

# Latitudinal clines in *Drosophila melanogaster*: body size, allozyme frequencies, inversion frequencies, and the insulin-signalling pathway

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## Abstract

Many latitudinal clines exist in *Drosophila melanogaster*: in adult body size, in allele frequency at allozyme loci, and in frequencies of common cosmopolitan inversions. The question is raised whether these latitudinal clines are causally related. This review aims to connect data from two very different fields of study, evolutionary biology and cell biology, in explaining such natural genetic variation in *D. melanogaster* body size and development time. It is argued that adult body size clines, inversion frequency clines, and clines in allele frequency at loci involved in glycolysis and glycogen storage are part of the same adaptive strategy. Selection pressure is expected to differ at opposite ends of the clines. At high latitudes, selection on *D. melanogaster* would favour high larval growth rate at low temperatures, and resource storage in adults to survive winter. At low latitudes selection would favour lower larval critical size to survive crowding, and increased male activity leading to high male reproductive success. Studies of the insulin-signalling pathway in *D. melanogaster* point to the involvement of this pathway in metabolism and adult body size. The genes involved in the insulin-signalling pathway are associated with common cosmopolitan inversions that show latitudinal clines. Each chromosome region connected with a large common cosmopolitan inversion possesses a gene of the insulin transmembrane complex, a gene of the intermediate pathway and a gene of the TOR branch. The hypothesis is presented that temperate *D. melanogaster* populations have a higher frequency of a 'thrifty' genotype corresponding to high insulin level or high signal level, while tropical populations possess a more 'spendthrift' genotype corresponding to low insulin or low signal level.

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## Introduction

Differences in animal body size exist between species, between populations within a species, between individuals within a population, and as a result of different environments. We know much about patterns in the change of body size in evolution, but next to nothing about the genetic and developmental changes that cause size differences between related species (Conlon and Raff 1999). We know much about repeatable differences in body size between *Drosophila melanogaster* populations, and about environ-

mental effects on body size, but the genetic and developmental background has not been sufficiently explored to provide a concise account of the mechanisms leading to those differences. We know body size within populations has a heritable component, but not which genes contribute to such genetic differences in body size. In *D. melanogaster*, not only body size but many other traits, ranging from wing shape to molecular chaperones, vary both within and among populations. A major problem in evolutionary biology is to assess whether all this genetic variation in different traits is distributed independently, or whether some patterns of co-occurring genetic variants in many systems can be discerned. Here we will examine what natural variation occurs on the one hand, and what

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is known about physiological and developmental-genetic mechanisms influencing body size or growth on the other hand. Our aim is to arrive at a working hypothesis for the possible adaptive role and mechanisms of body-size variation in *D. melanogaster* in nature.

### Genetic variation in populations from contrasting climates

#### Body size

*D. melanogaster* adults from temperate populations are larger than *D. melanogaster* adults from tropical populations when raised at the same temperature. This cline in adult body size has been found on most continents where it has been looked for. Possibly the first cline in adult body size to be described was that between Africa and Europe (David 1975). From the outset the cline was shown not only to be a local phenomenon, but worldwide, as flies originating from the island Reunion in the Indian Ocean had a body size that was intermediate to African and European flies, and was on the latitudinal cline. Tropical African flies are small sized; flies from equatorial Congo and from Tanzania are smaller than flies from France (Delpuech *et al.* 1995; Noach *et al.* 1996), Denmark or the Netherlands (Bochdanovits and de Jong 2003c,d). A corresponding cline extends from the Baltic to Central Asia, again leading to larger flies at cooler temperatures (Imasheva *et al.* 1994). A similar latitudinal cline in body size was found along the east coast of North America (Coyne and Beecham 1987), with small-sized flies in the state of Florida and larger-sized flies in Maine. The west coast of North America seems not to have been sampled continuously, but *D. melanogaster* from Costa Rica are smaller than *D. melanogaster* from the state of Washington in the US (Bochdanovits and de Jong 2003a). In Australia a cline in body size is present along the east coast (James *et al.* 1995, 1997), from Queensland to Tasmania. In South America a cline in body size is present but might take a rather sudden step towards the tropics (van't Land *et al.* 1999). The range of climate zones is less in southern Africa, but a body-size cline in *D. melanogaster* is nevertheless present (Capy *et al.* 1993; Gilchrist and Partridge 1999). A latitudinal cline in body size seems not to be present in India or Japan (David *et al.* 1976).

Body size may be measured as fresh weight, wing length, wing area or thorax length. Temperate populations are larger than tropical populations in all these measures, as well as in wing/thorax ratio (Noach *et al.* 1996). Wing/thorax ratio is related to flight capacity: larger wing/thorax ratio translates into better flying capacity (Petavy *et al.* 1997), implying temperate populations would be better flyers. The difference in mean body size between tropical and temperate populations is genetic but differs in genetic detail between continents (Bochdanovits and de Jong 2003a; Gilchrist and Partridge 1999).

#### Development

The larger size in temperate *D. melanogaster* populations has various causes. There is not a single way to reach large size. In the character of choice for many measurements, wing size, a strong latitudinal cline exists that can be due to either a cline in cell size or a cline in cell number. Higher cell number in temperate populations was found in Australia (James *et al.* 1995, 1997), in Europe versus Africa (De Moed *et al.* 1997), and in South America (Zwaan *et al.* 2000). Cell size is smaller too in tropical populations (James *et al.* 1995; De Moed *et al.* 1997). Cell size seems to play a smaller role in the latitudinal cline in wing size than cell number (Zwaan *et al.* 2000). Development time also differs between temperate and tropical populations. Temperate populations often develop faster or grow faster than tropical ones when reared at the same temperature (James and Partridge 1995; De Moed *et al.* 1998). Nevertheless, the correlation between development time and thorax size is not significant over all populations of the Australian cline, the cline for which the most data are available (James *et al.* 1995; Worthen 1996). The pattern of faster growth and larger size in temperate populations and slower growth and smaller size in tropical populations is better substantiated if only the end populations of a cline are taken.

Larvae from Australian populations that had evolved at high latitudes were found to use limited food more efficiently, so that the overall adult body size achieved was larger. The increases in growth efficiency found in populations from high latitudes could explain their increased body size and more rapid development (Robinson and Partridge 2001). Minimum weight necessary for pupation is another growth parameter of populations. After transferring developing larvae from their food to agar, so that they have to try to complete development on stored resource, larval weight at which 50% of the larvae survive to pupate is scored as the minimum weight necessary for pupation. The higher efficiency in temperate populations is accompanied by a higher minimum weight to reach adulthood (De Moed *et al.* 1999; Robinson and Partridge 2001). Interrupted larval growth leads to different use of stored resources. In temperate populations, stored resources are preferentially used to generate a larger adult body, even at some risk to larval survival. In tropical populations, stored resources are allocated to larval survival rather than to larger adult size. In temperate populations the allocation involved glycogen level (Bochdanovits and de Jong 2003d).

#### Larval survival, adult traits

Larval survival is higher in a population from Denmark than in a population from Panama. So is adult longevity when adults are fed (Bochdanovits and de Jong 2003c). Adult longevity was higher in temperate and tropical

Australian populations than in subtropical populations, when overwintering at fairly low outside temperature in Melbourne (Mitrovski and Hoffmann 2001). However, no difference in longevity in overwintering was found between temperate and tropical Australian populations when overwintering outside in temperate Melbourne (Mitrovski and Hoffmann 2001) or in tropical Cairns (Hoffmann *et al.* 2003).

The number of ovarioles is higher in temperate populations than in tropical populations; a similar linear cline in ovariole number is present over all continents (Capy *et al.* 1993). African and French populations had identical optimum temperature for ovariole development (Delpuech *et al.* 1995), indicating higher fecundity in temperate populations at all developmental temperatures.

Mass-specific metabolic rate showed some clinal pattern in the one instance in which it was examined. Metabolic rates of Australian populations increased with latitude when measured at 18°C but not at 25°C (Berrigan and Partridge 1997). In the same study walking speed was slower for the larger temperate flies, in agreement with the earlier study by van Dijken *et al.* (1985) on a West African and a Spanish population.

#### Starvation resistance

Starvation resistance has been found to differ between *Adh* and *a-Gpdh* genotypes (Oudman *et al.* 1994); given that the allele frequencies at these loci show latitudinal variation, one would expect a latitudinal difference in starvation resistance. Indeed, a latitudinal cline for starvation resistance and desiccation resistance is present in Indian populations of *D. melanogaster* (Karan *et al.* 1998). The starvation and desiccation clines go in opposite directions: starvation survival is highest nearest the equator, desiccation survival is highest in the relatively northern populations. In Australia, however, genetic variation in starvation resistance was larger within populations than between populations (Hoffmann *et al.* 2001), and no cline was found. Similarly, no cline for starvation resistance was found in South American populations (Robinson *et al.* 2000).

#### Allozymes

Many enzymes possess several alleles that all reach intermediate frequencies in natural populations: the populations are polymorphic for allozymes. Allozyme frequency clines have been studied for Europe/Africa and North America. Often, the different allozymes prove to differ in enzyme activities. In the enzymes phosphoglucomutase (PGM), alcohol dehydrogenase (ADH), and *a*-glycerophosphate dehydrogenase (glycerol-3-phosphate dehydrogenase, GPDH), the allozyme with higher enzyme activity at lower temperature is found at higher frequency in higher-latitude temperate populations of *D. melanogaster* (Eanes

1999; Verrelli and Eanes 2001b). It is surmised that the higher ADH and *a*-GPDH activities facilitate lipid storage. Higher lipid storage might increase longevity or fecundity. Higher PGM activity is strongly related to higher glycogen content in adult flies (Verrelli and Eanes 2001a). For the enzymes glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD), the allozyme with lower enzyme activity is found at higher frequency at higher latitudes. The coupled *G6pd* and *6Pgd* allele frequencies appear to define a polymorphism for decreasing pentose shunt function with decreasing enzyme activity at increasing latitudes. Increased pentose shunt flux is coupled to increased lipid synthesis and decreased glycogen synthesis (Eanes 1999). Together these polymorphisms in *Pgm*, *G6pd* and *6Pgd* suggest a higher flux to glycogen synthesis in northern populations. Higher glycogen levels are found in temperate populations than in tropical populations. Higher glycogen levels in larvae correspond to larger adult body size, correlating over population mean values (figure 1). In temperate populations larval glycogen is preferentially used to promote adult body size (Bochdanovits and de Jong 2003d). A tendency for increased metabolic storage of glycogen seems to be an adaptation to temperate regions.

#### Microsatellite frequencies

Microsatellites show allelic differences in number of microsatellite repeats, and the repeat frequencies behave as allelic frequencies. Gockel *et al.* (2001) investigated the repeat frequencies at 19 microsatellite loci over the cline in eastern Australia. Five of the 19 microsatellite loci showed a latitudinal cline in frequency: the frequency of a particular repeat number allele showed significant linear regression on latitude. These microsatellite loci are listed with their chromosomal locations in table 1.

