



Pergamon

Journal of Insect Physiology 48 (2002) 231–237

*Journal
of
Insect
Physiology*

www.elsevier.com/locate/jinsphys

Temperature modulates epidermal cell size in *Drosophila melanogaster*

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Received 29 August 2001; received in revised form 29 October 2001; accepted 30 October 2001

Abstract

Most ectotherms show increased body size at maturity when reared under colder temperatures. In principle, temperature could produce this outcome by influencing growth, proliferation and/or death of epidermal cells. Here we investigated the effects of rearing temperature on the cell size and cell number in the wing blade, the basitarsus of the leg and the cornea of the eye of *Drosophila melanogaster* from two populations at opposite ends of a South American latitudinal cline. We found that, in both strains of *D. melanogaster* and in both sexes, a decrease in rearing temperature increases the size of the wings, legs and eyes through an effect on epidermal cell size, with no significant change in cell number. Our results indicate that temperature has a consistent effect on cell size in the *Drosophila* epidermis and this may also apply to other cell types. In contrast, the evolutionary effects of temperature on the different organs are not consistent. We discuss our findings in the context of growth control in *Drosophila*. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Temperature; Body size; Cell size; Growth; *Drosophila*

1. Introduction

It has long been known that ectothermic organisms develop more slowly but show increased body size at maturity, when they are reared under colder temperatures (Alpatov, 1930; Ray, 1960; von Bertalanffy, 1960). In a comprehensive review of the literature, Atkinson (1994) found that over 80% of experimental studies demonstrate an inverse relationship between rearing temperature and adult body size. This pattern has been found in protists, plants and several animal phyla, such as rotifers, nematodes, molluscs and chordates. It has been most thoroughly documented across the arthropods and, particularly, within both the hemimetabolous and holometabolous insects. For example, when *Drosophila melanogaster* eggs were laid at 25 °C and then reared at either 16.5 or 29 °C, the resulting flies from the colder

temperature were 9% and 35% larger in their thorax length and wing area, respectively (French et al., 1998). There is, as yet, no comprehensive mechanistic explanation for this widespread developmental response to rearing temperature (Partridge and French, 1996; Atkinson, 1994).

The adult body size of an insect depends on the final number and size of the cells in the surface epidermal layer that secretes the cuticular exoskeleton. In principle, the rearing temperature could affect the epidermis through influences on the growth, the division and/or the death of cells (for reviews of size regulation see Conlon and Raff, 1999; Stern and Emlen, 1999; Day and Lawrence, 2000). There have been several studies of the cellular basis of temperature effects on size in the fruitfly *D. melanogaster*, but only in the wing blade where each epidermal cell secretes a cuticular trichome (Dobzhansky, 1929). Trichome counts demonstrate that a decrease in rearing temperature increases the wing size through an increase in cell size, with little or no effect on final cell number (Alpatov, 1930; Robertson, 1959; Delcour and Lints, 1966; Masry and Robertson, 1979;

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Partridge et al., 1994; DeMoed et al., 1997a,b). Here we have extended the study of the effects of rearing temperature to the epidermal cell size and cell number in the basitarsus of the leg and in the cornea of the eye.

In addition to the developmental response, temperature can also cause the evolution of changes in the genetic control of body size. Laboratory populations of *Drosophila* kept at low temperature evolve an increased body size, when reared at a standard temperature (Anderson, 1973; Cavicchi et al. 1985, 1989; Partridge et al., 1994). At least in the wing blade of *D. melanogaster*, this results from an increase in cell size (Cavicchi et al., 1985; Partridge et al., 1994). Thermal evolution seems also to occur in natural populations. In many species of animal (including fruit flies) there are clear geographical clines in body size, with populations from the higher latitudes producing the larger individuals, even when all are reared in standard conditions (for a review see Partridge and French, 1996). Several species of *Drosophila* show such genetic clines in body size across different continents (Misra and Reeve, 1964; Stalker and Carson, 1947; David and Bocquet, 1975; James et al., 1995; Pegueroles et al., 1995; Zwaan et al., 2000). The cellular basis of the size differences has been studied in the Australian and South American *D. melanogaster* clines—again, only in the wing blade—and cell size has been shown to contribute significantly, although to different extents in the two clines (James et al., 1995; Zwaan et al., 2000). Here we investigate differences in cell size and cell number in the wing, leg and eye, between two divergent populations from the South American latitudinal cline (Zwaan et al., 2000).

