THE ROYAL SOCIETY

doi 10.1098/rspb.2001.1787

Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions

Paul Mitrovski and Ary A. Hoffmann*

Centre for Environmental Stress and Adaptation Research, La Trobe University, Bundoora, Victoria 3086, Australia

Patterns of climatic adaptation in drosophila and other insects have largely been inferred from laboratory comparisons of traits that vary clinally. Here, we extend this research to comparisons under semi-natural conditions. To test for clinal variation in reproductive patterns and survival over winter, *Drosophila melanogaster* populations were initiated from seven collection sites along the eastern coast of Australia, ranging from tropical to temperate regions. The fecundity and survival of these populations were monitored in field cages at a temperate location until all adults had died more than 5 months later. Total fecundity showed a curvilinear relationship with latitude, due to higher egg production by high- and low-latitude populations. Adults from temperate locations survived winter conditions better than those from subtropical populations but not tropical ones. There was a linear cline in the timing of egg production: temperate populations produced eggs later than populations from lower latitudes. This cline is likely to be adaptive because egg-to-adult viability experiments indicated that only eggs laid in spring developed successfully to the adult stage. There was no evidence for climatic adaptation in the immature stages. The adult mortality rate increased gradually over winter, and in some populations was also correlated with the minimum ambient temperature. These results indicate that adaptation to winter conditions in *D. melanogaster* has involved shifts in reproductive patterns.

Keywords: clines; *Drosophila*; life-history traits; delayed reproduction; stress

1. INTRODUCTION

When faced with stressful climatic conditions, insects and other organisms can adapt by increasing their survival of the stress (Hoffmann & Parsons 1991) and by altering their life history to maximize reproductive output (Sibly & Calow 1989). Life-history changes might include shifts in reproductive effort, the timing of reproduction or the rate of maturation. Much of the evidence for climatic adaptation comes from clinal patterns in traits. For instance, in drosophila there are a number of clinal patterns in the survival of desiccation and starvation stress that are thought to reflect geographical variation in these stresses (Karan *et al*. 1998). Clinal patterns in size (Coyne & Beecham 1987; James *et al*. 1995; Huey *et al*. 2000), developmental time (James & Partridge 1995) and egg retention (Bouletreau-Merle *et al*. 1982) are also thought to be adaptive.

However, while these clinal patterns suggest traits that may be affected by climatic adaptation, the link is indirect in the absence of additional supporting evidence. One problem is that the life-history patterns and stress responses of organisms are invariably measured in the laboratory. While this makes it easier to rear and test organisms under controlled conditions, and thereby ascertain that differences between clinal populations are heritable, it is often unclear whether the stresses and conditions experienced in the laboratory reflect those in the field. This makes it difficult to draw conclusions about the nature of selection under field conditions and, ultimately, the evolution of different survival and life-history patterns. In contrast, some transplant experiments in plants have been particularly successful at identifying the impact of climatic selective factors in the field when provenances from a broad range of geographical locations are used (e.g. Loik & Noble 1993).

Here, we extend the traditional approach to studying clinal variation in insects using *Drosophila melanogaster* populations held in field population cages. We test for clinal shifts in survival and reproductive patterns over winter, which is the most stressful condition seasonally experienced by *D. melanogaster* in temperate areas, as evidenced by marked reductions in population size (McKenzie & Parsons 1974; Nielsen & Hoffmann 1985). *D. melanogaster* overwinters at the adult stage (Izquieredo 1991), possibly aided by a reproductive diapause triggered when the adults are recently eclosed (Saunders *et al*. 1989). Thus, overwintering in *D. melanogaster* provides an opportunity to test for adaptive clinal variation in reproductive patterns and survival in adults.

To test for clinal variation, replicate population cages were initiated with adults originating from seven locations along the east coast of Australia, spanning tropical and temperate regions. Populations were monitored for more than 5 months at a temperate locality for fecundity, mortality and offspring viability.

2. MATERIAL AND METHODS

(a) *Experimental procedure*

Flies were collected by fruit baiting in March–April 2000 from locations along the east coast of Australia ranging in latitude from 16°25′ S to 41°9′ S. Seven mass-bred populations were generated from the offspring of 10 inseminated females collected at each location. These populations were reared for one generation at 19 °C under continuous light before being used

*Author for correspondence (a.hoffmann@latrobe.edu.au).

to establish field cages (field parents were held for several days before providing offspring to ensure that only a total of two generations elapsed before setting up the flies in the field).