#### Inversions

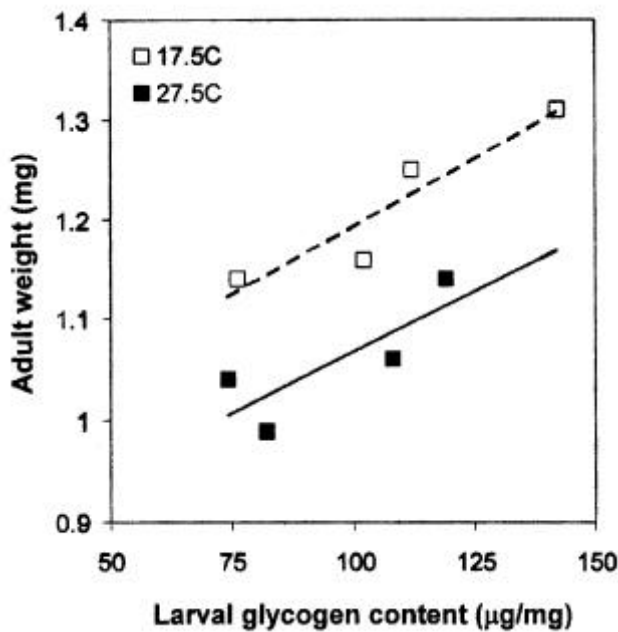
Many chromosome inversions have been found in natural populations (Lemeunier *et al.* 1986; Lemeunier and Aulard 1992). Usually, natural populations are polymorphic for a range of inversions. The inversions have been categorized in classes indicating their occurrence. Seven inversions belong to the class 'common cosmopolitan'. These inversions occur in every population studied for inversions, and are at varying but occasionally high frequencies. Four common cosmopolitan inversions are found in all natural populations (table 2; compare table IX from Lemeunier *et al.* 1986 p. 205), and often at high frequencies. In North America and Australia the frequencies of these inversions are highly correlated with latitude, being much higher in the more equatorial populations of both continents (Mettler *et al.* 1977; Knibb *et al.* 1981). Inversions *In(2L)t* and *In(2L)NS* seem alternatives, as well as

*In(3R)P* and *In(3L)M*, and *In(3R)P* and *In(3R)K*. The inversion *In(2L)t* is found from Africa to Europe too, decreasing in frequency with latitude, as well as in South America (van't Land *et al.* 2000). The *Gpdh* (*a-Gpdh*) locus is found within the chromosomal inversion range, the locus *Adh* just outside it. Inversion *In(2L)t* is highly associated with the *Gpdh<sup>F</sup>* allele and the *Adh<sup>S</sup>* allele, whereas the standard *St* chromosomal arrangement shows all combinations of *Gpdh* alleles and *Adh* alleles (Malpica *et al.* 1987). In experimental populations, the inversion *In(2L)t* possessed a higher frequency at higher tempera-

ture (van Delden and Kamping 1989, 1991). Homozygote *In(2L)t* genotypes have a relatively low body weight and longer developmental time (van Delden and Kamping 1991), whereas individuals heterozygous for *In(2L)t* have highest fitness after heat stress (Kamping and van Delden 1999b). The change in inversion frequency over latitudes can be easily understood as a consequence of changes in balancing selection; in each population, the heterozygote would have highest relative fitness, but the relative fitnesses of the two homozygotes change. The fitness of the *In(2L)* homozygote would increase with higher temperature, and the fitness of the *St* homozygote decrease, leading to a change in *In(2L)* inversion frequency over latitude.

Selection on the other inversions is not as clear as that on *In(2L)t*. None of the current neutrality tests provides evidence for direct selection on the inversion *In(3L)P* (Hasson and Eanes 1996). Apart from the latitudinal cline in *In(3R)P* frequency, only the persistent correlation of this inversion with *Drosophila* body size is suggestive of selection (Betran *et al.* 1998; Weeks *et al.* 2002). The locus *Pgm* is within the inversion *In(3L)P*, but no *Pgm* alleles have been found that were specific for the inversion (Verrelli and Eanes 2000). The four common cosmopolitan inversions all decrease in frequency with latitude. The two sets of inversions on the same chromosome showed slight linkage disequilibrium towards coupling, but no general deviation from gametic equilibrium has been shown convincingly (Knibb *et al.* 1981).

Inversions are described and characterized by their breakpoints. What genes are contained in the inversion can be approximated using the gene maps in FlyBase (<http://flybase.org>), but this has not been determined directly. It is not known whether one inversion carries the same alleles of all its genes over all its range. Using a comparison between *Drosophila* species, Caceres and co-workers (Caceres *et al.* 1997, 1999) argued that long inversions represent alternative selected blocks of chromosome. If this argument could be accepted, it would imply that the common cosmopolitan inversions that show clear



**Figure 1.** Relation between larval mass-specific glycogen content just prior to pupation and adult fresh weight. At 17.5°C larvae are 200 h old, at 27.5°C larvae are 96 h old. A point refers to one of the populations sampled in the following countries: Congo, Panama, Denmark, Sweden.

**Table 1.** Microsatellite loci showing a latitudinal cline in repeat frequency in Australian populations (Gockel *et al.* 2001), and the chromosomal locations of some genes of the insulin-signalling pathway in *Drosophila melanogaster*.

Microsatellite	AC004759	DMU14395	DMTRXIII	DMU25686	AC008193
<i>R</i> <sup>2</sup>	0.323	0.597	0.340	0.763	0.499
Location	38E1-E9	65D1-D3	88B3	93F	94D
Gene	<i>Idgf1</i> , . . . , <i>Idgf3</i>	<i>Ilp1</i> , . . . , <i>Ilp5</i>	<i>Akt1</i>	<i>InR</i>	<i>Tsc1</i>
Location	36A1	67C8	89B2	93D4	95E4
Gene		<i>S6k</i>			
Location		64E8-11			

After correction for multiple comparisons, latitude explained a significant proportion of the total variation in repeat frequency for these five microsatellite marker loci, out of 19 loci tested.

latitudinal clines are stable in allelic content and selected. That is, they form a coadapted gene complex.

### Within-population genetic variation

Only in one *D. melanogaster* population have body size, development time, allelic variation at the *Adh* and *Gpdh* loci, microsatellite repeat frequency and inversion frequency been studied together (Weeks *et al.* 2002). This study used an extensive collection of isofemale lines from Coffs Harbour, in the middle range of the Australian cline along the eastern coast. Body size and development time were determined independently and genetically. The *Adh<sup>S</sup>* allele was related to longer development time, as had been found earlier (Oudman *et al.* 1991 and references therein), but not to body size. The inversion *In(2L)t* and the *Gpdh* locus were not correlated with body size or development time, in contrast with results from experiments starting from a European base population (van Delden and Kamping 1989, 1991; Oudman *et al.* 1992). Body size was strongly correlated with three allele frequencies: with allele frequency at the heat-shock-resistance *hsr-omega* locus, located on chromosome arm 3R at chromosomal position 93D6-7, and with frequency at the microsatellite loci DMU25686 and AC008193, at chromosomal locations 93F and 94D respectively. Alleles at the three loci proved highly correlated. These three loci are within the region spanned by *In(3R)P*, but the allelic content of this inversion and of the standard chromosome are not known. Therefore the relation between the inversion and body size could not be determined.

A relation between the inversion *In(3R)P* and body size in the Australian cline follows from other data too. Weeks *et al.* (2002) used one population from the middle of the Australian eastern coast cline. Gockel *et al.* (2002) analysed the genetic differences in body size from populations at the endpoints of this Australian latitudinal cline and found that the largest effect on body size was associated with a location on chromosome arm 3R. Their inbred lines, differing strongly in body size and representing the endpoints of the cline, were fixed for the *St* and *In(3R)P*

chromosomes (mentioned in Weeks *et al.* 2002). This implies that the *In(3R)P* inversion was related to small body size, and the standard chromosome sequence to larger body size.

### Selection

#### Adaptation in nature

*D. melanogaster* is by origin a West African species (Lachaise *et al.* 1988), and is more genetically variable within Africa than elsewhere (Kauer *et al.* 2002). North America seems to have been colonized from Europe rather than from Africa (David and Cappy 1988). At least in the Caribbean, recent admixture from Africa seems likely, as African and Caribbean flies have the same female pheromone (Ferveur *et al.* 1996; Coyne *et al.* 1999). and some correspondence in microsatellites (Caracristi and Schlotterer 2003). Nowadays, fruit exports might well transport *D. melanogaster* to distant parts of the globe, as microsatellite similarity of flies caught in Tasmania to flies from Israel rather than to flies from Australia seems to indicate (Agis and Schlotterer 2001). Whether *D. melanogaster* found in northern Europe represent a resident population remains in doubt: *D. melanogaster* can be caught in Sweden and Finland in summer, but body size and body-size plasticity suggest that such flies might represent recent immigration due to fruit imports (G. de Jong (Finland), Z. Bochdanovits (Sweden), unpublished data).

The stability of a latitudinal cline depends upon the existence of resident populations. Any cline in *D. melanogaster* in North America or South America must have been established within the past 400 years; the Australian cline presumably within the past 100 years. A similar latitudinal cline in wing size was established in two decades in *D. subobscura* (Huey *et al.* 2000), showing the strength of selection on body size. The occurrence of repeated and corresponding clines on several continents in identical traits indicates selection. Inversion-frequency clines and allozyme-frequency clines are as prevalent as body-size clines. Inversion frequency, allozyme frequency and adult body size vary in the course of the year with temperature (Kamping and van Delden 1999a). The genetic size in-

**Table 2.** Common cosmopolitan inversions in *Drosophila melanogaster* (after Lemeunier and Aulard 1992) and chromosomal location of insulin-signalling pathway genes (FlyBase)

Inversion	Chromosome range	Frequency correlation with latitude		Insulin-signalling pathway genes
		North America	Australia	
<i>In(2L)t</i>	22D3-E1; 34A8-9	- 0.92	- 0.84	<i>chico, Pten, Tor</i>
<i>In(2L)NS</i>	23E2-3; 35F1-2			<i>chico, Pten, Tor</i>
<i>In(2R)NS</i>	52A2-B1; 56F9-13	- 0.90	- 0.67	
<i>In(3L)P</i>	63C; 72E1-2	- 0.74	- 0.74	<i>S6k, Ilp1, . . . , Ilp5, Pi3K68D</i>
<i>In(3L)M</i>	66D; 71D			<i>Ilp1, . . . , Ilp5, Pi3K68D</i>
<i>In(3R)P</i>	89C2-3; 96A1-19	- 0.80	- 0.72	<i>Pi3K92E, InR, Tsc1</i>
<i>In(3R)K</i>	86F1-87A1; 96F11-97A1			<i>Akt1, Pi3K92E, InR, Tsc1</i>

crease in temperate populations is regarded as direct evidence for evolutionary temperature adaptation in body size—whatever the exact mechanism might be. Populations from the wet tropics, the original environment for *D. melanogaster*, might be present and reproducing year-round. In temperate populations, adults overwinter (Izquierdo 1991), and this presents a challenge for living long on little food. Experiments done at La Venta, Spain (43°N), showed that adults surviving through the natural temperature fluctuations were able to lay eggs to produce a natural spring generation early May (Izquierdo 1991). Average winter temperature was 8.5°C, but minimum mean monthly temperature could be as low as 3.5°C, including freezing nights.

