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Clinal variation in *Drosophila serrata* for stress resistance and body size

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Summary

Clines for size and stress resistance traits have been described for several *Drosophila* species and replicable clines across different species may indicate climatic selection. Here we consider clines in stress resistance traits in an Australian endemic species, *D. serrata*, by comparing levels of variation within and among isofemale lines initiated with flies collected from the eastern coast of Australia. We also consider clinal variation in chill coma recovery, a trait that has recently been shown to exhibit high levels of variation among *Drosophila* species. Patterns were compared with those in the cosmopolitan species *D. melanogaster* from the same area. Both desiccation and starvation resistance showed no clinal pattern despite heritable variation among isofemale lines. In contrast chill coma resistance exhibited a linear cline in the anticipated direction, resistance increasing with latitude. Body size was measured as wing length and body weight. Both traits showed geographic variation and strong non-linear clines with a sharp reduction in size in the tropics. These results are discussed in the context of climatic selection and evolutionary processes limiting species borders.

1. Introduction

A number of studies have investigated clinal variation for stress resistance traits in *Drosophila* species (Hoffmann & Harshman, 1999). Latitudinal clines for desiccation and starvation resistance exist for several *Drosophila* species on the Indian subcontinent (Karan *et al*., 1998*a*). However, these clines may not occur on other continents or in other species. For instance, Robinson *et al*. (2000) failed to show a cline for starvation resistance in *D. melanogaster* from the west coast of South America while Hoffmann *et al*. (2001) also found no clinal variation for this trait and desiccation resistance in *D. melanogaster* from eastern Australia.

In contrast, clinal patterns for size-related traits in Drosophila do appear consistent across continents as well as species. For instance, body size in *D. melanogaster* increases with latitude in several areas including eastern Australia, North America, Europe, Africa and Asia (Capy *et al*., 1993; Imasheva *et al*., 1994; James *et al*., 1995). The same clinal pattern existed for wing length in *D. subobscura* in Europe and also became evident in North America a few years after it was colonized by this species (Huey *et al*., 2000).

At this stage it is not clear what evolutionary factors are responsible for clinal patterns in *Drosophila*. Some authors have suggested that mean temperature changes along clines are the main selective factor. For size in particular, temperature is thought to be responsible for repeatable clinal patterns across continents, and laboratory data support this conjecture (e.g. Partridge *et al*., 1994) although direct field evidence is lacking. Variation in levels of gene flow may also influence clinal patterns and perhaps explain their presence on only some continents or in some species. For instance, geographic differences due to selection may be relatively weaker in cosmopolitan species because of gene flow from the movement of fruit by humans.

Here we investigate clinal patterns in an Australian endemic species, *D. serrata*, a species largely confined to eastern Australia (Jenkins & Hoffmann, 2001). *D. serrata* is attracted to fruit bait but is not found in orchards or close to human habitation and utilizes a variety of other resources for breeding sites (Jenkins & Hoffmann, 2001). Thus this species is unlikely to be

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influenced by human-assisted movement of fruit. *D. serrata* has a distinct border just south of Sydney and has been the subject of intense investigations to determine evolutionary factors limiting species borders (Jenkins & Hoffmann, 1999; Jenkins & Hoffmann, 2000).

We consider both desiccation and starvation resistance, as well as recovery from a short exposure to cold stress ('chill coma recovery') because this trait appears effective in discriminating among *Drosophila* species (Gibert *et al*., 2001). Cold resistance is of particular interest because both ecological analyses (Jenkins & Hoffmann, 2001) and shifts in cold resistance across seasons at border populations (Jenkins & Hoffmann, 1999) suggest that this trait is directly or indirectly associated with the southern *D. serrata* border. Finally we consider two measures of body size to determine whether size follows the same pattern of clinal variation in eastern Australia as in *D. melanogaster* (James *et al*., 1995). To assess geographic patterns in these traits, we compare variation among isofemale lines within populations with variation among populations from different locations. This approach allows the strength of any clinal patterns to be compared with levels of heritable variation within populations (Hoffmann *et al*., 2001).

