PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Testing evolutionary hypotheses about species borders: patterns of genetic variation towards the southern borders of two rainforest *Drosophila* and a related habitat generalist

Belinda van Heerwaarden, Vanessa Kellermann, Michele Schiffer, Mark Blacket, Carla M Sgrò and Ary A Hoffmann

Proc. R. Soc. B 2009 **276**, doi: 10.1098/rspb.2008.1288 first published online 25 February 2009

PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2009) **276**, 1517–1526
doi:10.1098/rspb.2008.1288
Published online 25 February 2009

Testing evolutionary hypotheses about species borders: patterns of genetic variation towards the southern borders of two rainforest *Drosophila* and a related habitat generalist

Belinda van Heerwaarden[1,*], Vanessa Kellermann[1], Michele Schiffer[2], Mark Blacket[1], Carla M. Sgrò[3] and Ary A. Hoffmann[1]

[1]*Department of Genetics, Centre for Environmental Stress and Adaptation Research (CESAR), University of Melbourne, Parkville, 3010 Victoria, Australia*
[2]*Department of Zoology, University of Melbourne, Parkville, 3010 Victoria, Australia*
[3]*School of Biological Sciences, Monash University, Clayton, 3800 Victoria, Australia*

Several evolutionary hypotheses help explain why only some species adapt readily to new conditions and expand distributions beyond borders, but there is limited evidence testing these hypotheses. In this study, we consider patterns of neutral (microsatellite) and quantitative genetic variation in traits in three species of *Drosophila* from the *montium* species group in eastern Australia. We found little support for restricted or asymmetrical gene flow in any species. In rainforest-restricted *Drosophila birchii*, there was evidence of selection for increased desiccation and starvation resistance towards the southern border, and a reduction in genetic diversity in desiccation resistance at this border. No such patterns existed for *Drosophila bunnanda*, which has an even more restricted distribution. In the habitat generalist *Drosophila serrata*, there was evidence for geographic selection for wing size and development time, although clinal patterns for increased cold and starvation resistance towards the southern border could not be differentiated from neutral expectations. These findings suggest that borders in these species are not limited by low overall genetic variation but instead in two of the species reflect patterns of selection and genetic variability in key traits limiting borders.

Keywords: range limits; gene flow; genetic variation; heritability; climate; selection

1. INTRODUCTION

When there are no identifiable geographic barriers limiting the distribution of species, a variety of biotic (e.g. competition or predation) and abiotic (e.g. resource availability, physiological limits, demography or climate) factors may prevent further range expansion (Hoffmann & Blows 1994; Gaston *et al.* 2008). Climatic variables are thought to be important in dictating distributional limits in many ectotherms, because species only survive within a narrow range of conditions (Chown *et al.* 2002). Climatic distribution modelling and detailed ecological experiments can help identify traits involved in limiting the distribution of species (Sutherst *et al.* 2007; Phillips *et al.* 2008). However, this type of information does not answer a long-standing evolutionary question: why do species fail to adapt to ecological conditions beyond their border thereby preventing a continual range expansion?

There are several hypotheses to explain why adaptation beyond the border of a species does not occur (Hoffmann & Blows 1994; Kirkpatrick & Barton 1997; Bridle & Vines 2007; Eckert *et al.* 2008). Genetic variation may be generally low in border populations, as adverse conditions at the border reduce population size, decreasing genetic variation through drift (Hoffmann & Blows 1994). Restricted gene flow will reduce the movement of alleles from central to border populations, further exacerbating the effects of drift on the levels of genetic variation and adaptive potential in border populations (Hoffmann & Parsons 1997). Consequently, lower genetic variation in neutral markers coupled with greater genetic differentiation should be evident within marginal populations (Eckert *et al.* 2008). The heritability of traits under selection may be low at border populations as a result of selection fixing favoured alleles, decreasing additive genetic variance, or as a result of increased environmental variance due to heterogeneous conditions experienced by border populations (Hoffmann & Blows 1994). If selection is involved in reducing heritable variation, changes in trait mean are expected towards the border as the selection gradient becomes steeper as populations are at a physiological limit (Blows & Hoffmann 1993). Heritable variation may also be generally low in populations owing to physiological constraints or a loss of gene function due to decay under relaxed selection, whereby obvious changes in the mean or neutral genetic variation may not be apparent (Hoffmann & Kellermann 2006).

* Author for correspondence (b.vanheerwaarden@pgrad.unimelb.edu.au).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2008.1288 or via http://rspb.royalsocietypublishing.org.

One contribution of 17 to a Special Issue 'Geographic range limits of species'.

Received 9 September 2008
Accepted 20 November 2008

Table 1. Evolutionary hypotheses to account for limits to species ranges and predictions (based partly on Hoffmann & Parsons 1997).

	predictions			
hypotheses	low 'neutral' genetic variation	low h^2 in traits limiting borders	clinal variation towards border	genetic interactions restricting evolution
low heritability in traits limiting borders due to directional selection at borders		+	+	
low overall genetic variation in border populations	+	+		
low heritability in traits limiting borders due to physiological constraints/genetic decay		+		
asymmetric gene flow from the central to border populations			+	
adaptation restricted by trait interactions				+
changes in independent characters required for adaptation beyond the border			+	

Asymmetrical gene flow may also play a role in preventing expansion beyond the border of a species distribution. High levels of gene flow from large central populations to small border populations can dilute the presence of locally adapted genotypes in border populations and prevent range expansion (Garcia-Ramos & Kirkpatrick 1997; Kirkpatrick & Barton 1997). Signatures of asymmetrical gene flow include differences in effective population size and/or the difference in the proportion of private alleles between populations (Beerli & Felsenstein 2001; Kennington *et al.* 2003).

Another set of hypotheses deals with multiple trait effects. Negative genetic correlations between traits under directional selection can impede evolution in the traits at borders, even when sufficient genetic variation is present (Lande & Arnold 1983; Price & Langen 1992; van Tienderen & de Jong 1994; Barton & Partridge 2000). Furthermore, hidden pleiotropic effects inducing stabilizing selection on one character may hinder directional selection on an otherwise neutral trait (Baatz & Wagner 1997). Positive genetic correlations between traits may also constrain evolution in the direction of selection if small trait values are favoured in one trait and large trait values are favoured in the other correlated trait (Blows & Hoffmann 2005). There is relatively little empirical evidence of trait correlations limiting evolution, but detection of these effects generally requires large experiments and information on all traits under selection.

