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## Genetic structure of fragmented November moth (Lepidoptera: Geometridae) populations in farmland

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Habitats are now becoming increasingly fragmented throughout the world due to intense cultivation. As a consequence, populations of some animals with low mobility have become isolated, thus increasing the risk of inbreeding and local extinction. In Britain, weakly flying geometric moths of the genus *Epirrita* are a good model species with which to test the genetic effects of habitat fragmentation on insect populations. Genetic variation within and between populations of two *Epirrita* species captured using a network of light traps at two spatial scales (local and national) was assessed using allozyme electrophoresis, with particular reference to the local scale (the 330-ha arable farm estate at Rothamsted, Hertfordshire, in southern Britain). Populations sampled widely in England and Wales displayed low (but statistically significant) levels of genetic differentiation for both species ( $F_{st} = 0.0051$ – $0.0114$  and  $0.0226$  for *E. dilutata* and *E. christyi*, respectively). However, analysis of large samples of *E. dilutata* from four small woods at Rothamsted revealed low ( $F_{st} = 0.0046$ ) but significant differentiation, indicating that gene flow was restricted, even at this very small scale. It was concluded that small intervening patches of farmland (often a few fields width) were enough to prevent genetic homogeneity. The close similarity between more distant *Epirrita* populations was considered to be a result of historical, rather than recurrent gene flow, as genetic equilibrium between drift and gene flow is unlikely over such scales. © 2003 The Linnean Society of London. *Biological Journal of the Linnean Society*, 2003, 78, 467–477.

**ADDITIONAL KEYWORDS:** allozymes – conservation genetics – *Epirrita dilutata* – *Epirrita christyi* – molecular markers – habitat fragmentation – population genetic structure.

### INTRODUCTION

Many insect species are declining in industrialized countries (Heath, Pollard & Thomas, 1984; Falk, 1994; van Swaay & Warren, 1999; Asher *et al.*, 2001). The pressures of modern agriculture and increased urbanization has led to a decrease in the total area of many natural and semi-natural habitats, through destruction or changes in management regimes, and what remains is often highly fragmented (e.g. Kirby & Thomas, 1994). This fragmentation may lead to isolation of the populations resident within these fragments, particularly if a species has poor powers of

dispersal. Those species that are prone to regular or intermittent local extinction and rely on recolonization to persist within a region (i.e. have a metapopulation structure) will decline if habitat fragmentation prevents dispersal between patches (Hanski, Kuussaari & Nieminen, 1994). Indeed this appears to be the pattern of decline in many well studied butterfly species in Britain (Thomas, Thomas & Warren 1992). Where gene flow is restricted, habitat fragmentation will also affect the population genetic structure of a species (Saccheri *et al.*, 1998). In the absence of gene flow (and/or stabilizing selection), natural selection will tend to favour adaptation to local conditions and populations will differentiate due to genetic drift. If populations are small (and thus the effect of genetic drift great) both factors may lead to the loss of genetic variation within populations. In the short term selection may increase fitness, but in the long term it may

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reduce the evolutionary potential of the population, leaving it susceptible to environmental change or reducing its potential for expansion. For instance, there is evidence that complete isolation of some British butterfly populations has led to a reduction in dispersal ability (Dempster, 1991): as emigrants rarely encounter or colonize suitable habitats and immigrants never enter the population, selection favours less mobile individuals.

Recent studies at Rothamsted (Hertfordshire, UK) on macrolepidoptera diversity and spatial population distribution at the 'farmland scale' have indicated that, for many species, strong population density gradients can occur over very short distances, often at the boundaries of different land-use types (Woiwod & Thomas, 1993). Not surprisingly, populations of tree feeding species such as *Epirrita* spp. have, by far, their highest densities in the fragments of woodland distributed on the farm and are very rare in open arable land (Fig. 1). *Epirrita* spp. may therefore be good representatives of a wide range of taxa occupying fragments of habitat, which formally occurred in large continuous areas.