2. Materials And Methods

(i) Stocks

Most stocks used in the experiments were collected in March–April 2000 when 13 populations of *Drosophila serrata* were collected between Terrigal (33°25′ S) in New South Wales and Mossman (16°28′ S) in northern Queensland (Fig. 1). A second collection of seven populations was made in November 2000 between Innisfail (17°30′ S) and Cape Tribulation (16°02′ S) to boost the number of lines in northern populations, which were not well represented in the original collection. Up to 10 isofemale lines were set up for each population and these were maintained at 25 °C under constant light at a population size of at least 100 flies per line. Lines were reared on a sugar (1·6% w/v), agar (3·2%), yeast (3·2%) and potato (1·6%) medium that was always treated with antibiotics (2% dihydrostreptomycin and 0·6% penicillin added to the medium surface) and an antifungal agent (Nipagin: 0·14% w/v). No live yeast was added. Lines were tested for the different traits when they were between 3 and 13 generations removed from the field.

We also undertook crosses between some pairs of isofemale lines from the same location (5–9 crosses in total) to monitor any potential inbreeding depression effects associated with setting up the isofemale lines. These F1 'cross' lines were tested along with the isofemale lines for stress resistance and size measurements. However, since no differences between these lines and the isofemale lines from the same location were detected, data for the crosses are not considered further. The absence of inbreeding depression is not surprising given that the lines were expanded rapidly following one generation of sib mating, and is consistent with the results of comparisons between isofemale lines and crosses in *D. melanogaster* (Hoffmann *et al*., 2001).

(ii) Traits

To measure starvation and desiccation resistance, males and females from the isofemale lines were mated in pairs (five pairs per line) and left to oviposit for 2 d. By setting up pairs of flies we ensured that larvae developed under low-density conditions (10–50 adults emerged per vial with 25 ml of medium). Offspring were collected and aged for 1 day in vials. Ten male and 10 female offspring were selected randomly under CO_2 from each line and left to recover for a day. Both starvation and desiccation stresses were measured on individual flies 3 days after eclosion. For desiccation resistance 2–10 isofemale lines per location were tested, while for starvation resistance we only scored a maximum of 5 lines per location.

For starvation resistance, adults (4 or 5 of each sex per line) were placed individually in 1·5 ml Eppendorf tubes containing 0.5 ml of agar medium (2.5% w/w). These tubes had been pierced on each side with a needle to allow for air movement. The tubes were then placed in sealed glass tanks containing water reservoirs to maintain humidity close to 100%. Mortality was scored every 6 h.

To measure desiccation resistance, 5 females were placed individually in 5 ml glass vials and the vials covered with gauze. These were then placed in a sealed glass tank containing silica gel generating a relative humidity of 18–20%. Mortality was scored every hour.

To evaluate chill coma recovery, females (3 days old) were placed individually in 42 ml glass vials which were then immersed in a 10% glycol solution cooled to 0 °C. The vials were removed after 8 h and recovery time was scored for the individual females. Flies were considered to have recovered when they were able to stand up. Four females per line were tested, and 3–10 lines were tested per location.

To determine wing length, wings of individuals from the desiccation experiment were mounted on slides. An image of the right wing (except where damaged) was then captured using a Wild M38 microscope, attached to a Panasonic WV-GP460 digital camera, using tpsDig version 1·2 written by F. James Rohlf. Landmarks were obtained from the images and used to compute wing length (along the

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Fig. 1. Map of Australia showing collection sites for the *D. serrata* populations.

third longitudinal vein from its intersection of the anterior cross vein to the wing tip).

Finally, to assess weight, offspring of pair matings raised in 42 ml vials were collected and aged for 7–9 days before being weighed (as non-virgins). The sexes were separated prior to weighing and individuals of each sex were weighed as a group. Adults were immobilized by freezing at −80 °C for 2–2·5 min prior to weighing. The number of flies emerging from a vial varied between 10 and 50 individuals, and flies were therefore weighed in groups ranging from 2 to 28 per sex. A covariance analysis on average weight was initially used to test whether population differences for weight depended on offspring number. However, the covariate was not significant, indicating that the average weight of individual flies was not altered within this density range.