Despite the fact that these hypotheses have been around for some time, there is still a paucity of data to evaluate them. Patterns of asymmetrical gene flow have only been examined in a few species and rarely in the context of borders (Riechert 1993; Raymond & Marquine 1994; Kennington *et al.* 2003). Despite predictions for lower population sizes in border populations, it is still debatable whether population sizes are in fact lower in marginal populations (Sagarin & Gaines 2002). Some studies have found lower levels of neutral genetic variation in marginal populations (Johansson *et al.* 2006; Eckert *et al.* 2008; York *et al.* 2008); however, these differences were not always striking and reflect changes in population size rather than evolutionary potential (Hoffmann & Willi 2008). Furthermore, often changes in genetic variation or population size are not evident (Wang *et al.* 2002; Garner *et al.* 2004; Eckert *et al.* 2008). Levels of genetic variation in quantitative traits have only been linked to borders in a limited number of studies (Hoffmann *et al.* 2003; Kellermann *et al.* 2006). In plants, a few studies have used transplants to demonstrate traits related to borders (Eckhart *et al.* 2004; Griffith & Watson 2005), but such studies are rare, particularly across multiple species. Clinal patterns of molecular markers and quantitative traits provide a way of testing border hypotheses (table 1), but few studies have simultaneously compared patterns across different levels, or considered multiple species.

Here, we describe new data and use published data to investigate patterns of quantitative and neutral molecular variation across the geographic distribution of three closely related *Drosophila* species differing in their level of ecological specialization and distribution. Clinal patterns for several climatic traits have been identified in the previous studies of two of these species, *Drosophila birchii* Dobzhansky and Mather and *Drosophila serrata* Malloch (Hallas *et al.* 2002; Hoffmann *et al.* 2003; Griffiths *et al.* 2005), suggesting that the traits are under natural selection and may be involved in limiting the southern border of these species. However, such clinal patterns may also be the result of demographic history or genetic drift in isolated populations (Vasemagi 2006).

Comparisons between molecular markers and quantitative traits may clarify whether demographic factors or selection is involved in population differentiation along clines (Gockel *et al.* 2001) and also indicate whether small population sizes and/or asymmetrical gene flow are found at species borders. Small population sizes at the border should be reflected by the lower levels of variation in microsatellite markers, although a reduction in variation in neutral markers reflects changes in population size rather than changes in heritable variation for quantitative traits (Hoffmann & Willi 2008). If selection is underlying divergence, we predict differentiation will be observed in the quantitative traits but not the microsatellite markers. However, if genetic drift or

demographic history is causing divergence, high microsatellite differentiation is expected and clinal patterns in quantitative traits are not expected to exceed variation explained by the microsatellite alleles (Gockel *et al.* 2001). If additive genetic variance in traits under selection is low or if gene flow is swamping differentiation, limited divergence in the microsatellite markers (low F_{ST} values) and quantitative traits are expected.

2. MATERIAL AND METHODS

New data on clinal variation in several quantitative traits and microsatellite loci in *Drosophila bunnanda* Schiffer & McEvey and microsatellite variation across the southern and northern populations of *D. serrata* were added to the extensive data on microsatellite clinal data in *D. birchii* (Schiffer *et al.* 2007), and quantitative stress/morphological/life-history traits in *D. birchii* and *D. serrata* (Hallas *et al.* 2002; Hoffmann *et al.* 2003; Sgrò & Blows 2003; Griffiths *et al.* 2005; Schiffer *et al.* 2007) to investigate patterns of quantitative and neutral molecular variation across the geographic distribution of these three species. Distributed in Papua New Guinea (PNG), and along the east coast of Australia, with a southern border at Wollongong (figure 1), *D. serrata* is considered a habitat generalist (Schiffer & McEvey 2006). By contrast, *D. birchii* is restricted to rainforest fragments in PNG and northeast Australia and has a relatively narrow distribution with a southern border at Byfield in north Queensland, 1278 km north of the *D. serrata* border (Schiffer & McEvey 2006; figure 1). Similar to *D. birchii*, the recently described *D. bunnanda* is restricted to rainforest patches in northeast Queensland, although its southern border (Townsville) is 561 km further north of the *D. birchii* border and it is not known whether populations are present in PNG (figure 1; Schiffer & McEvey 2006).

(a) *Taxon sampling*

Drosophila serrata samples for the microsatellite analysis were collected using banana baits at nine locations (table 1 in the electronic supplementary material), spanning a large proportion of this species' geographic range along the east coast of Australia (figure 1). These populations were collected in February and March of 2006, except for the Cooktown population, which was collected in May 2004. A total of 400 *D. bunnanda* samples were collected using banana baits at 10 locations (table 1 in the electronic supplementary material) throughout the species distribution in northeast Queensland (figure 1). Flies were collected during three field trips conducted between March and May spanning three consecutive years (2004–2006). All molecular variation estimates for *D. birchii* were taken from Schiffer *et al.* (2007). The flies collected from the field were stored in 100 per cent ethanol at −20°C prior to extraction. DNA was extracted with the Chelex method, as described in Magiafoglou *et al.* (2002).

(b) *Microsatellite variation*

Drosophila bunnanda and *D. serrata* samples were each screened for six polymorphic microsatellite markers (one x-linked and five autosomal markers) as previously described in Magiafoglou *et al.* (2002) and Schiffer *et al.* (2004, 2007; see table 2 in the electronic supplementary material). Polymerase chain reaction (PCR) of each locus was performed in 10 µl reactions containing 1 µl of template DNA, 1.5 mM of $MgCl_2$, 2 mM of dNTPs and 0.2 pmol μl^{-1} of each primer and 0.3 pmol μl^{-1} of the forward primer labelled with LI-COR IRDye 700 or 800. Bands were detected using electrophoresis on a 6 per cent acrylamide gel on a LI-COR Global IR2 automated DNA analyser. Alleles were scored by comparing their sizes with the standards that were distributed across the gel in lanes adjacent to the samples.

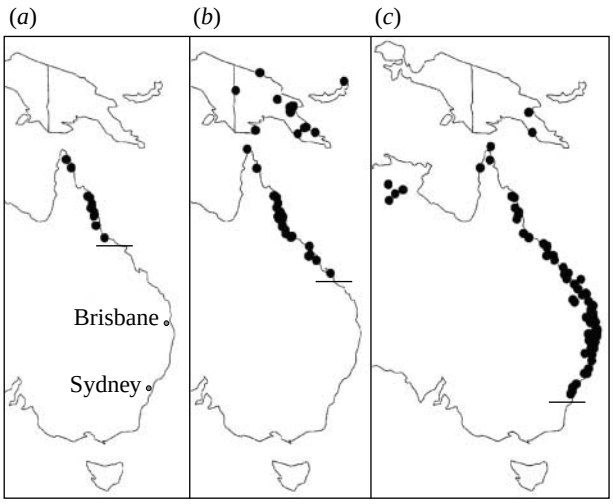

Figure 1. Geographic range of (*a*) *D. bunnanda*, (*b*) *D. birchii* and (*c*) *D. serrata* along the east coast of Australia (modified from Schiffer & McEvey 2006). Southern borders are indicated by horizontal lines.

