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Genetic diversity in butterflies: interactive effects of habitat fragmentation and climate-driven range expansion

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Some species are expanding their ranges polewards during current climate warming. However, anthropogenic fragmentation of suitable habitat is affecting expansion rates and here we investigate interactions between range expansion, habitat fragmentation and genetic diversity. We examined three closely related Satyrinae butterflies, which differ in their habitat associations, from six sites along a transect in England from distribution core to expanding range margin. There was a significant decline in allozyme variation towards an expanding range margin in *Pararge aegeria*, which has the most restricted habitat availability, but not in *Pyronia tithonus* whose habitat is more widely available, or in a non-expanding 'control species' (*Maniola jurtina*). Moreover, data from another transect in Scotland indicated that declines in genetic diversity in *P. aegeria* were evident only on the transect in England, which had greater habitat fragmentation. Our results indicate that fragmentation of breeding habitats leads to more severe founder events during colonization, resulting in reduced diversity in marginal populations in more specialist species. The continued widespread loss of suitable habitats in the future may increase the likelihood of loss of genetic diversity in expanding species, which may affect whether or not species can adapt to future environmental change.

Keywords: climate change; habitat fragmentation; butterfly; allozyme

1. INTRODUCTION

Species ranges are not static and many species have undergone marked changes in their distributions during recent climate warming (Warren *et al.* 2001). Quaternary studies show that shifting distributions in response to global climate changes is not a new phenomenon for insects (Coope 1995). What is new, however, is the large-scale anthropogenic loss of natural habitats. In order for insects to track climate, they must be able to expand their ranges across modern, patchy landscapes (Stone & Sunnucks 1993).

During range expansion, reduced genetic diversity often occurs in populations at range margins (e.g. during post-glacial expansion, Schmitt & Seitz 2002). These genetic changes arise as a consequence of repeated founder events, population bottlenecks and/or genetic drift in small populations. Reduced genetic diversity has also been reported in populations occupying fragmented habitats (e.g. Berwaerts *et al.* 1998), and such effects may be more severe in the future as habitats continue to be lost and remaining populations become increasingly isolated. However, the impacts of current climate-driven range expansion on genetic diversity have not been considered. Moreover any potential interactive effects of range expansion through fragmented habitats on genetic diversity have not been examined.

This study investigates patterns of genetic variation in two species of Satyrinae butterfly (*Pararge aegeria* and *Pyronia tithonus*) which over the past 60 years have been expanding their British distributions (see electronic supplementary material). These two species have broadly similar dispersal ability but differ in their habitat associations; *P. aegeria* is primarily restricted to woodland whereas *P. tithonus* occurs in a wider variety of habitats (Hill *et al.* 2001*b*). We compare patterns of genetic diversity in these expanding species with that of a closely related 'control' species (*Maniola jurtina*) which has similar ecology but is not currently expanding its range (Asher *et al.* 2001). We test the hypotheses that genetic diversity declines towards the range margin in expanding species and that declines are more marked in the habitat specialist.

2. MATERIAL AND METHODS

(a) *Insect material*

During summer 2001 and 2002, adult male butterflies from the three study species were sampled along a transect running from distribution core to range margin in England (table 1). *P. aegeria* was additionally sampled along a transect in Scotland.

(b) *Allozyme analysis*

Half the abdomen was homogenized in 60 µl 50 mM Tris–HCl pH 8.0 and centrifuged at 10 000*g* for 15 min at 4 °C. The supernatant was then run on cellulose acetate plates (Helena, Beaumont, TX) at room temperature at 200 V for 15–40 min. Individual allozyme protocols were taken from Searle (1985) and Hebert & Beaton (1993) with minor modifications. The enzymes isocitrate dehydrogenase (ID, EC 1.1.1.41), aconitase (ACO, EC 4.2.1.3), adenylate kinase (AK, EC 2.7.4.3) and glucose-6-phosphate dehydrogenase (GPD, EC 1.1.1.49) were screened using 40 mM Tris–10 mM citrate pH 7.6 as a buffer, glucose phosphate isomerase (GPI, EC 5.3.1.9) and phosphoglucomutase (PGM, EC 2.7.5.1) were screened using 25 mM Tris–0.19 mM glycine pH 8.5, and glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1) was screened using 35 mM sodium phosphate pH 6.3 as a buffer. Three enzymes had more than one locus (ID, ACO, GOT) and so we screened 10 loci in total. Allozyme scores were analysed using software PopGene v.1.32 and TFPGA v.1.3.