(iii) Analysis

To test for differences among locations, nested ANOVAs (with isofemale line nested within location) were undertaken. Variance components were computed using maximum likelihood with SPSS for Windows 10·0. These were used to compare the relative amount of variation within and among populations. We also computed intraclass correlations and isofemale heritabilities following Hoffmann & Parsons (1988) to examine levels of variation within geographic regions. Isofemale heritabilities (H) are computed from the variance components within and between lines adjusted for group size where appropriate using

$$H = \frac{S_A^2}{S_A^2 + xs^2},$$

where S_A^2 is the variance component among lines, s^2 is the component of variance within lines and x is the group size. These estimates need to be treated cautiously because there can be a non-genetic correlation among individuals within the same group. To ensure the same number of isofemale lines per location for this analysis, we randomly deleted some lines from some locations prior to analysis and also deleted locations for which only a few lines were available.

Latitudinal patterns were tested with regression analysis. A linear equation was first fitted to test for linear effects, and quadratic and cubic components were then added to test for curvilinear relationships. Associations with different climatic variables as well as latitude were also tested, using multiple regression. Climatic data for each collection site were obtained from Climatic Averages Australia (www.bom.gov.au) by identifying the nearest weather station to the site. We considered annual daily maximum temperature, daily January maximum, annual daily minimum temperature, daily July minimum, average humidity at 0900 hours, average humidity at 1500 hours, total rainfall, and the annual number of rain days. Forward selection was used in the regression analysis. The variable accounting for most of the variance was first

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fitted, and then the variable accounting for the most residual variance, and so on. Latitude was also included in the regression analysis as some environmental variables (especially daylength) are tightly associated with latitude and we wanted to separate daylength effects from temperature/humidity effects.

We also examined the correlations between desiccation and starvation resistance using isofemale line means. These traits are partly correlated in *D. melanogaster* (Hoffmann & Parsons, 1993; Hoffmann *et al.*, 2001) and we wanted to test for this association in *D. serrata*. Correlations computed from isofemale line means are similar to those based on family means and may approximate genetic correlations as long as isofemale line scores are not confounded by inbreeding depression. This correlation was computed by considering all isofemale lines without correcting for locality effects and also after adjusting all line means for differences in resistance among localities (i.e. we considered deviations from the locality mean rather than the actual values).

3. Results

(i) Stress resistance traits

Nested ANOVAs indicate that locations differed significantly for chill coma recovery but not for starvation or desiccation resistance (Table 1). Differences among lines were significant for all resistance traits. Line effects accounted for 7% to 25% of the variance. In the case of chill coma recovery, location effects accounted for a higher proportion of the variance than line effects, but for the other traits line effects were more important. Chill coma recovery times were relatively longer for lines from northern sites.

Latitudinal patterns were evident for chill coma recovery and male starvation but not for desiccation resistance and female starvation (Table 2). At higher latitudes, chill coma recovery times decreased, reflecting a higher level of resistance (Fig. 2). Male starvation resistance showed a weak tendency to increase with latitude (Fig. 3).

Isofemale line heritabilities for starvation resistance were 16% and 18% for males and females respectively. The estimate for desiccation resistance was 28% and for chill coma recovery it was 14%. The estimate for desiccation resistance is similar to that obtained from selection experiments with *D. serrata* for this trait (Blows & Hoffmann, 1993).

Regression analysis indicated that no climatic variables were significantly associated with desiccation resistance. For female starvation resistance, the analysis indicated a weak association with average minimum temperature ($b = -0{\cdot}91 \pm 0{\cdot}43$, $P = 0{\cdot}05$), while male starvation resistance was associated with

Table 1. *Nested analyses of variance comparing differences among lines and locations for* D. serrata *tested for resistance to desiccation, starvation, chill coma recovery and wet weight*