Estimations of genetic variation, such as observed heterozygosity and allelic richness, were calculated with FSTAT v. 2.9.3 (Goudet 1995). GENEPOP v. 4.0.7 (Rousset 2008) was used to examine whether there was linkage disequilibrium between loci (which was not evident). Levels of population differentiation (F_{ST}) were examined for each species using FSTAT v. 2.9.3 (Goudet 1995). Significance of overall F_{ST} values were tested with randomization tests in FSTAT v. 2.9.3 (Goudet 1995). Mantel tests (implemented in Genetic Analysis in Excel (GENALEX) v. 6.1 (Peakall & Smouse 2006) with F_{ST} values ($F_{ST}/1-F_{ST}$) obtained from GENEPOP v. 4.0.7 (Rousset 2008) were undertaken to examine whether there were associations between genetic and geographic distance. To investigate whether there was asymmetrical gene flow between populations, we used the software package MIGRATE v. 3.0 (Beerli & Felsenstein 2001) to estimate the effective number of migrants ($4Nm$, where N is the effective population size and m is the migration rate) entering and leaving each population per generation. This program calculates maximum-likelihood estimates of migration rates and sub-population size between pairs of populations using coalescent theory, and assumes populations have a constant effective population size through time, the rate of mutation is constant and populations exchange migrants with constant rates per generation (Beerli & Felsenstein 2001). Simulations have shown the ability of MIGRATE to accurately detect the levels of gene flow when genetic diversity is high and the level of migration is moderate (Abdo *et al.* 2004). We relied on default search settings, using the microsatellite stepwise mutation model and running MIGRATE three times to verify the consistency of our results. To avoid biases in gene flow due to differences in sample size, we randomly trimmed the dataset to make population sizes as equal as possible. As our samples included both males and females, analyses of population structure, genetic diversity and

migration rates were undertaken only with the data from the autosomal loci.

(c) *Quantitative data*

For *D. serrata*, development time estimates were taken from Sgrò & Blows (2003), while desiccation, starvation and cold resistance estimates were taken from Hallas *et al.* (2002). Cold and desiccation resistance for *D. birchii* and *D. bunnanda* were estimated from isofemale lines with populations collected using banana baits in 2004 from eight and five populations, respectively (table 1 in the electronic supplementary material); methods for these estimates are described below. Other quantitative trait estimates for *D. birchii* including a second estimate for desiccation and cold resistance were taken from Hoffmann *et al.* (2003) and Griffiths *et al.* (2005). The isofemale lines were each initiated from a field-inseminated female and maintained at a population size of 50–100 flies. Isofemale lines were maintained on a potato–yeast–sucrose–agar media at 25°C with constant light. All assessments were completed within five generations of lines being established from field females. Desiccation resistance and chill coma recovery were assayed in eight and five populations of *D. birchii* and *D. bunnanda*, respectively, with 10–25 isofemale lines assayed per population (table 1 in the electronic supplementary material). All lines were reared at a standard density by placing 20 eggs into 50 ml vials with eggs collected from watch glasses containing a treacle–yeast–agar media coated with live yeast to stimulate oviposition. For each individual isofemale line, five replicate vials were initiated. Desiccation and cold resistance were estimated with a single individual female from each vial in order to eliminate common vial effects. The flies emerging from these vials were collected over a 48 hour period, left for a day to allow mating and sexed via CO_2 anaesthesia. Flies were then left to recover and tested for stress resistance between 5 and 7 days of age.

Desiccation resistance was estimated by placing the vials into a desiccator containing silica gel producing 10–15 per cent relative humidity. The flies were scored every hour for knock-down until all flies had succumbed to the effects of desiccation. This trait discriminates between *Drosophila* species from natural habitats that differ in the levels of aridity (Parsons 1982).

Chill coma recovery was estimated by scoring the time to recovery from a cold shock. Individuals were placed into vials that were then submerged in a cold bath containing ethylene glycol, with the tank remaining at 0°C throughout the period of exposure. The flies were submerged for 2 hours at which point the vials were removed and the flies scored for chill coma recovery. All flies had entered a chill coma in this exposure period. This trait differentiates between *Drosophila* species from tropical and temperate environments (Gibert & Huey 2001) and also exhibits clinal variation in *Drosophila melanogaster* from eastern Australia (Hoffmann *et al.* 2002).

(d) *Analysis*

To investigate whether there was evidence of clinal patterns in both microsatellite loci and quantitative traits, a regression of most common allele (MCA) frequency/trait mean and latitude was undertaken. The MCA was obtained using GENALEX v. 6.1 (Peakall & Smouse 2006) and the MCA frequency was calculated by dividing the number of times the MCA was found within a population by the total number of alleles sampled within a population (two alleles per individual for autosomal loci and one allele per individual for x-linked loci). A linear equation was first fitted to test for linear effects, and quadratic, cubic and exponential components were then added to test for curvilinear relationships. To determine whether latitudinal patterns for quantitative traits were stronger than those for the neutral markers, reflecting selection, the procedure outlined in Gockel *et al.* (2001) was followed, involving a comparison of the explanatory power of latitude for the quantitative and molecular variation. Briefly, confidence intervals for R^2 coefficients of determination were obtained from the empirical distributions of quantitative traits or molecular markers by bootstrapping with 1000 iterations. Values from the regressions involving quantitative traits were obtained by resampling the data for isofemale lines, while the values for each microsatellite locus were obtained by resampling the data for individuals from the populations. For the molecular data, mean R^2 values over all loci were computed and 95% CI values determined from the 1000 R^2 bootstrapped means. Resampling and bootstrapping were performed using the Microsoft Excel add-in program POPTOOLS v. 2.7.5 (Hood 2006). Latitudinal variation in quantitative traits was considered to be different from microsatellite loci variation when the lower 95% CI value for the quantitative trait did not overlap with the average upper 95% CI value for the microsatellite markers.

Measures of quantitative genetic variation (coefficient of intra-class correlation, t) were calculated for each population as outlined in Hoffmann & Parsons (1988). The coefficient of intra-class correlation is a measure of the difference in the within- and between-line variances of isofemale lines and may include non-additive components of variation. Latitudinal patterns for t were tested using regression analyses.

3. RESULTS

(a) Drosophila serrata

In this species, a significant association with latitude was found for wing size, development time, female chill coma recovery time and male starvation resistance, with resistance/mean values for these traits increasing towards the southern border (figure 2). No clinal associations with latitude were detected for female desiccation and starvation resistance (table 2). The latitudinal patterns observed in wing size, development time, cold resistance and male starvation resistance are unlikely to be the result of low levels of gene flow between populations, as there was little evidence of microsatellite genetic differentiation in the populations of *D. serrata* collected across a similar latitudinal cline, with low F_{ST} values (0.005 ± 0.003, $p = 0.139$) and only a weak positive correlation between genetic and geographic distance ($p = 0.049$). Other studies have also found low estimates of differentiation in the southern field populations and the northern laboratory mass-bred populations (southern: $F_{ST} = 0.002 \pm 0.001$, $p = 0.018$, (Magiafoglou *et al.* 2002); northern: $F_{ST} = 0.002 \pm 0.001$, $p = 0.018$, (Chenoweth & Blows 2008)). Little asymmetrical gene flow was detected between populations (table 3 in the electronic supplementary material); there was some evidence for asymmetrical gene flow from the southern border population of Wollongong to the nearby population at Terrigal, but this was in the opposite direction to that expected if gene flow from the central populations was swamping marginal populations (table 3 in the electronic supplementary material).

