Functional Ecology 2004
18, 700–706

Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability

A. AYRINHAC,*‡ V. DEBAT,* P. GIBERT,*§ A.-G. KISTER,* H. LEGOUT,* B. MORETEAU,* R. VERGILINO* and J. R. DAVID*†

**Laboratoire Populations, Génétique, et Evolution, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette Cedex, France, ‡University of Groningen, Evolutionary Genetics Group, 9750 AA Haren, The Netherlands, and §Laboratoire Biométrie, Biologie Evolutive, Université Lyon I, 69622 Villeurbanne Cedex, France*

Summary

1. According to their geographical distribution, most *Drosophila* species may be classified as either temperate or tropical, and this pattern is assumed to reflect differences in their thermal adaptation, especially in their cold tolerance. We investigated cold tolerance in a global collection of *D. melanogaster* by monitoring the time adults take to recover from chill coma after a treatment at 0 °C.

2. Flies grown at an intermediate temperature (21 °C) showed a significant linear latitudinal cline: recovery was faster in populations living in colder climates.

3. The role of growth temperature was analysed in a subset of tropical and temperate populations. In all cases, recovery time decreased when growth temperature was lowered, and linear reaction norms were observed. This adaptive phenotypic plasticity explained more than 80% of the total variation, while genetic latitudinal differences accounted for less than 4%.

4. The beneficial effect observed in adults grown at a low temperature contrasts with other phenotypic effects which, like male sterility, appear as harmful and pathological. Our results point to the difficulty of finding a general interpretation to the diversity of plastic responses that are induced by growth temperature variations.

Key-words: Chill coma, cold tolerance, latitudinal cline, reaction norms, recovery time

Functional Ecology (2004) **18**, 700–706

Introduction

For ectotherm species, such as insects, temperature has long been recognized as a major environmental factor responsible for species abundance and geographic distribution (Andrewartha & Birch 1954; Precht, Christophersen & Hensel 1955; Cossins & Bowler 1987; Leather, Walters & Bale 1993). Ambient temperature varies according to daytime and season, so that natural populations are often exposed to heat or cold stress (Gibbs, Perkins & Markow 2003). The capacity to adapt to and tolerate such stresses is crucial for the persistence of populations (Hoffmann & Parsons 1991, 1997; Addo-Bediako, Chown & Gaston 2000; Chown, Addo-Bediako & Gaston 2002; Hoffmann, Sorensen & Loeschcke 2003; Klok & Chown 2003). In temperate countries, species must tolerate cold conditions during winter and have developed a diversity of adaptive mechanisms to do so, including the occurrence of diapause and the production of antifreeze compounds (Leather *et al.* 1993; Graham, Walker & Davies 2000).

Drosophilid species form convenient models with which to investigate ectotherm responses to stress because they are found across a full range of habitats from the Arctic to the equator. *Drosophila melanogaster* is a cosmopolitan species, and its broad geographical range is accompanied by genetic variation in a diversity of traits, which, in most cases, vary progressively with latitude (David & Capy 1988): this pattern suggests that local climate and especially temperature is the selective factor. Latitudinal clines have been demonstrated for several morphological traits (e.g. Capy, Pla & David 1993) and also for traits directly related to fitness, such as viability, rate of development, larval competitive ability, egg production and tolerance to stresses (heat, cold, desiccation, starvation) (Stanley & Parsons 1981; Boulétreau-Merle *et al.* 1982; Davidson 1990; James & Partridge 1995, 1998; Guerra *et al.* 1997; Karan *et al.* 1998; Hoffmann *et al.* 2003).

A specific problem in thermal adaptation investigations arises from the fact that most morphological and

†Author to whom correspondence should be addressed. E-mail: david@pge.cnrs-gif.fr

physiological traits exhibit a large amount of phenotypic plasticity related to growth temperature. For several morphological traits, the response curves are now well described and adaptive variations in the shape of the reaction norms are known across species or geographical populations (see David, Gibert & Moreteau 2004). However, plasticity in fitness traits is less commonly investigated because measurements at the adult stage include variation, which is a consequence of both growth and experimental temperature (Huey *et al*. 1999).