Experiments on overwintering were done on Australian clinal populations (Mitrovski and Hoffmann 2001; Hoffmann *et al.* 2003). Adult flies from many populations of the latitudinal cline were kept outside in cages on food in Melbourne (37.5°S latitude). Winter temperature in Melbourne is not low: cage temperature was reported to average about 10°C over winter. Minimum temperature could be as low as 3°C, but no freezing nights were reported. Longevity of adult flies differed between populations, subtropical populations having lowest longevity. No eggs that were laid in winter survived. High-latitude temperate populations had higher egg-laying capacity at the beginning of spring (Mitrovski and Hoffmann 2001). Over a tropical winter, with temperatures averaging 20°C in the cages, temperate populations had earlier and higher egg production than tropical populations (Hoffmann *et al.* 2003). This is in agreement with the cline in ovariole number, where temperate flies have more ovarioles than tropical flies (Capy *et al.* 1993; Delpuech *et al.* 1995).

In the experiments the adult flies in outside cages were fed, and the Melbourne temperature was relatively high. Circumstances for adult flies to get through a European winter might be harsher, with scarcely any food and lower temperatures. Izquierdo (1991) found that 50–60% of flies were able to survive a period of 45 days at 4°C, and were able to lay viable eggs afterwards. Below 6°C flies rely on survival without feeding, and only above 12°C is full activity resumed (David *et al.* 1983). In natural temperate populations, therefore, selection on starvation resistance and longevity is likely to be high.

#### **Experimental evolution**

In several laboratories, *D. melanogaster* populations have been split, and the replicate populations maintained for several to many years under different constant-temperature conditions. The outcome is identical over labs. Under laboratory evolution, populations that are maintained for a considerable number of generations at low temperature are genetically larger than populations kept for the same period at high temperature (Cavicchi 1978; Huey *et al.* 1989, 1991; Cavicchi *et al.* 1991; Santos *et al.* 1994).

Larger body size evolved in the lab under cold temperatures was accompanied by larger cell size (Partridge *et al.* 1994a), larger number of eggs (Partridge *et al.* 1995), faster larval development (James and Partridge 1995), higher larval efficiency (Neat *et al.* 1995), higher metabolic rate (Berrigan and Partridge 1997) and larger egg size (Azevedo *et al.* 1996). Some experimental populations showed higher fitness when tested in their selection environment than in other environments (Partridge *et al.* 1994b; Nunney and Cheung 1997).

In these experiments, populations were kept at different temperatures and were provided with similar rearing conditions. But as is well known, populations raised at higher temperature stabilize at higher numbers under laboratory conditions. Standard rearing practice leads to more crowded and moist cultures at high temperature; low-temperature rearing implies lower density and drier food conditions, by autonomous population regulation. A major difference in larval crowding is therefore confounded with the difference in temperature. To side-step this problem we started experimental-evolution lines at fixed temperatures (17.5°C and 27.5°C), and on poor and rich food, from the same Netherlands population. After 10 generations, body size proved to be largest under the cold-temperature, poor-food experimental-evolution line. Tested at 17.5°C, the rich-food selection lines developed slower than the poor-food selection lines; at 27.5°C no difference in development time was found (Bochdanovits and de Jong 2003b), implying that crowding in experimental temperature adaptation cultures has to be controlled to avoid confounding the effect of temperature with that of crowding.

The differences in body size and its correlated characters are very similar between temperate and tropical populations on the one hand, and between cold-adapted and warm-adapted lab populations on the other hand. Higher temperature selects for smaller size, and lower temperature selects for faster and more efficient development and larger size. In both natural and experimental populations the main mechanism to reach larger size seems higher efficiency of larval growth in cold-adapted populations. This higher efficiency in cold-adapted populations is underscored by the good performance of the low-temperature, poor-food experimental-evolution line (Bochdanovits and de Jong 2003b).

#### **Selection lines**

The earliest artificial selection experiments in *D. melanogaster* might have been on body size. Selection for larger thorax size or wing size leads to generally larger body size, longer development time at the same growth rate, higher critical weight, and lower fecundity. Selection for smaller size leads to lower growth rate and lower critical weight (Partridge *et al.* 1999, and references therein). The males from a larger-bodied selection line possessed a

mating advantage over males from the corresponding control line and small-bodied selection line, but only at the lower rearing and test temperature (Reeve *et al.* 2000). Similarly, females from the large-bodied selection line lived significantly longer and produced more offspring over their lifetime than females from the control line and small-bodied selection line, but again only when reared and tested in the colder environment (McCabe and Partridge 1997). In an experiment on the effect of larval competition, individuals from a selection line for large thorax size had an increasing disadvantage with increasing larval density, compared to the control line (Santos *et al.* 1994). However, high larval density by itself does not select for small body size (Santos *et al.* 1997).

Laboratory selection on development time can be direct or indirect. Direct selection for faster development led to shorter development time and smaller adult size (Nunney 1996; Prasad *et al.* 2000), as well as higher early fecundity (Chippindale *et al.* 1997). Indirect selection on development time by way of high adult mortality led to a shorter development time, smaller adult size and higher early fecundity than in a population with low adult mortality, at identical larval density (Gasser *et al.* 2000; Stearns *et al.* 2000). In such selection experiments on life-history traits, which trait showed a correlated response differed between experiments, depending upon any additional environmental constraint and any differences in genetic variation that might be present (Ackermann *et al.* 2001; Chippindale *et al.* 2003).

Several experiments have selected for higher starvation resistance in *D. melanogaster*, and adults responded to selection for starvation resistance through a genetically determined increase in both lipid and carbohydrate content (Chippindale *et al.* 1996; Djawdan *et al.* 1998). Adult flies were larger in the starvation-resistance lines and developed slower (Chippindale *et al.* 1996; Djawdan *et al.* 1998; Harshman *et al.* 1999). As an immediate (phenotypic) response to starvation, intermediary metabolism enzyme activities decreased. In contrast, enzyme activities, notably of G6PD, changed in response to selection, with enzyme activities associated with lipid biogenesis being increased (Harshman *et al.* 1999). Thus, starvation evoked the evolution of higher enzyme activity, while the phenotypic response to starvation was lowered enzyme activity. The net effect is a similar metabolism of the flies over the environments to which they are adapted.

### Comparison

Selection lines and natural populations show a fairly consistent difference in their association between development rate, growth rate and body size. Natural populations at the opposite ends of clines all have a pattern of larger flies together with faster growth and smaller flies together with lower growth rate. Development rate is iden-

tical between temperate and tropical populations, or higher in larger flies from temperate populations. Selection lines for development time, body size or starvation resistance always associate fast development with small body size and large body size with slow development. The implication seems to be that selection in nature does not correspond to selection in the laboratory: in nature selection is either not on body size or development time itself, or on a combination of traits rather than on a single trait, body size.

On the other hand, selection lines and natural populations might show similar patterns. Longevity is higher in larger-sized flies but only at the lower temperature, both in natural populations and in selection lines for size. Experimental evolution under high and low temperature shows many of the life-history patterns found in tropical and temperate populations. It is clear that temperature is ultimately responsible but the details of the selection pressures are far from clear, especially as larger flies might have a consistent fitness advantage as adults. Any adaptive explanation of larger flies under a colder climate immediately provokes the persistent question why tropical *D. melanogaster* are small (cf. Blanckenhorn 2000). A fly from a tropical population is quite different from a fly of a similar size from a temperate population. Perhaps molecular studies might provide illumination on the connection between traits.

### Pathways signalling growth

Of the known metabolic pathways involving growth factors, the insulin-signalling pathway (figure 2) emerges as the primary one for both cell proliferation and cell growth (Britton and Edgar 1998; Hipfner and Cohen 1999; Oldham and Hafen 2003). An exhaustive genetic dissection in *D. melanogaster* has validated the role of the insulin-signalling pathway in growth control (Saucedo and Edgar 2002; Oldham and Hafen 2003), and this pathway is now seen as a major regulator of growth in this species (Johnston and Gallant 2002). The insulin-signalling pathway is highly conserved in multicellular animals, and next to its role in growth is also a major player in the determination of metabolism and nutrient storage.

Converging on to the insulin-signalling pathway is the TOR pathway. TOR means target of rapamycin, a name that originates from the original discovery in yeast of the gene *Tor*, as the gene attacked by the drug rapamycin. The TOR pathway regulates cell growth (cell size) rather than cell proliferation (cell number) (Schmelzle and Hall 2000). TOR might function as a nutrient sensor, in particular as an amino acid sensor, and the TOR pathway plays a prominent role in regulating cell growth in response to nutrients (Cardenas *et al.* 1999; Zhang *et al.* 2000). In *Drosophila* larvae, TOR stimulates growth of the endo-

replicating tissues rather than of the mitotic diploid tissues (Britton and Edgar 1998; Oldham *et al.* 2000).