2. Materials and methods

2.1. Genotypes

In these experiments, we used stocks of *D. melanogaster* derived from two South American populations collected by van't Land and van Putten in 1995: one from Chile (PM, latitude 42° S) and another from Ecuador (GU, latitude 2° S). Stocks were kept in bottles at 25 °C for one year before these experiments were carried out and they differed in adult size (PM flies having larger wings than GU flies) but not in their development rate (van't Land et al., 1999).

The possibility of maternal effects was eliminated by rearing flies from each population at low density and at a temperature of either 25 or 18 °C, and then using these as parents of the experimental flies. The parental flies (from each population and each temperature) were then transferred to small yeasted laying pots and eggs were collected over a 3-h period. The first instar larvae hatching from these eggs were then used to set up standard, low-density cultures in vials of yeasted food medium at

the appropriate temperature (25 or 18 °C). For later measurement of leg cell size (see below), some third instar larvae were removed, sexed and then reared in separate vials, while the other larvae were allowed to develop undisturbed to adulthood.

2.2. Measurements

We measured organ size and epidermal cell size in the wing, in the eye and in the proximal tarsal segment (the basitarsus) of the leg. Two-day-old adult flies were preserved in 70% ethanol, and area measurements could then be made of individual wing cells, each of which secretes a cuticular trichome (Dobzhansky, 1929), and of the group of four cone cells which secrete the corneal lens of the eye ommatidium (Wolff and Ready, 1993). Leg cell measurements, however, must be made at late pupal stage, directly on the epidermal cells before they die (Held, 1979). Independent samples of 10 animals of each sex, genotype (PM and GM) and temperature treatment (18 and 25 °C) were measured for the different size traits, and each cell area measurement was repeated three times per individual organ and then averaged to give a mean cell area.

For leg cell measurements, early pupae of known sex were transferred to a slide covered with double-sided tape and allowed to develop (for 35 h at 25 °C and for 74 h at 18 °C) to reach early 'pharate adult' stage (Bainbridge and Bownes, 1981). Then the pupae were extracted from the puparium, pierced in the thorax, fixed in Perenyi's fluid and stained with Schiff's reagent (Held, 1979). Mesothoracic legs of each pupa were dissected and mounted in DPX, and the anterior sides of the basitarsi were photographed at $\times 200$ magnification, using a Nikon CV-M300 video camera attached to a PowerMacintosh. The length and anterior area of each basitarsus was measured using Object-Image version 1.60 (Vischer et al., 1994). The central region of each basitarsus was photographed at $\times 1000$ in about 30 sections, the nuclei contained in a circular area of 250 μm^2 were counted and the average cell area was calculated by dividing 250 μm^2 by the nuclear count. An index of the total number of cells in the anterior basitarsus was calculated by dividing the area by the average cell area.

For measurement, one wing from each fly was removed, fixed in propanol, mounted in Aquamount, photographed at $\times 50$ magnification and the wing area was measured using Object-Image. A standard region (in the posterior medial cell, equidistant from the fourth and fifth longitudinal veins and the posterior cross vein) was photographed at $\times 400$ magnification, and the trichomes contained within a circular 12 500 μm^2 area were counted. Average cell area was calculated by dividing 12 500 μm^2 by the trichome count, and an index of the total number of cells on the dorsal wing surface was calculated by dividing the wing area by its average cell area.