Field cages consisted of a clear plastic cylinder (radius 140 mm × height 120 mm) with fabric mesh windows (80 mm × 90 mm) on either side to allow air movement. Cages were open at one end and a nylon stocking was attached to the opening, enabling the transfer of flies and food cups. A plastic cup (70 mm × 50 mm) containing a laboratory medium (agar, dried yeast and sucrose) and two antibiotics (dihydrostreptomycin and penicillin G) was placed inside each cage for egg laying. To avoid damage from soil organisms, each cage was suspended within an inverted basket (550 mm × 410 mm) *ca.* 80 mm from the ground. Baskets were positioned in the shade of a wattle tree (*Acacia sophorae*) in a reserve at La Trobe University near Melbourne.

Flies were used in the cages 2 days after eclosion, and each cage was established with 17 males and 17 females. The ageing period after eclosion meant that adults would not have entered the reproductive diapause described by Saunders *et al.* (1989), which requires cold exposure within the first few hours after eclosion, although in other experiments the proportion of females from eastern Australian populations showing this diapause is low (R. Hallas, unpublished data). We set up 12 cages for each population at the start of winter on 6 June. This establishment date was selected because until late May adults eclose in large numbers from breeding sites in the Melbourne environs (Sgrò & Hoffmann 1998).

Food cups were replaced every 3 or 4 days. At this stage, adult mortality was also scored by removing dead flies from the cages. Cups were held at 4 °C until eggs (and hatched egg cases) could be counted. The experiment was continued until all the flies originally introduced into the cages were dead at the end of spring in November. The ambient temperature was recorded at the experimental site every hour for the duration of the experiment (6 June to 28 November) using a data logger (TinyTalk II, Hastings Data Loggers Pty Ltd, Port Macquarie, NSW, Australia). Preliminary measurements indicated that the temperature inside the cages was within 1 °C of that outside the cages.

Eggs laid in the field cages were collected once a month (June to October) to measure viability. Ten eggs from a single cage were transferred to a vial (100 mm × 24 mm) containing 25 ml fresh medium, and covered with a fabric mesh allowing aeration. These vials were held outside in field cages identical to those used for the adults. Vials were monitored weekly to check for larvae and pupae and to score adult eclosion. We set up 96 vials in both June and July, 94 vials in August, 33 vials in September and 24 vials in October. The egg-to-adult viability and egg production were used to estimate fitness (see § 3b).

(b) *Analysis*

We initially investigated the mortality rate and reproductive rate over time to examine changes in these rates after the cages were established. To examine mortality patterns, we computed the mortality rate at time x, μ_x, as $-\ln(N_{x+1}/N_x)$, where N is the number of flies alive at a particular time (Promislow *et al.* 1996). The mortality rate was computed separately for each cage and adjusted to account for the number of days in the scoring interval. Rates were then averaged across replicate cages for each population (figure 1*a*). Only a few flies (< 6%) survived 144 days after the cages were established, so mortality rates beyond this time were not considered.

Stepwise multiple regression was used to examine the associations between mortality rate and three temperature variables (mean, maximum and minimum temperature within the mortality interval) as well as the time since the cages were established. The data indicate an initially high level of mortality in the second interval after the cages were set up (figure 1*a*). This coincided with a period of low temperature, but may also have reflected mortality associated with the initial establishment of flies in the cages, particularly as later periods of even lower temperatures did not coincide with equivalent increases in mortality. Therefore, only data recorded after the second scoring interval were considered in the regression analysis. Regressions were performed on log-transformed variables (to improve the distribution of residuals) as well as untransformed data; the outcomes of these analyses were almost identical so only the regressions on untransformed data are presented. This analysis should be viewed with some caution because cages were repeatedly measured. The densities of flies in the cages would have changed, and selection could also have influenced the genetic constitution of each cage population over time.

We also examined changes in the rate of egg production by surviving females. This rate was computed for each interval and expressed as the number of eggs per live female per day.