Trait	Source	d.f.	MS	Significance of F	Variance component[a] (%)
Desiccation resistance (time to death in hours)	Location	20	34·88	0·315	0·5
	Line	152	30·53	< 0·001	24·8
	Error	657	11·87		74·7
Starvation resistance (females) (time to death in hours)	Location	20	620·95	0·665	0
	Line	68	748·16	< 0·001	6·8
	Error	374	402·25		93·2
Starvation resistance (males) (time to death in hours)	Location	20	171·10	0·720	0
	Line	63	338·45	< 0·001	9·9
	Error	353	190·59		90·1
Chill coma recovery (in minutes)	Location	20	1102·09	< 0·001	16·7
	Line	148	224·64	< 0·001	13·2
	Error	615	122·74		70·1
Wing length (females, in mm) $\times 10^{-4}$	Location	20	3123·13	< 0·001	85·8
	Line	79	62·67	< 0·001	7·3
	Error	368	10·69		6·9
Wing length (males, in mm) $\times 10^{-4}$	Location	20	752·31	< 0·001	88·8
	Line	56	28·13	< 0·001	2·6
	Error	283	10·92		8·6
Weight (females, in g) $\times 10^{-4}$	Location	20	68·84	< 0·001	52·1
	Error	120	8·75		47·9
Weight (males, in g) $\times 10^{-4}$	Location	20	48·29	< 0·001	45·0
	Error	120	8·16		55·0

[a] Variance components are maximum likelihood estimates.

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Table 2. *Regression analysis testing for associations between latitude and the traits based on isofemale line means*

	Linear component		Quadratic component		
Trait	$b \pm$SE	P	$b \pm$SE	P	Intercept
Starvation (females)	0·331 ± 0·249	0·186	–	–	80·95
Starvation (males)	0·541 ± 0·187	0·005	–	–	62·28
Desiccation	0·033 ± 0·044	0·455	–	–	14·68
Chill coma recovery	−0·682 ± 0·127	< 0·001	–	–	17·681
Wing length (females)	0·1144 ± 0·0076	< 0·001	−0·0019 ± 0·0002	< 0·001	−0·241
Wing length (males)	0·0693 ± 0·0155	< 0·001	−0·0012 ± 0·0003	< 0·001	0·334
Wet weight (females) × 10^{-4}	1·121 ± 0·146	< 0·001	−0·020 ± 0·003	< 0·001	−3·49
Wet weight (males) × 10^{-4}	0·788 ± 0·147	< 0·001	−0·014 ± 0·003	< 0·001	−2·58

Linear, quadratic and cubic components were considered in the regression analyses. Cubic terms were never significant, while quadratic components were only significant for the size traits.

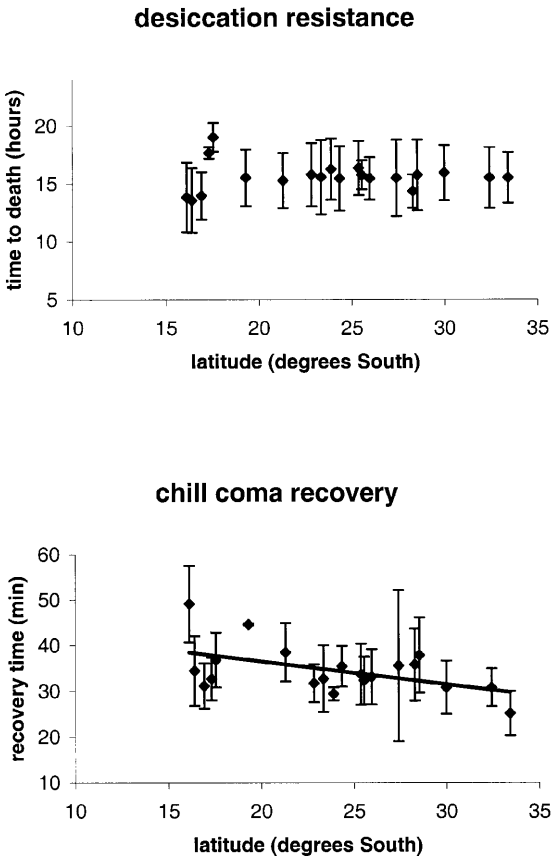

Fig. 2. Resistance to desiccation and cold shock in *D. serrata* populations from different latitudes. Each point represents the mean of 2–10 isofemale lines. Error bars are standard deviations based on isofemale line means, which had been estimated from 4 or 5 individuals. The linear regression line for chill coma recovery is indicated.