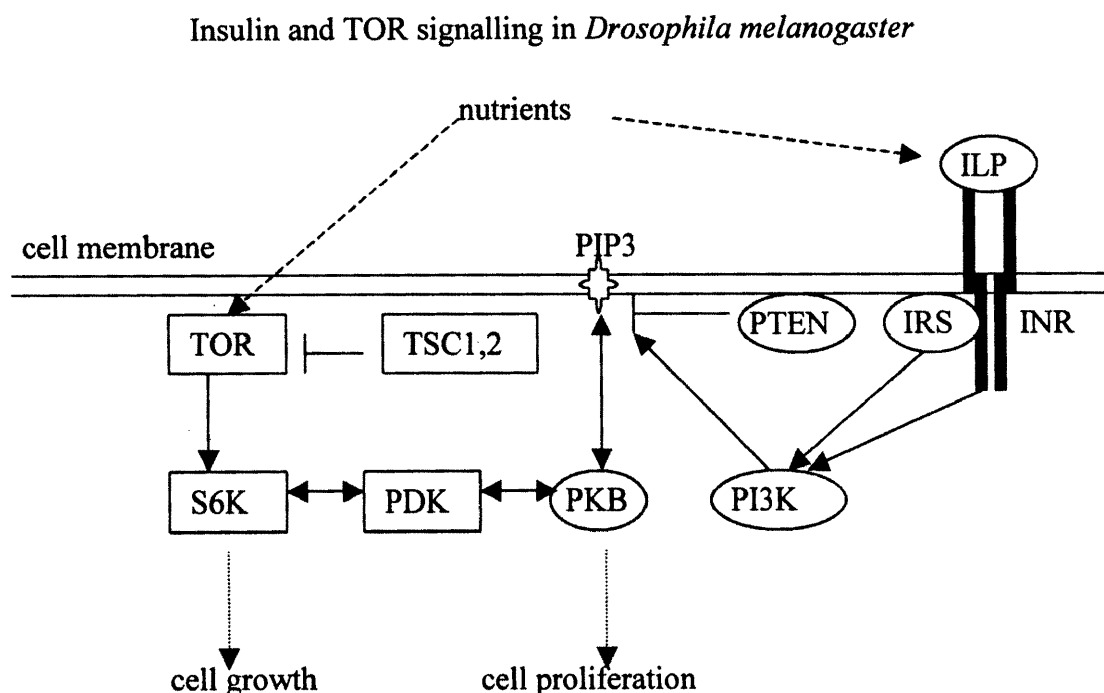
#### Pathway genes

Genetic studies in *D. melanogaster* have highlighted the conservation of the insulin-signalling pathway in all multicellular animals, and we now have a good picture of pathway connections and possible pathway influence on cell growth and cell proliferation (Oldham and Hafen 2003; figure 2 here). The insulin-signalling pathway plays an essential role in controlling body, organ and cell size. The pathway involves the *Drosophila* insulin genes, i.e. those for the insulin-like receptor InR (gene *InR*), insulin receptor substrate (IRS; gene *chico*), the lipid kinases PI(3)K (genes *Pi3K21B*, *Pi3K59F*, *Pi3K68D*, *Pi3K92E*), PTEN (gene *Pten*), Akt/PKB (gene *Akt1*), PDK (gene *Pk61C*) and S6K (gene *S6k*; = RPS6-p70-protein kinase), and the eIF4F complex (eukaryotic initiation factor; genes *eIF-4A*, *eIF-4E* and *eIF-4G*). Seven genes coding for insulin-like peptides (ILP) have been identified (named *Ilp1*, . . . , *Ilp7*) (Brogiolo *et al.* 2001). Of these peptides, especially ILP2 shows a strong interaction with InR in determining body size. In addition to the ILPs, four factors have been isolated that are able to cooperate with

human insulin in promoting proliferation of cultured *Drosophila* cells (Kawamura *et al.* 1999; Bryant 2001). These factors, the imaginal-disc growth factors (IDGFs), affect imaginal-disc size, and might act in a manner similar to competence factors in mammals (Johnston and Gallant 2002) or as lectins (Bryant 2001). The four IDGFs are coded for by the genes *Idgf1*, *Idgf2*, *Idgf3* and *Idgf4*. Two more genes of the *Idgf* gene family have been identified, *Chit* and *Idgf5*. The genes *Idgf1* and *Idgf3* have been sequenced extensively, and show much nucleotide polymorphism within and between populations and species; *Idgf3* seems to be evolving neutrally, but some polymorphism in *Idgf1* might be under balancing selection (Zurovcova and Ayala 2002). The TOR pathway converges on the insulin-signalling pathway at S6K (Oldham and Hafen 2003; figure 2 here). Its genes are *Tor* itself, and two genes corresponding to human tumour suppressor genes, the genes *Tsc1* and *Tsc2* (= *gigas*), that might be involved in suppression of TOR action (Gao and Pan 2001).

#### Mutation effects

The identification of pathways in cell biology proceeds through screening of mutants to identify loss-of-function



**Figure 2.** The insulin and TOR pathways (after Oldham and Hafen 2003, *Trends Cell Biol.* **13**, 79–85). Model of insulin–IGF and TOR signalling in *Drosophila melanogaster*. All these components display growth alterations when mutated. Abbreviations: ILP, insulin-like peptides 1–7, genes *Ilp1*, . . . , *Ilp7*; INR, insulin receptor, gene *InR*; IRS, insulin receptor substrate, gene *chico*; PI3K, phosphoinositide 3-kinase, gene *Pi3K92E*; PDK, phosphoinositide-dependent kinase, gene *Pk61C*; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKB, protein kinase B, gene *Akt1*; S6K, S6 kinase, gene *S6k*; TOR, target of rapamycin, gene *Tor*; TSC, tuberous sclerosis complex, genes *Tsc1* and *Tsc2*.

effects. Loss-of-function mutations in any of the insulin-signalling pathway components lead to a reduction of cellular and organismal growth rate. Overexpression of inhibitory components slows growth rates. Overexpression of *Drosophila* insulin-like peptide-2 (ILP2) increases organismal size (Brogiolo *et al.* 2001). Ablation of the insulin-producing neurosecretory cells leads to developmental delay, growth retardation and elevated carbohydrate levels in larval haemolymph. Such larvae with reduced ILP concentration possessed an average combined glucose and trehalose level of 38% above normal. The glucose and trehalose levels returned to normal when ILP2 was provided systemically by a transgene *Ilp2* (Rulifson *et al.* 2002). This experiment established the *in vivo* identity of *Drosophila* insulin.

The phenotypes of loss-of-function mutations in the insulin-signalling pathway of *D. melanogaster* suggest that this pathway is a conserved system for coordination of metabolism, reproduction and lifespan, which allows phenotypic adjustment to environmental differences (Garofalo 2002). Downregulation of signalling strength along the insulin-signalling pathway when nutrients are limited would suppress nutrient storage and cell growth, and allow nutrient mobilization by tissues such as the fat body. Nutrient intake would lead to the stimulation of insulin-like receptors and PI(3)K activity, and this in turn would promote anabolic metabolism, nutrient storage, cell growth, and cell cycle progression (Britton *et al.* 2002). Another area yet to be examined in *D. melanogaster* is the role of the insulin-signalling pathway in regulation of the enzymes of glucose storage and utilization. This function of insulin is also probably conserved (Garofalo 2002)

Mutations along the insulin branch, from insulin to PDK, influence both cell size and cell number. Perhaps their major influence is on cell number. Mutations along the TOR branch (TSC, TOR, S6K) influence only cell size.

### Nutrition

The insulin-signalling pathway connects nutrition to metabolism and to growth. Mutations in several insulin-signalling pathway components phenocopy the effects of starvation—small adult, larval delay. This supports the idea that nutritional status plays an important role in the control of insulin-signalling pathway signalling, and ILP levels (Brogiolo *et al.* 2001; Britton *et al.* 2002). The influence of different nutrition can be shown in experiments with larvae that possess different levels of insulin pathway signalling. Normally, DNA endoreplication and cell growth ceases in the gut and fat body cells within 1 to 2 days of starvation on 20% sucrose (Britton and Edgar 1998). Inactivating the pathway did not result in DNA endoreplication or cell growth, but activating insulin pathway signalling led to cell growth and higher DNA content in the starving larvae. Hyperactivating insulin

pathway signalling in larvae on 20% sucrose led to larval starvation: the median survival time was up to 5 days shorter. Therefore activating insulin pathway signalling caused continuing growth despite starvation sensitivity at the organismal level (Britton *et al.* 2002). Inhibiting insulin pathway signalling in larvae leads to developmental arrest and phenocopies the cellular and organismal effects of starvation even in presence of abundant larval food; under starvation conditions, such larvae survived as well as controls (Britton *et al.* 2002).

Food deprivation reduces the activity of the insulin-signalling pathway *in vivo*. Third-instar larvae suddenly deprived of food have decreased *Ilp3* and *Ilp5* expression in their brain. Part of the nutrient-dependent regulation of growth is mediated by the regulated expression of the *Ilp3* and *Ilp5* genes in the medial neurosecretory cells in the *Drosophila* brain. The medial neurosecretory cells project their axon terminals into the ring gland where ecdysone and juvenile hormone (JH) are synthesized, suggesting that in *D. melanogaster* secretion of ecdysone or JH or both is influenced by insulin (Ikeya *et al.* 2002). Dwarf flies carrying *InR* mutations have an 80% decrease in the level of JH (Tatar *et al.* 2001).

### Longevity and fecundity

Homozygote *chico*<sup>1</sup>/*chico*<sup>1</sup> are sterile dwarfs while heterozygote *+/chico*<sup>1</sup> flies have normal size and are partially fertile. Both homozygotes and heterozygotes of the null mutation *chico*<sup>1</sup> live longer than wild-type flies (Clancy *et al.* 2001); longevity and sterility therefore do not depend upon dwarf size. Insulin receptor mutations differed in their effect on longevity. Most viable mutants seemed dwarf and short-lived, but one heterozygote combination of two mutant alleles proved long-lived and female sterile (Tatar *et al.* 2001). Homozygote mutants for *S6k* are sterile too. Lower expression of the insulin-like peptide gene *Ilp2* leads to lower female fecundity (Ikeya *et al.* 2002). The insulin-signalling pathway in *Drosophila* is strongly homologous to that in mouse, *C. elegans* and man (Garofalo 2002). This would indicate that higher insulin or insulin pathway signalling levels would increase fecundity and decrease longevity, and lower insulin or insulin pathway signalling levels would decrease fecundity and increase longevity (Drummond-Barbosa and Spradling 2001).

### Mutants versus natural variation

All studies discussed above used loss-of-function mutations or overexpression of a transgene. Many of these mutations are lethal in the larval stage, others cause pupal delays or adult sterility. Some mutations were found by techniques involving mutagens and mitotic crossing over to generate a mutant cell clone for examination while most tissues of the individual are wild type and the indi-

vidual is viable. These artificial major-effect mutations can be used to elucidate pathway components, but are not naturally occurring. No natural major-effect mutations of insulin genes or any of the pathway genes are known (but many homozygote lethals exist in the region of the insulin genes (FlyBase)). The lack of major viable mutants delayed the discovery of the insulin-like peptide genes until 2001, when they were found by BLASTing a basic insulin pattern against the *Drosophila* genome sequence (Brogiolo *et al.* 2001). Naturally occurring genetic variation somewhere along the insulin-signalling pathway potentially would provide a link between molecular signalling mechanisms and quantitative-genetic variation in growth and body size. Such natural genetic variation would represent minor effects in molecular terms. It might be timing of expression of genes, level of RNA synthesis, protein level, affinity between the different proteins involved, etc. Could any such natural variation plausibly exist?

### The insulin-signalling pathway and body size in *Drosophila melanogaster*

In this section, we present two hypotheses, and discuss circumstantial evidence that is consistent with these hypotheses. The first hypothesis is that genetic variation in body size in or between natural populations of *D. melanogaster* is caused by genetic variation along the insulin-signalling pathway or the TOR pathway or both. The second hypothesis is that geographically temperate and cold-adapted laboratory populations of *D. melanogaster* possess stronger signalling activity along the insulin-signalling pathway. Many mutants influence body size, but the insulin-related genes provide patterns that correspond to the patterns in natural variation. In the following discussion, organismal and molecular observations are juxtaposed to highlight their correspondence.

