 0 1	3 0 0	0 1 0	Sinervo 1990; Sinervo and Licht 1991; Sinervo et al. 1992; Hill 1993; Sinervo and Doughty 1996
Artificial selection:					
Insecta (4)	2 0 0	0 0 1	1 0 0	2 0 1	Zarapkin 1935; Parsons 1964‡; Bakker 1969; Harvey 1985‡; Sota 1993; Azevedo, McCabe, Zwaan and Partridge, unpubl.
Maternal phenotypic correlations:					
Insecta (1)	. . .	1 0 0	. . .	1 0 0	Rossiter 1991
Tetrapoda (3)	. . .	0 1 0	1 0 0	1 1 0	Newman 1988†; Potti and Merino 1994

Entries 'x y z' are the number of species showing a significant positive correlation, no significant correlation, or a significant negative correlation between egg size and a given offspring fitness component. When more than one study was available for a single species their results were combined.

§ Other fitness components. 1) Within populations: positive correlation with dispersal ability in the gypsy moth (Capinera and Barbosa 1976); positive correlations with fertilization rate under sperm limitation in two sea urchin species (Levitan 1996); positive correlations with hatchling sprint speed and stamina in side-blotched lizards (Sinervo 1990; Sinervo and Huey 1992). 2) Experimental manipulation of egg size: positive correlation with hatchling sprint speed but no correlation with stamina in side-blotched lizards (Sinervo 1990; Sinervo and Huey 1992); no correlation with ovariole number in a locust (Verdier 1957). † Neonate size used instead of egg size. ‡ Selection on egg size.

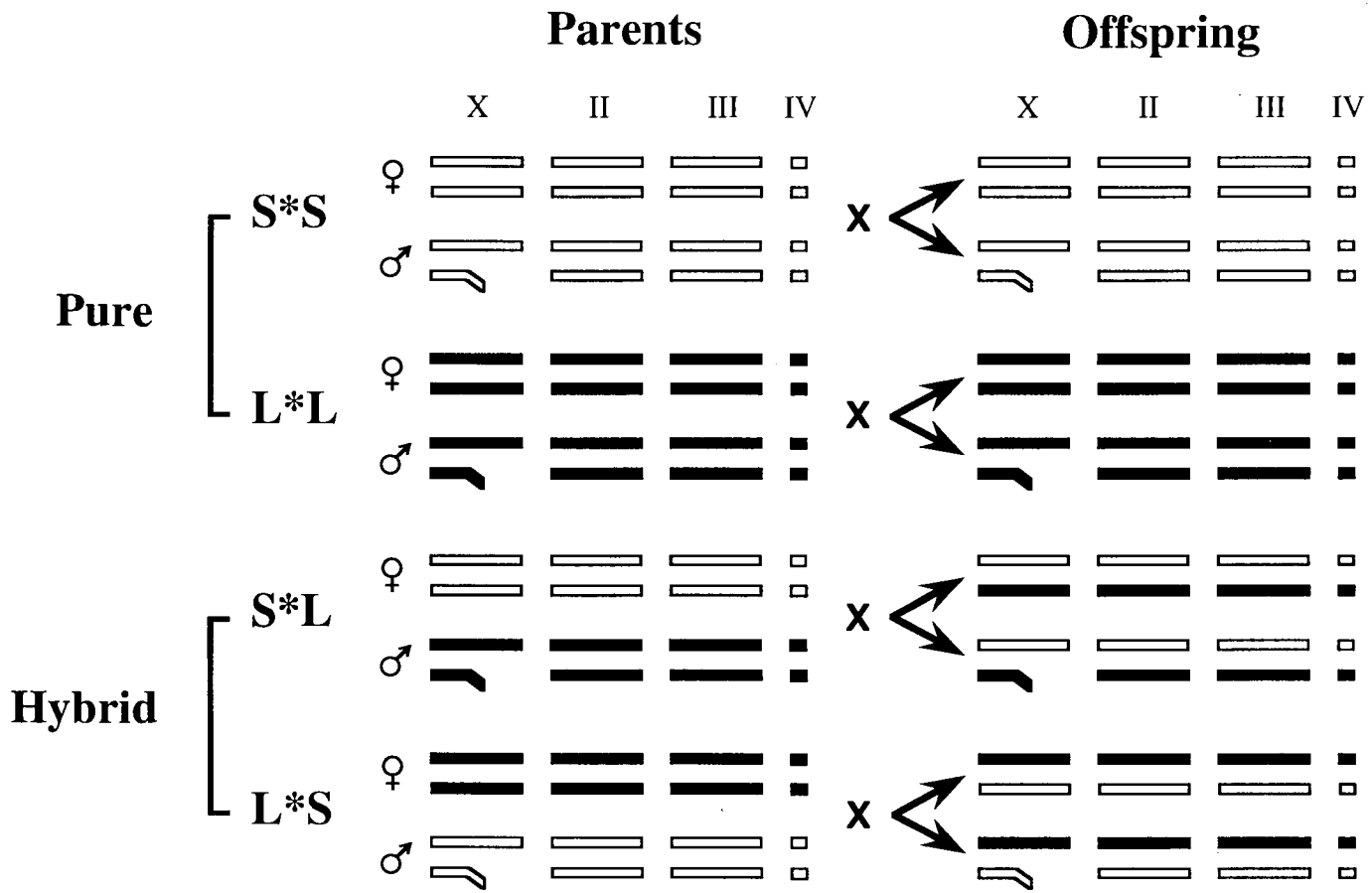

Figure 7.1 Diagram of the genetic constitution of parents and offspring in crosses within and among populations of *Drosophila melanogaster*. Notation in crosses: female*male population.

Table 7.2 Studies that detected maternal effects on life history traits in *Drosophila* by crosses between populations but did not investigate the effect of egg size.

Offspring trait		Species	Source
Embryonic respiration rate		*D. melanogaster*	Fourche 1959
Embryonic development rate		*D. subobscura*	Clarke et al. 1960
Larval development rate		*D. ananassae*	Moriwaki and Tobari 1963
Larval development rate		*D. pseudoobscura*	Poulson 1934
Pupal development rate	†	*D. pseudoobscura*	Poulson 1934
Preadult development rate	†	*D. melanogaster*	David 1955
Preadult development rate		*D. mercatorum*	Williams 1987
Preadult development rate	†	*D. subobscura*	McFarquhar and Robertson 1963
Larval viability		*D. melanogaster*	David 1955; Barnes 1984
Larval competitive ability		*D. melanogaster*	McGill et al. 1973
Thorax length	†	*D. subobscura*	McFarquhar and Robertson 1963
Wing size	†	*D. melanogaster*	Hersh and Ward 1932; Cavicchi et al. 1985
Wing size	†	*D. pseudoobscura*	Anderson 1966
Female fecundity	†	*D. simulans*	Watson and Hoffmanm 1996
Female fertility	†	*D. melanogaster*	Hiraizumi 1961

† Female offspring only.

Table 7.3 Summary of studies of maternal inheritance using reciprocal crosses between populations which differed in egg size.

Offspring trait		Populations	Crosses	Source
Streblospio benedicti (Polychaeta)				
Larval survival		+	+	Levin et al. 1991
Planktonic period		+	0	Levin et al. 1991
Larval size		+	0	Levin et al. 1991
Adult size	†	+	0	Levin et al. 1991
Offspring egg size	†	+	0	Levin et al. 1991
Mesocyclops edax (Crustacea)				
Adult size	†	+	0	Allan 1984
Offspring egg size	†	+	0	Allan 1984
Drosophila funebris				
Adult size	†	–	0	Zarapkin 1934
Drosophila melanogaster				
Larval weight (48-72h)		+	+	Bakker 1969
Adult size (72h feeding)	†	+	+	Bakker 1969
Offspring egg size	†	+	0	Warren 1924

Entries are the signs of the correlations between egg size and the offspring character among parental populations or reciprocal crosses.

† Female offspring only.

7.3 Materials and Methods

7.3.1 Populations

Four pairs of outbred populations of *Drosophila melanogaster* were chosen for displaying large differences in egg size. These differences were established prior to the experiments described here (Chapter 6; R. Azevedo, J. McCabe, B. Zwaan and L. Partridge, unpublished results). In each pair, the populations laying large and small eggs will be termed L and S, respectively.

Australian lines (AU).—HS and BH were defined, respectively, as L and S (Table 2.1). This experiment was done 18 months after they were collected (Section 2.1.1). L flies had larger thorax and wing sizes than S flies, but the lines did not differ in cell size or development time (James and Partridge 1995; James et al. 1995; Chapter 3).

South American lines (SA).—PM and GU were defined, respectively, as L and S (Table 2.2). The experiments described below were done one year after the populations were collected (Section 2.1.2). L flies had larger wings than S flies, but both developed at the same rate (J. Van't Land, P. Van Putten, B. Zwaan, H. Villarroel, A. Kamping and W. Van Delden, unpublished results).

Lines selected for wing size (WS).—The large and small selected lines were defined, respectively, as L and S (Section 2.1.4). Selection was carried out for 24 generations at 25°C before this experiment was done. L flies had larger wing areas, cell areas and cell numbers than S flies (B. Zwaan and L. Partridge, unpublished data).