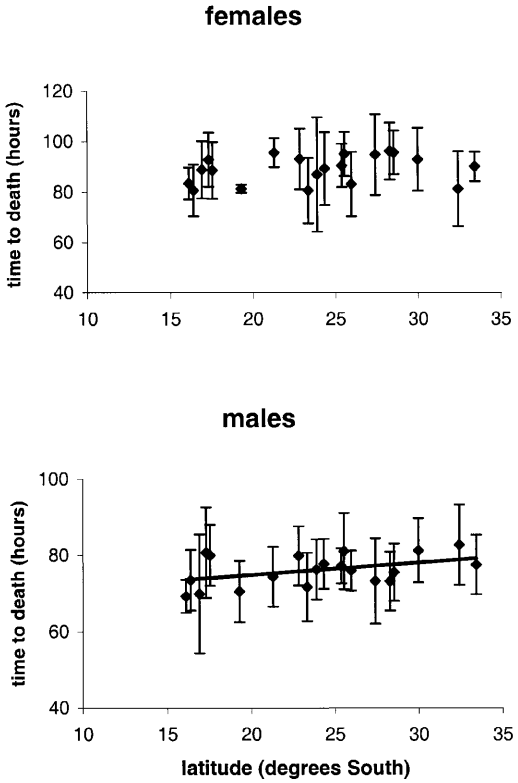

Fig. 3. Starvation resistance of females and males from *D. serrata* lines originating from different latitudes. Each point represents the mean of 2–5 isofemale lines. Error bars are standard deviations based on isofemale line means, which had been estimated from 4 or 5 individuals. The linear regression linking resistance to latitude was significant only for the male data.

the minimum July temperature ($b = -0{\cdot}67 \pm 0{\cdot}19$, $P = 0{\cdot}003$). Starvation resistance levels may therefore be linked to low-temperature conditions. Cold resistance showed a strong association with the minimum July temperature ($b = -0{\cdot}73 \pm 0{\cdot}16$, $P > 0{\cdot}001$), and the residual values remaining after this variable had been fitted showed a weak association with 0900 hours humidity ($b = 0{\cdot}38 \pm 0{\cdot}16$, $P = 0{\cdot}03$).

Isofemale line means for female desiccation and starvation resistance were positively correlated although the correlation was fairly low ($r = 0{\cdot}21$, $N = 76$, $P = 0{\cdot}04$). This suggests a weak association between resistance to these stresses. After correcting

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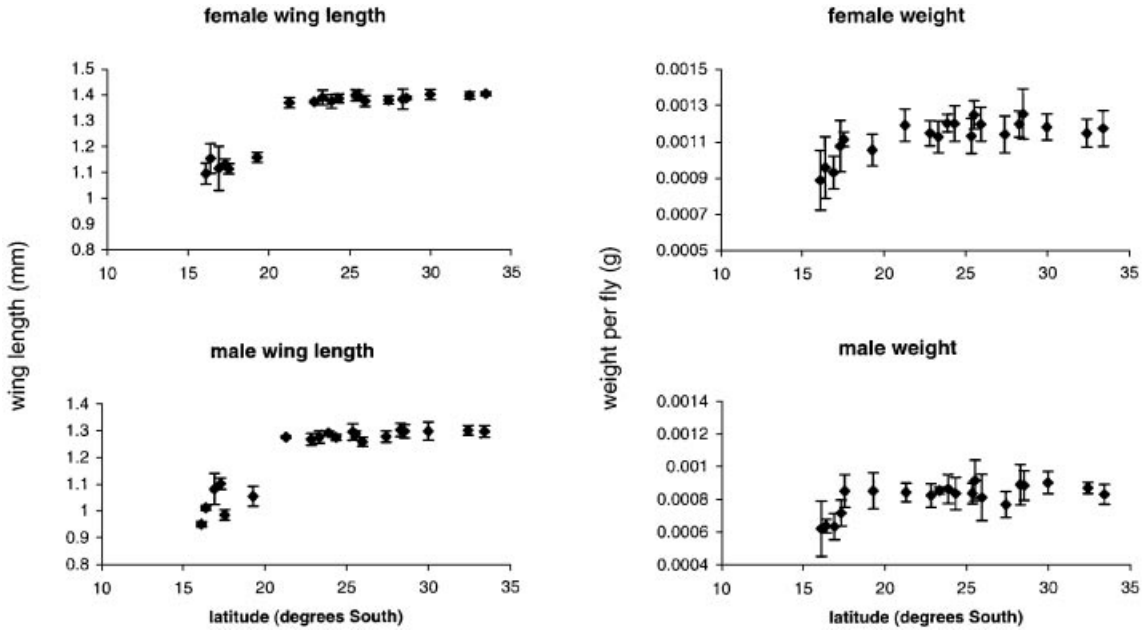