In the present work, we analyse cold tolerance in *D. melanogaster* with a recently developed assay (David *et al*. 1998), that measures the time needed for recovering from chill coma after cold treatment. Recovery from chill coma induced at 0 °C provided a clear-cut discrimination between temperate and tropical species (Gibert *et al*. 2001). Comparison of geographical populations in nine cosmopolitan species also showed that temperate strains recovered faster than tropical ones, suggesting a genetic adaptation (Gibert *et al*. 2001). Such adaptive genetic variations were confirmed and latitudinal clines were found in Australian populations of two species, *D. melanogaster* (Hoffmann, Anderson & Hallas 2002) and *D. serrata* (Hallas, Schiffer & Hoffmann 2002). Here we investigate a global set of populations of *D. melanogaster* and demonstrate a latitudinal cline which might exist on all continents. We also analyse the role of growth temperature and show that most adaptive variability arises from phenotypic plasticity. By changing the developmental temperature the adult phenotype varies from cold tolerance (as found in temperate species) to cold sensitivity (as found in purely tropical ones).

Table 1. List of the 24 populations of *D. melanogaster* investigated with the year of collection, their (North or South) latitude of origin, mean recovery time ± standard error. Standard deviations are calculated using the number of independent measures ($n = 4{\cdot}65 \pm 0{\cdot}41$ per population)

Populations	Year of collection	Latitude	Mean ± SE (minutes)	*n*
Sao Tomé	2000	0°20 N	44·4 ± 4·7	8
Nairobi (Kenya)	2000	1°16 S	49·6 ± 2·9	4
Mombasa (Kenya)	2000	4°02 S	40·8 ± 2·1	5
Brazzaville (Congo)	1999	4°15 N	43·9 ± 8·5	3
Pointe Noire (Congo)	1999	4°46 N	39·4 ± 3·3	3
French Guyana	1996	5°09 N	45·6 ± 4·9	5
Cotonou (Benin)	1998	6°21 N	58·4 ± 1·2	4
Weita (Australia)	1997	12°4 S	33·9 ± 2·89	3
Bahia (Brazil)	1999	12°59 S	38·6 ± 3·4	3
Mananara (Madagascar)	2000	16°10 S	44·1 ± 1·9	4
Campinas (Brazil)	2000	22°53 S	39·6 ± 3·7	4
Rio (Brazil)	2001	22°54 S	44·7 ± 2·6	4
Rohtak (India)	1998	28°53 N	33·3 ± 7·8	3
Marrakech (Morocco)	2001	31°38 N	46·0 ± 0·1	2
Montevideo (Uruguay)	2000	34°51 S	40·2 ± 1·7	5
Chincoteague (USA)	2001	37°55 N	40·6 ± 2·5	4
Capri (Italy)	2000	40°33 N	31·0 ± 2·4	5
Foissac (France)	2001	44°02 N	25·3 ± 1·3	5
Bordeaux (France)	2001	44°51 N	32·2 ± 2·5	8
Besançon (France)	2000	47°15 N	27·1 ± 2·9	4
Seattle (USA)	2000	47°56 N	34·0 ± 1·8	5
Prunay (France)	2001	48°41 N	29·0 ± 6·0	4
Draveil (France)	2001	48°44 N	32·6 ± 1·9	11
Helsinki (Finland)	>5 years	60°10 N	33·9 ± 1·2	4
Total	–	–	38·64 ± 7·81	110

Materials and methods

STRAINS INVESTIGATED

We investigated 24 different strains from around the world, ranging from the equator to 60° of latitude (see Table 1). With a few exceptions (Table 1) all strains had been kept in the laboratory for less than 3 years before the start of experiments. All strains were founded from at least 10 pairs of wild-collected flies, and sometimes much more. They were kept in culture bottles at 20 ± 1 °C and the population size was at least 50 pairs in each generation.

EXPERIMENTAL PROCEDURES

Groups of adults (about 20 pairs) were taken from the mass strains and used as parents for the production of experimental flies. Each group oviposited at 21 °C for a short duration (6–8 h) in culture bottles (110 ml) containing a cornmeal–agar medium seeded with live yeast. A piece (about 5 g) of a killed yeast medium (David & Clavel 1965) was added to each bottle to improve the feeding quality and reduce possible larval crowding effects. With this procedure, the number of adults in each bottle was fewer than 500. After emergence, adults were transferred to fresh food and kept for 2–4 days before the experiment. All 24 strains were studied following development at 21 °C.