The aim of the present study was to determine whether fragmentation of woodland within the agroecosystem has led to population genetic substructuring of *Epirrita* at the local scale, whilst at the same time, assessing population genetic structure at the national

scale. The Rothamsted Light Trap network (Woiwod & Harrington, 1994) was employed to catch insects, both at a small spatial scale on the Rothamsted estate (330 ha), and at the national scale (England and Wales). Thereafter, genetic variability of individual moths was assessed using electrophoretic markers (Loxdale & Lushai, 1998) from which estimates of population genetic structure be calculated, as has been done for many insect species (cf. Daly, 1989; Loxdale, 1994; Loxdale & Lushai, 1999, 2001; Mallet, 2001). It was hoped that collection of this type of data, by providing further insights into how insect population structure of species of low vagility is influenced by habitat fragmentation, will prove useful in the conservation of species that are currently widely distributed, as well as those that are endangered.

## MATERIAL AND METHODS

### STUDY INSECTS

The November moth *Epirrita dilutata* Denis & Schiffmüller and its congener the pale November moth *E. christyi* Allen (Lepidoptera: Geometridae) are two closely related moths that are single brooded and widely distributed in the British Isles (Skinner, 1984), although *E. christyi* occurs more locally. The larvae of both species are polyphagous, feeding on a wide range



**Figure 1.** Density distribution of *Epirrita dilutata* in 1991 on the Rothamsted estate ( $\text{Log}_{10}$  number of individuals +1) based on 26 light traps (indicated by white dots). The positions of the four woodland sites, where population densities were greatest, are outlined in white (WHS = White Horse Spinney). Black lines indicate arable and grassland field boundaries. The area shown measures approximately  $1.4 \times 1.9$  km.

of trees and shrubs (e.g. *Betula*, *Quercus*, *Fagus*, *Craetagus*, *Ulmus* and *Corylus*). The overwintering eggs hatch in April and the larvae feed until June when they pupate in the soil (Harrison, 1920). The weakly flying adults emerge in autumn, from late September to mid November and are readily captured using standard Rothamsted light traps with 200 W tungsten bulbs (Williams, 1948), representing a significant proportion of the catch at these particular flight times.

#### INSECT COLLECTIONS

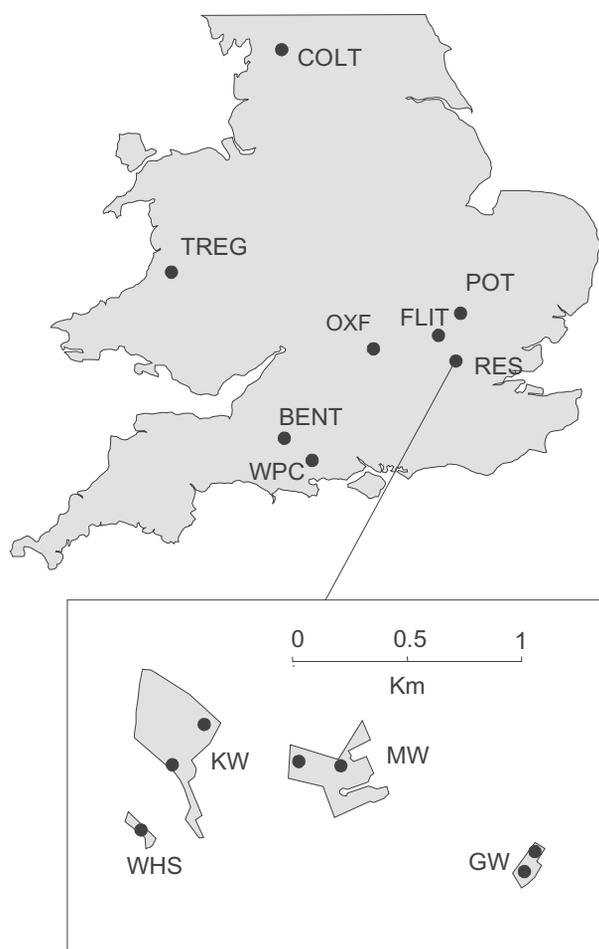
Samples of *E. dilutata* were obtained between 1990 and 1992 from four woodland sites at Rothamsted Experimental Station (RES) using standard Rothamsted light traps (Williams, 1948). These sites were: White Horse Spinney (WHS); Knott Wood (KW); Manor Wood (MW); and Geescroft Wilderness (GW) (Fig. 1). At three sites (KW, MW and GW), two light traps were operated, thus providing replicate samples for comparison. Light traps were operated daily throughout the flight period. Samples of *E. christyi* were available from only two RES sites (KW and MW). A further 19 traps distributed on the estate were also in operation, but sample sizes for both species were too low to provide meaningful data for genetic analysis.