#### Cell number and cell size

Body size is larger in temperate populations owing to higher cell number and, to a lesser extent, cell size (James *et al.* 1995; De Moed *et al.* 1997). Cell size is higher at lower temperature and under better feeding conditions (De Moed *et al.* 1997). Major loss-of-function mutations in genes of the insulin-signalling pathway (*Ilps*, *InR*, *chico*, *PI3k*, *Pten*, *Akt1*) in *D. melanogaster* lead to decreased body size as well as decreased cell size and decreased cell number. Overexpression of *Ilps*, *InR*, *Pi3k92E* and *Akt1* leads to larger animals owing to increased cell size (Britton *et al.* 2002). The TOR–S6K branch influences cell size, subject to nutrient status. Mutations in TOR inhibit growth (Schmelzle and Hall 2000; Zhang *et al.* 2000). TOR responds positively to the presence of amino acids and induces upregulation of translation

(Oldham and Hafen 2003). Differences in mean body size between populations from opposite ends of a cline might well be caused by differences in signalling strength along the insulin-signalling pathway. The temperate populations would represent higher signalling strength. Temperature and food effects might be due to the TOR branch.

#### Larval growth efficiency

Larvae in cold-adapted populations grow faster (De Moed *et al.* 1998; James and Partridge 1998) and more efficiently (Neat *et al.* 1995; Robinson and Partridge 2001). Fast growth is correlated with the *Adh<sup>FF</sup>* genotype, and perhaps with the *Gpdh<sup>SS</sup>* genotype; the inversion *In(2L)t*, associated with *Adh<sup>SS</sup> Gpdh<sup>FF</sup>*, might correspond to slower growth (van Delden and Kamping 1989, 1991; Oudman *et al.* 1992). Nutrient intake leads to the stimulation of insulin receptors and PI(3)K activity, and this in turn promotes anabolic metabolism, nutrient storage, cell growth and cell cycle progression (Britton *et al.* 2002). Higher signalling levels along the insulin-signalling pathway will therefore promote growth. TOR seems a good candidate to regulate efficiency, as it responds to nutrient availability. Differential sensitivity due to natural TOR genetic variation might be a good candidate to regulate efficiency. The allozyme genotypes might represent differences in effective nutrient status. Since TOR and the *Adh* locus are fairly closely linked in the standard chromosome arrangement (table 3), and the inversion *In(2L)t* is associated with *Adh<sup>SS</sup> Gpdh<sup>FF</sup>*, direct genetic associations might be possible here.

#### Larval starvation

Third-instar larvae of temperate populations that are suddenly deprived of food have low survival probability, but if they survive the resulting adult is larger for the same larval weight than larvae from tropical populations, which have higher probability to reach adulthood, but produce smaller adults for the same larval weight (Bochdanovits and de Jong 2003d). The inference is that stored larval resources are differentially used, temperate populations aiming at large adults even if compromising larval survival and tropical populations aiming at larval survival even if the resulting adult is small. Inhibiting the insulin-signalling pathway phenocopies the cellular and organismal effects of starvation when the larvae are fed, whereas activating this pathway bypasses the nutritional requirement for cell growth, leading to both cell growth and high larval mortality on a starvation diet (Britton *et al.* 2002). The inference might be that activating the insulin-signalling pathway promotes development of a large adult at the risk of larval death under starvation conditions. Thus, in larvae deprived of food, the effect of activating the insulin-signalling pathway is similar to that found in larvae from temperate populations.

**Nutrient storage**

Third-instar larvae from temperate populations store more glycogen relative to their weight than larvae from tropical populations. In temperate populations, genetic variation leads to enzymes geared to a flux towards glycogen (Eanes 1999; Verrelli and Eanes 2001b). Increased lipid metabolism is present too but proceeds by a different enzymatic pathway. Insulin stimulates storage of glycogen and lipid (Saltiel and Kahn 2001; Garofalo 2002). Stimulating the insulin-signalling pathway enhances nutrient (glycogen and lipid) storage in the developing larva

(Britton *et al.* 2002). Enzyme genotypes associated with higher glycogen content would imply hormonal regulation towards glycogen storage.

**Genetic association**

Genetic localization of quantitative-genetic variation in body size corresponds to the genetic localization of the insulin-signalling pathway genes. Of course, a chromosome location contains very many genes, many with unknown function, and correspondence in chromosomal localization is, at most, suggestive of possible causation.

**Table 3.** Chromosomal location of insulin-signalling pathway genes and some metabolism-related genes in *Drosophila melanogaster* (source: FlyBase). Gene locations in inversions are printed in bold.

Gene name	Abbreviation	Chromosome					
		X	2L	2R	3L	3R	4
6-Phosphogluconate dehydrogenase	<i>6Pgd</i>	2D4					
insulin-like peptide 7	<i>Ilp7</i>	3E2					
insulin-like protein 6	<i>Ilp6</i>	3A1					
Imaginal disc growth factor 4	<i>Idgf4</i>	9A3					
Glucose-6-phosphate dehydrogenase	<i>G6pd (Zw)</i>	18D13					
Phosphoinositide 3-kinase at 21B	<i>Pi3K21B</i>		21B8				
Eukaryotic initiation factor 4A	<i>eIF-4A</i>		<b>26B2</b>				
Glycerophosphate dehydrogenase	<i>Gpdh</i>		<b>26A3</b>				
chico	<i>chico (dIRS)</i>		<b>31B1</b>				
–	<i>Pten</i>		<b>31B4-5</b>				
Target of rapamycin	<i>Tor</i>		<b>34A4</b>				
Alcohol dehydrogenase	<i>Adh</i>		35B3				
Imaginal disc growth factor 1	<i>IDGF-1</i>		36A1				
Imaginal disc growth factor 2	<i>IDGF-2</i>		36A1				
Imaginal disc growth factor 3	<i>IDGF-3</i>		36A1				
(insulin-like growth factor receptor)	<i>CG10702</i>		37B13				
Phosphoglucose isomerase	<i>Pgi</i>		44F6				
Inhibitor of apoptosis 2	<i>Iap2</i>			<b>52D2</b>			
Chitinase-like = Idgf 6	<i>Chit</i>			<b>53C11</b>			
Imaginal disc growth factor 5	<i>Idgf5</i>			<b>55C</b>			
Phosphoinositide 3-kinase at 59F	<i>Pi3K59F</i>			59E4-F1			
Protein kinase 61C	<i>Pk61C (PDK)</i>				61B1		
Adipokinetic hormone-like	<i>Akh</i>				<b>64A7-8</b>		
RPS6-p70-protein kinase	<i>S6k</i>				<b>64E8-11</b>		
Eukaryotic initiation factor 4E	<i>eIF-4E</i>				<b>67B2</b>		
insulin-like protein 1	<i>Ilp1</i>				<b>67C8</b>		
insulin-like protein 3	<i>Ilp3</i>				<b>67C8</b>		
insulin-like peptide 4	<i>Ilp4</i>				<b>67C8</b>		
insulin-like peptide 5	<i>Ilp5</i>				<b>67C9</b>		
Insulin-related peptide = Ilp2	<i>IRP (Ilp2)</i>				<b>67C8</b>		
Phosphoinositide 3-kinase at 68D	<i>Pi3K68D</i>				<b>68D4-6</b>		
Phosphogluconate mutase	<i>Pgm</i>				<b>72D8</b>		
gigas = Tuberous sclerosis complex 2	<i>gig=TSC2</i>				76F2		
Ras homologue expressed in brain	<i>Rheb</i>					83B2	
Glycogen synthase	<i>GlyS</i>					88E1	
(CG4006)	<i>Akt1 (PKB)</i>					89B3	
Phosphoinositide 3-kinase at 92E	<i>Pi3K92E (Dp110)</i>					<b>92F3</b>	
Insulin-like receptor	<i>InR</i>					<b>93E4</b>	
Tuberous sclerosis complex 1	<i>Tsc1</i>					<b>95E4</b>	
(insulin-like growth factor binding complex)	<i>CG11910</i>					96D2-3	
Eukaryotic initiation factor 4G	<i>eIF-4G</i>						102F5

**QTL and microsatellites:** A chromosome exchange showed that the major genes for the body-size difference between the ends of the Australian cline and the ends of the South American cline are located on the third chromosome (Gockel *et al.* 2002). For both populations, a further QTL (quantitative trait loci) study indicated the right arm, chromosome 3R (Gockel *et al.* 2001; Calboli *et al.* 2003). The genes for insulin-like peptides are on chromosome arm 3L, the insulin receptor gene and *Akt1* on 3R. Allele frequencies of repeats at five microsatellite loci showed a significant latitudinal cline. These microsatellites are found at chromosome positions that make them clearly linked to the genes for IDGF, insulin and the insulin receptor (table 1). Genetic variation for body size within the Australian Coffs Harbour population proved strongly correlated with the *hsr-omega* locus at chromosomal location 93D4 and the microsatellite loci DMU25686 and AC008193, at chromosomal locations 93F and 94D respectively (Weeks *et al.* 2002). These three loci are within the region spanned by *In(3R)P*.

**Inversions and pathway genes:** The genes mentioned in the insulin-signalling pathway (figure 2) are those for insulin-like peptide 1 to 5 (genes *Ilp1*, . . . , *Ilp5*), insulin-

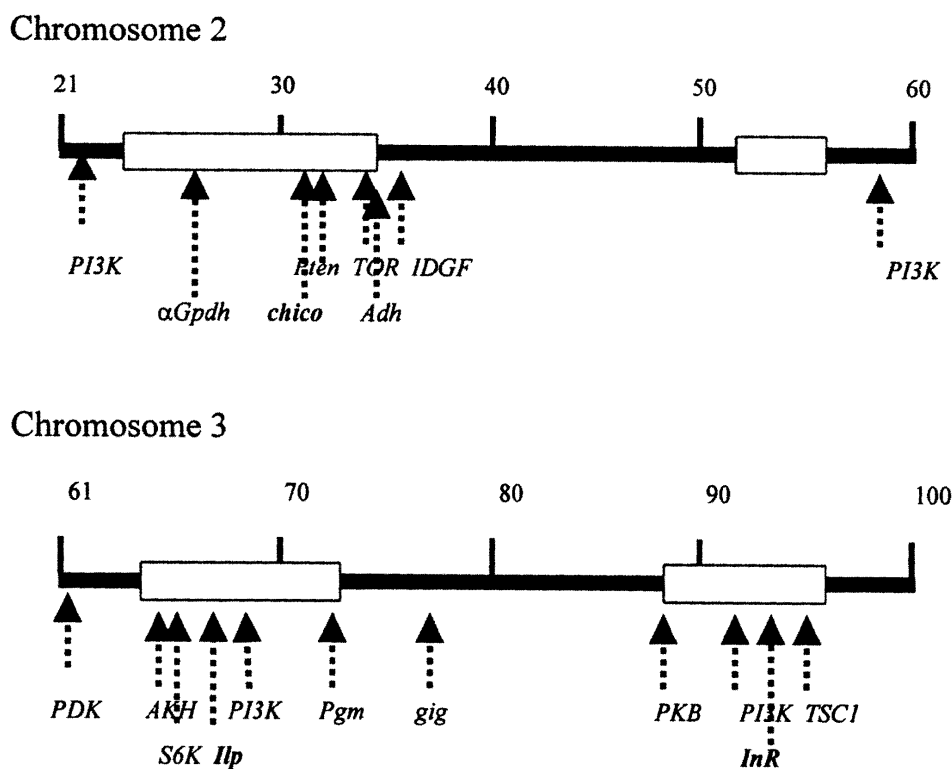
like receptor (gene *InR*), insulin receptor substrate (*chico*), the lipid kinases PI(3)K (genes *Pi3K21B*, *Pi3K59F*, *Pi3K68D*, *Pi3K92E*), PTEN (gene *Pten*), Akt/PKB (gene *Akt1*), PDK (gene *Pk61C*) and S6K (gene *S6k*; = RPS6-p70-protein kinase). The genes of the TOR branch are *Tor*, *Tsc1* and *Tsc2*. The locations of these genes are not randomly distributed over the chromosomes. The cytological locations of genes involved in the insulin-signalling pathway are given in figure 3 and in table 3 together with the locations of some other genes that have been mentioned. Genes in these pathways are associated with the chromosomal locations of the three larger common cosmopolitan inversions (figure 3).