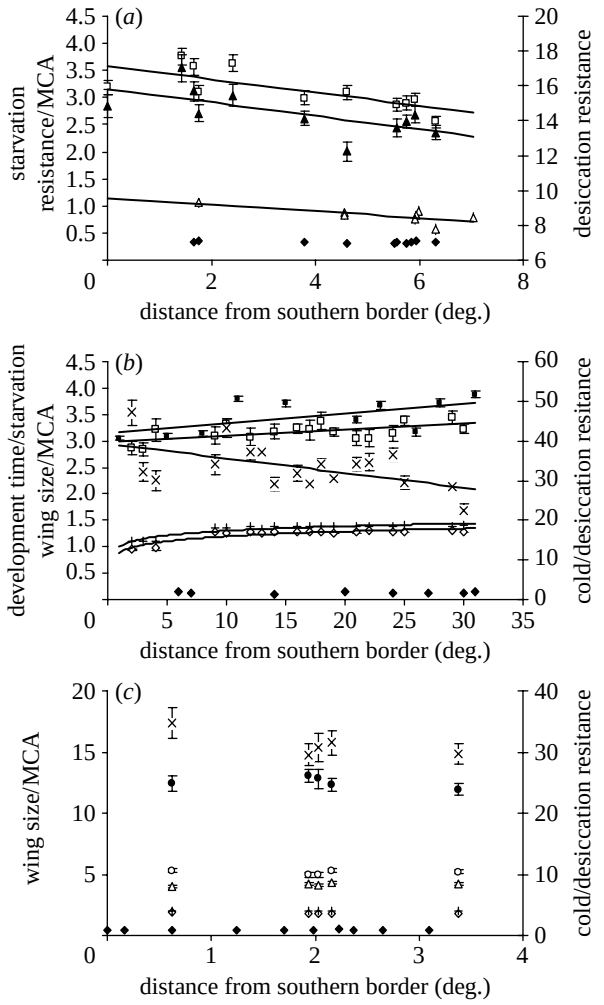

Figure 2. Clinal patterns for stress, morphological and life-history traits in (*a*) *D. birchii* (open squares, starvation; filled diamonds, MCA; filled triangles, desiccation 2002; open triangles, desiccation 2004), (*b*) *D. serrata* (open squares, starvation, males; pluses, wing size, females; open diamonds, wing size, males; filled squares, development time; filled diamonds, MCA; crosses, cold) and (*c*) *D. bunnanda* (pluses, wing size, females; open diamonds, wing size, males; filled diamonds, MCA; open triangles, desiccation, males; crosses, cold, males, open circles, desiccation, females; filled circles, cold, females). Error bars are 1 s.e.

To investigate whether patterns in the traits were due to selection rather than genetic drift, we compared the proportion of variation explained by latitude for the traits with the level of variation in MCA frequency in the microsatellite markers. With the exception of locus D34, which showed a highly significant negative relationship with latitude ($R^2=0.728$, $p=0.003$), no significant associations with latitude were found for MCA frequency in *D. serrata* (table 2 in the electronic supplementary material). Overall, the average variation in the microsatellite markers explained by latitude was 20.9 per cent (table 2 in the electronic supplementary material); when D34 was excluded, only 9.9 per cent of the variation in the MCA was explained by latitude (table 2). Despite significant increases in male starvation and female cold resistance towards the southern border, the coefficient of determination for the latitudinal patterns observed in these traits was not higher than for the microsatellite markers, because the lower confidence interval for all of these traits overlapped with the upper confidence interval for the microsatellite markers (table 2), even when D34 was excluded. As such, the strength of the latitudinal patterns in these traits does not exceed neutral expectations based on the microsatellite markers sampled in the current study. By contrast, the coefficient of determination for the latitudinal association with wing size and development time was higher than that of the microsatellite markers, with no overlap between the lower and upper 95 per cent confidence intervals of the coefficients of determination when D34 was excluded (table 2). Thus, it appears that the increases in these traits towards the southern border are likely to be due to selection. High levels of genetic variation were found in *D. serrata* across all microsatellite loci (table 2 in the electronic supplementary material). There was some evidence for changing levels of genetic diversity with latitude (figure 3). A significant quadratic relationship with latitude was observed for allelic diversity, with a slight decrease in diversity in the southern border populations in comparison with mid-latitude populations. No significant association was found between latitude and the coefficient of intra-class correlation for any of the quantitative traits (data not shown).

(b) *Drosophila birchii*

There was a linear association between latitude and both desiccation (marginally non-significant in flies from the 2004 collection) and starvation resistance in *D. birchii* females. Resistance in both traits increased towards the southern border (figure 2). No association with latitude was found for cold or heat resistance, suggesting that climatic selection for thermal tolerance is not occurring over the latitudinal distribution of *D. birchii*. Unlike in *D. serrata*, there was no significant association between wing size and latitude in *D. birchii* females. Low levels of microsatellite genetic differentiation between the populations of *D. birchii* ($F_{ST}=0.002$) and a lack of evidence for isolation by distance ($p=0.178$) suggest that low levels of gene flow are not responsible for population differentiation in desiccation or starvation resistance. There was no evidence for a latitudinal association for MCAs at any of the microsatellite loci (table 2 in the electronic supplementary material). Furthermore, the proportion of variation explained by latitude for desiccation and starvation resistance was found to exceed the proportion of variation explained by latitude for microsatellite variation (table 2), suggesting that selection may be responsible for divergence in mean resistance across latitude.

Despite a lack of evidence for restricted or asymmetrical gene flow into the southern populations of *D. birchii* (table 3 in the electronic supplementary material), there was a significant decline in allelic diversity in the microsatellite markers in the northern and southern populations, suggesting that population sizes may be larger in the central populations (figure 3). However, the reduction in allele diversity in these populations was minimal and this pattern was not evident for the expected heterozygosity (figure 3). The coefficient of intra-class correlation for desiccation resistance in females collected in 2004 showed a significant exponential association with latitude, with the coefficient of intra-class correlation decreasing towards the southern border ($R^2=0.734$, $p=0.014$), while a significant increase towards the

Table 2. Regression analysis of the effects of latitude on population means for the traits, including confidence intervals for the proportion of variation explained by latitude. (Significant associations are italicized.)