To investigate large scale geographical variation, samples of *E. dilutata* were also obtained from Flitwick (Bedfordshire), Colt Park (North Yorkshire) and Tregaron (Dyfed) in 1991, and from Potton Wood (Bedfordshire) in 1992 and Oxford (Oxfordshire) in 1993. Samples of *E. christyi* were obtained from Tregaron in 1991, Potton Wood in 1992 and 1993, White Parish Common and Bently Wood (both in Wiltshire) in 1993. Samples from Colt Park and Tregaron were collected using Rothamsted light traps of the Rothamsted Insect Survey national light-trap network (Woiwod & Harrington, 1994). Samples from other sites were collected using either mercury vapour light alone or in combination with collection by net. The *Epirrita* collection sites are shown in Figure 2. Moths required for electrophoretic work were stored at  $-80^{\circ}\text{C}$ .

#### ELECTROPHORESIS

The wings and genitalia of the moths were removed and kept for later identification. Moths were then homogenized and stored in liquid nitrogen as multiple 7- $\mu\text{L}$  aliquots following the methods described by Wynne & Brookes (1992). Electrophoresis was undertaken using the Helena cellulose acetate system (see Wynne, Loxdale HD & Brookes, 1992).

A total of 17 enzymes (representing approximately 21 putative loci) were screened (at least 40 individuals per locus per species) for polymorphic and discrimina-



**Figure 2.** Map of England and Wales showing sampling sites for *Epirrita* spp. Colt Park (COLT); Tregaron (TREG); Flitwick Wood (FLIT); Potton Wood (POT); Rothamsted Experimental Station (RES); Oxford (OXF); White Parish Common (WPC); Bently Wood (BENT). RES sites were: White Horse Spinney (WHS); Knott Wood (KW); Manor Wood (MW); and Geescroft Wilderness (GW).

tory enzymes suitable for population and taxonomic use, respectively. These were: adenylate kinase (AK; EC 2.7.4.3), fructose-1,6-diphosphate (FDP; EC 3.1.3.11), glutamate dehydrogenase (GDH; EC 1.4.1.3), glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), glycerol-3-phosphate dehydrogenase ( $\alpha$ GPD; EC 1.1.1.8), hexokinase (HK; EC 2.7.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), lactate dehydrogenase (LDH; EC 1.1.1.27), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), mannose-phosphate isomerase (MPI; EC 5.3.1.8), peptidases (PEP-A and PEP-D, using substrates leucyl-glycine and phenyl-alanine, respectively) (PEP; EC 3.4.11), phosphoglucose isomerase (PGI; EC 5.3.1.9), 6-phosphogluconate

dehydrogenase (6PGD; EC 1.1.1.44) and phosphoglucose mutase (PGM; EC 2.7.5.1). The running buffers used were 50 mM Tris-citrate, pH 7.8 (for 6PGD), 100 mM Tris-citrate, pH 8.2 (for AK, FDP, GDH, G6PDH,  $\alpha$ GPD, HK, IDH, LDH, MDH, ME and MPI) and 25 mM Tris-glycine, pH 8.5 (for GOT, PEP-A, PEP-D, PGI and PGM). For most enzymes, the duration of the run was 20 min, the exceptions being G6PDH (30 min) and 6PGD (40 min). Staining recipes were used directly or modified from Richardson, Baverstock & Adams (1986).

Of the enzymes screened, only GOT (*Got-f*), G6PD, PGI and PGM displayed polymorphism of a clear Mendelian basis. The remainder were either monomorphic ( $\alpha$ GPD, HK, MDH, PEP-A and PEP-D), displayed polymorphism that proved difficult to interpret (IDH, ME, MPI and 6PGD), were poorly resolved or stained weakly (AK, FDP, GDH, *Got-s* and LDH). After the initial screening, all samples were tested for the variable enzymes (GOT, PGI, PGM and G6PD) and a limited number for the monomorphic enzymes ( $\alpha$ GPD, HK, PEP-A, PEP-D and MDH).

The computer program BIOSYS-1 (Swofford & Selander, 1981) was used to calculate allele and genotype frequencies and measure genetic distance (Nei, 1978). Allele frequency variance measures (Wright, 1951; Weir & Cockerham, 1984), and deviations from Hardy-Weinberg (H-W) equilibrium were analysed using the program GENEPOP (version 3.2a; Raymond & Rousset, 1995).