The influence of developmental temperature was analysed in a subset of nine strains, four from temperate and five from tropical origin. Oviposition took place at 21 °C as above, and culture bottles were transferred, after removing the parents, to constant temperature incubators regulated at ±0·1 °C. Seven experimental temperatures were utilised: 12, 14, 17, 21, 25, 28 and 31 °C. After emergence, adults were transferred to fresh food and kept at their growth temperature before being subjected to cold stress.

RECOVERY TIME (RT) FROM COLD TREATMENT

Adults aged 2–4 days were transferred without anaesthesia in empty glass vials which were immediately set in a box containing melting ice, that is at a temperature of 0 °C (see David *et al*. 1998). The number of flies was not precisely controlled, but was about 50. Duration of cold treatment was always 16 h. For measuring recovery time, adults are placed into a Petri dish at room temperature (22–24 °C). At the beginning, all flies are in chill coma and unable to move. The transfer to room temperature permits a progressive recovery, starting by

the capacity to move the tarsi, then the legs and finally to stand up. We considered a fly recovered from chill coma when it could stand on its legs: the fly was then removed from the Petri dish, and the time elapsed from the beginning noted as an estimate of RT. For each group, the mean RT was calculated and used as a basic observation.

In *D. melanogaster*, RT is slightly longer in males than in females (David *et al*. 1998), but the difference is small and the values in both sexes from the same batch are highly correlated. For simplicity we decided to pool the data of males and females, as in other investigations (Gibert *et al*. 2001; David *et al*. 2003). We know (David *et al*. 1998) that RT is prone to uncontrolled variations when different batches of flies from the same strain and the same age, grown at the same temperature but in different vials, are investigated. For that reason, as in previous works (Gibert *et al*. 2001; David *et al*. 2003) we always repeated the analysis several times on the same strain but on different batches of adults and in different generations. The accuracy with which the mean RT of a strain is known depends not on the total number of flies observed, but on the number of independent repeats (David *et al*. 1998).

Results

GENETIC VARIATION IN RELATION TO LATITUDE OF ORIGIN

The list of the 24 investigated populations is given in Table 1. For each population, several independent repeated measurements were made (range 2–11, average 4·65 ± 0·41). As in previous work (David *et al*. 1998), we found significant variations between independent repeats on the same strain, so that the mean of each population is defined by the number of repeats and not by the number of flies. ANOVA (not shown) revealed a highly significant heterogeneity among investigated populations. We further investigated the origin of these variations by analysing the relationship between recovery time and latitude. A highly significant negative linear regression was found, shown in Fig. 1 (slope = −0·284 ± 0·056, $P < 0·001$, intercept = 45·81 ± 1·79, $P < 0·001$). Figure 1 reveals a large variability of the populations around the linear regression. We tested the possible influence of a quadratic component but this effect was not significant (ANCOVA, $P = 0·25$). The general latitudinal cline reflects an overall adaptive genetic variation: populations living in tropical places recover more slowly and are more sensitive to cold than temperate populations.

Population level analyses of this nature assume the populations are genetically independent and not connected by migration, although this is not always the case (Felsenstein 2002). We think that population non-independence is unlikely to be important for two reasons. First, geographically distant populations were selected, which should minimize connection due to human activities. Second, we investigate an adaptive trait that is maintained by local climate selection, sufficiently strong to suppress the effects of incoming migrants.

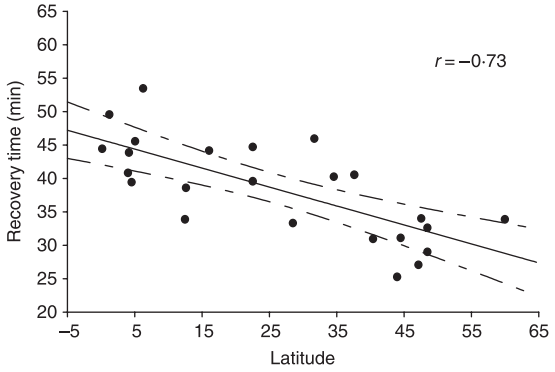

Fig. 1. Relationship between recovery time and latitude for 24 populations of *D. melanogaster*. The linear regression is shown as well as its confidence intervals.