For eye measurements, adult heads were dissected and boiled in 1 M potassium hydroxide to remove the internal tissue. The eyes were photographed at $\times 50$ magnification in standard orientation and their areas were measured using Object-Image. Then the average area of 32 ommatidial facets in the middle of the eye was estimated using Object-Image, by measuring the area of a triangle containing the centres of nine ommatidial facets on each side. An index of the total number of ommatidial facets was then calculated by dividing the area of the eye by its average facet area.

3. Results

3.1. Effects of sex, genotype and rearing temperature on organ size

Our data show that the wings, tarsi and eyes all differ significantly in size between sexes, between the equatorial (GU) and temperate (PM) populations, and between rearing temperatures of 25 and 18 °C (Table 1;

Fig. 1). Higher order interactions between these effects were non-significant, with the exception of a significant genotype-by-temperature interaction for wing size: the difference among genotypes, averaged across sexes, was 14% at 25 °C and 21% at 18 °C (Table 1; Fig. 1). Females were about 20% larger than males in all three organs, flies from the PM strain were bigger than those from the GU strain and all flies were larger when reared at 18 °C (Table 2). However, the relative magnitudes of the effects of genotype and temperature differed among organs (Table 2): both effects were strongest in the wing ($\sim 20\%$), intermediate in the leg ($\sim 10\%$) and weakest in the eye ($\sim 5\%$).

3.2. Cellular basis of the effects of sex, genotype and rearing temperature

The effect of rearing temperature on the size of all three organs is mediated solely by changes in cell size; cell number does not change significantly between temperatures (Tables 1 and 2). This is confirmed by the observation that the response to temperature for every organ, genotype and sex has a slope of approximately 1

Table 1

Factorial ANOVAs on organ size, cell size and cell number including temperature, genotype and sex as fixed main effects. The variables were \log_{10} -transformed to satisfy the assumption of homogeneity of variances

Source of variation	df	Organ size		Cell size		Cell number	
		SS	F-ratio	SS	F-ratio	SS	F-ratio
Wings							
Genotype (G)	1	0.114332	160.06***	0.000926	1.59	0.094687	111.42***
Temperature (T)	1	0.163579	229.00***	0.127392	219.14***	0.002259	2.66
Sex (S)	1	0.250515	350.70***	0.097936	168.47***	0.035182	41.40***
G×T	1	0.005123	7.17**	0.001542	2.65	0.001043	1.23
G×S	1	0.000509	0.71	0.000253	0.44	0.000044	0.05
T×S	1	0.000256	0.36	0.000487	0.84	0.000037	0.04
G×T×S	1	0.000008	0.01	0.000092	0.16	0.000153	0.18
Error	72	0.051431		0.041855		0.061189	
Legs							
Genotype (G)	1	0.036705	26.07***	0.023962	19.32***	0.001354	0.62
Temperature (T)	1	0.067163	47.70***	0.035108	28.31***	0.005153	2.38
Sex (S)	1	0.188150	133.62***	0.059349	47.86***	0.036154	16.67***
G×T	1	0.002214	1.57	0.001047	0.84	0.000216	0.10
G×S	1	0.000855	0.61	0.000001	0.00	0.000803	0.37
T×S	1	0.000977	0.70	0.000000	0.00	0.000950	0.44
G×T×S	1	0.000021	0.01	0.000331	0.27	0.000518	0.24
Error	72	0.101386		0.252974		0.177829	
Eye							
Genotype (G)	1	0.004484	4.27*	0.002393	4.68*	0.000326	0.36
Temperature (T)	1	0.017786	16.95***	0.012624	24.68***	0.000441	0.48
Sex (S)	1	0.144114	137.31***	0.021489	42.01***	0.054304	59.46***
G×T	1	0.000345	0.33	0.000179	0.35	0.000027	0.03
G×S	1	0.000976	0.93	0.000056	0.11	0.000565	0.62
T×S	1	0.001020	0.97	0.001441	2.82	0.000036	0.04
G×T×S	1	0.000031	0.03	0.000015	0.03	0.000090	0.10
Error	72	0.075567		0.036828		0.065755	

Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

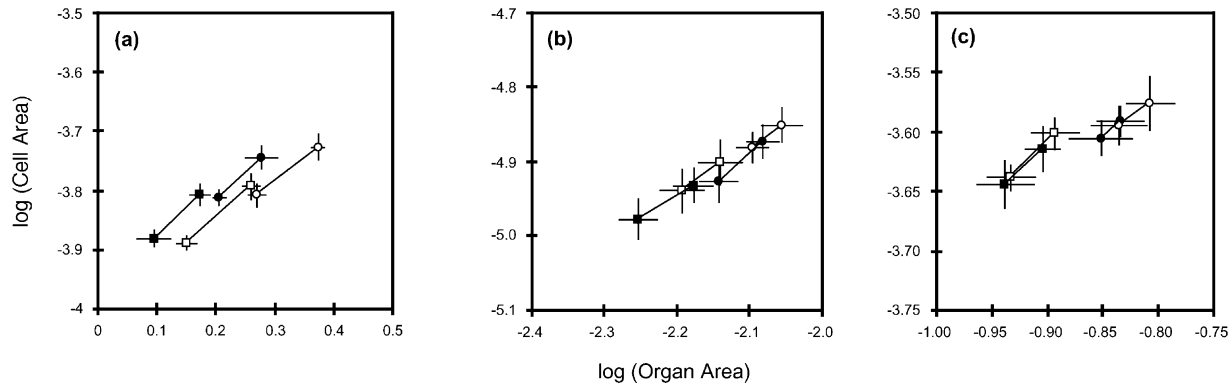


Fig. 1. Cell area and organ area for the different genotypes, sexes and treatments: (a) wing; (b) leg; (c) eye. ●, GU females; ■, GU males; ○, PM females; □, PM males. Data from the two experimental temperatures are connected by lines. Values are means and 95% confidence intervals of \log_{10} -transformed measurements. The X and Y axes of each plot are scaled so that a slope of 1 (indicating that the change in organ size is entirely based on cell size) corresponds to a 45° diagonal.

Table 2

Average magnitudes of the effects of temperature, genotype and sex on organ size and cell size in the three structures studied (difference in size between the two states as a proportion of the average size)

Organ	Magnitude of effect (%)		
	Genotype (PM–GU)	Temperature (change per °C)	Sex (F–M)
Wing			
Organ size	17.8	–3.0	25.6
Cell size	1.9	–2.6	15.9
Cell number	15.7	–0.3	9.7
Leg			
Organ size	9.9	–1.9	22.1
Cell size	8.0	–1.4	12.4
Cell number	1.8	–0.5	9.4
Eye			
Organ size	3.6	–0.9	19.5
Cell size	2.8	–0.9	7.7
Cell number	0.8	–0.1	12.1

in the log cell size vs. log organ size plots given in Fig. 1 (for details see Zwaan et al., 2000, pp. 340–341). On average, cell area in the wing, leg and eye declined by 2.6%, 1.4% and 0.9%, respectively, for a 1 °C rise in rearing temperature (Table 2).

Sexual dimorphism in the size of wings, tarsi and eyes is caused by differences in both cell size and cell number (Tables 1 and 2; Fig. 1). The cellular basis of the size differences between the two geographical populations is not consistent, however, for the different organs: the wings differ only in cell number, whereas the legs and the eyes differ in the size, but not in the number, of their cells (Tables 1 and 2; Fig. 1).