Cell number selected populations (CN).—One large and one small selected line were defined, respectively, as L and S (Section 5.3.2). The lines were selected for 11 generations before this experiment was done. L flies had larger wings with more cells and developed more slowly than S flies (J. McCabe and L. Partridge, unpublished manuscript).

7.3.2 Rearing Methods

Egg volume, development time and adult weight.—Each pair of populations was tested in turn, at the temperature at which it was kept (AU and CN at 18°C; SA and WS at 25°C). Flies for the crosses were obtained by a reduced design (Section 2.2.5). This was necessary since the environmental conditions experienced by the females affect the size of their eggs (Chapter 6). Adults emerging from these cultures were collected as virgins and kept in vials for 5 days at 25°C, or 8 days at 18°C. I did not find any evidence that there were non-virgin females in these samples. The 4 possible crosses within and among (reciprocal) populations were done: S*S, L*L, S*L and L*S (notation: female line

crossed with male line). Each cross, with ~40 couples, was set-up in two laying pots with grape juice and agar medium and a dab of live yeast paste. Eggs were collected from females aged ~6 days at 25°C, or ~10 days at 18°C, in consecutive laying periods: AU 3x3.5 hours, SA 4x2.5 hours, WS 3x2.5 hours, CN 4x3.5 hours (Section 2.2.4).

In the AU crosses, 110 eggs in each cross were individually measured, and then transferred to a labelled small culture vial 7mg of live yeast in suspension. In the crosses with the SA, WS and CN populations, about 100-150 unmeasured eggs in each cross were individually reared as above, and 50 eggs in each cross were separately measured within 24 hours. The AU larvae were checked for pupation time. In the SA, WS and CN crosses I measured total development time. The females emerging in all the crosses were weighed. Only female development times and body weights were considered in the analyses.

Larval competition.—This experiment was done on the flies of the SA crosses reared for the previous experiment. Eggs were collected from females aged ~10 days in 4 consecutive 3-hour laying periods (Section 2.2.4). Simultaneously, eggs were sampled in petri dishes with grape juice medium and live yeast paste directly from a cage population with the eye-colour mutation *sepia* (*se*) on a Dahomey genetic background. A series of duo-cultures was set up for each cross in small vials containing 17.5mg of live yeast in suspension. Four larval density levels with 10, 20, 30 and 40 larvae/vial were used, with a constant 2:3 ratio wild:*se* and 12 replicates (15 in the first density level). The numbers of *se* and of male and female wild-type adults were counted. The wild-type females were weighed.

Egg and hatchling weights, egg viability and hatching time, and larval feeding rate.— This experiment was done on the SA populations at 25°C. Eggs were collected from each population into 4 bottles in moderate densities 100-200 eggs per bottle (Section 2.2.4). Adults resulting from these cultures were collected as virgins and kept in vials for 5 days. Again, no evidence for non-virginity was detected. The 4 possible crosses within and among (reciprocal) populations were done in random single pairs: S*S, L*L, S*L and L*S (notation: female line crossed with male line). This ensured that all the females used (~40 per population) were mated and laid fertile eggs, which was important for the egg viability test.

The experimental pairs of each cross were mixed in a laying pot with grape juice and agar medium and a dab of live yeast paste. The adults were kept in the laying pots for 5-15 days with daily changes of food. For the assays of different characters, eggs were collected in consecutive 3-hour laying periods (Section 2.2.4). 1) Egg and hatchling weights, 2) egg viability and hatching time and 3) larval feeding rate were measured in separate experiments on larvae of unknown sex, but all experimental

animals were reared to adulthood to test for variations in sex ratio among crosses. In a separate experiment, eggs hatching at different times were reared to adulthood to test for sex dimorphism in embryonic development rate.

Egg volume in the offspring.—Before mixing the couples produced in the previous experiment in laying pots (6-7 days age), the females were separated from the males and allowed to lay eggs individually in vials with yeasted grape-juice medium for 18 hours. From each of 16 females/cross I measured 4 eggs. Of the remaining eggs of each female, two groups of four eggs were transferred to culture vials containing 20mg yeast suspension, and allowed to develop at 25°C. After eclosion the F1 females were allowed to mate with males of the same cross. After 6 days, these females were again separated from the males and allowed to lay eggs in yeasted grape-juice medium for 12 hours . Samples of 5 eggs from each of 12 females per cross were measured.

7.3.3 Measurements

Egg size.—See section 2.3.4. Sometimes the eggs were stored at 6°C for a few hours to delay hatching.

Larval and preadult development time.—Pupation time was measured by recording the number of larvae forming a puparium every 4 hours (~2% resolution). Total development time was measured by timing the adult eclosions to the nearest 30 minutes at 25°C, and to the nearest hour at 18°C (~0.2% resolution).

Adult dry weight.—The females were collected into 1ml centrifuge tubes within 36 hours of emergence and frozen at –20°C. The measurements were done on flies dried in an oven at 50°C for 3 hours: the tubes were opened during the drying and then closed to prevent the flies from absorbing humidity (~0.5% resolution) (Section 2.3.5).

Larval competitive ability and sex ratio.—Let a and b be the number of wild-type and mutant (*se*) flies emerging in each vial, respectively. Larval competitive ability was defined as $2.5 \cdot a/(a+b)$. This means that if a:b are in a 4:6 ratio (the original ratio in the larvae) the wild-types are said to have a competitive ability of 1. The proportion of wild-type females was also recorded.

Egg and hatchling fresh weights.—Eggs and 2-5 hours old first instar larvae were collected with a needle, transferred to a fresh petri-dish with grape juice and agar medium, washed with distilled water using a trimmed paint-brush, and dried with tissue paper. Groups of 5 eggs or 4 larvae from each cross were weighed (~5% resolution) (Section 2.3.5).

Embryonic development time and egg viability.—The number of hatched eggs was recorded every 20 minutes by counting the empty egg shells (~1.5% resolution).

Egg viability was defined as the proportion of hatched eggs relative to the total number of eggs in each lay; this measure assumes that fertilization rates were constant in different crosses.

Larval feeding rate.—The method used was similar to that developed by Sewell and colleagues (1975). Groups of 5 first instar larvae (6-12 hours old) from each cross were transferred in turn to a fresh petri-dish with grape juice and agar medium, over which a live yeast suspension (2mg yeast/100ml water) had been spread with a paintbrush, to a depth allowing the larvae to move freely. The larvae were allowed to recover from the transfer for 2 minutes and were then observed for a test period of continuous feeding. The *(T)* time needed for 50 cephalopharyngeal retractions was measured in s and a feeding rate index was defined as the number of retractions per minute (3000/*T*) (~2.5% measurement error).

7.3.4 Statistical analyses

All characters were analysed by fitting linear models to the data (Section 2.4.1). I compared each character among populations (L*L vs. S*S) and among reciprocal crosses (L*S vs. S*L). The comparisons among reciprocal crosses tested the presence of a maternal genetic effect of egg size.

Egg volume in the SA crosses was inverted and square-rooted, adult weight in the competition experiment was log-transformed, hatching time (SA) was raised to the fifth power, larval development time (AU) was squared, and preadult development time was inverted (WS and CN) or inverted and cubed (SA) (Section 2.4.1). All other traits were kept untransformed. After transformation, the variances were homogeneous among crosses for all characters (O'Brien, Brown-Forsythe and Levene tests, $P > 0.05$). Standardised residuals for egg volume, egg and hatchling weight, adult weight and larval feeding rate were normally distributed (Shapiro-Wilk test, $P > 0.05$) and, therefore, these traits were analysed by fitting linear models with normal errors by least-squares. Transformed development times showed distributions that deviated significantly from normality due to outliers (see below), the grouped structure of the data (several events at each time) and, in the case of preadult development time, the diurnal rhythm of emergence (Bakker and Nelissen 1963). To increase the power of my analyses I fitted linear models with normal, Weibull and logistic error distributions by maximum-likelihood and compared their respective negative log-likelihoods. In all cases the logistic error distribution produced the best fit, followed by the normal distribution, so that all development time characters were analysed by logistic linear models.

To analyse the data from the larval competition experiment (larval competitive ability, sex ratio at emergence and adult female dry weight), I included the effect of

larval density level in the linear models. The experimental design of some traits included a random blocking factor (e.g. lay for development times). The significance of the blocking variable was first assessed in an analysis including all the crosses; if the factor was found to be significant then it was used to correct the data from each cross, before the final comparisons.

Some outliers were evident in the development time data sets. In embryonic development times these were mostly early hatching eggs, which had probably been retained by their mothers. In contrast, the pupation or eclosion time data sets contained slow developing outliers, which probably suffered accidents (e.g. desiccation by wandering onto the glass surface of the vial or starvation by burrowing too deeply into the agar medium). I excluded the outliers from the analyses. The product-limit empirical distributions of times were estimated and then I removed any individuals occurring beyond intervals greater than 7 standard deviations of the mean time (2-3% of the samples). The parameter estimates and significance tests from the linear models on the trimmed data sets were checked against those of non-parametric alternatives (Kaplan-Meyer product limit estimates and Wilcoxon test) on the full data sets, and were found to be quantitatively very similar (results not shown).