PHENOTYPIC PLASTICITY ACCORDING TO GROWTH TEMPERATURE

The previous experiments were all undertaken on flies reared at 21 °C. Morphological traits are, however, highly plastic in relation to growth temperature (David *et al*. 2004) and we checked whether chill coma recovery time would be influenced by thermal conditions during development. We chose to analyse populations from the two ends of the cline, i.e. in four temperate populations from France, and four Afrotropical populations. We also investigated an Indian subtropical population from Rohtak, near New Delhi. Our results revealed a major effect of growth temperature: RT was always very short following development at 12 °C, and increased steadily when higher temperatures were used. This phenomenon is illustrated (Fig. 2) by the distributions of recovery time for three growth temperatures (12, 21 and 31 °C) for two populations from Kenya and France. The figure shows that there is no overlap between 12 and 21 °C although an overlap may exist between the distributions at 21 and 31 °C in the Kenyan population. It is also clear that an increase in mean value is accompanied by an increase in the variance of the distribution. To ensure homogeneity of variances, data were log-transformed before being submitted to ANOVA (Table 2). Calculations revealed highly significant effects due to population, temperature and their interaction. More significantly, the major portion of the variance (80%) was due to temperature, i.e. to phenotypic plasticity, while genetic differences between populations accounted for only 3·7%. We subsequently analysed the shape of the response curves (recovery time as a function of growth temperature), that is the reaction norms, using a polynomial model (see David *et al*. 1997). It turned out that the best fit was provided by a linear regression, and the results of these regressions

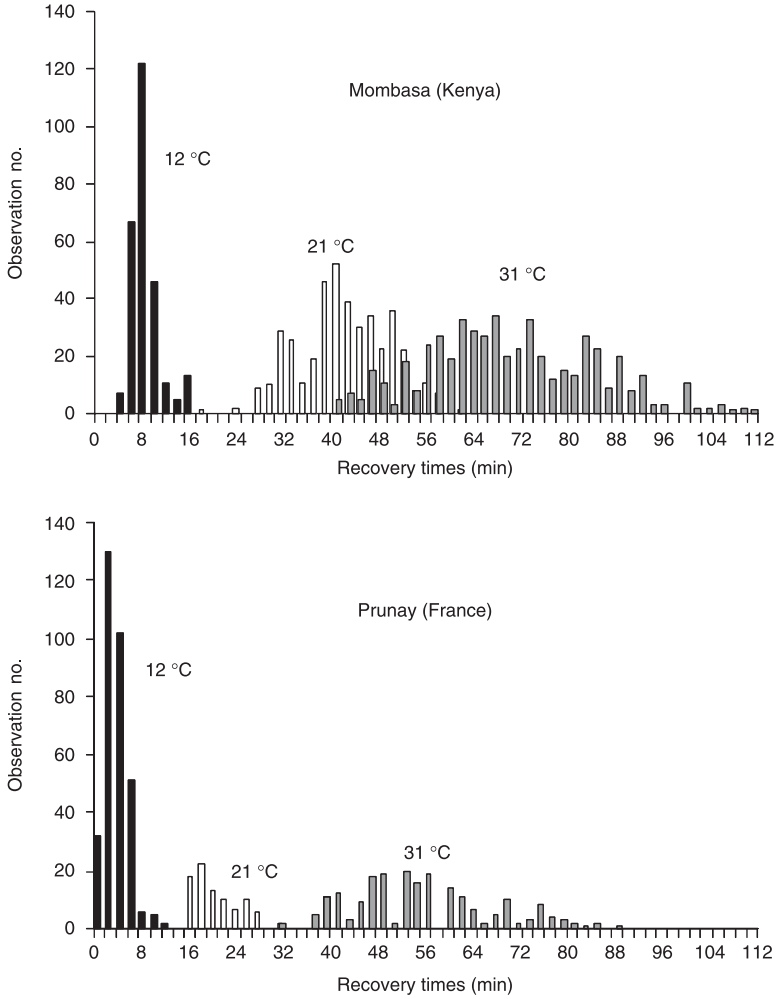

Fig. 2. Distribution of individual recovery times for three growth temperatures (12, 21 and 31 °C) in two populations: a tropical population from Mombasa (Kenya) and a temperate one from Prunay (near Paris, France).