species	trait	slope	R^2	*p*-value	lower CI	upper CI
D. serrata	cold, females[a]	−0.586	0.306	*0.017*	0.168	0.412
	desiccation, females[a]	−0.016	0.004	0.798	0.000	0.082
	starvation, females[a]	0.234	0.053	0.372	0.000	0.258
	starvation, males[a]	0.486	0.365	*0.010*	0.044	0.524
	development time[b]	0.034	0.404	*0.048*	0.264	0.540
	wing size, males[a]	0.133[c]	0.907	*<0.001*	0.865	0.931
	wing size, females[a]	0.117[c]	0.928	*<0.001*	0.887	0.941
	microsatellites[d]	−10.130	0.209	—	0.036	0.222
D. birchii	cold, females 2002[e]	0.030	0.003	0.868	0.000	0.224
	cold, females 2004	0.032	0.002	0.924	0.000	0.009
	desiccation, females 2002[f]	0.334	0.478	*0.018*	0.266	0.611
	desiccation, females 2004	0.197	0.560	0.051	0.159	0.781
	starvation, females[e]	2.854	0.461	*0.022*	0.310	0.557
	heat, females[e]	0.082	0.086	0.380	0.000	0.309
	development time, females[e]	1.560	0.250	0.117	0.087	0.415
	development time, males[e]	1.073	0.141	0.255	0.026	0.293
	wing size, females[e]	−0.290	0.009	0.777	0.000	0.126
	microsatellites[g]	1.857	0.078	—	0.028	0.144
D. bunnanda	cold, females	0.332	0.142	0.532	0.000	0.806
	cold, males	1.785	0.685	0.084	0.019	0.929
	desiccation, females	0.067	0.033	0.772	0.000	0.514
	desiccation, males	−0.170	0.380	0.268	0.003	0.846
	wing size, females	1.983	0.323	0.317	0.071	0.605
	wing size, males	1.571	0.206	0.443	0.065	0.606
	microsatellites	−0.797	0.061	—	0.023	0.166

[a]Data from Hallas *et al.* (2002).
[b]Data from Sgrò & Blows (2003).
[c]Logarithmic relationship.
[d]Excluding locus D34.
[e]Data from Griffiths *et al.* (2005).
[f]Data from Hoffmann *et al.* (2003).
[g]Data from Schiffer *et al.* (2007).

southern border was evident in the coefficient of intra-class correlation for cold resistance in females collected in 2004 ($R^2=0.874$, $p=0.002$; figure 4).

(c) Drosophila bunnanda

There was no significant evidence for an association with latitude for any of the quantitative traits examined in the preliminary analysis of five populations of *D. bunnanda* (figure 2, table 2). There was a trend for cold resistance in males to increase towards the southern border (figure 2); however, this association was not significant. There was also no evidence of population differentiation, with a low F_{ST} value ($F_{ST}=0.001\pm0.002$, $p=0.060$) and no patterns suggesting isolation by distance ($p=0.248$). Furthermore, little asymmetrical gene flow was detected between the southern populations, although there was evidence for asymmetrical gene flow in some mid-latitude populations (table 3 in the electronic supplementary material). There were no significant associations between latitude and MCA frequency in a more comprehensive analysis of microsatellite variation in 10 populations collected across most of this species' distribution (table 2 in the electronic supplementary material). The proportion of variation explained by latitude in all quantitative traits in *D. bunnanda* did not exceed the proportion of variation explained by the microsatellite markers examined in this study (table 2). A significant quadratic association with latitude was observed for heterozygosity, with the lower

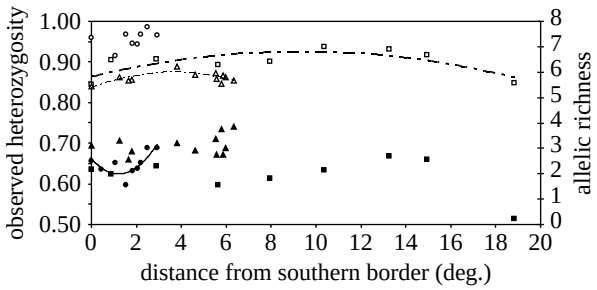

Figure 3. Correlation between levels of observed heterozygosity and allelic diversity with distance from the southern border in *D. birchii*, *D. serrata* and *D. bunnanda*. Open symbols, allelic diversity; filled symbols, observed heterozygosity. Only significant regression lines are indicated.

levels of heterozygosity observed in mid-latitude populations (figure 3). However, no clinal changes in the coefficient of intra-class correlation for wing size, cold or desiccation resistance were observed (data not shown).

4. DISCUSSION

Adaptation beyond the border of a species may be limited by low genetic variation, high or low gene flow, environmental influences and/or trait interactions. Past empirical studies have focused on demographic hypotheses for species borders, comparing neutral genetic

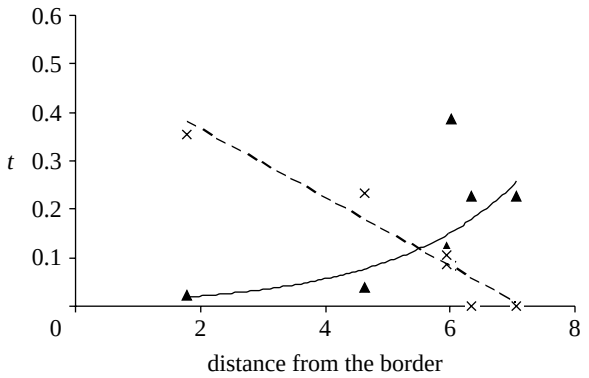

Figure 4. Clinal patterns for the coefficient of intra-class correlation (t) for cold (crosses) and desiccation (triangles) resistance in *D. birchii*.

variation in the border and central populations (Eckert *et al.* 2008), rather than identifying and examining patterns of variation in putative quantitative traits. We found no strong evidence for restricted or asymmetrical gene flow from the central to southern populations in any of the three species, indicating that gene flow is unlikely to be involved in limiting adaptation beyond the border of these species, although simulation studies have found a reduction in accuracy for detecting asymmetrical gene flow when the levels of population differentiation are extremely low or high (Abdo *et al.* 2004). A small reduction in allelic diversity in the northern and southern populations was found in both *D. birchii* and *D. serrata* (figure 3), suggesting that population sizes may be larger in the central populations; however, decreases in allelic diversity were small, and in *D. serrata*, the levels of heterozygosity were higher in the southern populations, indicating that low population size is unlikely to reduce the ability of these populations to respond to selection pressures at the border. The pattern of increase in heterozygosity at higher latitudes for *D. serrata* is the opposite to that observed for *D. melanogaster* populations from the east coast of Australia (Kennington *et al.* 2003) and is unusual, in that many species show greater genetic diversity closer to the equator, possibly owing to the persistence of larger long-term populations during the periods of climatic instability (Hewitt 2000, 2004). The lack of clear patterns for the decreases in neutral variation in *D. birchii*, *D. serrata* or *D. bunnanda* suggest that low population size is not influencing the southern border of these species.