I calculated the Spearman rank correlation coefficients within crosses between egg volume, body weight and development time, correcting the significance tests in each cross by a sequential Bonferroni procedure (Rice 1989).

7.4 Results

The means and 95% confidence limits of the response variables were estimated for each cross, using transformed and corrected data, according to the appropriate error distribution, and then back-transformed to their original form (Figures 7.2-7.9). Details of the comparisons between populations and between crosses are presented in Table 7.4. In the following, L and S refer to the parental populations and L*L, S*S, L*S and S*L refer to the offspring from the crosses.

7.4.1 Egg size and offspring development time and adult weight

In the AU crosses, L females laid significantly larger female eggs than S females (Figure 7.2; Table 7.4). I did a three-way analysis of variance with sex of the eggs, maternal and paternal population as crossed fixed effects. Only maternal population had a significant effect on egg volume ($F_{[1,356]}$=111.29, P<0.001). The remaining main effects and interactions did not significantly affect egg size. In the SA, WS and CN crosses, L females also laid significantly larger eggs than S females (Figure 7.2; Table 7.4).

In the AU, SA and WS sets of crosses, L*L females developed at the same rate as S*S females; in the CN crosses, L*L females took a significantly longer time to develop to adulthood than S*S females. In the AU, SA and CN reciprocal crosses, L*S females developed significantly faster than S*L females. In the WS crosses, L*S females developed faster than S*L females, but the difference was not significant (Figure 7.3; Table 7.4). Laying period had a significant effect on hatching time in all groups of crosses ($P \leq 0.01$).

In all crosses, L*L females were significantly heavier than S*S females. In the CN reciprocal crosses, L*S females were significantly heavier than S*L females. However, in the remaining pairs of reciprocal crosses there were no significant differences in female weight (Figure 7.4; Table 7.4).

Egg volume was not significantly correlated with either pupation time or adult weight in any cross (no consistent trend in sign). In all crosses adult weight was positively correlated to larval development time (corrected probabilities combined over all crosses, $P=0.003$) (results not shown).

7.4.2 Larval competition

Variation in larval competitive ability, sex ratio and female weight was first investigated by fitting linear models to the data from all crosses including the effects of cross, density level and cross by density interaction. Larval competitive ability was found to vary significantly with larval density ($\chi^2_{[3]}=102.4$, $P<0.001$) and cross ($\chi^2_{[3]}=102.4$, $P<0.001$) without a significant interaction ($\chi^2_{[9]}=12.29$, $P>0.1$) (Figure 7.5A). The sex ratio of wild-type flies was not significantly heterogeneous among crosses and density levels (all factors $P>0.05$, results not shown). Female weight varied significantly with larval density ($F_{[3,123]}=231.7$, $P<0.001$) and cross ($F_{[3,123]}=6.40$, $P<0.0001$), with no significant interaction ($F_{[9,114]}=0.77$, $P>0.5$) (Figure 7.5B). To compare the larval competitive abilities and adult weights among populations and among reciprocal crosses, I fitted separate models with the effects of density and cross. L*L larvae were significantly better competitors and the females attained a significantly higher weight than S*S larvae, but the L*S and S*L crosses did not differ significantly in either larval competitive ability or female dry weight (Table 7.4).

7.4.3 Egg and hatchling weight

The variation in these traits was identical to that in egg volume. L females laid significantly heavier eggs, that hatched into heavier larvae, compared to S females (Figure 7.6; Table 7.4). The eggs and larvae that survived to adulthood (91%) showed no difference in sex ratio among crosses ($P>0.2$).

7.4.4 Embryonic development time and egg viability

L*L eggs took significantly longer to hatch than S*S eggs, whereas L*S eggs hatched significantly faster than S*L eggs (Figure 7.7A; Table 7.4). Laying period had a significant effect on hatching time ($\chi^2_{[7]}$=29.06, P<0.001). I found no evidence for sexual dimorphism for hatching time (results not shown).

L*L eggs were significantly less viable than S*S eggs, but L*S eggs were significantly more viable than S*L eggs (Figure 7.7B; Table 7.4). The hatched larvae that survived to adulthood (88%) showed no difference in sex ratio among crosses (P>0.4), suggesting that the differences were not caused by differential mortality of one sex.

7.4.5 Larval feeding rate

L*L first instar larvae fed at the same rate as S*S larvae. However, L*S larvae showed a significantly higher feeding rate than S*L larvae (Figure 7.8; Table 7.4). Measurement group (i.e. time of the day) had a highly significant effect on larval feeding rate ($F_{[11,225]}$=32.32, P<0.001). The experimental larvae that survived to adulthood (94%) showed no difference in sex ratio among crosses (P>0.1) suggesting that the differences among the reciprocals were not caused by differences in feeding rate between males in each cross.

7.4.6 Offspring egg size

The egg volumes of each female in each generation were averaged for analyses. In the parental generation, L females laid significantly larger eggs than S females (Figure 7.9A; Table 7.4). In the offspring, L*L females laid significantly larger eggs than S*S females, but the females from the reciprocal crosses laid eggs of similar size (Figure 7.9B; Table 7.4).

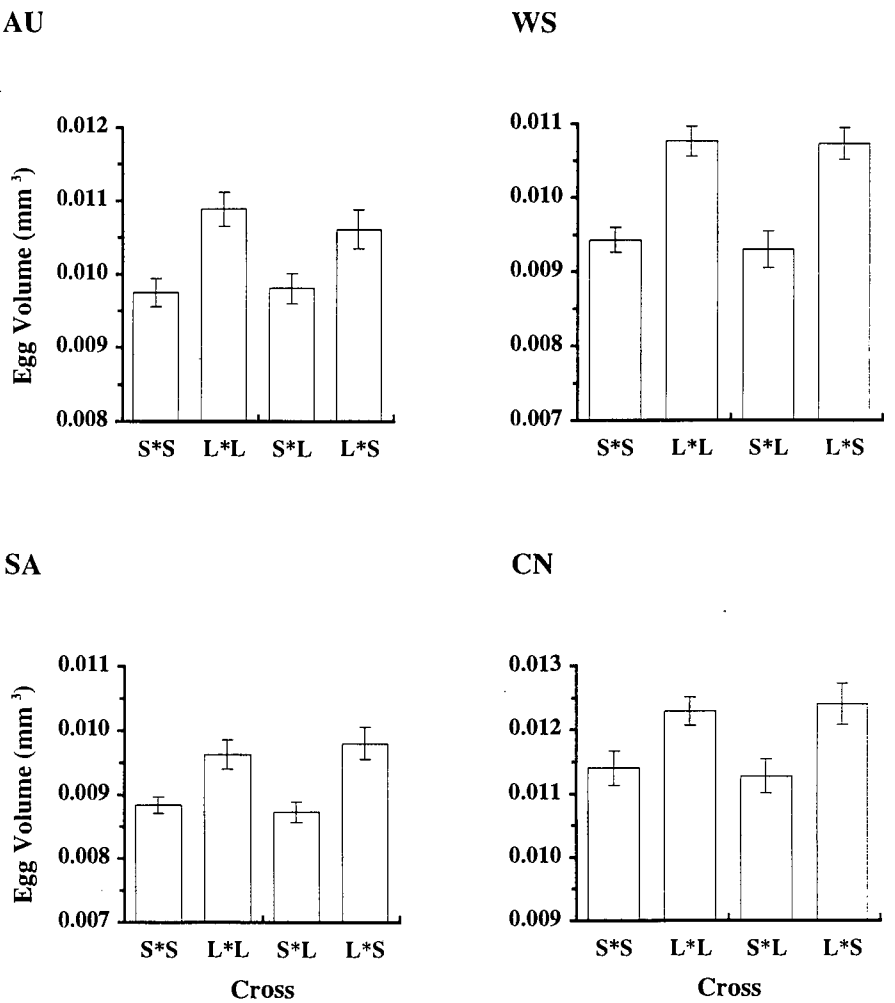

Figure 7.2 Volume of the eggs from crosses within and among 4 different pairs of populations (AU, Australian, female eggs only; SA, South American; WS, wing size selection; CN, cell number selection). Values are means and 95% confidence limits. Notation in crosses: female*male population.