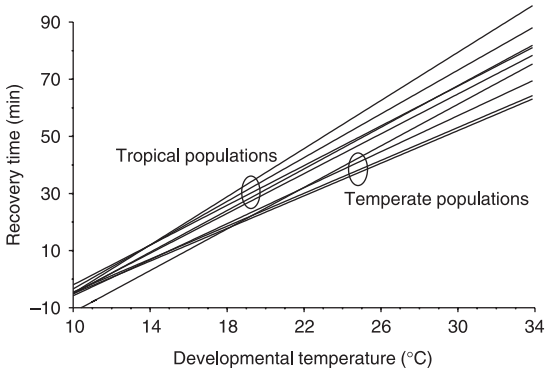

Fig. 3. Linear relationship between recovery time and developmental temperature for four temperate and five tropical populations of *D. melanogaster*. Only regression lines are given for the sake of simplicity.

Table 2. Results of a mixed model ANOVA with population as random and temperature as fixed effect on adult recovery time. Repeats (number of vials) are nested into population and temperature. Data were log-transformed to homogenize variances

	df	MS	*F*	Variation explained (%)
Population (1)	8	71·53	32·66***	3·7
Temperature (2)	6	2101·3	278·31***	80·7
Repeat (1 * 2)	203	2·19	24·33***	2·8
Interaction 1 * 2	48	7·55	3·45***	2·3
Error	18 238	0·09		10·5

***$P < 0{\cdot}001$.
df, degree of freedom; MS, mean square; *F*, variance ratio.

are given in Table 3 and shown in Fig. 3. For all populations, the slopes are significantly positive. The average value for the temperate populations is $3{\cdot}11 \pm 0{\cdot}17$, significantly less than the average ($3{\cdot}78 \pm 0{\cdot}11$) obtained for the tropical populations (see legend of Table 3). A lesser slope in the temperate populations suggests that their reactivity to temperature might be less than that of the tropical populations. But the mean values of the two sets of populations are also different (Table 3) so that a scaling effect is possible. To check this possibility, we estimated, for each population, a relative magnitude of plasticity by calculating the coefficient of variation among the seven experimental temperatures. As seen in Table 3, coefficients of variation (CVs) were significantly greater for the temperate than for the tropical populations, suggesting a higher reactivity to growth temperature in temperate flies.

Discussion

Recovery time after a cold shock at 0 °C is a laboratory assay aimed at measuring the cold sensitivity of a given *Drosophila* strain. Like most laboratory assays, it does not correspond to a situation occurring in nature. So the question is: are our results relevant to natural selection and adaptation? We believe that our assay provides a positive answer for four main reasons.

First it has been shown (Gibert *et al*. 2001) that recovery from chill coma induced at 0 °C permits an unambiguous classification of species as either tropical or temperate. Second, a brief survey (Gibert *et al*. 2001) revealed that this distinction between tropical and temperate species is also observed in other insect orders, suggesting a need for more extensive comparisons. Third, we have shown here that genetic variations among world populations of *D. melanogaster* vary according to a latitudinal cline. Populations living at high latitudes under temperate climates recover more quickly (i.e. are more cold tolerant) than tropical populations. Fourth, and still more interestingly, we show here that recovery time is a plastic trait, depending on developmental temperature. The results are in the expected direction for an adaptive plasticity: a development at low (12 °C) temperature considerably increases the cold tolerance of adults so that *D. melanogaster* (an Afrotropical species) approaches the values typical of purely temperate species (Gibert *et al*. 2001).

Table 3. Results of linear regression analysis applied to the nine investigated populations. Comparisons of the two geographical groups are made with ANOVA ($F_{1,7}$). Slopes are significantly steeper in tropical populations ($P = 0{\cdot}018$), and this difference does not allow a comparison of intercepts. Mean values of recovery time (RT) are calculated for the average development temperature of 21·1 °C, and difference is highly significant ($P = 0{\cdot}0001$). Coefficients of variation (CV) are calculated between the seven experimental temperatures, and the plasticity is more pronounced in the temperate populations ($P = 0{\cdot}045$)