To examine associations of traits with latitude, cage means were treated as data points. Latitudinal patterns were tested for total reproduction, as measured by total egg output, mean longevity and the time taken to achieve half the egg output in a cage. Both the total egg output and the timing of egg output will depend on the cage mortality and the laying rate of surviving females. The rate of egg output was steady for the first 98 days after the cages were established, then abruptly increased (figure 1*b*); therefore, the mean laying rate was computed separately for these two intervals (up to 98 days and after 98 days) by averaging the laying rates across observation periods within these intervals regardless of the number of females contributing within this period. Both linear and quadratic components were considered in the regressions.

Finally, for viability, the proportions of adults emerging from vials were compared using non-parametric Kruskal–Wallis tests to overcome the problem of the data being highly skewed.

3. RESULTS

(a) *Mortality and fecundity patterns*

In the stepwise regressions on mortality rate, the time since the cages were set up accounted for most of the variation in each of the populations and was always entered first. Changes in mortality rates are, therefore, likely to have reflected senescence rather than temperature. All regression coefficients were positive (table 1), reflecting an increase in mortality rate with the time since the cages were established. The only other variable that affected mortality rate was the minimum temperature within an interval, which was significant for three populations. For each of these populations (as well as those where the regression coefficients did not differ significantly from zero) the coefficient was negative, suggesting an increase in the mortality rate when temperatures were particularly low.

As noted in § 2b, the egg-laying rate (figure 1*b*) initially tended to be constant, but showed a sharp increase *ca.* 98 days after the cages were set up. When the data before and after this sudden change were considered separately, there was no evidence from regression analyses (not shown) for an association between fecundity rate and

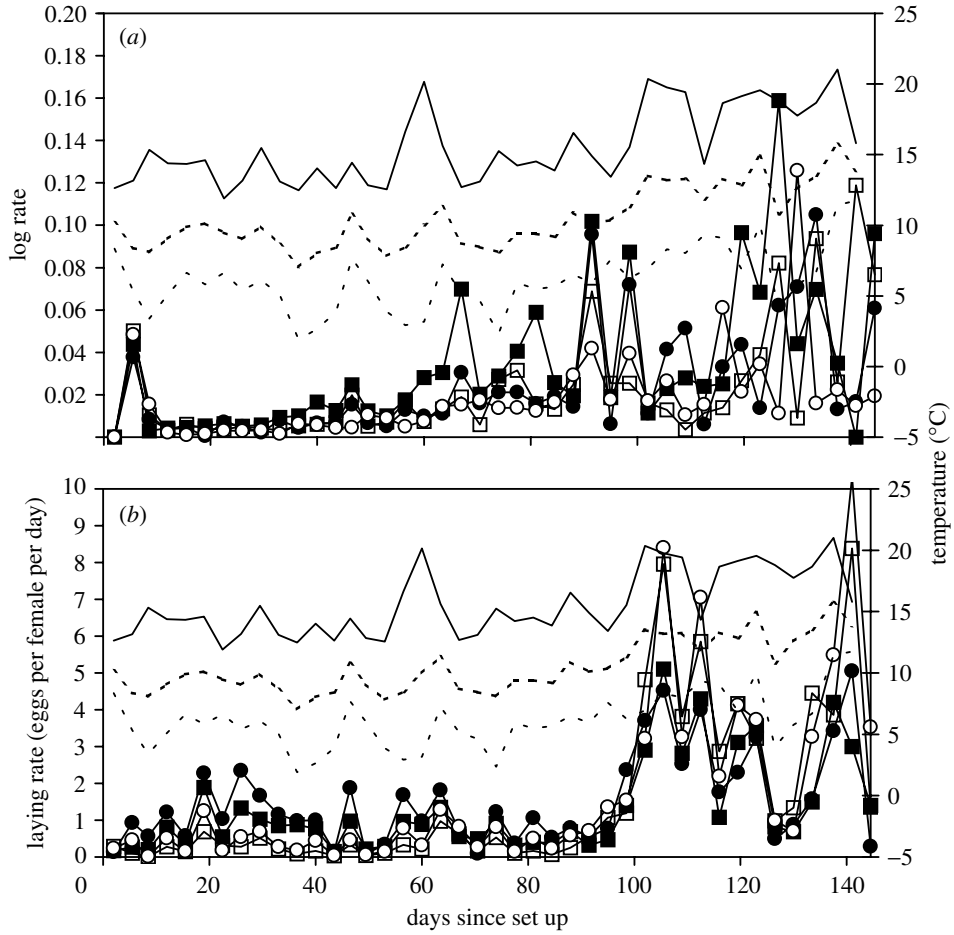

Figure 1. (*a*) Mortality rate (sexes combined) and (*b*) rate of egg laying by females at different times after the cages were set up. The minimum (bottom stippled line), maximum (solid line) and average (top stippled line) temperatures recorded during the laying or mortality interval are also presented. Data are provided for the two southernmost high latitude (Launceston (open circles) and Melbourne (open squares)) and the two northernmost low latitude (Mossman (filled circles) and Innisfail (filled squares)) populations. Mortality rate is as defined in § 2b.