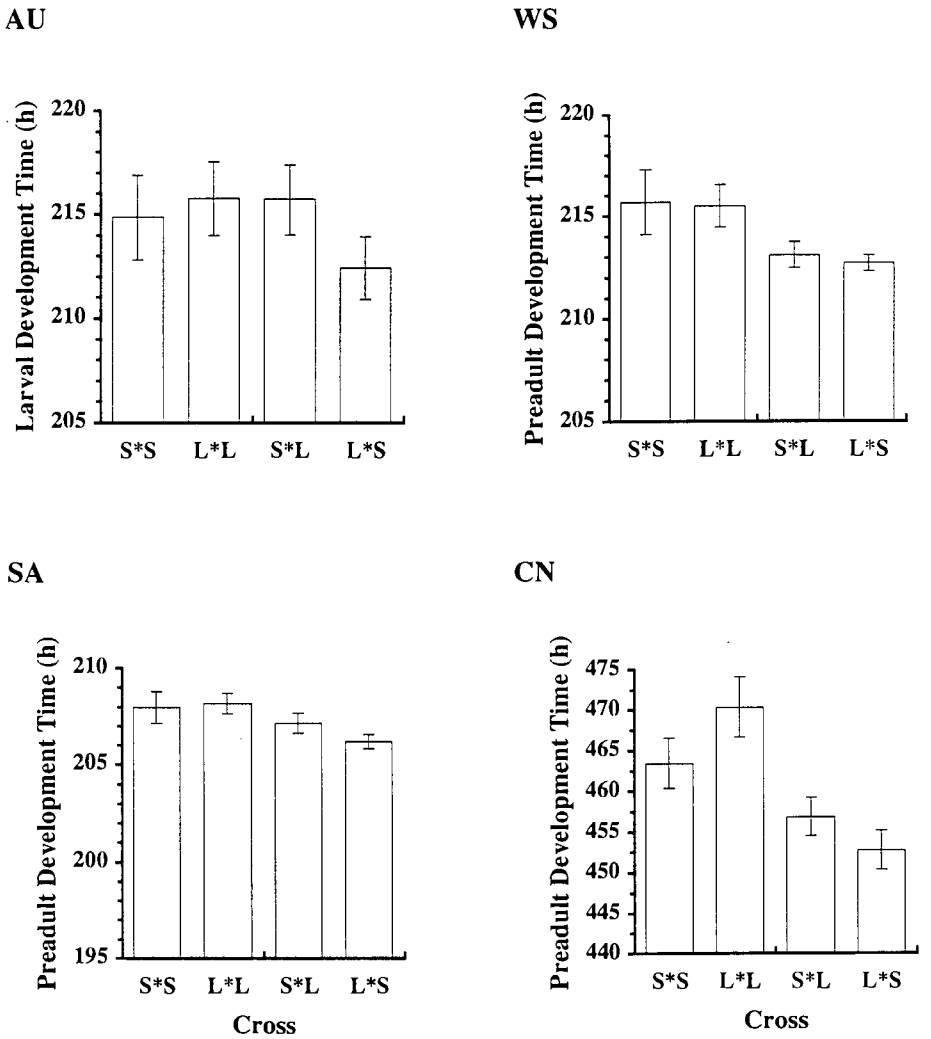

Figure 7.3 Development time of female offspring from crosses within and among 4 different pairs of populations (AU, Australian; SA, South American; WS, wing size selection; CN, cell number selection). Values are means and 95% confidence limits estimated by maximum likelihood using a logistic error distribution. Notation in crosses: female*male population.

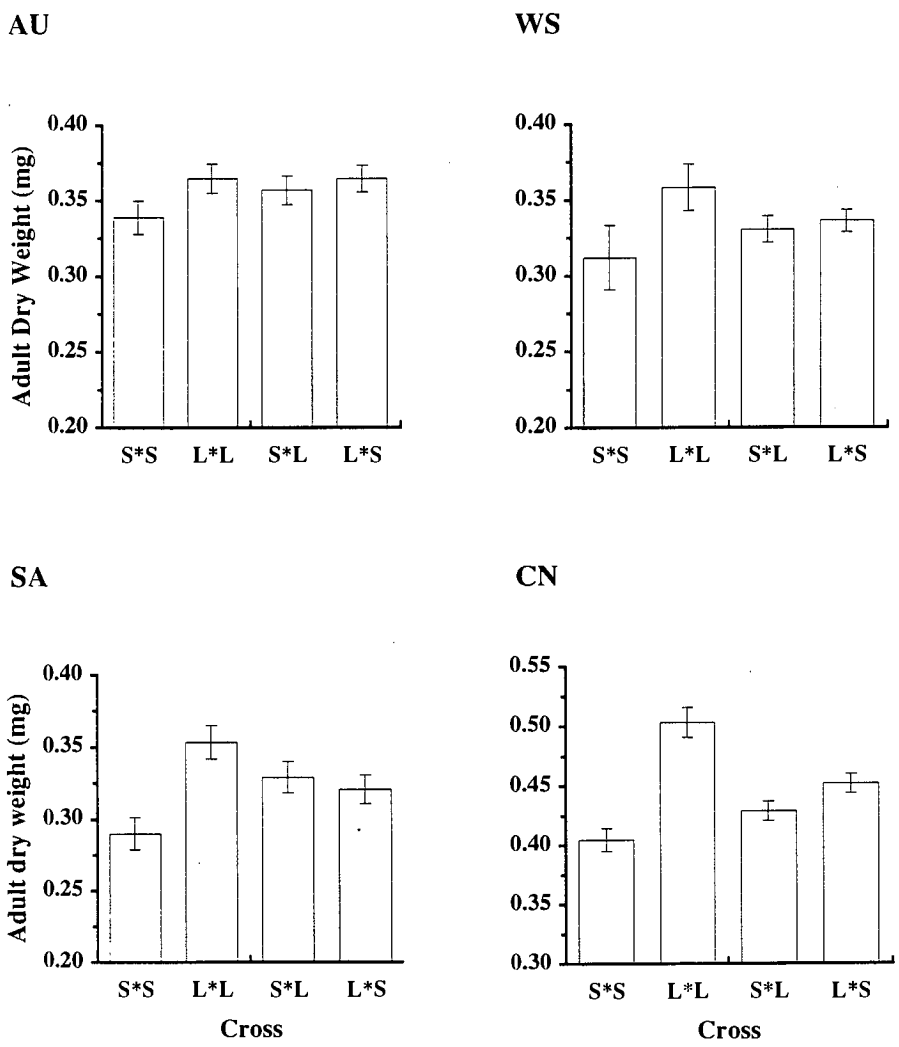

Figure 7.4 Dry weight of female offspring from crosses within and among 4 different pairs of populations (AU, Australian; SA, South American; WS, wing size selection; CN, cell number selection). Values are means and 95% confidence limits. Notation in crosses: female*male population.

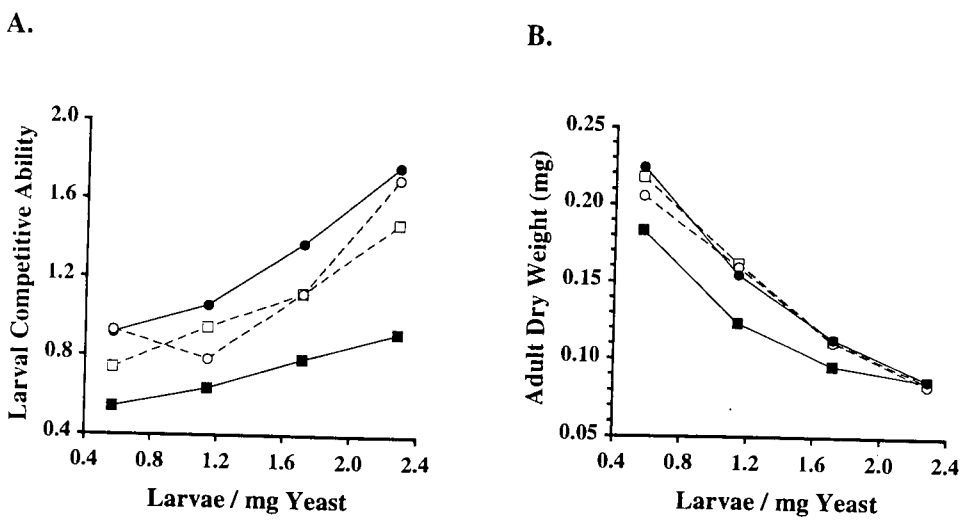

Figure 7.5 Larval competition experiment with offspring from crosses within and among the SA populations against a se mutant strain. (A) Larval competitive ability of wild-type against se estimated by a generalized linear model with a binomial error distribution. (B) Dry weight of wild-type females. Values are geometric means. Notation in crosses: female*male population. L*L, ■ S*S, □ L*S, and ○ S*L.