Climate	Strain	Intercept	Slope	Mean RT	CV
Temperate	Prunay	−31·94	2·79***	27·04	75·9
	Bordeaux	−47·89	3·64***	29·06	92·7
	Draveil	−37·28	3·15***	29·31	77·9
	Foissac	−33·60	2·85***	26·65	77·2
	Mean ± SE	−37·68 ± 3·10	3·11 ± 0·17	28·02 ± 0·68	80·9 ± 3·4
Tropical	Sao Tomé	−41·64	3·83***	39·33	70·8
	Mombasa	−39·57	3·75***	39·71	68·3
	Mananara	−36·72	3·48***	36·85	69·1
	Rohtak	−41·53	3·64***	35·42	76·6
	Pointe Noire	−46·99	4·21***	42·01	72·8
	Mean ± SE	−41·29 ± 1·50	3·78 ± 0·11	38·66 ± 1·15	71·5 ± 1·3

To our knowledge, this is the first time that a linear reaction norm based on development according to growth temperature has been observed in *Drosophila*. Other fitness traits, such as viability, offspring production, male fertility or adult longevity, exhibit a generally sharp decrease at both ends of the thermal range (Cohet & David 1978; David *et al*. 1983; Pétavy *et al*. 2001; Chakir *et al*. 2002). In such cases, phenotypes may be interpreted as pathological, reflecting the deleterious effects of extreme developmental temperatures. However, in the case of cold tolerance plasticity clearly has a beneficial effect. In this respect, chill coma recovery appears to support the beneficial acclimation hypothesis (Leroi, Bennett & Lenski 1994) but it could also be interpreted as 'colder is better' (Huey *et al*. 1999). Analogous trade-offs between cold tolerance and fertility were also observed after acclimation of *D. melanogaster* adults at 11 °C (Bubliy *et al*. 2002). The fact that a given environment may result in pathological effects on some fitness traits but in beneficial effects on others illustrates the complexity and diversity of plastic responses, and the difficulty of generalized interpretations.

In a temperate country such as France, autumn generations do develop at temperatures close to 12 °C. This is shown by examining morphometric traits and knowing their reaction norms. The two most informative traits are female abdomen pigmentation and wing/thorax ratio (David *et al*. 1994; Pétavy *et al*. 2002) which both exhibit monotonically decreasing reaction norms. A low developmental temperature in adult flies collected in October and November is inferred by the observation of a very dark pigmentation and high wing/thorax ratio (J. R. David, unpublished observations). During winter, adults developed at a low temperature probably overwinter in sheltered places, although this is not well understood (Izquierdo 1991). They are probably submitted to repeated cold stresses and the capacity to recover rapidly is an advantage, which is also correlated to a better survival (J. R. David, unpublished observations).

From an evolutionary point of view, a consensus exists that *D. melanogaster* is native to tropical Africa (David & Capy 1988) and that it could have reached Europe several millennia ago, independently of modern human transportation (Lachaise *et al*. 1988). The cold adaptation seen in temperate populations may be the consequence of a long-lasting directional selection. The observation of a similar adaptive plasticity in the ancestral tropical populations is, however, unexpected since temperatures approaching 0 °C and able to induce a chill coma are not encountered in nature. Two opposite kinds of explanation may be proposed. First, we may assume that plasticity, which presumably implies some specific changes in the nervous system (see David *et al*. 1998), is a by-product of some more general functional changes related to growth temperature. For example, a lowering of temperature in ectotherms increases the level of unsaturated fatty acids, so that the fluidity of cell membranes is kept stable (Cossins & Bowler 1987; Hazel 1995). In *Drosophila*, it has been shown in several species that acclimation to low temperature resulted in a qualitative change in the proportion of monoenes and diens, and also in a decrease in the length of fatty acids (Ohtsu, Kimura & Katagiri 1998). In this respect, chill coma plasticity might be considered as an exaptation, according to Gould & Vrba (1982). Second, it is also possible that *D. melanogaster*, in spite of its Afrotropical origin, was already adapted to cold. Cold temperatures are encountered in the mountains of tropical countries and the occurrence of mountain populations in that species is known. Indeed, the collection of a wild living male in a natural habitat at an altitude of 3000 m was already mentioned (David & Tsacas 1981). In other words, *D. melanogaster* could have been somehow preadapted to the colonization of temperate countries by first colonizing afrotropical mountains. To examine this hypothesis, it would be interesting to investigate colonizing species which, like *D. ananassae* (Morin *et al*. 1997) or *Zaprionus indianus* (Karan, Moreteau & David 1999), are readily transported by humans, but are restricted to tropical places and apparently unable to tolerate cold winters.

References

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* **267**, 739–745.