On chromosome arm 2L, *chico*, *Pten*, and *Tor* are located in the region of *In(2L)t*; *Pi3K21B* is before and *Idgf1*, *Idgf2*, *Idgf3* are just after *In(2L)t*.

On chromosome arm 3L, *S6k*, *Ilp1*, . . . , *Ilp5* and *Pi3K68D* are located in the region of *In(3L)P*; *Pk61C* is before and *Tsc2 (gigas)* is after *In(3L)P*.

On chromosome arm 3R, *Pi3K92E*, *InR* and *Tsc1* are located in the region of *In(3R)P*; *Akt1* is just before *In(3R)P*.

The locations and surroundings of the three longer common universal inversions all have at least one gene of



**Figure 3.** Location of the insulin-signalling pathway genes on the cytological map of *Drosophila melanogaster* chromosomes 2 and 3. The locations of the inversions are indicated as boxes. Genes within an inversion range are drawn in the standard order.

each major pathway component: insulin receptor transmembrane, insulin intermediate path and TOR branch. The *In(2L)t* region boasts *chico*, *Pten* and *Tor*. The *In(3L)P* region has *Ilp1*, ..., *Ilp5*, *Pi3K68D* and *S6k*. The *In(3R)P* region possesses *InR*, *Akt1* and *Pi3K92E*, and *Tsc1*. Again, this might be chance. However, it might provide much scope for genetically independent regulation of pathway activity.

**Body size, inversions, and pathways:** At least for the Australian populations, body size is genetically linked to markers at chromosomal locations 93–94, near the position of the gene for the insulin-like receptor. This position is within the range of the *In(3R)P* inversion. Inversions and body size all show repeated latitudinal clines. A possible inference is that natural genetic variation that has something to do with the insulin receptor causes genetic variation in body size. Whether that natural genetic variation pertaining to the insulin receptor would be receptor sensitivity to insulin, receptor efficiency, or receptor density in the membrane remains an open question.

### Summary

All the evidence discussed so far is circumstantial, and suggests that genetic body size variation might be caused by genetic variation somewhere along the insulin-signalling pathway. Specifically, lower signalling strength implies smaller flies with lower nutrient storage and, therefore, higher nutrient availability for immediate activity. Higher insulin levels or higher signalling strength implies larger flies, and higher nutrient storage. The great unknown here is natural variation in signal strength. Whether differences in signal strength would originate in differences in nucleotide composition, gene expression levels, gene expression timing or protein levels is absolutely unknown; however, this is a major question if evolutionary differences in development rate and body size are to be attacked.

The distribution of genes over the major chromosome arms argues for extensive possibilities for genetic adjustment of signal strength. Perhaps body size itself would have to do with the insulin-signalling pathway, and growth rate with the TOR branch. A possibility is that chromosome arm 2L is the major region for genes influencing development rate. The enzyme loci *Adh* and *aGpdh* have repeatedly been associated with development time (van Delden and Kamping 1989, 1991; Oudman *et al.* 1992). The loci *Tor* and *Idgf1*, ..., *Idgf3* might influence growth in a way that influences growth rate, as TOR primarily stimulates growth of the endoreplicating tissues (Britton and Edgar 1998; Oldham *et al.* 2000). In contrast, the *In(3R)P* region might be the major region for genetic variation in body size (Weeks *et al.* 2002), and body size might depend upon signalling strength channelled through the insulin receptor.

### Discussion

Evolutionary biologists and molecular geneticists have taken distinct approaches to study body size, and have come to surprisingly different kinds of understanding of the control of body size in insects (compare Stern 2001). Evolutionary biologists have concentrated on natural genetic variation, and on adaptive explanations of repeatable geographic variation within a species. *Drosophila melanogaster* with genetically larger body size are commonly found in temperate populations and in cold-adapted laboratory populations. Larger body size evolved under lower temperature goes together with faster growth and higher ovariole number. The patterns are repeatable and clear, but a molecular explanation is lacking.

In contrast, molecular geneticists have searched for genes that result in larger or smaller organisms when mutated. One pathway in particular, the insulin-signalling pathway, has attracted considerable attention because defects in this pathway cause clear effects on the final size of adult *D. melanogaster*. Of the identified evolutionarily conserved metazoan pathways, the insulin-signalling pathway is the major pathway influencing cell size, cell number and organismal size (Saucedo and Edgar 2002; Oldham and Hafen 2003). As the insulin-signalling pathway influences metabolism as well as growth, longevity and fecundity, a study of variation surrounding the insulin-signalling pathway promises to provide the necessary junction between genes and evolution of body size. A big challenge for the future is to marry molecular, physiological and evolutionary studies of growth to unravel the apparently complex role of genes, such as those involved in insulin signalling, in the control of insect growth (Stern 2001).

The genes of the insulin-signalling pathway might not be the only set of genes influencing such life-history traits as body size, fecundity and longevity. Extensive studies looking for quantitative trait loci (QTL) for life-history traits have so far only been undertaken using recombinant inbred lines (RIL) derived from crosses between the laboratory strains Oregon-R and 2b; the markers were given by many *roo* transposons with known cytological location. QTL for female reproductive success were located at the tip of the X chromosome between markers at cytological locations 1B and 3E (compare table 3), and on the left arm of chromosome 2 in the 30D–38A cytological region. Ovariole number QTL mapped to cytological intervals 62D–69D and 98A–98E, both on the third chromosome. The regions harbouring QTL for female reproductive success and ovariole number were also identified as QTL for longevity in previous studies with these lines (Wayne *et al.* 2001). Male longevity and female ovariole number were negatively correlated among RIL. Density and sex greatly influenced QTL for longevity (Leips and MacKay 2000). Over studies, QTL for longe-

vity have been found at cytological locations 1B, 3E, 4F, 7D, 9A, 19A on the X chromosome; at 33E, 34E, 38E, 46C, 48D, 50D, 57F on the second chromosome; and at 63A, 65D, 68B, 68C, 70C, 73D, 77A, 85F, 87F, 96F, 97D, 98A, 99B on the third chromosome (Leips and MacKay 2002). QTL near each other might well represent experimental noise. While some of these QTL positions roughly correspond to the cytological locations of the genes of the insulin-signalling pathway, some others don't. Notably, the tip of the X chromosome, the region 30–38 and the region 62–69 would correspond to the region for the insulin-signalling pathway; but 96–99 has no known insulin pathway genes.

The insulin-signalling pathway clearly has a large impact on *Drosophila* growth, but it cannot be the whole story. On the physiological side, insect growth is regulated by hormonal pulses and a hormonal cascade (Nijhout 1994). Of particular interest is the release of a small pulse of prothoracicotropic hormone (PTTH) at the start of the third larval stage. This PTTH release is related to nutrition, but the signalling system is unknown. PTTH promotes ecdysteroid secretion but is itself inhibited by JH. An ecdysone pulse precedes larval moulting and signals the end of growth before pupation. It is becoming increasingly likely that insulin-like peptides tag on into this system. The medial neurosecretory cells of the *Drosophila* brain in which the genes *Ilp3* and *Ilp5* are expressed project their axon terminals into the ring gland where ecdysone and JH are synthesized. This suggests that in *D. melanogaster* secretion of ecdysone or JH or both is influenced by insulin, and they interact in larval growth (Ikeya *et al.* 2002). In *D. melanogaster* adults too, insulin is implicated in larger regulatory networks involving ecdysone. Insulin stimulates oogenesis and ecdysone production in females, and is involved with molecular chaperones (Tatar *et al.* 2003). Especially, a larva has to know when to stop growing (Stern 2003). The signal to prepare for eventual metamorphosis is likely to be the PTTH pulse early in the third larval stage, long before expression of *Ilp2* stops in the middle of the third larval stage (Arbeitman *et al.* 2002) and long before the ecdysone signal to start pupation. The interaction between the molecular signalling system of the insulin pathway and the hormonal signalling system determines larval growth rate and development, but the very diverse data are only now being explored and coordinated (Nijhout 2003; Stern 2001).

An adaptive hypothesis concerning the relation between insulin-signalling pathway and geographic variation in body size might be that the temperate populations represent a 'thrifty' genotype at relatively high insulin level, or a relatively high level of signalling along the pathway. A relatively high insulin level would lead to larger body size and higher fecundity. A relatively high insulin level would lead to relatively high storage of nutrients, which

would be necessary for winter survival and high egg laying in a cool spring. The nutrients concerned would be produced by high-activity allozyme variants of PGM, ADH and  $\alpha$ -GPDH, and not shunted off by high-activity allozyme variants of 6PGD and G6PD. Such allozyme variants are found in high frequency in temperate populations. A relatively high insulin level would however lead to lower longevity. High longevity would have to depend upon other pathways, notably those that have to do with starvation and lipid storage. Whether higher insulin pathway signalling levels themselves would lead to faster larval growth, or whether fast growth would depend as much upon the TOR branch is not at all clear. The complementary hypothesis would be that tropical populations represent a 'spendthrift' genotype at lower insulin level or at a lower level of signalling along the insulin pathway. A relatively low insulin level or insulin signalling level would lead to lower storage of nutrients but higher starvation resistance of larvae, and small body size. However, it might have more resources available for immediate activity, and therefore excel in larval competitive ability or male territorial activity. Low insulin activity promotes longevity rather than fecundity, and male longevity might be important in sexual selection.

What we need are studies of *Drosophila melanogaster* body size that connect molecular, physiological and evolutionary studies. Such studies would show gene regulation in natural populations, and the genetic localization of natural genetic variation for body size.