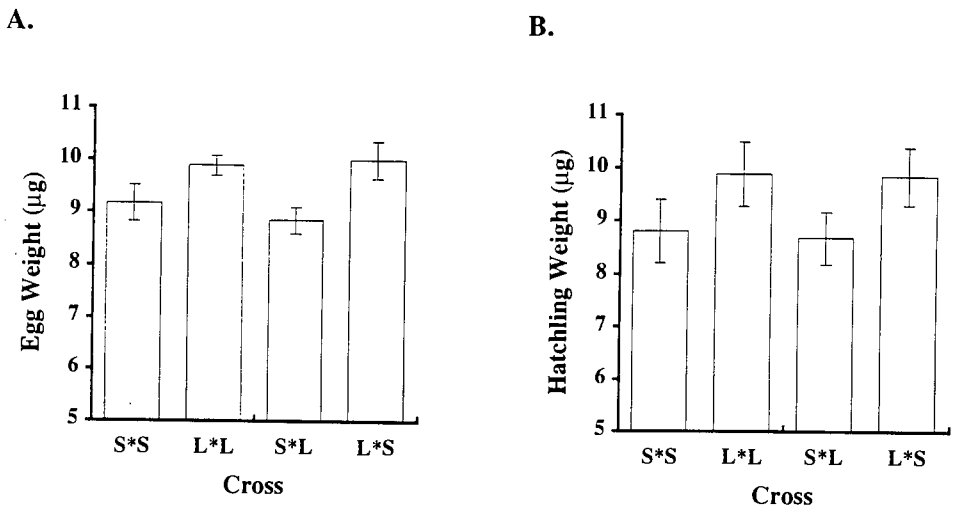

Figure 7.6 Weight of eggs (A) and first instar larvae (B) from crosses within and among the SA populations. Values are means and 95% confidence limits. Notation in crosses: female*male population.

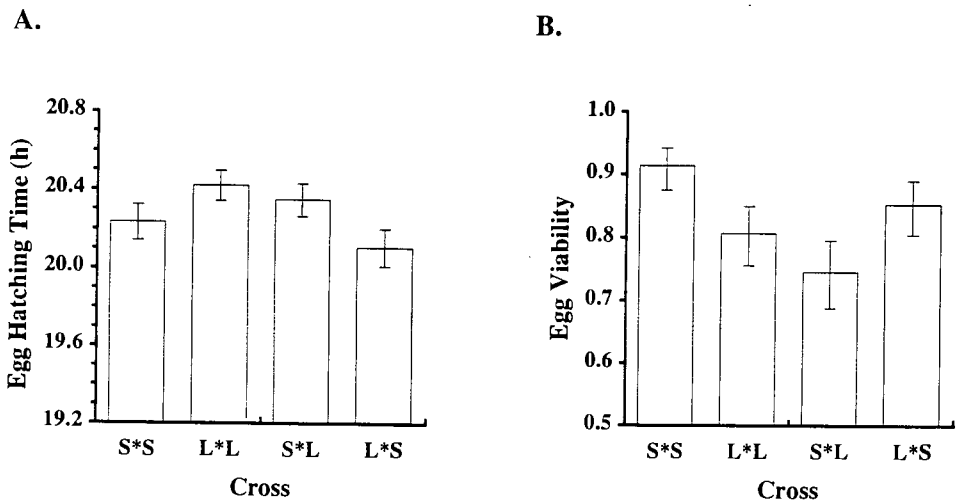

Figure 7.7 Hatching time (A) and viability (B) of eggs laid by crosses within and among the SA populations. Values are means and 95% confidence limits estimated by maximum likelihood using (A) logistic and (B) binomial error distributions. Notation in crosses: female*male population.

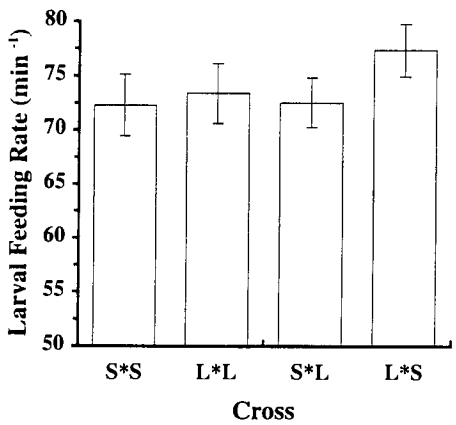

Figure 7.8 Larval feeding rate of first instar larvae from crosses within and among the SA populations. Values are means and 95% confidence limits. Notation in crosses: female*male population.

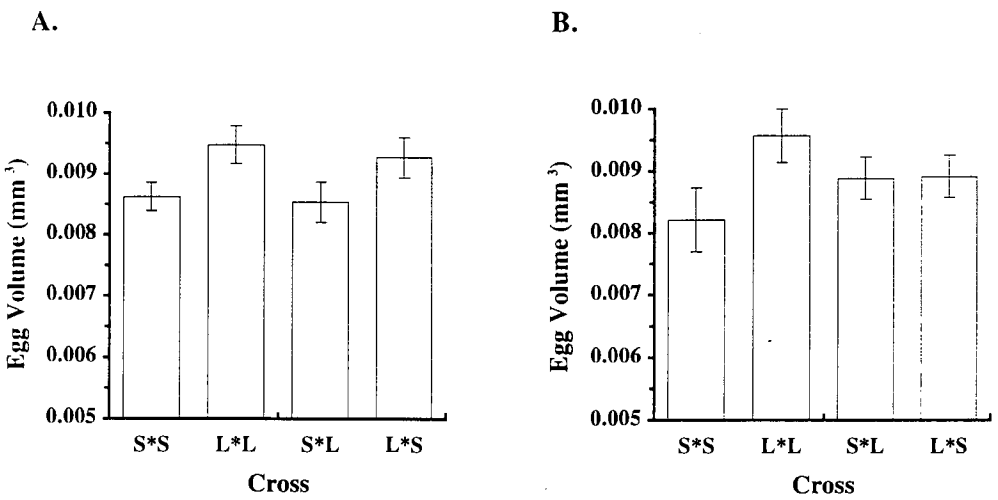

Figure 7.9 Volumes of eggs from crosses within and among the SA populations (A) and by the offspring from those crosses (B) . Values are means and 95% confidence limits. Notation in crosses: female*male population.

Table 7.4 Comparisons of offspring life history characters between populations and between reciprocal crosses. Generalized linear models with normal (N), logistic (L) or binomial (B) error distributions were fitted. Significance tests were done by t -, F - or likelihood ratio χ^2 tests. Δ is the magnitude of the difference (L*L – S*S or L*S – S*L) expressed as a percentage of the trait mean (values of Δ were replaced by zeroes when P >0.3) and n is the sample size.

					Populations (L*L – S*S)			Crosses (L*S – S*L)		
Character		Lines	Errors	Test	Δ	n	Statistic	Δ	n	Statistic
Parental egg volume	†	AU	N	t	11.0	87	7.60 ***	7.9	96	4.88 ***
Parental egg volume		SA	N	t	8.5	100	6.17 ***	11.6	100	7.42 ***
Parental egg volume		WS	N	t	13.2	100	10.12 ***	14.3	100	8.74 ***
Parental egg volume		CN	N	t	7.5	100	5.16 ***	9.5	100	5.45 ***
Parental egg weight		SA	N	t	7.6	20	4.14 ***	12.3	20	6.02 ***
Embryonic development time		SA	L	χ^2	0.9	469	8.92 **	-1.2	413	13.00 ***
Egg hatchability		SA	B	χ^2	-12.4	552	7.80 ***	13.4	525	7.10 ***
Hatchling weight		SA	N	t	11.6	24	2.80 *	12.6	24	3.48 **
Larval feeding rate		SA	N	t	0	120	.56	6.4	120	2.91 ***
Larval competitive ability		SA	B	χ^2	54.8	104	64.04 ***	0	102	.75

Larval development time	†	AU	L	χ^2	0	86	.40	-1.5	95	8.21 **
Preadult development time	†	SA	L	χ^2	0	86	.34	-0.5	85	7.88 **
Preadult development time	†	WS	L	χ^2	0	79	.02	0	108	1.04
Preadult development time	†	CN	L	χ^2	1.5	135	7.44 **	-0.9	169	5.39 *
Adult weight	†	AU	N	*t*	7.3	84	3.53 ***	2.2	89	1.21
Adult weight	†	SA	N	*t*	19.7	74	7.69 ***	-2.6	80	1.14
Adult weight	†	WS	N	*t*	13.8	73	3.69 ***	0	103	.95
Adult weight	†	CN	N	*t*	21.7	106	12.60 ***	5.3	150	3.96 ***
Adult weight in competition	†	SA	N	*F*	8.3	45	13.71 ***	0	85	.65
Offspring egg volume	†	SA	N	*t*	15.1	24	4.15 ***	0	26	.15

† Female offspring only. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

7.5 Discussion

My experiments showed that egg size had positive effects on embryonic viability and development rate, hatchling weight, larval feeding rate, and larval and preadult development rates. Since the environments experienced by the females from each pair of crossed lines and by their offspring were equivalent and replicated, we detected maternal genetic effects of egg size. No consistent effects of egg size were detected on larval competitive ability, adult weight or offspring egg size. In general, the maternal effects of egg size could not be predicted from the patterns of covariation among parental populations in any of the characters. This meant that the differences in life history among populations were mostly determined by zygotic effects. Phenotypic correlations within crosses were also inadequate tests of the effects of egg size, presumably due to confounding effects of measurement error and variation in maternal condition within crosses. This proves that my method is a good way of disentangling the causes and consequences of variation in egg size while controlling for zygotic genetic effects, unlike phenotypic correlations within and among populations or artificial selection.