3. RESULTS

(a) *Comparison among species*

In total, we analysed material from 272 individuals of *P. aegeria* (expanding specialist) from nine sites, and 106 individuals of *P. tithonus* (expanding generalist) and 99 individuals of *M. jurtina* (control species) from six sites (see electronic supplementary material for summary data). In *M. jurtina*, all 10 loci were polymorphic and were characterized by a total of 34

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Table 1. Location of collecting sites in England and Scotland. Species code MJ, *M. jurtina* (control); PA, *P. aegeria* (expanding specialist); PT, *P. tithonus* (expanding generalist). Location of sample sites is based on the UK Ordnance Survey grid, numbers in brackets give distances (in km) east and north, respectively, from a location beyond the southwest limit of Britain.

site number	species collected	location	ordnance survey 10-km grid reference	distance from core site (km)
		England		
1	MJ, PA, PT	Cheltenham	SP02 (400/220)	—
2	MJ, PA, PT	Nottingham	SK63 (460/330)	106
3	MJ, PA, PT	Doncaster	SE50 (450/400)	177
4	PA	Wentbridge	SE41 (440/410)	191
5	MJ, PA, PT	Selby	SE53 (450/430)	208
6	MJ, PA, PT	Wetherby	SE44 (440/440)	217
7	PT	Knaresborough	SE35 (430/450)	230
8	MJ	Barton	NZ20 (420/500)	283
		Scotland		
9	PA	Inverness	NH64 (260/840)	—
10	PA	Forres	NH96 (290/860)	31
11	PA	Buckie	NJ46 (340/860)	85

alleles. Both *P. tithonus* and *P. aegeria* were monomorphic at two loci and the remaining eight polymorphic loci were characterized by a total of 22 and 26 alleles, respectively. Results suggest that overall there was random mating within populations of each species (only seven out of 110 possible site/locus combinations were not in Hardy–Weinberg equilibrium and these occurred in all species and several site/locus combinations). Populations of the control species *M. jurtina* had higher allelic diversity (mean number of alleles per locus, Kruskal–Wallis, $\chi^2_2 = 15.37$, $p < 0.001$; effective number of alleles, $\chi^2_2 = 15.15$, $p = 0.001$), and higher expected heterozygosity ($\chi^2_2 = 15.40$, $p < 0.001$) than either of the expanding species. There was a decline in genetic diversity across species such that *M. jurtina* > *P. tithonus* > *P. aegeria*.

Measures of genetic diversity are known to be sensitive to sample size. In this study there were significant but *negative* relationships between sample size and our measures of genetic diversity (regression analyses, $p < 0.01$) because *P. aegeria* was sampled most but had much lower genetic diversity overall (mean expected heterozygosity per locus over all individuals: *P. aegeria* = 0.08; *P. tithonus* = 0.18; *M. jurtina* = 0.21). Within species, there was no relationship between sample size and any of our measures of genetic diversity ($p > 0.2$ in all cases) for any species.

(b) *Changes in genetic diversity during range expansion*

In England, there was a significant decline in the mean number of alleles per locus from distribution core to range margin in *P. aegeria* (expanding specialist; linear regression, $F_{1,4} = 19.25$, $p = 0.012$, $R^2 = 0.83$; figure 1) but not in either of the other two species. Further evidence for greater genetic differentiation among sites in *P. aegeria* comes from the observation that allele frequencies differed among sites at six loci in *P. aegeria* but at only one locus in *P. tithonus* and *M. jurtina* (Fisher's R×C contingency test). However, there was no relationship between either the expected heterozygosity within populations or effective number of alleles per locus and distance