Fig. 4. Geographic patterns for body size in *D. serrata* as measured by wet weight and wing length. For wing length, data points represent the means of 2–6 isofemale lines. For body weight, one vial of 10 flies was tested per line and there were 2–8 lines per latitude. Error bars are standard deviations based on isofemale line means.

for locality effects, a similar correlation between these stresses was obtained ($r = 0{\cdot}19$, $N = 76$, $P = 0{\cdot}05$).

(ii) Size traits

For body weight, location effects were evident for both sexes (Table 1), but the relative magnitude of line and location effects could not be determined as only one replicate per line was weighed. The regression of latitude on weight showed a non-linear pattern, the quadratic component (but not the cubic component) being significant (Table 2). The wet weight of both sexes changed little at the higher latitudes but decreased sharply at lower latitudes (Fig. 4). In multiple regressions examining associations with latitude and climatic variables, only latitude showed a significant association with weight for both sexes.

For wing length, patterns were similar to those for weight. Non-linear associations with latitude were evident for both sexes (Table 2), reflecting a sharp decrease in size at latitudes of 20° S and lower. Differences among locations were substantial, the location term accounting for more than 80% of the variance (Table 1). As in the case of wet weight, significant associations were detected with latitude for both sexes but not for any of the climatic variables. Isofemale heritabilities for wing length were estimated as being 51% for females and 23% for males.

4. Discussion

The data indicate geographic patterns for cold resistance and size in *D. serrata* across a latitudinal gradient from 36° S to 16° S. The size cline is non-linear and indicates a sharp decrease in the tropics around latitude 20° S. In contrast, we found no evidence for clinal patterns for desiccation and starvation stress in this species. These patterns are consistent with *D. serrata* findings from limited geographic comparisons of a few populations (Blows & Hoffmann, 1993; Jenkins & Hoffmann, 2000) and partly match those for *D. melanogaster* over a similar region. In *D. melanogaster*, size decreases towards the Equator along the eastern Australian coast (James *et al.*, 1995), while there is little pattern for desiccation and starvation resistance (Hoffmann *et al.*, 2001). Moreover, recent data (R. Hallas, unpublished) indicate a cline in chill coma recovery in *D. melanogaster* from eastern Australia, consistent with results from a more limited comparison of cold resistance (Stanley & Parsons, 1981) in Australian populations of *D. melanogaster* and *D. simulans*. Thus parallel patterns occur in these species despite the fact that *D. melanogaster* is cosmopolitan and probably a recent arrival in Australia, whereas *D. serrata* is an endemic species common in areas of native vegetation rather than human activities (Jenkins & Hoffmann, 2001).

Despite these similarities, there is one important difference between the size clines of *D. melanogaster* and *D. serrata*. The *D. serrata* cline is non-linear and shows a rapid decrease in size in the tropics. In contrast, most of the changes in the *D. melanogaster* size cline occur at higher latitudes (James *et al.*, 1995). The nature of selection on size in these two species may therefore be different. While temperature has been suggested as the main factor influencing the size cline in *D. melanogaster* (Partridge *et al.*, 1994), there

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is no association in *D. serrata* between size and temperature variables from weather stations near the collection sites. Size is associated with some traits in *D. serrata* (Hercus & Hoffmann, 1999) but it remains to be seen what selective factors influence this trait. In other *Drosophila* species size selection may be mediated by the impact of wing size relative to body size on flight capacity (Azevedo *et al*., 1998) as well as by the impact of size on development time (e.g. Prasad *et al*., 2000). Size patterns may also be unrelated to selection and instead reflect cryptic northern and southern subspecies of *D. serrata*. However, in the laboratory there is no evidence of postmating reproductive isolation between lines from these areas (Blows, 1993).