Egg volume and weight, and hatchling weight (2-5 hours) were found to be determined by maternal genotype, without any noticeable effect of paternal genotype. This is not surprising since, in *Drosophila,* the egg is formed prior to fertilisation. However, I could not detect a consistent or significant influence on adult weight in 3 out of 4 crosses. Thus, the maternal effect on offspring size was overcome by zygotic effects. This is supported by Bakker's (1969) observation that, although the maternal effect on offspring size was detectable up to 72 hours, the effect decreased with age: the difference between the reciprocal crosses relative to that between the parental populations was consecutively 23%, 16% and 12%, at 48 hours, 52 hours and 72 hours, respectively. Evidence that the maternal component of variation in offspring size declined with age has been found in other animals by quantitative genetic analyses (Newman 1988; Cheverud et al. 1983; Atchley et al. 1980). The other adult trait, offspring egg size, was also determined by zygotic effects but not by maternal inheritance. An absence of maternal effects of egg size on offspring egg size was also observed in *D. melanogaster* (Warren 1924) and other invertebrates (Allan 1984; Levin et al. 1991).

I observed positive effects of egg size on egg hatchability, rate of embryonic development and feeding rate of young hatchlings (6-12 hours). Since I do not know the sex of the individuals involved, it is possible that X-chromosome differences between the males in the reciprocal crosses may have affected the variation in these traits. However, comparisons among the parental populations suggest that zygotic differences

among males actually counteracted the effect of egg size for all traits: egg viability and development rate in the parental populations were negatively related to egg size, and larval feeding rate did not differ among the parental populations. I also did not find any evidence for differential sampling of males in the reciprocal crosses or for sexual dimorphism in embryonic development time. Therefore, the differences among reciprocal crosses in egg viability and hatching time and larval feeding rate were probably caused by the differences in egg size.

Egg size consistently showed a positive effect on development rate (in the same direction in 4 crosses, but non-significant in one). The repeatability of the result in independent crosses, regardless of the covariation between the traits in the parental populations, provides good evidence that the observed differences were caused by the maternal effect of egg size, rather than by random environmental variation (Stone 1947). Further corroboration was provided by David (1961) who found no differences in preadult development rate between reciprocal crosses of populations that differed in that trait but not in egg size. In the SA crosses, the difference in embryonic development time between the reciprocal crosses (14±8min) could explain the maternal genetic effect of egg size on preadult development time (56±38min). However, this result could reflect insuficient power in my experiments to test this relationship. The effect of egg size on hatchling size and feeding rate can probably confer an additional advantage to the developing larva.

My results revealed that relatively large differences in egg size (8-14%) can have a small but significant effect on preadult development rate (~0.5%). In *D. melanogaster*, the effect of egg size on development time probably has a magnitude comparable to that of nuclear genetic effects in a single generation. The response to artificial selection for fast development at 25°C produced a mean divergence of ~0.6% per generation (14 generations) relative to the controls (Zwaan 1995; Nunney 1996). Furthermore, in a mutation accumulation experiment Mukai and Yamazaki (1971) showed that mutations in homozygous condition on the II chromosome caused an average delay of ~0.8% in eclosion time, at 25°C and high larval densities. It should also be noted that the effect of egg size on development time was probably underestimated in my study, since growth in isolation with food provided *ad libitum* increases the rate of development and reduces differences among genotypes. For example, the difference among the AU populations developing at low larval density (4 larvae/ml yeasted food) was 0.9% compared to 0.4% in isolation (James and Partridge 1995), and that between the CN populations at high density (40 larvae/ml yeasted food) was 4.7% compared to 1.5% (J. McCabe and L. Partridge, unpublished manuscript).

The effect of egg size on larval growth rate and feeding rate might have been expected to influence larval competitive ability (Bakker 1961, 1969; Burnet et al. 1977). It is possible that the effects of egg size on development rate and larval feeding rate were too small to have an impact on larval competitive ability. For example, larval competitive ability was found to respond to artificial selection on adult thorax length (Santos et al. 1992b, 1994; Partridge and Fowler 1993), but the divergence in preadult development time observed in that study was of ~4% (Partridge and Fowler 1993; Santos et al. 1994). The maternal effect of egg size on development rate could also have ecological significance. An increase in egg size might bring forward age at first reproduction, which could be favoured in periods of population expansion or of high adult mortality (Lewontin 1965; Charlesworth 1994).

My experiments do not provide direct evidence on the proximal mechanisms of maternal inheritance involved. The maternal inheritance observed could have a nutritional origin. Larger *Drosophila* eggs could have more nutrients, as has been shown in other invertebrates (e.g. Capinera et al. 1977; Clarke et al. 1991; Clarke and Gore 1992; Guisande and Gliwicz 1992; Bridges 1993). This is likely since larger eggs developed faster but did not show any reduction in weight at hatching. The energetic content of the eggs could have direct effects not only on the developing embryo but also on the larvae. In some insects, embryos developing in larger eggs have larger yolk reserves available during development and also retain a greater amount of yolk for consumption after embryonic diapause (Capinera et al. 1977) or hatching (Wellington 1965). The maternal effects of egg size are not caused by maternal body size, since the maternal effect was observed in crosses regardless of whether adult body size and egg size were positively (this study) or negatively (Bakker 1969) associated among parental lines.

In *Drosophila*, several agents can cause cytoplasmic incompatibility. Hoffmann and collaborators (1994) found that Australian populations of *D. melanogaster* were polymorphic for an infection by a *Wolbachia* microorganism that reduced egg hatchability by 15-30% in crosses of uninfected females to infected males. However, since my L population came from a region of low frequency of infection (15%) compared to the S population (85%), I would expect that S*L offspring would be fitter than L*S offspring (Hoffmann et al. 1994). Also, it is highly unlikely that cytoplasmic incompatibility agents with similar effects on preadult development time occurred in independently derived pairs of populations, including populations selected from the same base stock.

Despite my evidence for a selective advantage of larger eggs, egg size is probably under stabilizing selection in *D. melanogaster*, since the trait can respond to artificial

selection in both directions (Bell et al. 1955; Parsons 1964). I did not find any evidence for a fitness trade-off of egg size during the preadult period, and a trade-off between egg size and adult fitness is unlikely because adult traits are less susceptible to maternal effects (Tables 7.2 and 7.3). Egg size could be constrained by a negative genetic correlation with female fecundity, but no evidence for such a trade-off appears to have been sought in *Drosophila*. Alternatively, egg size might be constrained by additive genetic correlations with other offspring traits. This would happen if there was a negative covariation between the nuclear genetic effects of a fitness component and its effects via egg size (Riska et al. 1985; Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990). Maternal adult body size could constrain the evolution of egg size in such a way, because selection for large wing size can increase egg size (R. Azevedo, J. McCabe, B. Zwaan and L. Partridge, unpublished results) but there is strong evidence for a negative genetic correlation between adult body size and preadult development rate (Hillesheim and Stearns 1991; Partridge and Fowler 1993; Zwaan et al. 1995; Nunney 1996).

The effects of egg size on development time could be important in the thermal evolution of development time, since large egg size and fast development time appear to be favoured at cold temperatures in *Drosophila melanogaster* (Section 1.6.2; Chapter 6). However, egg size does not seem to play an important role in the thermal evolution of body size.

8

General Discussion

8.1 Developmental Effects of Temperature

The pattern of phenotypic plasticity of morphological and life-history characters in response to temperature to emerge from a variety of studies in *D. melanogaster* is clear (Table 8.1). Increasing temperature during development, decreased size of the eggs laid by females (as did temperature of egg laying, Chapter 6), increased preadult development and growth rates and growth efficiency, and decreased adult body size and cell size. Wing-thorax size ratio and relative wing length and width also decreased with increasing temperature during development. One obvious question that results from this is whether the phenotypic plasticity of body size in response to temperature is adaptive: or is larger body size especially advantageous at cold temperatures? As noted before, the similarity between the cellular bases of developmental and evolutionary responses to temperature suggests that a common mechanism could be involved in both processes which would imply adaptive phenotypic plasticity. However there is little direct evidence for this hypothesis.

Phenotypic plasticity in response to temperature may generate variation in fitness at different temperatures and this effect could involve body size. For example, territorial success (control for a food/oviposition resource) was greater in males reared at 25°C (small) than in those reared at 18°C (large), irrespective of the temperature at which they were tested (18°C or 27°C) (Zamudio et al. 1995). In contrast, flies that developed at 15°C did show a higher flight power output at that temperature than flies that developed at 22°C or 30°C, but not at the warmer temperatures (Barnes and Laurie-Ahlberg 1986). These two studies suggest that large body size may not be advantageous at all temperatures, but they have the problem that body size was not manipulated independently of other morphological and physiological traits with potential effects on fitness (e.g. wing-thorax size ratio in the case of flight ability). Likewise, thermal selection experiments indicate that large adult body size does not confer an advantage at all temperatures: adult fitness components changed so that populations selected at 16.5°C were fitter at 16.5°C than those selected at 25°C, but populations selected at 25°C were fitter at 25°C than those selected at 16.5°C, despite their smaller size (Partridge et al.

1995). In a more controlled approach, lines that were artificially selected for increased thorax length at 25°C were tested at 18°, 25° and 29°C for larval survival (Partridge and Fowler 1993). The results, again, showed no evidence for an unconditional advantage of large body size at any temperature: larvae from the large selected regime showed a decline in larval viability, and the disadvantage did not change with temperature.