4. Discussion

4.1. Rearing temperature, body size and cell size

In the two geographical strains of *D. melanogaster* and in both sexes, we have found that a decrease in rearing temperature increases the size of the wings, legs and eyes through an effect on epidermal cell size, with no significant change in cell number. It was important to extend the study to other organs, as the adult wing is a highly specialised structure, with cuticle secreted by a very squamous epidermis, and therefore may not be representative of the rest of epidermis. Indeed, it has been proposed that the trichome spacing estimates the degree

of flattening (rather than the size) of the epidermal cells (Kuo and Larsen, 1987). Our results indicate, however, that temperature has a consistent effect on cell size in the *Drosophila* epidermis and this may also apply to other cell types. Furthermore, there is some indication that an inverse relationship between temperature and cell size may be very general, as it has been reported in several cell types in a planarian (Romero and Baguna, 1991) and in the red blood cells of a fish (van Voorhies, 1996). Lower temperatures also increase cell size in the wings of other dipteran flies (V. French and L. Partridge, unpublished results). If this applies generally to insect epidermis, it will be most interesting to study cell size control in those atypical species, such as the cricket *Acheta* (Roe et al., 1980), in which body size is *decreased* by a reduction in rearing temperature.

Egg size also tends to be inversely related to temperature in several animal species including *D. melanogaster* (for a review see Azevedo et al., 1997). However, since mature oocytes are usually giant cells whose contents result from the metabolic activities of many other cells (both adjacent and remote), the mechanisms controlling their size might not be comparable to those of somatic cells.

4.2. Evolutionary temperature, body size and cell size

Many studies have found geographical size clines in *Drosophila*, with a consistent increase in body size with latitude (see Section 1). The results of laboratory thermal evolution experiments, and the demonstrations of genetic increases in size with increasing altitude (Stalker and Carson, 1948) and in cooler periods of the year (Stalker and Carson, 1949; Tantawy, 1964), have implicated temperature as a major selective agent in the evolution of the latitudinal clines. It was already known, however, that the cellular basis of the differences in wing size differs between the *D. melanogaster* clines in Australia and South America (Zwaan et al., 2000). Here we have shown that the size differences do not even have the same cellular basis in different organs: populations from the South American cline differ in wing size through differences in cell number, whereas it is cell size that differs in the tarsus and the eye. It is not clear why long-term exposure to different environmental conditions (including temperature) should favour genetic changes with different consequences for growth control in different regions of the epidermis.

4.3. Growth control and the effects of temperature

In *Drosophila*, the epidermis of the adult head and thoracic segments is formed by separate imaginal discs that arise within the embryonic segments and then grow steadily inside the developing larva, eventually to fuse together and replace the larval epidermis during meta-

morphosis. The wing imaginal disc, for example, grows from around 20 to 50 000 cells between hatching and pupariation (Bryant and Levinson, 1985), and then undergoes further cell divisions in the early pupa (Schubiger and Palka, 1987).

The control of growth of the imaginal discs (and thereby control of adult size) has generally been regarded as *intrinsic* to the discs, given full larval nutrition (Bryant and Simpson, 1984). Hence discs will not grow beyond their normal size, even in a larva with delayed pupariation, and an immature disc implanted into the permissive conditions of the adult abdomen will grow up to (but not beyond) the normal final cell number (Bryant and Levinson, 1985). The intrinsic control of growth is closely integrated with the intercellular signalling that establishes spatial patterns of cell fate within the developing disc. Hence the ectopic expression of the secreted signals encoded by genes such as *decapentaplegic* or *wingless* provokes both localised cell proliferation and the duplication of pattern (e.g. Zecca et al., 1996) and, conversely, cells that are unable to transduce these signals do not grow or divide (Edgar and Lehner, 1996). One of the targets of *wingless* is the transcription factor *Drosophila Myc* (Johnston et al., 1999). Loss of *dMyc* function slows down cellular growth and reduces cell size, whereas *dMyc* overexpression increases cell growth rate and cell size (Johnston et al., 1999).