Table 1. Regression analysis associating mortality rate with time since the cages were set up and temperature parameters.

(Only the coefficients for minimum temperature are shown because the other temperature parameters were not significant in any of the populations.)

	time since cage established		minimum temperature		
population	$b \pm$ s.e.[b]	p	$b \pm$ s.e.[a]	p	intercept
Mossman	5.25 ± 0.102	< 0.001	-3.52 ± 1.832	0.063	0.0051
Innisfail	6.95 ± 1.285	< 0.001	-5.26 ± 2.315	0.025	0.0128
Sarina	3.55 ± 0.764	< 0.001	-1.40 ± 1.377	0.316	0.0031
Rainbow Beach	4.46 ± 0.911	< 0.001	-3.97 ± 1.640	0.021	0.0121
Coffs Harbour	3.44 ± 0.881	< 0.001	-3.54 ± 1.586	0.032	0.0171
Melbourne	3.81 ± 0.860	< 0.001	-2.51 ± 1.550	0.114	0.0056
Launceston	3.43 ± 0.939	< 0.001	-0.65 ± 1.692	0.702	-0.0038

[a]Coefficients and standard errors $\times 10^{-4}$.
[b]Coefficients and standard errors $\times 10^{-3}$.

either the time since the cages were set up or any temperature variable.

(b) *Clinal patterns*

For total fecundity there was evidence of significant linear and quadratic components in regressions on latitude (table 2). Egg counts were highest for the two tropical populations and decreased with latitude, before increasing again for the two southernmost populations (figure 2*a*). In contrast, only the linear component was significant for the timing of reproduction, as measured by the time at which half the eggs in a cage had been laid.

Table 2. Associations between latitude and life-history parameters measured in cages.

(Each cage was treated as a datum point and latitude was considered as a linear and as a quadratic component in the analyses.)

	linear component		quadratic component		
trait	$b \pm$ s.e.	p	$b \pm$ s.e.	p	intercept
eggs per cage	-294.1 ± 43.88	< 0.001[a]	4.9 ± 0.76	< 0.001[a]	4707.6
time to 50% eggs	1.20 ± 0.219	< 0.001[a]	-0.005 ± 0.034	0.884	28.22
mean longevity	-4.22 ± 1.433	0.004[a]	0.08 ± 0.025	0.003[a]	118.92
egg production before 98.5 days	-0.164 ± 0.0211	< 0.001[a]	0.003 ± 0.0004	< 0.001[a]	2.836
egg production between 98.5 days and 144.5 days	0.081 ± 0.0206	< 0.001[a]	0.004 ± 0.003	0.171	0.416

[a]Remains significant after correction for multiple comparisons.

This trait showed an increase with latitude: tropical populations reproduced earlier than temperate ones (figure 2*b*).

The egg-laying patterns could be a result of changes in longevity or rates of egg laying by the surviving females. For longevity, only data pooled across sexes are presented because males and females showed identical patterns. A latitudinal association is indicated by significant quadratic and linear terms in the regressions (table 2). Mean longevity decreased from north to south, but increased sharply in the two southernmost populations (figure 2*c*). Thus, longevity patterns partly matched the patterns for total reproductive output but not those for the timing of reproduction.

There were significant linear and quadratic components for egg-laying rate in the first 98 days (table 2). Egg-laying rate declined in more southerly populations, but this decline levelled off below Sarina and there was a suggestion of a higher egg-laying rate in the Tasmanian population (figure 3*a*). Only a linear pattern was indicated by the regression analysis for egg-laying rate after 98 days; the egg-laying rate was relatively higher in the southern populations (figure 3*b*).