Table 8.1 Summary of the developmental and evolutionary responses to low temperatures of morphological and life history traits in *Drosophila melanogaster*.

		Evolution	
Character	Development	Laboratory	Nature
Egg size	+	+	+
Development rate	–	0 / +	+
Growth rate	–	+	?
Growth efficiency	–	+	+
Body size	+	+	+
Wing-thorax size ratio	+	0 / +	+
Relative wing length	+	?	0
Relative wing width	+	?	+
Cell size	+	+	[+]
Cell number	[+]	0	+

Comparisons between the effects of development at low temperatures (Partridge et al. 1994a, 1994b; Neat et al. 1995; Crill et al. 1996; Chapters 4 and 5) and the outcomes of evolution at low temperatures in the laboratory (Partridge et al. 1994a, 1994b; Neat et al. 1995; James and Partridge 1995; Van't Land et al., unpubl.; Chapter 6) and in the field (James et al. 1995; James and Partridge 1995; Chapters 3-6). Entries indicate an increase (+), decrease (–), or no response (0), in the trait. Entries in square brackets indicate weak response. Multiple entries indicate contradictory studies. Question marks indicate that no evidence exists.

The adaptive nature of phenotypic plasticity in relation to temperature could be inferred from the patterns of response of plasticity itself to natural and artificial selection. However, no evidence that phenotypic plasticity responded to thermal selection was found in studies of latitudinal variation (Coyne and Beecham 1987; Chapter 4) and thermal evolution in the laboratory (Partridge et al. 1994a) in *D. melanogaster*. A more explicit test would consist in studying the correlated responses in fitness components to artificial selection for different levels of plasticity. This could be done in *D. melanogaster*, since phenotypic plasticity of body size in response to temperature has been shown to be heritable and to respond to artificial selection (Scheiner and Lyman 1989, 1991).

8.2 Evolutionary Response to Temperature

Evolutionary responses to selection at different temperatures are also well known for *Drosophila melanogaster* (Table 8.1). Although they are analogous to the developmental responses to temperature for egg size, wing size and cell size (at least in the laboratory) and other morphological traits, they differ markedly for development and growth rates and growth efficiency.

Although selection on adult body size could explain some of the patterns generated by thermal evolution (e.g. Chapters 5 and 6), it is not clear what selection pressures would favour large body size at low temperatures. Resistance to high temperature and to desiccation increase with body size, but this would predict a different pattern of latitudinal variation in body size, i.e. selection for large body size near the equator and not at high latitudes (Tantawy and Mallah 1961; Levins 1969). Resistance to high temperatures, by itself, is also an unlikely selection force to account for thermal evolution since Oudman and colleagues (1988) did not find a relationship between body size and the ability to withstand high temperatures, when humidity was kept high. Also, the hypothesis that large body size is adaptive at low temperatures since it reduces the rate of heat exchange, is an unlikely force in *Drosophila*, where surface effects predominate, and the impact of metabolic heat on the regulation of body temperature is expected to be negligible (Stevenson 1985). Body size could also be favoured at high latitudes by selection on flight performance, if dispersal was more important in temperate habitats, compared to tropical ones, due to environmental unpredictability: larger flies could be better able to resist starvation and fatigue during flight (Roff 1977; Dingle et al. 1980). However, this cannot adequately explain thermal evolution of body size in the laboratory, since selection for dispersal ability is absent from cage environments.

The occurrence of rapid larval development at higher latitudes and in laboratory evolution at cold temperatures suggests that rates of larval development and/or growth are important targets of thermal selection. This is especially likely for two reasons: on the one hand, at a single temperature, there seems to be strong directional selection for fast growth (Clarke et al. 1961; Robertson 1963; Sewell et al. 1975; Burnet et al. 1977; Partridge and Fowler 1993) and, on the other, selection on body size is expected to decrease larval development and growth rates (Partridge and Fowler 1993). In *D. melanogaster*, body size and development rate were negatively correlated among populations, but the association was not significant when the effect of latitude was removed (James et al. 1995; Chapter 6, Table 6.3), arguing that the cline in one character did not result from a correlated response to selection on the other character (i.e. that. different genes were responsible for the geographic variation in the two traits). The observed patterns might be explained either by differential selection on each character at different latitudes, or by selection on a third character correlated with both development time and body size causing a variable response at different latitudes.

Cooler environments may be permissive or selective of the evolution of more rapid larval growth, and that this may be responsible for the evolutionary change in adult body size in response to temperature. Indeed, growth efficiency has been shown to increase under laboratory thermal selection (Neat et al. 1995). However, it is not clear why lower temperatures should select for more efficient growth, since growth efficiency should be advantageous at all temperatures. Some suggestions have been made for possible trade-offs across temperatures between growth efficiency and other activities (e.g. somatic maintenance, detoxification) but, at present, these proposals make assumptions which lack real empirical or theoretical support (Atkinson 1994; Partridge and French 1996). It would be interesting to know if the geographic populations differ in other traits related to growth (e.g. critical size for pupariation, larval feeding rate, growth efficiency).

Density dependent selection could be a component in the process of thermal selection. Populations from temperate regions, with seasonal environments, undergo frequent episodes of population expansion, which are expected to favour early maturation (Lewontin 1965; Charlesworth 1994). Although we do not know the precise effects of temperature on levels of intra- and inter-specific competition in nature, there is some indication that, in *Drosophila* populations, productivity and densities increase with temperature (Birch et al. 1963; Davis et al. 1995; Partridge et al. 1995). Therefore, tropical populations of *Drosophila* may spend more time at carrying capacity, subjected to density-dependent population regulation and high levels of competition (David and Capy 1982). The selection pressures on the life history in such conditions will depend

on the impact of mortality at different ages (Charlesworth 1994). The effects of an equal increase in mortality at each age class are similar to those of a high rate of population growth. However, when mortality is especially high for juvenile stages, selection for early breeding will be relaxed. These predictions are generally confirmed by laboratory density-dependent selection experiments. Evolution at high larval densities has been shown to decrease body size and larval preadult development rate and feeding rate, but without increasing growth efficiency (Mueller 1988, 1990; Bierbaum et al. 1989; Roper et al. 1996). Therefore, selection at low temperatures could permit the evolution of higher growth efficiency if it reduced the impact of larval competition and the need for the metabolically costly activities associated with high competitiveness (Partridge and French 1996). This hypothesis could be tested in the laboratory by comparing the intensity of larval competition in population cages and controlled density cultures, kept at different temperatures. The importance of density-dependent selection in thermal evolution should also be investigated by keeping populations at different temperature and densities.

8.3 Maternal Effects and Thermal Evolution

The experiments done in much of my laboratory work on populations under thermal selection (geographic and laboratory lines), were designed so as to minimize the impact of maternal effects on the traits to be examined (Section 2.2.5). Although this "washing out" of maternal effects has been recently criticised by Bernardo (1996a), it reflects the experimentalist's effort to control and simplify a complex biological system. In fact, although maternal effects have been shown to influence the evolution of other traits, they are usually difficult to control and predict (see Chapter 7 for references). However, in the light of recent work, it is clear that an important component of research into *Drosophila* thermal evolution should be the study of maternal effects (e.g. Huey et al. 1995; Crill et al. 1996; Watson and Hoffman 1996; Chapter 7).

One of the aspects that has been largely neglected in discussions of thermal adaptation has been the possible impact of maternal effects of temperature on body size and larval life history characters. Maternal effects could be important in thermal evolution since they could act as cross-generational phenotypic plasticity (Roach and Wulff 1987; Bernardo 1996a). I found that egg size has important maternal genetic effects on offspring fitness (Chapter 7), and that females laid eggs of different sizes, depending on the temperature at which they developed and laid their eggs (Chapter 6). In addition, Crill and collaborators have reported that *D. melanogaster* females that developed at 18°C laid larger eggs at 22°C than females that developed at 25°C (Crill et

the same study, it was also shown that the offspring of females that developed at the lower temperature were heavier and had lower knock-down temperatures than those of females that developed at the higher temperature (Crill et al. 1996).

Egg size responded to thermal selection, with larger eggs evolving at lower temperatures, both in geographic and laboratory populations (Chapter 6; Table 8.1). Egg size itself could be an important target of thermal selection but, at present, there is little evidence to accept this hypothesis. Selection for increased egg size has been shown to increase body size as a correlated response, but the response in growth rate has not been studied (see Chapter 7 for references). Alternatively, egg size may have evolved as a correlated response to selection on other traits (e.g. growth efficiency). This would be possible since egg size has been found to increase in response to artificial selection for both large body size and fast development rate (see Chapter 7 for references). In addition, through its maternal genetic effects, egg size could itself potentiate the responses in hatching time, development time and larval feeding rate during thermal selection. The possible role of temperature maternal effects of egg size in thermal evolution requires further study. It would be interesting to know if egg size responded to selection for increased growth efficiency and if it had a maternal genetic effect on that trait (Chapter 7).