It has now become clear that there is also an *extrinsic* control of imaginal disc growth, mediated by the highly-conserved insulin signalling pathway (Oldham et al., 2000; Leever, 2001). Manipulations of intracellular kinase transduction from the insulin receptor (DInr) can lead to increases or decreases in wing size, through effects on both cell size and cell number (Leever et al., 1996; Weinkove et al., 1999). Similarly, reducing DInr activity approximately halves body size, reducing both cell size and cell number in both the wing and the eye, whereas overexpression of the receptor in the developing eye increases both the size and number of its cells (Brogiolo et al., 2001). Several genes have now been identified that code for insulin-like peptides (putative ligands for DInr) and are expressed specifically within the brain, the ventral nerve cord, the gut or, in one case (DILP2), in the imaginal discs. Strikingly, overexpression of DILP2 greatly enlarges the adult, again by increasing both cell size and cell number (Brogiolo et al., 2001). One target of insulin signalling, the ribosomal protein S6 kinase, affects cell size but not cell number (Montagne et al., 1999). The insulin signalling pathway is thought to regulate growth in response to environmental factors such as the availability of nutrients (Oldham et al., 2000; Drummond-Barbosa and Spradling, 2001).

Cell cycle regulators appear to be the downstream targets of both intrinsic and extrinsic factors that ultimately regulate cell growth rate, cell size and proliferation (Neufeld et al., 1998). However, cell cycle genes do not,

themselves, regulate organ size. For example, overexpression of the transcriptional regulator *dE2F* during imaginal disc development speeds up the cell cycle and results in discs of normal size with more, smaller cells; similarly, overexpression of *RBF*, an inhibitor of *dE2F*, slows down the cell cycle and results in a disc of normal size with fewer, larger cells (Neufeld et al., 1998).

Despite recent progress in understanding the genetic basis of growth control in *Drosophila* developing organs, the way in which the different processes interact remains largely unknown. We believe that the developmental effect of temperature on cell size provides an important model system for the study of growth control. Rearing temperature has a very consistent effect on the size of ectothermic animals (Atkinson, 1994) and, arguing from the present results from different regions of *Drosophila* epidermis (and from other sparse evidence), this may typically occur by modulation of cell size. Identifying the elements of growth control that are modulated by developmental temperature and by thermal selection remains a challenge for future research.

Acknowledgements

We thank Lewis Held, Jr, and David Stern for advice on the histology and Armand Leroi for help with the microscopy. This research was funded by the Biotechnology and Biological Sciences Research Council.