## References

- Ackermann M., Bijlsma R., James A. C., Partridge L., Zwaan B. J. and Stearns S. C. 2001 Effects of assay conditions in life history experiments with *Drosophila melanogaster*. *J. Evol. Biol.* **14**, 199–209.
- Agis M. and Schlotterer C. 2001 Microsatellite variation in natural *Drosophila melanogaster* populations from New South Wales (Australia) and Tasmania. *Mol. Ecol.* **10**, 1197–1205.
- Arbeitman M. N., Furlong E. E. M., Imam F., Johnson E., Null B. H., Baker B. S., Krasnow M. A., Scott M. P., Davis R. W. and White K. P. 2002 Gene expression during the life cycle of *Drosophila melanogaster*. *Science* **297**, 2270–2275.
- Azevedo R. B. R., French V. and Partridge L. 1996 Thermal evolution of egg size in *Drosophila melanogaster*. *Evolution* **50**, 2338–2345.
- Berrigan D. and Partridge L. 1997 Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. *Comp. Biochem. Physiol.* **A118**, 1301–1307.
- Betran E., Santos M. and Ruiz A. 1998 Antagonistic pleiotropic effect of second-chromosome inversions on body size and early life-history traits in *Drosophila buzzatii*. *Evolution* **52**, 144–154.
- Blanckenhorn W. U. 2000 The evolution of body size: What keeps organisms small? *Q. Rev. Biol.* **75**, 385–407.
- Bochdanovits Z. and de Jong G. 2003a Co-variation of larval gene expression and adult body size in natural populations of *Drosophila melanogaster*. *Mol. Biol. Evol.* **20**, 1760–1766.

- Bochdanovits Z. and de Jong G. 2003b Experimental evolution in *Drosophila melanogaster*: interaction of temperature and food quality selection regimes. *Evolution* **57**, 1829–1836.
- Bochdanovits Z. and de Jong G. 2003c Temperature dependence of fitness components in geographical populations of *Drosophila melanogaster*: Changing the association between size and fitness. *Biol. J. Linn. Soc.* **80**, 717–725.
- Bochdanovits Z. and de Jong G. 2003d Temperature dependent larval resource allocation shaping adult body size in *Drosophila melanogaster*. *J. Evol. Biol.* **16**, 1159–1167.
- Britton J. S. and Edgar B. A. 1998 Environmental control of the cell cycle in *Drosophila*: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* **125**, 2149–2158.
- Britton J. S., Lockwood W. K., Li L., Cohen S. M. and Edgar B. A. 2002 *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev. Cell* **2**, 239–249.
- Broggiolo W., Stocker H., Ikeya T., Rintelen F., Fernandez R. and Hafen E. 2001 An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* **11**, 213–221.
- Bryant P. J. 2001 Growth factors controlling imaginal disc growth in *Drosophila*. *Cell Cycle Dev.* **237**, 182–199.
- Caceres M., Barbadilla A. and Ruiz A. 1997 Inversion length and breakpoint distribution in the *Drosophila buzzatii* species complex: Is inversion length a selected trait? *Evolution* **51**, 1149–1155.
- Caceres M., Barbadilla A. and Ruiz A. 1999 Recombination rate predicts inversion size in diptera. *Genetics* **153**, 251–259.
- Calboli F. C. F., Kennington W. J. and Partridge L. 2003 QTL mapping reveals a striking coincidence in the positions of genomic regions associated with adaptive variation in body size in parallel clines of *Drosophila melanogaster* on different continents. *Evolution* **57**, 2653–2658.
- Capy P., Pla E. and David J. R. 1993 Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *Drosophila simulans*. 1. Geographic variations. *Genet. Sel. Evol.* **25**, 517–536.
- Caracristi G. and Schlotterer C. 2003 Genetic differentiation between American and European *Drosophila melanogaster* populations could be attributed to admixture of African alleles. *Mol. Biol. Evol.* **20**, 792–799.
- Cardenas M. E., Cutler N. S., Lorenz M. C., Di Como C. J. and Heitman J. 1999 The TOR signaling cascade regulates gene expression in response to nutrients. *Genes Dev.* **13**, 3271–3279.
- Cavicchi S. 1978 Investigation on early divergence between populations of *Drosophila melanogaster* kept at different temperatures. *Genetica* **48**, 81–87.
- Cavicchi S., Giorgi G., Natali V. and Guerra D. 1991 Temperature related divergence in experimental populations of *Drosophila melanogaster*. 4. Fourier and centroid analysis of wing shape and relationship between shape variation and fitness. *J. Evol. Biol.* **4**, 141–159.
- Chippindale A. K., Chu T. J. F. and Rose M. R. 1996 Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* **50**, 753–766.
- Chippindale A. K., Alipaz J. A., Chen H. W. and Rose M. R. 1997 Experimental evolution of accelerated development in *Drosophila*. 1. Developmental speed and larval survival. *Evolution* **51**, 1536–1551.
- Chippindale A. K., Ngo A. and Rose M. R. 2003 The devil in the details of life-history evolution: instability and reversal of genetic correlations during selection on *Drosophila* development. *J. Genet.* **82**, 133–145 (this issue).
- Clancy D. J., Gems D., Harshman L. G., Oldham S., Stocker H., Hafen E., Leevers S. J. and Partridge L. 2001 Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* **292**, 104–106.
- Conlon I. and Raff M. 1999 Size control in animal development. *Cell* **96**, 235–244.
- Coyne J. A. and Beecham E. 1987 Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* **117**, 727–737.
- Coyne J. A., Wicker-Thomas C. and Jallon J. M. 1999 A gene responsible for a cuticular hydrocarbon polymorphism in *Drosophila melanogaster*. *Genet. Res.* **73**, 189–203.
- David J. R. 1975 Evolution of a cosmopolitan species: genetic latitudinal clines in *Drosophila melanogaster* wild populations. *Experientia* **31**, 164–166.
- David J. R. and Capy P. 1988 Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet.* **4**, 106–111.
- David J. R., Bocquet C. and Pla E. 1976 New results on the genetic characteristic of the Far East race of *Drosophila melanogaster*. *Genet. Res.* **28**, 253–260.
- David J. R., Allemand R., Van Herreweghe J. and Cohet Y. 1983 Ecophysiology: abiotic factors. In *The genetics and biology of Drosophila* (ed. M. Ashburner, H. L. Carson and J. N. Thompson), vol. 3d, pp. 105–170. Academic Press, London.
- De Moed G. H., de Jong G. and Scharloo W. 1997 The phenotypic plasticity of wing size in *Drosophila melanogaster*: the cellular basis of its genetic variation. *Heredity* **79**, 260–267.
- De Moed G. H., de Jong G. and Scharloo W. 1998 The energetics of growth in *Drosophila melanogaster*: Effect of temperature and food conditions. *Neth. J. Zool.* **48**, 169–188.
- De Moed G. H., Kruitwagen C. L. J. J., de Jong G. and Scharloo W. 1999 Critical weight for the induction of pupariation in *Drosophila melanogaster*: genetic and environmental variation. *J. Evol. Biol.* **12**, 852–858.
- Delpuech J., Moreteau B., Chice J., Pla E., Vouidibio J. and David J. R. 1995 Phenotypic plasticity and reaction norms in temperate and tropical populations of *Drosophila melanogaster*: ovarian size and developmental temperature. *Evolution* **49**, 670–675.
- Djawan M., Chippindale A. K., Rose M. R. and Bradley T. J. 1998 Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol. Zool.* **71**, 584–594.
- Drummond-Barbosa D. and Spradling A. C. 2001 Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* **231**, 265–278.
- Eanes W. F. 1999 Analysis of selection on enzyme polymorphisms. *Annu. Rev. Ecol. Syst.* **30**, 301–326.
- Ferveur J. F., Cobb M., Boukella H. and Jallon J. M. 1996 Worldwide variation in *Drosophila melanogaster* sex pheromone: Behavioural effects, genetic bases and potential evolutionary consequences. *Genetica* **97**, 73–80.
- Gao X. S. and Pan D. J. 2001 TSC1 and TSC2 tumour suppressors antagonize insulin signaling in cell growth. *Genes Dev.* **15**, 1383–1392.
- Garofalo R. S. 2002 Genetic analysis of insulin signaling in *Drosophila*. *Trends Endocrinol. Metab.* **13**, 156–162.
- Gasser M., Kaiser M., Berrigan D. and Stearns S. C. 2000 Life-history correlates of evolution under high and low adult mortality. *Evolution* **54**, 1260–1272.
- Gilchrist A. S. and Partridge L. 1999 A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. *Genetics* **153**, 1775–1787.
- Gockel J., Kennington W. J., Hoffmann A., Goldstein D. B. and Partridge L. 2001 Nonclinality of molecular variation implicates selection in maintaining a morphological cline of *Drosophila melanogaster*. *Genetics* **158**, 319–323.