In the viability experiments, none of the eggs, from any of the populations, set up in June, July or August developed to the adult stage. When we examined the vials it was evident that some of the eggs had hatched, and there were even a few pupae in some vials, which had not eclosed. In contrast, adults did successfully emerge from vials set up in September and October. For September the mean viability was 48% and for October it was 90%. Kruskal–Wallis tests on the proportions of flies that emerged provided no evidence of viability differences between the populations (September: $\chi^2 = 7.14$, d.f. $= 6$, $p = 0.307$; October: $\chi^2 = 11.61$, d.f. $= 6$, $p = 0.072$) and regressions (not shown) did not indicate any latitudinal patterns for this trait.

We used the egg-laying patterns and viability data to obtain an approximate estimate of the total fitness of the population over winter by assuming that the viability of all eggs laid in September was 48% and that of all eggs laid after September was 90%. This estimate provides an indication of R_0, the reproductive output of a population over a generation (Stearns 1992). Latitudinal patterns for this estimate (figure 3*c*) suggest that the two southernmost populations had the highest fitness. The difference in fitness between the temperate and subtropical populations was particularly marked, being around four- to fivefold. Unfortunately, we were unable to compute r (the intrinsic rate of increase) because we looked at only one offspring generation, although a similar pattern is likely because only the eggs produced after winter developed to the adult stage.

4. DISCUSSION

The clinal variation in the timing of reproduction that we have detected in the population cages is likely to be adaptive. Only offspring produced after August survived to the adult stage, and therefore any delay in reproduction increases fitness over winter. The late reproduction in temperate populations, compared with subtropical populations, is partly related to a higher rate of late egg production by females and partly to an increase in longevity. In contrast, tropical populations survived winter conditions as well as temperate populations, and, in this case, late reproduction reflected only rates of egg production by live females. These results match those from a more limited comparison of two populations (Melbourne, Innisfail) undertaken over winter the previous year (Olsen *et al*. 2001).

The mortality data indicate that the abilities of adults from tropical and temperate populations to survive winter conditions are similar. This suggests that populations did not differ in adult cold tolerance, and contrasts with laboratory findings of experiments using populations from eastern Australia (Stanley & Parsons 1981; but see Hoffmann & Watson 1993). We did find that mortality rates increased in several populations when temperatures were particularly low, suggesting that adult survival is under selection. This trait responds to laboratory selection, although its heritability is relatively low (Watson & Hoffmann 1996). Perhaps laboratory assays of adult cold resistance are not relevant to survival under field conditions, particularly as they are undertaken with young flies, whereas overwintering involves the survival of old flies.

We found no evidence that immature stages from temperate populations were more likely to survive cold conditions than those from other areas. As noted by Izquieredo (1991), immatures die at different stages of development over winter and none survive to the adult stage. Thus, we found no evidence for adaptation involving egg-to-adult viability, despite genetic variation for this trait in the laboratory (Tucic 1979).

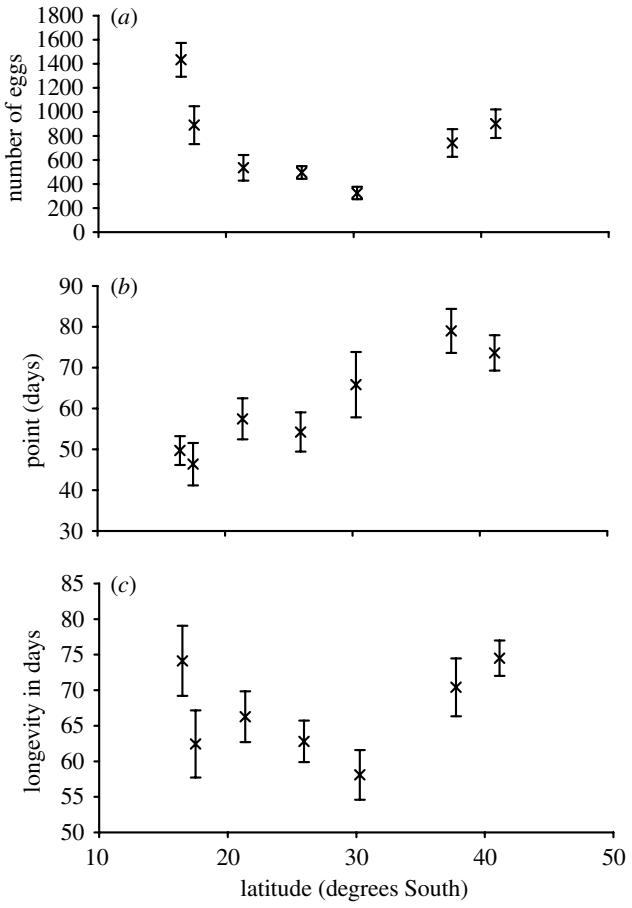

Figure 2. Cage means for (*a*) total egg output, (*b*) the midpoint of egg output and (*c*) longevity (sexes combined) plotted against latitude.