References

- Alpatov, W.W., 1930. Phenotypical variation in body and cell size of *Drosophila melanogaster*. *Biological Bulletin* 58, 85–103.
- Anderson, W.W., 1973. Genetic divergence in body size among experimental populations of *Drosophila pseudoobscura* kept at different temperatures. *Evolution* 27, 278–284.
- Atkinson, D., 1994. Temperature and organism size: a biological law for ectotherms? *Advances in Ecological Research* 25, 1–58.
- Azevedo, R.B.R., French, V., Partridge, L., 1997. Life history consequences of egg size in *Drosophila melanogaster*. *American Naturalist* 150, 250–282.
- Bainbridge, S.P., Bownes, M., 1981. Staging the metamorphosis of *Drosophila melanogaster*. *Journal of Embryology and Experimental Morphology* 66, 57–80.
- Broggiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., Hafen, E., 2001. An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Current Biology* 11, 213–221.
- Bryant, P.J., Simpson, P., 1984. Intrinsic and extrinsic control of growth in developing organs. *Quarterly Review of Biology* 59, 387–415.
- Bryant, P.J., Levinson, P., 1985. Intrinsic growth control in the imaginal primordia of *Drosophila* and the autonomous action of a lethal mutation causing overgrowth. *Developmental Biology* 107, 355–363.
- Cavicchi, S., Guerra, D., Giorgi, G., Pezzoli, C., 1985. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. *Genetics* 109, 665–689.
- Cavicchi, S., Guerra, D., Natali, V., Pezzoli, C., Giorgi, G., 1989. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. II. Correlation between fitness and body dimensions. *Journal of Evolutionary Biology* 2, 235–251.
- Conlon, I., Raff, M., 1999. Size control in animal development. *Cell* 96, 235–244.
- David, J.R., Bocquet, C., 1975. Similarities and differences in latitudinal adaptation of two *Drosophila* sibling species. *Nature* 257, 588–590.
- Day, S.J., Lawrence, P.A., 2000. Measuring dimensions: the regulation of size and shape. *Development* 127, 2977–2987.
- Delcour, J., Lints, F.A., 1966. Environmental and genetic variations of wing size, cell size and cell division rate, in *Drosophila melanogaster*. *Genetica* 37, 543–556.
- DeMoed, G.H., DeJong, G., Scharloo, W., 1997a. Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. *Genetical Research* 70, 35–43.
- DeMoed, G.H., DeJong, G., Scharloo, W., 1997b. The phenotypic plasticity of wing size in *Drosophila melanogaster*: the cellular basis of its genetic variation. *Heredity* 79, 260–267.
- Dobzhansky, T., 1929. The influence of the quantity and quality of chromosomal material on the size of the cells in *Drosophila melanogaster*. *Wilhelm Roux' Archiv für Entwicklungsmechanik* 115, 363–379.
- Drummond-Barbosa, D., Spradling, A.C., 2001. Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Developmental Biology* 231, 265–278.
- Edgar, B.A., Lehner, C.F., 1996. Developmental control of cell cycle regulators: a fly's perspective. *Science* 274, 1646–1652.
- French, V., Feast, M., Partridge, L., 1998. Body size and cell size in *Drosophila*: the developmental response to temperature. *Journal of Insect Physiology* 44, 1081–1089.
- Held, L.I. Jr., 1979. Pattern as a function of cell number and cell size on the second-leg basitarsus of *Drosophila*. *Roux's Archives of Developmental Biology* 187, 105–127.
- James, A.C., Azevedo, R.B.R., 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., Partridge, L., 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* 146, 881–890.
- Johnston, L.A., Prober, D.A., Edgar, B.A., Eisenman, R.N., Gallant, P., 1999. *Drosophila myc* regulates cellular growth during development. *Cell* 98, 779–790.
- Kuo, T., Larsen, E., 1987. The cellular basis of wing size modification in *Drosophila*: the effects of the miniature gene, crowding, and temperature. *Developmental Genetics* 8, 91–98.
- Leevers, S.J., Weinkove, D., MacDougall, L.K., Hafen, E., Waterfield, M.D., 1996. The *Drosophila* phosphoinositide 3-kinase Dp110 promotes cell growth. *The EMBO Journal* 15, 6584–6594.
- Leevers, S.J., 2001. Growth control: invertebrate insulin surprises. *Current Biology* 11, R209–R212.
- Masry, A.M., Robertson, F.W., 1979. Cell size and number in the *Drosophila* wing. III. The influence of temperature differences during development. *Egyptian Journal of Genetics and Cytology* 8, 71–79.
- Misra, R.K., Reeve, E.C.R., 1964. Clines in body dimensions in populations of *Drosophila subobscura*. *Genetical Research* 5, 240–256.
- Montagne, J., Stewart, M.J., Stocker, H., Hafen, E., Kozma, S.C., Thomas, G., 1999. *Drosophila* S6 kinase: a regulator of cell size. *Science* 285, 2126–2129.
- Neufeld, T.P., de la Cruz, A.F.A., Johnston, L.A., Edgar, B.A., 1998. Coordination of growth and cell division in the *Drosophila* wing. *Cell* 93, 1183–1193.
- Oldham, S., Böhni, R., Stocker, H., Brogiolo, W., Hafen, E., 2000. Genetic control of size in *Drosophila*. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* 355, 945–952.