8.4 The Cellular Basis of Body Size

Cell size has been shown to evolve in response to thermal selection in laboratory populations and, less strongly, in geographic populations (Chapters 3 and 4; Table 8.1). As mentioned before, this is unexpected if body size is the principal target of thermal selection (Section 1.6.1) and could, in turn, indicate that cell size itself is a target of thermal selection. The occurrence of thermal selection on cell size in the wing would be evolutionarily interesting, because there have been suggestions that cell size is under stabilising selection. In *Drosophila* and other organisms, changes in genome size resulted in correlated changes in cell size (e.g. Gates 1909; Dobzhansky 1929; Held 1979; Cavalier Smith 1985; Nurse 1985). However, some taxonomically diverse, long-established polyploids have shown an evolutionary reversion of cell size to the ancestral value (Nurse 1985). Whether cell size itself is adaptive at different temperatures could be investigated by selecting on cell size while keeping wing size constant.

The fact that thermal evolution of wing size in geographic populations showed a predominant effect of cell number could mean that thermal selection in nature targeted wing size more intensely. This hypothesis could also explain why thermal evolution in nature has led to a weaker differentiation in preadult development time: laboratory

selected lines showed a higher divergence in larval development time, when measured at 16.5°C (~9%), than the extremes of the cline when measured at 18°C (~6%) (James and Partridge 1995); also, a recently studied South American cline showed no latitudinal differentiation in preadult development rate (Section 2.1.2). Alternatively, cell number could have evolved as a correlated response to selection on wing shape, since cell proliferation and differentiation may be genetically associated (Section 1.3).

It would be interesting to investigate the role of cell size in differentiation among *Drosophila* species with different thermal habitats. Stevenson and colleagues (1995) have shown that species of Hawaiian *Drosophila* differ in both cell size and cell number in the eyes, legs and wings (but see below). However, a detailed comparative analysis of the cellular basis of body size evolution in drosophilids has not been done.

The wing intervein regions used to estimate cell size may not be representative of the cellular constitution of other tissues (even in the epidermis), since they consist of large, flattened cells whose major function is to provide an aerodynamic surface. In addition, the flattening of epidermal cells is variable so that cell area may not be a reliable estimator of cell volume (Kuo and Larsen 1987). The study of the cellular basis in other *Drosophila* tissues would, therefore, be interesting in the context of the thermal evolution of body size.

Drosophila larval cells are not suitable for investigation since they do not divide (Section 1.2). Other adult tissues have been studied for their cellular bases. The fact that most epidermal cells die after secreting the adult cuticle makes this technically difficult. One possibility is to estimate the density of pupal cells directly by histologic procedures (e.g. adipose tissue, Butterworth and Bodenstein (1968); leg epidermis, Held 1979). Alternatively, one might find other systems where cell size can be estimated indirectly from adult morphology. For example, in the femur there are trichomes which probably also correspond to individual cells (D. Stern, personal communication). Held (1979) proposed another system whereby, in the second leg basitarsus, cell size correlated with the number of bristles and that cell number correlated with the spacing of bristles. However, this hypothesis, although developmentally plausible, was only qualitatively supported by analyses of mutants with large effects on body size; his statistical analysis of small changes in body size in response to manipulations of larval nutrition was flawed and did not provide good evidence for the proposed relationships. Held's system should be further investigated in relation to thermal evolution. A similar argument applies to the suggestion of a relationship between cell number and bristle number in the abdomen (e.g. Busturia et al. 1994). Another system that could be useful in estimating cell size in the abdomen was described by Madhavan and Madhavan (1979, pp. 25-27): 72h after pupariation, epidermal cells

of the dorsum and ventrum in each segment were arranged in transverse rows so that in every row some cells produced 3-5 cuticular trichomes, which suggests that row spacing is related to cell size. Finally, the sizes of photoreceptors can be estimated from the sizes of the facets of the eye (Stevenson et al. 1995).

To understand more about the developmental mechanisms of body size evolution, it would be interesting to be able to count cell numbers in developing imaginal discs. This would allow the study of the effect of temperature on growth rate and rate of cell proliferation. Unfortunately the methods available at present (e.g. histological analysis, clonal analysis using X-ray-induced mitotic recombinations, cells counts in dissociated discs) are extremely variable and are, therefore, not suited for quantitative studies (Bryant and Simpson 1984; L. Partridge, personal communication).

8.5 The Genetic Basis of Thermal Evolution

The genetic basis of the observed clines in *D. melanogaster* could involve the segregation of the inversion *In(2L)t* which is known to increase in frequency towards the equator in natural populations of *D. melanogaster* from different continents (Inoue et al. 1984; Anderson et al. 1987). This inversion was shown to confer a survival advantage at high temperatures (Van Delden and Kamping 1989) and to slow down development and decrease body size at a range of temperatures (Van Delden and Kamping 1991). It also decreased in unreplicated laboratory evolution at lower temperatures (20° and 25°C) relative to selection at 29.5°C (Van Delden and Kamping 1991).

If we consider the cline in this inversion detected in Australia (Anderson et al. 1987) we would predict that body size would not change much between 15° and 35°S since the inversion frequencies were relatively constant (~20%) and that the decrease in body size would be more marked between 35° and 45° where *In(2L)t* declines markedly in frequency (<10%). This prediction matches the observations qualitatively (Chapter 3). However it is not entirely consistent with the cline in larval development time which showed a different shape from that of body size (James and Partridge 1995).

The causality of these patterns is further complicated by the fact that there are also latitudinal clines in the polymorphisms of 2 enzyme loci (*Adh* and *αGpdh*, with *F* and *S* alleles increasing with latitude respectively) (Oakeshott et al. 1982) which are in linkage disequilibrium with *In(2L)t* (Anderson et al 1987). In controlled experiments with experimental strains containing different combinations of *Adh* and *αGpdh* alleles, but with similar genetic background and lacking the *In(2L)t* inversion, the loci were shown to have significant effects on preadult development time, body weight and resistance to high temperature, and the loci also interacted with each other and with

temperature in complex ways (Oudman et al. 1991, 1992). Therefore it is very difficult, if not impossible, to predict the precise patterns in the clines of different traits directly from the allele and inversion frequencies in natural populations.

Modern techniques of mapping quantitative trait loci (QTLs) can now be used to detect loci of major effect on body size and other life history traits (Tanksley 1993). If such QTLs exist for the life history traits involved in thermal selection, they may be used directly in studies of natural and laboratory populations to provide a more complete understanding of the genetic basis of thermal evolution in *Drosophila*. In particular, such information could help elucidate which traits are the targets of thermal selection.

8.6 Thermoperiod and Photoperiod: Important Selective Agents in Nature?

Constant temperatures and constant photoperiod in the laboratory are not adequate models of natural conditions. The possibility that thermo- and photoperiod might have developmental and evolutionary effects on body size has never been investigated in *D. melanogaster*, and should be a major task for the future. This knowledge would be especially relevant for a proper interpretation of the outcome of laboratory evolution at constant temperatures.

Thermoperiod has been shown to have an environmental effect on preadult development time in *D. melanogaster* (Siddiqui and Barlow 1972) and other insects (Messenger and Flitters 1959; Hagstrum and Hagstrum 1970; Hagstrum and Leach 1973; Behrens et al. 1983; Ochieng'-Odero 1991; Brakefield and Mazzotta 1995); thermoperiod has also been shown to have environmental effects on body size in several insects (Beck 1983b, 1986; Behrens et al. 1983; Ochieng'-Odero 1991) (reviews: Beck 1983a; Ratte 1985). Photoperiod has also been shown to have developmental effects on growth in several insects (Ruberson et al. 1991; Lanciani 1992; Blackenhorn and Fairbairn 1995; Brakefield and Mazzotta 1995; Fantinou et al. 1996) and to interact with temperature and thermoperiod to produce such effects (Beck 1986; Ochieng'-Odero 1991; Corkum and Hanes 1992).

Photoperiod, in relation to season-length, has been proposed as an important agent of selection on life history traits, in insect populations with long generation times (Roff 1980, 1992). At lower latitudes, the amount of time available for growth and reproduction (day·degrees) is extended and, therefore, selection for increased size at maturity is expected, generating a size cline in the opposite direction to those produced by thermal selection (e.g. Masaki 1967; Mousseau and Roff 1989; Blackenhorn and

Fairbairn 1995). Thermoperiod is also a plausible agent of selection on development time in insects (Taylor 1981).

8.7 Challenge for the Future

The patterns generated by thermal evolution of phenotypic plasticity and genetic differentiation in morphological and life history traits require further investigation. Uncovering their genetic and developmental bases should both improve our understanding of phenotypic adaptation and of the effects of temperature on living systems. I have pointed to our inadequate understanding of the roles played in life history evolution by maternal inheritance (Section 8.3), and by spatio-temporal variation in thermo- and photoperiods (Section 8.6). We also require more detailed knowledge of the ecological context of thermal selection in *Drosophila*, particularly with respect to the relationship between food availability, larval density and thermal regime. It is also important to determine whether the *Drosophila* system is an adequate model for thermal evolution in other insects.

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