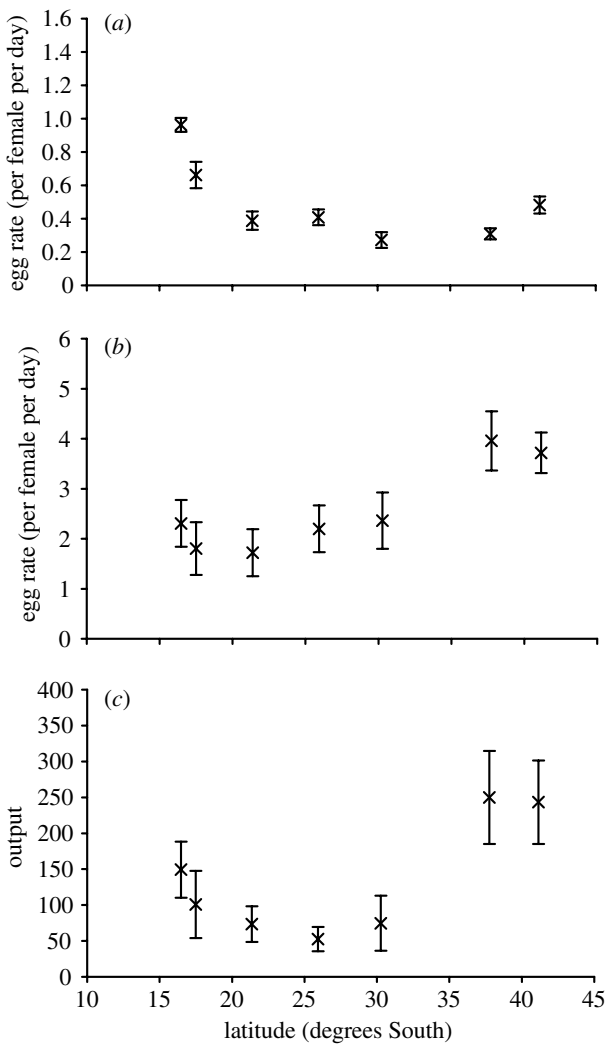

Figure 3. Cage means for the egg-laying rate per live female in (*a*) the early period and (*b*) the late period, and (*c*) total fitness plotted against latitude. The early period encompasses the first 96 days after the cages were established, while the late period covers subsequent egg laying until day 144. Total fitness was determined as the sum of the number of eggs produced each month multiplied by the mean egg-to-adult viability for that month.

It is not clear why late egg production is relatively higher in temperate populations. There may exist some form of reproductive diapause that is triggered some time after adult emergence, in contrast to the diapause described by Saunders *et al.* (1989), which requires exposure to cold conditions immediately after eclosion. Alternatively, there may be a shift to late reproduction in temperate populations that is not dependent on winter conditions. To test this, reproductive patterns would need to be examined under different sets of conditions. Some data on the reproductive patterns of the Melbourne and Innisfail populations under winter tropical conditions were collected by Olsen *et al.* (2001) over a 2 week period. They found that, while total egg output was similar in the two populations over this period, the tropical flies had a relatively higher output over the first few days, suggesting changes in reproductive patterns under favourable as well as under winter conditions.

In laboratory studies, early reproduction and adult longevity are often negatively correlated (e.g. Rose 1984). It has also been known for a long time that in the laboratory there is a close association between body size and reproductive output (Chaing & Hodson 1950; Robertson 1957). These constraints do not appear to have influenced the clinal patterns we detected. Selection appears to have favoured increased longevity and a high rate of early reproduction in the tropics. The steep and repeatable cline in size in *D. melanogaster* along the eastern coast of Australia (James *et al.* 1995) contrasts with the patterns for reproductive output that we detected. Therefore, patterns of early reproduction, longevity and size across populations do not appear to be constrained by the genetic associations between traits evident within populations.