- Partridge, L., Barrie, B., Fowler, K., French, V., 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* 48, 1269–1276.
- Partridge, L., French, V., 1996. Thermal evolution of ectotherm size: why get big in the cold? In: Johnston, I.A., Bennett, A.F. (Eds.), *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge, pp. 265–292.
- Pegueroles, G., Papaceit, M., Quintana, A., Guillén, A., Prevosti, A., Serra, L., 1995. An experimental study of evolution in progress: clines for quantitative traits in colonizing and Palearctic populations of *Drosophila*. *Evolutionary Ecology* 9, 453–465.
- Ray, C., 1960. The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology* 106, 85–108.
- Robertson, F.W., 1959. Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. *Genetics* 44, 869–896.
- Roe, R.M., Clifford, C.W., Woodring, J.P., 1980. The effect of temperature on feeding, growth and metabolism during the last larval stadium of the female house cricket *Acheta domesticus*. *Journal of Insect Physiology* 26, 639–644.
- Romero, R., Baguna, J., 1991. Quantitative cellular analysis of growth and reproduction in fresh-water planarians (Turbellaria, Tricladida). 1. A cellular description of the intact organism. *Invertebrate Reproduction and Development* 19, 157–165.
- Schubiger, M., Palka, J., 1987. Changing spatial patterns of DNA replication in the developing wing of *Drosophila*. *Developmental Biology* 123, 145–153.
- Stalker, H.D., Carson, H.L., 1947. Morphological variation in natural populations of *Drosophila robusta* Sturtevant. *Evolution* 1, 237–248.
- Stalker, H.D., Carson, H.L., 1948. An altitudinal transect of *Drosophila robusta* Sturtevant. *Evolution* 2, 295–305.
- Stalker, H.D., Carson, H.L., 1949. Seasonal variation in the morphology of *Drosophila robusta* Sturtevant. *Evolution* 3, 330–343.
- Stern, D.L., Emlen, D.J., 1999. The developmental basis for allometry in insects. *Development* 126, 1091–1101.
- Tantawy, A.O., 1964. Studies on natural populations of *Drosophila*. III. Morphological and genetic differences of wing length in *Drosophila melanogaster* and *D. simulans* in relation to season. *Evolution* 18, 560–570.
- van't Land, J., van Putten, P., Zwaan, B., Kamping, A., van Delden, W., 1999. Latitudinal variation in wild populations of *Drosophila melanogaster*: heritabilities and reaction norms. *Journal of Evolutionary Biology* 12, 222–232.
- van Voorhies, W.A., 1996. Bergmann size clines: a simple explanation for their occurrence in ectotherms. *Evolution* 50, 1259–1264.
- Vischer, N.O.E., Huls, P.G., Woldringh, C.L., 1994. Object-Image: an interactive image-analysis program using structured point collection. *Binary* 6, 160–166.
- von Bertalanffy, L., 1960. Principles and theory of growth. In: Nowinski, W.N. (Ed.), *Fundamental Aspects of Normal and Malignant Growth*. Elsevier, Amsterdam, pp. 137–259.
- Weinkove, D., Neufeld, T.P., Twardzik, T., Waterfield, M.D., Leever, S.J., 1999. Regulation of imaginal disc cell size, cell number and organ site by *Drosophila* class I-A phosphoinositide 3-kinase and its adaptor. *Current Biology* 9, 1019–1029.
- Wolff, T., Ready, D.F., 1993. Pattern formation in the *Drosophila* retina. In: Bate, M., Martínez-Arias, A. (Eds.), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, New York, pp. 1277–1325.
- Zecca, M., Basler, K., Struhl, G., 1996. Direct and long-range action of a wingless morphogen gradient. *Cell* 87, 833–844.
- Zwaan, B.J., Azevedo, R.B.R., James, A.C., Van't Land, J., Partridge, L., 2000. Cellular basis of wing size variation in *Drosophila melanogaster*: a comparison of latitudinal clines on two continents. *Heredity* 84, 338–347.