However, latitudinal patterns in traits related to climatic responses suggest genetic variation and/or interactions in these traits may be important in the limiting borders of these species. The rainforest-restricted *D. birchii* has a relatively low level of desiccation resistance and cold resistance compared with other more widely distributed *Drosophila* species (Hoffmann & Parsons 1997), and these traits may be involved in limiting the southern border of *D. birchii*, which occurs within an area where the lower levels of ambient humidity and colder conditions are encountered. Low levels of heritable variation for these traits in the southern populations reflecting selection might then be expected (table 1). This was the pattern found for desiccation resistance but not cold resistance. A lack of clinal variation in chill coma recovery time suggests that this trait is not under strong selection in the border populations of *D. birchii*. The coefficient of intra-class correlation for this trait increased towards the southern border, suggesting that genetic variation for cold resistance is high in the southern populations, and that low genetic variance for this trait is unlikely to limit adaptation to cooler conditions beyond the southern border. Nevertheless, as this measure of genetic variance includes non-additive as well as additive genetic variance, family studies are required to accurately assess the evolutionary potential of this trait. By contrast, desiccation resistance was consistently found to vary clinally in two independent collections and assessments (Hoffmann *et al.* 2003), with resistance increasing towards the southern border. High levels of gene flow between all populations, as well as stronger clinal patterns in desiccation resistance compared with microsatellite MCA, suggest that this pattern in desiccation resistance is not a consequence of drift but rather selection.

If selection is involved in limiting further adaptation to desiccation stress, genetic variance for this trait is predicted to decrease towards the southern border as favourable alleles become fixed, while a reduction in variation at neutral markers will only occur if selection is decreasing population sizes in border populations (Hoffmann & Willi 2008). In the current analysis, a dramatic decrease in the intra-class correlation for desiccation resistance, but not heterozygosity, was observed in the southern populations of *D. birchii*, suggesting that climatic selection may be influencing genetic variation in this trait. Previous family studies and selection experiments have shown very low heritable variation for desiccation resistance in the populations of *D. birchii* (Hoffmann *et al.* 2003; Kellermann *et al.* 2006), reinforcing the notion that clinal patterns reflect the action of selection and that this species has a limited ability to adapt to drier conditions beyond the southern border.

We also examined *D. birchii* for clinal patterns in starvation resistance, development time and wing size, which vary clinally in *D. melanogaster* (reviewed in Hoffmann & Weeks 2007). Clinal patterns for starvation resistance were stronger than those in MCA, suggesting that there is also geographic selection for this trait in *D. birchii*. Unlike in *D. melanogaster*, no clinal patterns were detected for development time or wing size. There is abundant genetic variation for wing size in *D. birchii* (Hoffmann *et al.* 2003; Kellermann *et al.* 2006), so genetic variation is unlikely to limit selection responses for this trait. Perhaps these traits may not be under strong climatic selection in *D. birchii*, particularly as the range of climatic conditions encountered within the distribution of *D. birchii* is much narrower than that encountered by *D. melanogaster*.

There was no evidence that selection on climatic traits reduced heritable genetic variance in *D. serrata*. No clinal patterns for desiccation resistance have been observed (Hallas *et al.* 2002). While cold resistance has been shown to vary clinally, with increases in resistance towards the southern border (Jenkins & Hoffmann 1999; Hallas *et al.* 2002), there was no decrease in the intra-class correlation for this trait towards the border. This is consistent with the previous evidence for the similar levels of heritable variation in cold mortality in field flies from the southern and central populations (Jenkins & Hoffmann 1999). High rates of gene flow among *D. serrata* populations

suggest that clinal patterns in cold resistance may be due to selection rather than demography, although this was not supported by the comparison between clinal patterns in the quantitative trait and microsatellite alleles. This may partly reflect the fact that one locus, in particular D34, exhibited a strong association with latitude. This locus is on the same chromosome arm as the *In(3R)a* chromosomal inversion in *D. serrata*, which encompasses 42 per cent of the chromosome and shows a significant latitudinal cline in the same direction as D34 (Stocker *et al.* 2004) and may indirectly influence patterns of the MCA at this locus. The absence of clinal patterns at the other microsatellite loci suggests that inversions are unlikely to be influencing latitudinal patterns or the levels of divergence in these markers. However, the presence of chromosomal inversions may limit the power to determine whether clinal patterns in cold in *D. serrata* are due to demography or selection. Latitudinal associations for starvation resistance were also not stronger than those for MCA frequency; however, clinal patterns for development time and wing size were stronger, indicating the action of climatic selection, which is consistent with the data for body size in *D. melanogaster* (Gockel *et al.* 2001).

These analyses and patterns do not help explain the southern border of *D. serrata*, and factors other than asymmetrical gene flow and low genetic variation in stress resistance traits may be involved. In the tests of experimental evolution for life-history traits performed under experimental conditions similar to those encountered beyond the southern border of this species, there was no evidence of adaptation in life-history traits after 20 generations of culture (Magiafoglou & Hoffmann 2003). There is some evidence for a trade-off between fecundity and cold resistance, with a reduction in fecundity in *D. serrata* individuals that showed increased cold resistance when kept in population cages past the southern border (Jenkins & Hoffmann 1999). Evolution in this species may be constrained by trait interactions, or perhaps a requirement for evolutionary shifts in multiple traits.

The rainforest-restricted *D. bunnanda* has the most restricted southern limit, located more than 500 km north of the southern border of *D. birchii*. This species shows a low level of tolerance and additive genetic variation for desiccation resistance similar to *D. birchii* (Kellermann *et al.* 2006; Van Heerwaarden *et al.* 2008). We found no evidence for clinal variation in cold and desiccation resistance or genetic variation for these traits in this species, suggesting that selection is unlikely to be depleting heritable variation for these traits in border populations. We also found high gene flow and a high level of variation in neutral genetic markers across the distribution of this species. Therefore, it is not the low levels of genetic variation due to small population size at the border that are preventing evolution to the conditions existing beyond the southern distribution of this species. Perhaps, as in the case of *D. birchii*, low genetic variance for desiccation resistance prevents adaptation to conditions outside rainforest environments, and a pattern of high gene flow across a narrow distributional (and climatic) range prevents the development of clinal patterns in this species. However, evolution over small spatial scales has been demonstrated in *D. melanogaster*, suggesting that factors other than high gene flow may be important (Collinge *et al.* 2006; Korol *et al.* 2006). A comparison of the slopes of latitudinal clines in quantitative traits between species along the same selection gradient (i.e. the same latitudinal scale) would help determine whether gene flow is swamping the effects of selection; however, we currently do not have enough data for such a comparison across all three species.

Although there has been a great deal of focus on demographic factors limiting adaptation beyond the border of populations, low levels of heritable variation in ecologically important traits may also play a role in dictating limits to the distribution of many species. At present, we do not know how important the low levels of heritable variation in quantitative traits under selection are in limiting species borders, because data on heritable variation in relevant traits have been rarely collected. The *Drosophila* data presented here suggest that low levels of quantitative trait variation may play a role in determining species borders even when the rates of gene flow are high and symmetrical and when the genetic architecture of traits differs.