Andrewartha, H.G. & Birch, L.C. (1954) *The Distribution and Abundance of Animals*. University of Chicago Press, Chicago, IL.

Boulétreau-Merle, J., Allemand, R., Cohet, Y. & David, J.R. (1982) Reproductive strategy in *Drosophila melanogaster*: significance of a divergence between temperate and tropical populations. *Oecologia* **53**, 323–329.

Bubliy, O.A., Riihimaa, A., Norry, F.M. & Loeschcke, V. (2002) Variation in resistance and acclimation to low-temperature stress among three geographical strains of

Drosophila melanogaster. *Journal of Thermal Biology* **27**, 337–344.

Capy, P., Pla, E. & David, J.R. (1993) Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. *Genetics Selection Evolution* **25**, 517–536.

Chakir, M., Chafik, A., Moreteau, B., Gibert, P. & David, J.R. (2002) Male sterility thermal thresholds in *Drosophila*: *D. simulans* appears more cold-adapted than its sibling *D. melanogaster*. *Genetica* **114**, 195–205.

Chown, S.L., Addo-Bediako, A. & Gaston, K.J. (2002) Physiological variation in insects: large scale patterns and their implications. *Comparative Biochemistry and Physiology B* **131**, 587–602.

Cohet, Y. & David, J. (1978) Control of the adult reproductive potential by preimaginal thermal conditions: a study in *Drosophila melanogaster*. *Oecologia* **35**, 319–342.

Cossins, A. & Bowler, K. (1987) *Temperature Biology of Animals*. Chapman & Hall, London and New York.

David, J.R. & Clavel, M.F. (1965) Interaction entre le génotype et le milieu d'élevage. Conséquence sur les caractéristiques du développement de la Drosophile. *Bulletin Biologique de France et Belgique* **99**, 369–378.

David, J.R. & Tsacas, L. (1981) Cosmopolitan, subcosmopolitan and widespread species: different strategies within the *Drosophilid* family (Diptera). *Comptes Rendus de la Société de Biogéographie* **57**, 11–26.

David, J.R., Allemand, R., Van Herrewege, J. & Cohet, Y. (1983) Ecophysiology: abiotic factors. *Genetics and Biology of Drosophila*, Vol. 3d (eds M. Ashburner, H.L. Carson & J.N. Thompson), pp. 105–170. Academic Press, New York.

David, J.R. & Capy, P. (1988) Genetic variation of *Drosophila melanogaster* natural populations. *Trends in Genetics* **4**, 106–111.

David, J.R., Moreteau, B., Gauthier, J.P., Pétavy, G., Stockel, J. & Imasheva, A. (1994) Reaction norm of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. *Genetics Selection Evolution* **26**, 229–251.

David, J.R., Gibert, P., Gravot, E., Pétavy, G., Morin, J.P., Karan, D. & Moreteau, B. (1997) Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *Journal of Thermal Biology* **22**, 441–451.

David, J.R., Gibert, P., Pla, E., Pétavy, G., Karan, D. & Moreteau, B. (1998) Cold stress tolerance in *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *Journal of Thermal Biology* **23**, 291–299.

David, J.R., Gibert, P., Moreteau, B., Gilchrist, G.W. & Huey, R.B. (2003) The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. *Functional Ecology* **17**, 425–430.

David, J.R., Gibert, P. & Moreteau, B. (2004) Evolution of reaction norms. *Phenotypic Plasticity: Functional and Conceptual Approaches* (eds T.J. De Witt & S.M. Scheiner), pp. 50–63. Oxford University Press, Cary, NC.

Davidson, J.K. (1990) Non-parallel geographic patterns for tolerance to cold and desiccation in *Drosophila melanogaster* and *Drosophila simulans*. *Australian Journal of Zoology* **38**, 155–161.

Felsenstein, J. (2002) Contrasts for a within-species comparative method. *Modern Developments in Theoretical Population Genetics* (eds M. Slatkin & M. Veuille), pp. 118–129. Oxford University Press, Oxford.

Gibbs, A.G., Perkins, M.C. & Markow, T.A. (2003) No place to hide: microclimates of Sonoran Desert *Drosophila*. *Journal of Thermal Biology* **28**, 353–362.

Gibert, P., Moreteau, B., Pétavy, G., Karan, D. & David, J.R. (2001) Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution* **55**, 1063–1068.