- Gockel J., Robinson S. J. W., Kennington W. J., Goldstein D. B. and Partridge L. 2002 Quantitative genetic analysis of natural variation in body size in *Drosophila melanogaster*. *Heredity* **89**, 145–153.
- Harshman L. G., Hoffmann A. A. and Clark A. G. 1999 Selection for starvation resistance in *Drosophila melanogaster*: physiological correlates, enzyme activities and multiple stress responses. *J. Evol. Biol.* **12**, 370–379.
- Hasson E. and Eanes W. F. 1996 Contrasting histories of three gene regions associated with In(3L)Payne of *Drosophila melanogaster*. *Genetics* **144**, 1565–1575.
- Hipfner D. R. and Cohen S. M. 1999 New growth factors for imaginal discs. *BioEssays* **21**, 718–720.
- Hoffmann A. A., Hallas R., Sinclair C. and Mitrovski P. 2001 Levels of variation in stress resistance in *Drosophila* among strains, local populations and geographic regions: Patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution* **55**, 1621–1630.
- Hoffmann A. A., Scott M., Partridge L. and Hallas R. 2003 Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. *J. Evol. Biol.* **16**, 614–623.
- Huey R. B., Partridge L. and Fowler K. 1989 Natural selection on thermal sensitivity of *Drosophila melanogaster*. *Am. Zool.* **29**, A141.
- Huey R. B., Partridge L. and Fowler K. 1991 Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution* **45**, 751–756.
- Huey R. B., Gilchrist G. W., Carlson M. L., Berrigan D. and Serra L. 2000 Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**, 308–309.
- Ikeya T., Galic M., Belawat P., Nairz K. and Hafen E. 2002 Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr. Biol.* **12**, 1293–1300.
- Imasheva A. G., Bublil O. A. and Lazebny O. E. 1994 Variation in wing length in Eurasian natural populations of *Drosophila melanogaster*. *Heredity* **72**, 508–514.
- Izquierdo J. 1991 How does *Drosophila melanogaster* overwinter? *Entomol. Exp. Appl.* **59**, 51–58.
- James A. C. and Partridge L. 1995 Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. *J. Evol. Biol.* **8**, 315–330.
- James A. C. and Partridge L. 1998 Geographic variation in competitive ability in *Drosophila melanogaster*. *Am. Nat.* **151**, 530–537.
- James A. C., Azevedo R. B. R. and Partridge L. 1995 Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. *Genetics* **140**, 659–666.
- James A. C., Azevedo R. B. R. and Partridge L. 1997 Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* **146**, 881–890.
- Johnston L. A. and Gallant P. 2002 Control of growth and organ size in *Drosophila*. *BioEssays* **24**, 54–64.
- Kamping A. and van Delden W. 1999a A long-term study on interactions between the Adh and alpha Gpdh allozyme polymorphisms and the chromosomal inversion In(2L)t in a seminatural population of *D. melanogaster*. *J. Evol. Biol.* **12**, 809–821.
- Kamping A. and van Delden W. 1999b The role of fertility restoration in the maintenance of the inversion In(2L)t polymorphism in *Drosophila melanogaster*. *Heredity* **83**, 460–468.
- Karan D., Dahiya N., Munjal A. K., Gibert P., Moreteau B., Parkash R. and David J. R. 1998 Desiccation and starvation tolerance of adult *Drosophila*: Opposite latitudinal clines in natural populations of three different species. *Evolution* **52**, 825–831.
- Kauer M., Zangerl B., Dieringer D. and Schlotterer C. 2002 Chromosomal patterns of microsatellite variability contrast sharply in African and non-African populations of *Drosophila melanogaster*. *Genetics* **160**, 247–256.
- Kawamura K., Shibata T., Saget O., Peel D. and Peter J. 1999 A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc cells. *Development* **126**, 211–219.
- Knibb W., Oakeshott J. and Gibson J. 1981 Chromosome inversion polymorphism in *Drosophila melanogaster*. I. Latitudinal clines and associations between inversions in Australasian populations. *Genetics* **98**, 833–837.
- Lachaise D., Cariou M. L., David J. R., Lemeunier F., Tsacas L. and Ashburner M. 1988 Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evol. Biol.* **22**, 159–225.
- Leips J. and MacKay T. F. C. 2000 Quantitative trait loci for life span in *Drosophila melanogaster*: Interactions with genetic background and larval density. *Genetics* **155**, 1773–1788.
- Leips J. and MacKay T. F. C. 2002 The complex genetic architecture of *Drosophila* life span. *Exp. Aging Res.* **28**, 361–390.
- Lemeunier F. and Aulard S. 1992 Inversion polymorphism in *Drosophila melanogaster*. In *Drosophila inversion polymorphism* (ed. C. Krimbas and J. R. Powell), pp. 339–405. CRC Press, Boca Raton.
- Lemeunier F., David J. R. and Tsacas L. 1986 The *melanogaster* species group. In *The genetics and biology of Drosophila*. (ed. M. Ashburner, H. L. Carson and J. N. Thompson), vol. 3e, pp. 147–256. Academic Press, London.
- McCabe J. and Partridge L. 1997 An interaction between environmental temperature and genetic variation for body size for the fitness of adult female *Drosophila melanogaster*. *Evolution* **51**, 1164–1174.
- Malpica J., Vasallo J., Frias A. and Fuentes-Bol F. 1987 On recombination among In(2L)t, aGpdh and Adh in *Drosophila melanogaster*. *Genetics* **115**, 141–142.
- Mettler L., Voelker R. and Mukai T. 1977 Inversion clines in populations of *Drosophila melanogaster*. *Genetics* **87**, 169–176.
- Mitrovski P. and Hoffmann A. A. 2001 Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions. *Proc. R. Soc. London* **B268**, 2163–2168.
- Neat F., Fowler K., French V. and Partridge L. 1995 Thermal evolution of growth efficiency in *Drosophila melanogaster*. *Proc. R. Soc. London* **B260**, 73–78.
- Nijhout H. 2003 The control of body size in insects. *Dev. Biol.* **261**, 1–9.
- Nijhout, H. F. 1994. *Insect hormones*. Princeton University Press, Princeton.
- Noach E. J. K., de Jong G. and Scharloo W. 1996 Phenotypic plasticity in morphological traits in two populations of *Drosophila melanogaster*. *J. Evol. Biol.* **9**, 831–844.
- Nunney L. 1996 The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: An example of a fitness trade-off. *Evolution* **50**, 1193–1204.
- Nunney L. and Cheung W. 1997 The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: Evidence for adaptive plasticity. *Evolution* **51**, 1529–1535.
- Oldham S. and Hafen E. 2003 Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control. *Trends Cell Biol.* **13**, 79–85.

- Oldham S., Montagne J., Radimerski T., Thomas G. and Hafen E. 2000 Genetic and biochemical characterization of dTOR, the *Drosophila* homolog of the target of rapamycin. *Genes Dev.* **14**, 2689–2694.
- Oudman L., van Delden W., Kamping A. and Bijlsma R. 1991 Polymorphism at the *Adh* and *a-Gpdh* loci in *Drosophila melanogaster*: effects of rearing temperature on developmental rate, body weight and some biochemical parameters. *Heredity* **67**, 103–115.
- Oudman L., van Delden W., Kamping A. and Bijlsma R. 1992 Interaction between the *Adh* and *a-Gpdh* loci in *Drosophila melanogaster*: adult survival at high temperature. *Heredity* **68**, 289–297.
- Oudman L., van Delden W., Kamping A. and Bijlsma R. 1994 Starvation resistance in *Drosophila melanogaster* in relation to the polymorphisms at the *Adh* and *a-Gpdh* loci. *J. Insect Physiol.* **40**, 709–713.
- Partridge L., Barrie B., Fowler K. and French V. 1994a Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**, 1269–1276.
- Partridge L., Barrie B., Fowler K. and French V. 1994b Thermal evolution of pre-adult life-history traits in *Drosophila melanogaster*. *J. Evol. Biol.* **7**, 645–663.
- Partridge L., Barrie B., Barton N. H., Fowler K. and French V. 1995 Rapid laboratory evolution of adult life-history traits in *Drosophila melanogaster* in response to temperature. *Evolution* **49**, 538–544.
- Partridge L., Langelan R., Fowler K., Zwaan B. and French V. 1999 Correlated responses to selection on body size in *Drosophila melanogaster*. *Genet. Res.* **74**, 43–54.
- Petavy G., Morin J. P., Moreteau B. and David J. R. 1997 Growth temperature and phenotypic plasticity in two *Drosophila* sibling species: probable adaptive changes in flight capacities. *J. Evol. Biol.* **10**, 875–887.
- Prasad N. G., Shakarad M., Gohil V. M., Sheeba V., Rajamani M. and Joshi A. 2000 Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time. *Genet. Res.* **76**, 249–259.
- Reeve M. W., Fowler K. and Partridge L. 2000 Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. *J. Evol. Biol.* **13**, 836–844.
- Robinson S. J. W. and Partridge L. 2001 Temperature and clinal variation in larval growth efficiency in *Drosophila melanogaster*. *J. Evol. Biol.* **14**, 14–21.
- Robinson S. J. W., Zwaan B. and Partridge L. 2000 Starvation resistance and adult body composition in a latitudinal cline of *Drosophila melanogaster*. *Evolution* **54**, 1819–1824.
- Rulifson E. J., Kim S. K. and Nusse R. 2002 Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. *Science* **296**, 1118–1120.
- Saltiel A. R. and Kahn C. R. 2001 Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799–806.
- Santos M., Fowler K. and Partridge L. 1994 Gene-environment interaction for body size and larval density in *Drosophila melanogaster*: an investigation of effects on development time, thorax length and adult sex ratio. *Heredity* **72**, 515–521.
- Santos M., Borash D. J., Joshi A., Bounlutay N. and Mueller L. D. 1997 Density-dependent natural selection in *Drosophila*: Evolution of growth rate and body size. *Evolution* **51**, 420–432.
- Saucedo L. J. and Edgar B. A. 2002 Why size matters: altering cell size. *Curr. Opin. Genet. Dev.* **12**, 565–571.
- Schmelzle T. and Hall M. N. 2000 TOR, a central controller of cell growth. *Cell* **103**, 253–262.
- Stearns S. C., Ackermann M., Doebeli M. and Kaiser M. 2000 Experimental evolution of aging, growth and reproduction in fruitflies. *Proc. Natl. Acad. Sci. USA* **97**, 3309–3313.
- Stern D. 2001 Body-size evolution: How to evolve a mammoth moth. *Curr. Biol.* **11**, R917–R919.
- Stern D. 2003 Body-size control: How an insect knows it has grown enough. *Curr. Biol.* **13**, R267–R269.
- Tatar M., Kopelman A., Epstein D., Tu M. P., Yin C. M. and Garofalo R. S. 2001 A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107–110.
- Tatar M., Bartke A. and Antebi A. 2003 The endocrine regulation of aging by insulin-like signals. *Science* **299**, 1346–1351.
- van Delden W. and Kamping A. 1989 The association between the polymorphisms at the *Adh* and *a-Gpdh* loci and the *In(2L)t* inversion in *Drosophila melanogaster* in relation to temperature. *Evolution* **43**, 775–793.
- van Delden W. and Kamping A. 1991 Changes in relative fitness with temperature among 2nd chromosome arrangements in *Drosophila melanogaster*. *Genetics* **127**, 507–514.
- van Dijken F., Stolwijk H. and Scharloo W. 1985 Locomotor activity in *Drosophila melanogaster*. *Neth. J. Zool.* **35**, 438–454.
- van't Land J., Van Putten P., Zwaan B., Kamping A. and van Delden W. 1999 Latitudinal variation in wild populations of *Drosophila melanogaster*: heritabilities and reaction norms. *J. Evol. Biol.* **12**, 222–232.
- van't Land J., Van Putten W. F., Villarroel H., Kamping A. and van Delden W. 2000 Latitudinal variation for two enzyme loci and an inversion polymorphism in *Drosophila melanogaster* from Central and South America. *Evolution* **54**, 201–209.
- Verrelli B. C. and Eanes W. F. 2000 Extensive amino acid polymorphism at the *Pgm* locus is consistent with adaptive protein evolution in *Drosophila melanogaster*. *Genetics* **156**, 1737–1752.
- Verrelli B. C. and Eanes W. F. 2001a Clinal variation for amino acid polymorphisms at the *Pgm* locus in *Drosophila melanogaster*. *Genetics* **157**, 1649–1663.
- Verrelli B. C. and Eanes W. F. 2001b The functional impact of *Pgm* amino acid polymorphism on glycogen content in *Drosophila melanogaster*. *Genetics* **159**, 201–210.
- Wayne M. L., Hackett J. B., Dilda C. L., Nuzhdin S. V., Pasyukova E. G. and MacKay T. F. C. 2001 Quantitative trait locus mapping of fitness-related traits in *Drosophila melanogaster*. *Genet. Res.* **77**, 107–116.
- Weeks A. R., McKechnie S. W. and Hoffmann A. A. 2002 Dissecting adaptive clinal variation: markers, inversions and size/stress associations in *Drosophila melanogaster* from a central field population. *Ecol. Lett.* **5**, 756–763.
- Worthen W. B. 1996 Latitudinal variation in developmental time and mass in *Drosophila melanogaster*. *Evolution* **50**, 2523–2529.
- Zhang H. B., Stallock J. P., Ng J. C., Reinhard C. and Neufeld T. P. 2000 Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes Dev.* **14**, 2712–2724.
- Zurovcova M. and Ayala F. J. 2002 Polymorphism patterns in two tightly linked developmental genes, *Idgf1* and *Idgf3*, of *Drosophila melanogaster*. *Genetics* **162**, 177–188.
- Zwaan B. J., Azevedo R. B. R., James A. C., van't Land J. and Partridge L. 2000 Cellular basis of wing size variation in *Drosophila melanogaster*: a comparison of latitudinal clines on two continents. *Heredity* **84**, 338–347.