We would like to thank Cherie Marks, Jennifer Griffiths, Rebecca Hallas, Jennifer Shirriffs and Rhonda Rawlinson for their technical assistance; Jon Bridle for some field lines and the Australian Research Council for funding via their Special Research Centre and Fellowship schemes (A.A.H., C.M.S.).

REFERENCES

Abdo, Z., Crandall, K. A. & Joyce, P. 2004 Evaluating the performance of likelihood methods for detecting population structure and migration. *Mol. Ecol.* **13**, 837–851. (doi:10.1111/j.1365-294X.2004.02132.x)

Baatz, M. & Wagner, G. P. 1997 Adaptive inertia caused by hidden pleiotropic effects. *Theor. Popul. Biol.* **51**, 49–66. (doi:10.1006/tpbi.1997.1294)

Barton, N. & Partridge, L. 2000 Limits to natural selection. *Bioessays* **22**, 1075–1084. (doi:10.1002/1521-1878(200012)22:12<1075::AID-BIES5>3.0.CO;2-M)

Beerli, P. & Felsenstein, J. 2001 Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proc. Natl Acad. Sci. USA* **98**, 4563–4568. (doi:10.1073/pnas.081068098)

Blows, M. W. & Hoffmann, A. A. 1993 The genetics of central and marginal populations of *Drosophila serrata*. I. Genetic variation for stress resistance and species borders. *Evolution* **47**, 1255–1270. (doi:10.2307/2409990)

Blows, M. W. & Hoffmann, A. A. 2005 A reassessment of genetic limits to evolutionary change. *Ecology* **86**, 1371–1384. (doi:10.1890/04-1209)

Bridle, J. R. & Vines, T. H. 2007 Limits to evolution at range margins: when and why does adaptation fail? *Trends Ecol. Evol.* **22**, 140–147. (doi:10.1016/j.tree.2006.11.002)

Chenoweth, S. F. & Blows, M. W. 2008 Q(st) meets the G matrix: the dimensionality of adaptive divergence in multiple correlated quantitative traits. *Evolution* **62**, 1437–1449. (doi:10.1111/j.1558-5646.2008.00374.x)

Chown, S. L., Addo-Bediako, A. & Gaston, K. J. 2002 Physiological variation in insects: large-scale patterns and their implications. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **131**, 587–602. (doi:10.1016/S1096-4959(02)00017-9)

Collinge, J. E., Hoffmann, A. A. & McKechnie, S. W. 2006 Altitudinal patterns for latitudinally varying traits and polymorphic markers in *Drosophila melanogaster* from eastern Australia. *J. Evol. Biol.* **19**, 473–482. (doi:10.1111/j.1420-9101.2005.01016.x)

Eckhart, V. M., Geber, M. A. & McGuire, C. M. 2004 Experimental studies of adaptation in *Clarkia xantiana*. I. Sources of trait variation across a subspecies border. *Evolution* **58**, 59–70. (doi:10.1554/02-450)

Eckert, C. G., Samis, K. E. & Lougheed, S. C. 2008 Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Mol. Ecol.* **17**, 1170–1188. (doi:10.1111/j.1365-294X.2007.03659.x)

Garcia-Ramos, G. & Kirkpatrick, M. 1997 Genetic models of adaptation and gene flow in peripheral populations. *Evolution* **51**, 21–28. (doi:10.2307/2410956)

Garner, T. W. J., Pearman, P. B. & Angelone, S. 2004 Genetic diversity across a vertebrate species' range: a test of the central-peripheral hypothesis. *Mol. Ecol.* **13**, 1047–1053. (doi:10.1111/j.1365-294X.2004.02119.x)

Gaston, K. J., Chown, S. L. & Evans, K. L. 2008 Ecogeographical rules: elements of a synthesis. *J. Biogeogr.* **35**, 483–500. (doi:10.1111/j.1365-2699.2007.01772.x)

Gibert, P. & Huey, R. B. 2001 Chill-coma temperature in *Drosophila*: effects of developmental temperature, latitude, and phylogeny. *Physiol. Biochem. Zool.* **74**, 429–434. (doi:10.1086/320429)

Gockel, J., Kennington, W. J., Hoffmann, A., Goldstein, D. B. & Partridge, L. 2001 Nonclinality of molecular variation implicates selection in maintaining a morphological cline of *Drosophila melanogaster*. *Genetics* **158**, 319–323.

Goudet, J. 1995 FSTAT (v. 1.2): a computer program to calculate *F*-statistics. *J. Hered.* **86**, 485–486.

Griffith, T. M. & Watson, M. A. 2005 Stress avoidance in a common annual: reproductive timing is important for local adaptation and geographic distribution. *J. Evol. Biol.* **18**, 1601–1612. (doi:10.1111/j.1420-9101.2005.01021.x)

Griffiths, J. A., Schiffer, M. & Hoffmann, A. A. 2005 Clinal variation and laboratory adaptation in the rainforest species *Drosophila birchii* for stress resistance, wing size, wing shape and development time. *J. Evol. Biol.* **18**, 213–222. (doi:10.1111/j.1420-9101.2004.00782.x)

Hallas, R., Schiffer, M. & Hoffmann, A. A. 2002 Clinal variation in *Drosophila serrata* for stress resistance and body size. *Genet. Res.* **79**, 141–148. (doi:10.1017/S0016672301005523)

Hewitt, G. M. 2000 The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907–913. (doi:10.1038/35016000)

Hewitt, G. M. 2004 The structure of biodiversity—insights from molecular phylogeography. *Front. Zool.* **1**, 1–16. (doi:10.1186/1742-9994-1-4)

Hoffmann, A. A. & Blows, M. W. 1994 Species borders: ecological and evolutionary perspectives. *Trends Ecol. Evol.* **9**, 223–227. (doi:10.1016/0169-5347(94)90248-8)

Hoffmann, A. & Kellermann, V. 2006 Revisiting heritable variation and limits to species distribution: recent developments. *Isr. J. Ecol. Evol.* **52**, 247–261. (doi:10.1560/IJEE_52_3-4_247)

Hoffmann, A. A. & Parsons, P. A. 1997 *Extreme environmental change and evolution*. Cambridge, UK: Cambridge University Press.

Hoffmann, A. A. & Parsons, P. A. 1988 The analysis of quantitative variation in natural populations with isofemale strains. *Genet. Sel. Evol.* **20**, 87–98. (doi:10.1051/gse:19880108)

Hoffmann, A. A. & Weeks, A. 2007 Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. *Genetica* **129**, 133–147. (doi:10.1007/s10709-006-9010-z)

Hoffmann, A. A. & Willi, Y. 2008 Detecting genetic responses to environmental change. *Nat. Rev. Genet.* **9**, 421–432. (doi:10.1038/nrg2339)

Hoffmann, A. A., Anderson, A. & Hallas, R. 2002 Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.* **5**, 614–618. (doi:10.1046/j.1461-0248.2002.00367.x)

Hoffmann, A. A., Hallas, R. J., Dean, J. A. & Schiffer, M. 2003 Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* **301**, 100–102. (doi:10.1126/science.1084296)

Hood, G. M. 2006. PopTools v. 2.7.5. See http://www.cse.csiro.au/poptools.