Gould, S.J. & Vrba, E.S. (1982) Exaptation – a missing term in the science of form. *Paleobiology* **8**, 4–15.

Graham, L., Walker, V. & Davies, P. (2000) Developmental and environmental regulation of antifreeze proteins in the mealworm beetle *Tenebrio molitor*. *European Journal of Biochemistry* **267**, 6452–6468.

Guerra, D., Cavicchi, S., Krebs, R.A. & Loeschcke, V. (1997) Resistance to heat and cold stress in *Drosophila melanogaster*: intra and inter population variation in relation to climate. *Genetics Selection Evolution* **29**, 497–510.

Hallas, R., Schiffer, M. & Hoffmann, A.A. (2002) Clinal variation in *Drosophila serrata* for stress resistance and body size. *Genetical Research* **79**, 141–148.

Hazel, J. (1995) Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annual Review of Physiology* **57**, 19–42.

Hoffmann, A.A. & and Parsons, P.A. (1991) *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.

Hoffmann, A.A. & Parsons, P.A. (1997) *Extreme Environmental Change and Evolution*. Cambridge University Press, London.

Hoffmann, A.A., Anderson, A. & Hallas, R. (2002) Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecology Letters* **5**, 614–618.

Hoffmann, A.A., Sorensen, J.G. & Loeschcke, V. (2003) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* **28**, 175–216.

Huey, R.B., Berrigan, D., Gilchrist, G.W. & Herron, J.C. (1999) Testing the adaptative significance of acclimation: a strong inference approach. *American Zoologist* **39**, 135–148.

Izquierdo, J.I. (1991) How does *Drosophila melanogaster* overwinter? *Entomologia Experimentalis et Applicata* **59**, 51–58.

James, A.C. & Partridge, L. (1995) Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. *Journal of Evolutionary Biology* **8**, 315–330.

James, A.C. & Partridge, L. (1998) Geographic variation in competitive ability in *Drosophila melanogaster*. *American Naturalist* **151**, 530–537.

Karan, D., Dahiya, N., Munjal, A.K., Gibert, P., Moreteau, B., Parkash, R. & 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.

Karan, D., Moreteau, B. & David, J.R. (1999) Growth temperature and reaction norms of morphometrical traits in a tropical Drosophilid: *Zaprionus indianus*. *Heredity* **83**, 398–407.

Klok, C.J. & Chown, S.L. (2003) Resistance to temperature extremes in sub-Antartic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* **78**, 401–414.

Lachaise, D., Cariou, M.L., David, J.R., Lemeunier, F., Tsacas, L. & Ashburner, M. (1988) Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evolutionary Biology* **22**, 159–225.

Leather, S., Walters, K. & Bale, J. (1993) *The Ecology of Insects Overwintering*. Cambridge University Press, Cambridge.

Leroi, A.M., Bennett, A.F. & Lenski, R.E. (1994) Temperature adaptation and competitive fitness: an experimental test of the Beneficial Acclimation Assumption. *Proceedings of the National Academy of Sciences of the USA* **91**, 1917–1921.

Morin, J.P., Moreteau, B., Pétavy, G., Parkash, R. & David, J.R. (1997) Reaction norms of morphological traits in *Drosophila*: adaptive shape changes in a stenotherm circumtropical species? *Evolution* **51**, 1140–1148.

Ohtsu, T., Kimura, M. & Katagiri, C. (1998) How *Drosophila* species acquire cold tolerance. Qualitative changes of phospholipids. *European Journal of Biochemistry* **252**, 608–611.

Pétavy, G., David, J.R., Gibert, P. & Moreteau, B. (2001) Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *Journal of Thermal Biology* **26**, 29–39.

Pétavy, G., Moreteau, B., Gibert, P. & David, J.R. (2002) Phenotypic plasticity of body pigmentation in *Drosophila*: influence of a developmental thermoperiodic regime in two sibling species. *Physiological Entomology* **27**, 124–135.

Precht, H., Christophersen, J. & Hensel, H. (1955) *Temperatur und Leben*. Springer, Berlin.

Stanley, S.M. & Parsons, P.A. (1981) The response of the cosmopolitan species, *Drosophila melanogaster*, to ecological gradients. *Proceedings of the Ecological Society of Australia* **11**, 121–130.

Received 15 January 2004; revised 30 March 2004; accepted 18 May 2004