Is the size cline in *D. serrata* steeper than in *D. melanogaster*? The steepness of the clines could be compared in several ways. One option is simply to compare changes in the means of the size traits, expressed as a percentage per degree latitude. For *D. serrata*, the overall change between the extremes of the latitudinal gradient is around 1·45% per degree latitude. This is more than twice the change (0·66%) observed in *D. melanogaster* computed from data in James *et al*. (1995). Given the non-linear nature of the *D. serrata* cline, we also examined changes along the steepest part of the cline, i.e. between the northernmost population and the population at 20° S. In this case, the change in *D. serrata* is 5%, substantially higher than changes for wing length in clines of *D. melanogaster* and of other *Drosophila* species (data from Capy *et al*., 1993; Imasheva *et al*., 1994; Karan *et al*., 1998*b*; Huey *et al*., 2000). Another option is to express cline steepness relative to the variance, evolvability or heritability of size in a population, given that these parameters can be related to the potential of a trait to respond to directional selection. Unfortunately, different measures of wing size are often used by researchers and data on trait variances are rarely provided in clinal studies, so we were unable to make this comparison. As heritability estimates for size in different *Drosophila* species (see Hoffmann, 2000) are similar to those estimated from isofemale lines of *D. serrata* in this study, the *D. serrata* size cline is also likely to be steep when expressed relative to heritable variation in this trait.

The lack of clinal variation in desiccation and starvation resistance contrasts with the findings by Karan, David and co-workers (e.g. Karan *et al*., 1998*a*) on clines in drosophilids in India. On this continent it appears that there are consistent increases in starvation resistance towards the tropics but opposing patterns for desiccation resistance. Limited population comparisons in other areas initially appear to support such patterns (Hoffmann & Harshman, 1999) but the present study, as well as those on *D. melanogaster* by Robinson *et al*. (2000) in South America and Hoffmann *et al*. (2001) in Australia, argue for different patterns on other continents. Presumably selective factors acting on these traits are inconsistent across continents due to different climatic conditions. For instance, in the Indian subcontinent, latitude is not strongly associated with average temperature (Karan *et al*., 1998*a*) unlike in Australia. Moreover, minimum humidity levels at high latitudes in India are lower than at any of the sites we sampled for *D. serrata* (28% in northern India versus 48% at one of our sites) so selection for desiccation resistance will be weaker in Australia.

In the regression analysis the cline in chill coma resistance in *D. serrata* was associated with daily minimum temperature, suggesting that cold may act as a direct or indirect selective force on this trait. In previous work on *D. serrata* populations near the southern border of this species, Jenkins & Hoffmann (1999) showed changing patterns of cold resistance (measured as adult mortality following a cold shock) between collections of flies before and after winter. Resistance in the most southerly population they examined was relatively higher than that of other populations after winter but not before winter. This pattern was established in two years and has since been repeated in an independent study (A. Magiafoglou, unpublished). Thus selection acts on cold resistance in some *D. serrata* populations, which is also consistent with the southern border of this species correlating closely with indices of cold stress (Jenkins & Hoffmann, 2001). Our results also demonstrate that chill coma recovery, which has previously been used in comparisons of *Drosophila* species (Gibert *et al*., 2001), provides a potential tool for investigating intraspecific variation in cold response.

In conclusion, geographic patterns for quantitative traits in the endemic species *D. serrata* along the eastern coast of Australia are similar to those of the cosmopolitan species *D. melanogaster* over the same region. Selection is presumably responsible for variation in cold resistance and size in *D. serrata* populations, and cold temperatures may select on chill coma resistance. It remains to be seen how these patterns in quantitative traits compare with levels of variation at nuclear molecular markers (cf. Gockel *et al*., 2001), and to identify field selection pressures that produce them.

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