While our results suggest that winter adaptation can be linked to life-history patterns, the population cage environment we have measured considers only a subset of the selective conditions likely to be experienced by flies in nature. There were no opportunities for competitive interactions for resources, including access to breeding sites and larval competition. The ability of flies to locate resources and escape predators was not considered in these cages. Nevertheless, our findings indicate that patterns of life-history traits have been altered by climatic

selection in *D. melanogaster*, leading to a delay in reproduction and an increase in overall fitness in temperate populations over winter. It remains to be seen whether these trait changes can be linked to genetic markers that show strong latitudinal differentiation over this geographical area (e.g. McColl & McKechnie 1999). The findings of this study, as well as those of Olsen *et al*. (2001), also highlight the benefits of studying climatic adaptation in drosophila with field cages.

This research was supported by the Australian Research Council via their Special Research Centre Program.

REFERENCES

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

Chaing, H. C. & Hodson, A. C. 1950 An analytical study of population growth in *Drosophila melanogaster*. *Ecol. Monogr.* **20**, 173–206.

Coyne, J. A. & Beecham, E. 1987 Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* **117**, 727–737.

Hoffmann, A. A. & Parsons, P. A. 1991 *Evolutionary genetics and environmental stress*. Oxford University Press.

Hoffmann, A. A. & Watson, M. 1993 Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. *Am. Nat.* **142**, S93–S113.

Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D. & Serra, L. 2000 Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**, 308–309.

Izquieredo, 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. *J. Evol. Biol.* **8**, 315–330.

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.

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.

Loik, M. E. & Noble, P. S. 1993 Freezing tolerance and water relations of *Opuntia fragilis* from Canada and the United States. *Ecology* **74**, 1722–1732.

McColl, G. & McKechnie, S. W. 1999 The *Drosophila* heat shock hsr-omega gene: an allele frequency cline detected by quantitative PCR. *Mol. Biol. Evol.* **16**, 1568–1574.

McKenzie, J. A. & Parsons, P. A. 1974 Numerical changes and environmental utilization in natural populations of *Drosophila*. *Aust. J. Zool.* **22**, 175–187.

Nielsen, K. M. & Hoffmann, A. A. 1985 Numerical changes and resource utilization in orchard populations of *Drosophila*. *Aust. J. Zool.* **33**, 875–884.

Olsen, K., Reynolds, K. T. & Hoffmann, A. A. 2001 A field cage test of the effects of the endosymbiont *Wolbachia* on *Drosophila melanogaster*. *Heredity*. (In the press.)

Promislow, D. E. L., Tatar, M., Khazaeli, A. A. & Curtsinger, J. W. 1996 Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. *Genetics* **143**, 839–848.

Robertson, F. W. 1957 Studies in quantitative inheritance. XI. Genetic and environmental correlations between body size and egg production in *D. melanogaster*. *J. Genet.* **55**, 428–443.

Rose, M. R. 1984 Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38**, 1004–1010.

Saunders, D. S., Henrich, V. C. & Gilbert, L. I. 1989 Induction of diapause in *Drosophila melanogaster*: photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. *Proc. Natl Acad. Sci. USA* **86**, 3748–3752.

Sgrò, C. M. & Hoffmann, A. A. 1998 Heritable variation for fecundity in field-collected *Drosophila melanogaster* and their offspring reared under different environmental temperatures. *Evolution* **52**, 134–143.

Sibly, R. & Calow, P. 1989 A life-cycle theory of responses to stress. *Biol. J. Linn. Soc.* **37**, 101–116.

Stanley, S. M. & Parsons, P. A. 1981 The response of the cosmopolitan species, *Drosophila melanogaster*, to ecological gradients. *Proc. Ecol. Soc. Aust.* **11**, 121–130.

Stearns, S. C. 1992 *The evolution of life histories*. New York: Oxford University Press.

Tucic, N. 1979 Genetic capacity for adaptation to cold resistance at different developmental stages of *Drosophila melanogaster*. *Evolution* **33**, 350–358.

Watson, M. J. O. & Hoffmann, A. A. 1996 Acclimation, cross-generation effects, and the response to selection for increased cold resistance in *Drosophila*. *Evolution* **50**, 1182–1192.