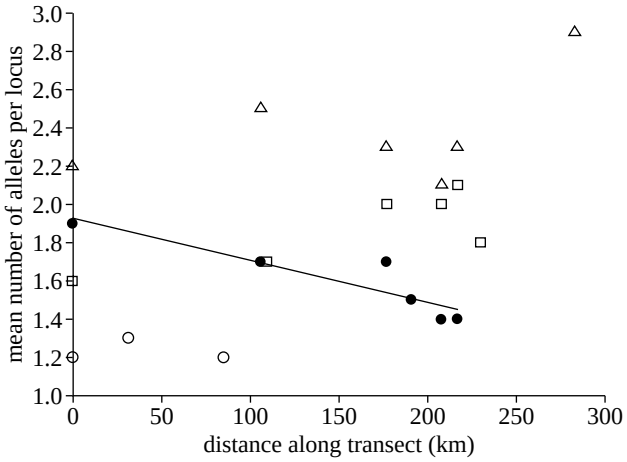

Figure 1. Relationship between mean number of alleles per locus and distance of populations along a transect from distribution core to range margin. *Pararge aegeria* (expanding specialist), circles (solid symbol, English sites; hollow symbols, Scottish sites), *Pyronia tithonus* (expanding generalist), squares; *Maniola jurtina* (control), triangles. Regression line shows the significant decline in genetic diversity in *Pararge aegeria* in England. No other relationship was significant.

from the distribution core for any of the species (linear regression; $p > 0.4$ in all cases), or any relationship with Nei's genetic distance between sites and site isolation (Mantel test; $p > 0.05$ for all three species). Wright's *F*-statistics showed little evidence of population sub-structuring in either *M. jurtina* ($F_{ST} = 0.006$, 95% CI = 0.003 to 0.019) or *P. tithonus* ($F_{ST} = 0.007$, 95% CI = −0.007 to 0.027) but the greatest population sub-structuring in *P. aegeria* ($F_{ST} = 0.102$, 95% CI = 0.043 to 0.166).

(c) *Comparison between transects in England and Scotland*

For the habitat specialist *P. aegeria* we compared patterns of genetic diversity along two transects in England and Scotland. Overall, English populations had higher genetic diversity (Mann–Whitney *U* tests; $p < 0.02$ for all measures of genetic diversity). By

contrast with England, there was no significant decrease in number of alleles per locus towards the range margin in populations in Scotland (linear regression, $p=0.7$). Populations in Scotland also had different allele frequencies compared with English populations and lower F_{ST} values indicating less sub-structuring in Scottish populations (Scotland, $F_{ST}=0.011$, 95% CI=0.001 to 0.016; England, $F_{ST}=0.102$, 95% CI=0.043 to 0.166).

4. DISCUSSION

This study found that the two expanding species *P. aegeria* and *P. tithonus* were less genetically diverse than *M. jurtina*. The British distributions of both expanding species contracted during the nineteenth century before subsequently re-expanding from the 1940s onwards (Asher *et al.* 2001) and so decreased genetic diversity in these species is likely to be due to population bottlenecks and repeated founder events occurring during recent range changes. The finding that *P. tithonus* (expanding generalist) was more genetically diverse than *P. aegeria* (expanding specialist) may reflect the differing impact of the landscape on the two species. All three study species colonized Britain after the last glacial maximum; although we have no information on historical allozyme diversities, current differences in genetic diversity among species are likely to be due to recent rather than historical range changes.

A decline in genetic diversity towards the range margin was detected in *P. aegeria*, the species with most restricted habitat requirements. By contrast, there was little evidence of genetic sub-structuring among populations in the other two species or among *P. aegeria* populations in Scotland where habitat availability is greater than in England (Hill *et al.* 2001*a*,*b*). However, there was little opportunity to detect declines in Scotland given that populations were virtually monomorphic. Thus, declines in genetic diversity during expansion were detected only in the more specialist species. Reduction in genetic diversity was evident only in terms of number of alleles per locus which is well known to be a sensitive measure of population bottlenecks (Maruyama & Fuerst 1985). Previous studies have shown that evolutionary increases in dispersal ability are evident at range margins in *P. aegeria* (Hill *et al.* 1999), but results from this study indicate that despite this there is still restricted gene flow among populations.

Allozyme frequencies have been shown to respond to selection and may reflect changes in factors likely to affect species' ability to track climate changes (Goulson 1993; Watt *et al.* 2003). Thus, there is a possibility that changes in allozyme diversity recorded in this study may also reflect different selection pressures acting on populations during range expansion, and this deserves more analysis. Our study has shown that anthropogenic habitat loss may reduce genetic diversity during range expansion. Given the rapidity of predicted climate warming for the future (IPCC 2001), any reduction in diversity and in species' ability to adapt to novel environmental changes could affect their long term persistence (Schmitt & Hewitt 2004).

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