Jenkins, N. L. & Hoffmann, A. A. 1999 Limits to the southern border of *Drosophila serrata*: cold resistance, heritable variation, and trade-offs. *Evolution* **53**, 1823–1834. (doi:10.2307/2640443)

Johansson, M., Primmer, C. R. & Merila, J. 2006 History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Mol. Ecol.* **15**, 975–983. (doi:10.1111/j.1365-294X.2006.02866.x)

Kennington, W. J., Gockel, J. & Partridge, L. 2003 Testing for asymmetrical gene flow in a *Drosophila melanogaster* body-size cline. *Genetics* **165**, 667–673.

Kellermann, V. M., van Heerwaarden, B., Hoffmann, A. A. & Sgrò, C. M. 2006 Very low additive genetic variance and evolutionary potential in two rainforest *Drosophila* species. *Evolution* **60**, 1104–1108. (doi:10.1554/05-710.1)

Kirkpatrick, M. & Barton, N. H. 1997 Evolution of a species' range. *Am. Nat.* **150**, 1–23. (doi:10.1086/286054)

Korol, A., Rashkovetsky, E., Iliadi, K. & Nevo, E. 2006 *Drosophila* flies in "Evolutionary Canyon" as a model for incipient sympatric speciation. *Proc. Natl Acad. Sci. USA* **103**, 18 184–18 189. (doi:10.1073/pnas.0608777103)

Lande, R. & Arnold, S. J. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226. (doi:10.2307/2408842)

Magiafoglou, A. & Hoffmann, A. 2003 Thermal adaptation in *Drosophila serrata* under conditions linked to its southern border: unexpected patterns from laboratory selection suggest limited evolutionary potential. *J. Genet.* **82**, 179–189. (doi:10.1007/BF02715817)

Magiafoglou, A., Carew, M. E. & Hoffmann, A. A. 2002 Shifting clinal patterns and microsatellite variation in *Drosophila serrata* populations: a comparison of populations near the southern border of the species range. *J. Evol. Biol.* **15**, 763–774. (doi:10.1046/j.1420-9101.2002.00439.x)

Parsons, P. A. 1982 Evolutionary ecology of Australian *Drosophila*: a species analysis. *Evol. Biol.* **14**, 297–350.

Peakall, R. & Smouse, P. E. 2006 GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295. (doi:10.1111/j.1471-8286.2005.01155.x)

Phillips, B. L., Chipperfield, J. D. & Kearney, M. R. 2008 The toad ahead: challenges of modelling the range and spread of an invasive species. *Wildl. Res.* **35**, 222–234. (doi:10.1071/WR07101)

Price, T. & Langen, T. 1992 Evolution of correlated characters. *Trends Ecol. Evol.* **7**, 307–310. (doi:10.1016/0169-5347(92)90229-5)

Raymond, M. & Marquine, M. 1994 Evolution of insecticide resistance in *Culex pipiens* populations: the Corsican paradox. *J. Evol. Biol.* **7**, 315–337. (doi:10.1046/j.1420-9101.1994.7030315.x)

Riechert, S. E. 1993 Investigation of potential gene flow limitation of behavioural adaptation in an aridlands spider. *Behav. Ecol. Sociol.* **32**, 355–363. (doi:10.1007/BF00183792)

Rousset, F. 2008 GENEPOP' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resour.* **8**, 103–106. (doi:10.1111/j.1471-8286.2007.01931.x)

Sagarin, R. D. & Gaines, S. D. 2002 Geographical abundance distributions of coastal invertebrates: using one-dimensional ranges to test biogeographic hypotheses. *J. Biogeogr.* **29**, 985–997. (doi:10.1046/j.1365-2699.2002.00705.x)

Schiffer, M. & McEvey, S. F. 2006 *Drosophila bunnanda*—a new species from northern Australia with notes on other Australian members of the montium subgroup (Diptera: Drosophilidae). *Zootaxa* **1333**, 1–23.

Schiffer, M., Carew, M. E. & Hoffmann, A. A. 2004 Molecular, morphological and behavioural data reveal the presence of a cryptic species in the widely studied *Drosophila serrata* species complex. *J. Evol. Biol.* **17**, 430–442. (doi:10.1046/j.1420-9101.2003.00657.x)

Schiffer, M., Kennington, W. J., Hoffmann, A. A. & Blacket, M. J. 2007 Lack of genetic structure among ecologically adapted populations of an Australian rainforest *Drosophila* species as indicated by microsatellite markers and mitochondrial DNA sequences. *Mol. Ecol.* **16**, 1687–1700. (doi:10.1111/j.1365-294X.2006.03200.x)

Sgrò, C. M. & Blows, M. W. 2003 Evolution of additive and nonadditive genetic variance in development time along a cline in *Drosophila serrata*. *Evolution* **57**, 1846–1851. (doi:10.1554/03-050)

Stocker, A. J., Foley, B. & Hoffmann, A. 2004 Inversion frequencies in *Drosophila serrata* along an eastern Australian transect. *Genome* **47**, 1144–1153. (doi:10.1139/g04-078)

Sutherst, R. W., Maywald, G. F. & Bourne, A. S. 2007 Including species interactions in risk assessments for global change. *Global Change Biol.* **13**, 1843–1859. (doi:10.1111/j.1365-2486.2007.01396.x)

Van Heerwaarden, B., Willi, Y., Kristensen, T. N. & Hoffmann, A. 2008 Population bottlenecks increase additive genetic variance but do not break a selection limit in rainforest *Drosophila*. *Genetics* **179**, 2135–2146. (doi:10.1534/genetics.107.082768)

van Tienderen, P. H. & de Jong, D. 1994 A general model of the relation between phenotypic selection and genetic response. *J. Evol. Biol.* 7, 1–12. (doi:10.1046/j.1420-9101.1994.7010001.x)

Vasemagi, A. 2006 The adaptive hypothesis of clinal variation revisited: single-locus clines as a result of spatially restricted gene flow. *Genetics* **173**, 2411–2414. (doi:10.1534/genetics.106.059881)

Wang, Y. Q., Zhang, Z. B. & Xu, L. X. 2002 The genetic diversity of central and peripheral populations of ratlike hamster (*Cricetulus triton*). *Chin. Sci. Bull.* **47**, 201–206. (doi:10.1360/02tb9048)

York, K. L., Blacket, M. J. & Appleton, B. R. 2008 The Bassian Isthmus and the major ocean currents of south-east Australia influence the phylogeography and population structure of a southern Australian intertidal barnacle, *Catomerus polymerus* (Darwin). *Mol. Ecol.* **17**, 1948–1961. (doi:10.1111/j.1365-294X.2008.03735.x)