## RESULTS

### BAND INTERPRETATION

Alleles for each polymorphic locus were designated alphabetically in order of increasing mobility from the cathode. An additional five alleles were noted, which had migration positions too close to other alleles to be scored consistently (two alleles close to *Pgi<sub>a</sub>*, one close to *Pgi<sub>b</sub>*, and two close to *Pgm<sub>c</sub>*) and subsequently were binned with the nearest named allele.

### GENETIC VARIATION IN *EPIRRITA* SPP.

In *E. dilutata*, 25% (3/12) and 33% (4/12) of the scorable loci were polymorphic at the 95% and 99% levels, respectively. Corresponding values for *E. christyi* were 25% (3/12) and 25% (3/12). Estimates of mean heterozygosity per locus were 0.134 for *E. dilutata* and 0.063 for *E. christyi*. The only locus which could be used to distinguish the two species with any degree of certainty was *G6pd*. In *E. christyi*, *G6pd* was fixed for allele *G6pd<sub>a</sub>*, whereas *E. dilutata* was almost fixed for allele *G6pd<sub>b</sub>* (with *G6pd<sub>a</sub>* and *G6pd<sub>c</sub>* occurring at low frequency). The absence of *G6pd<sub>b</sub>* and *G6pd<sub>c</sub>* in *E. christyi* and the low frequency of *G6pd<sub>a</sub>* in

*E. dilutata* meant that the chance of misidentification was about 0.6% using this enzyme alone. No evidence for the existence of linkage disequilibrium was found between any two of the three polymorphic loci ( $\leq 95\%$  criterion), indicating the independence of these loci. The genetic distance between the two species, based on four polymorphic and eight monomorphic loci, ranged from 0.144 to 0.171 with a mean ( $\pm$ SD) of 0.156 ( $\pm 6.3 \times 10^{-3}$ ).

### VARIATION IN *E. DILUTATA*

Data collected separately for the sexes (females comprised <10% of any sample) were combined as no heterogeneity was detected between them. For those sites with two sample sets (Knott Wood, Manor Wood and Geescroft Wilderness) analysis revealed no heterogeneity and the data were combined. Allele frequency data calculated for the samples from four woodland sites at Rothamsted and the five other British populations are presented in Table 1. In 68 (population  $\times$  locus) tests, six showed significant deviation from H-W expectations ( $P < 0.05$ ). However, over all loci, only one population (Knott Wood 1992) deviated significantly ( $P = 0.039$ ), indicating little deviation from random mating within populations.

### VARIATION AMONG *E. DILUTATA* POPULATIONS AT ROTHAMSTED

Amongst the four sites at Rothamsted, allele frequencies at the three polymorphic loci (95% criterion) were found to be broadly similar. Estimates of the level of genetic subdivision are consequently low ( $F_{st} < 0.008$ ). However significant heterogeneity was found among the populations in each of the three years studied (Table 2). Pairwise comparisons between populations revealed that most of the heterogeneity was due to differences between Geescroft Wilderness and the other three sites (Table 3). No temporal heterogeneity in allele frequency was found in the four populations at Rothamsted (Table 4), either for individual or over all loci (Fig. 3).

### LARGE SCALE GEOGRAPHICAL VARIATION IN *E. DILUTATA*

The allele frequencies for *Got-f*, *Pgi* and *Pgm* from sites sampled across Britain were similar to those sampled locally at Rothamsted. However tests revealed significant heterogeneity in allele frequencies at all three loci ( $P < 0.05$ ) and overall ( $P \ll 0.0001$ ). Estimates of  $F_{st}$  among the six sites (Flitwick Wood, Potton Wood, Colt Park, Tregaron, Oxford and Rothamsted) ranged from 0.0051 to 0.0114 depending on which population was used to represent

**Table 1.** Allele frequencies at the polymorphic enzyme loci of *Epirrita dilutata* populations at four woodland sites at Rothamsted for the years 1990–92 and five other British sites: Flitwick Wood (FLIT), Beds.; Pottton Wood (POT), Beds.; Colt Park (COLT), North Yorks.; Tregaron (TREG), Dyfed; and Oxford (OXF), Oxon. Also provided are observed (Obs) and expected (Exp) heterozygosity (Het) frequencies (\* indicates a significant deviation from Hardy–Weinberg expectations:  $P < 0.05$ )

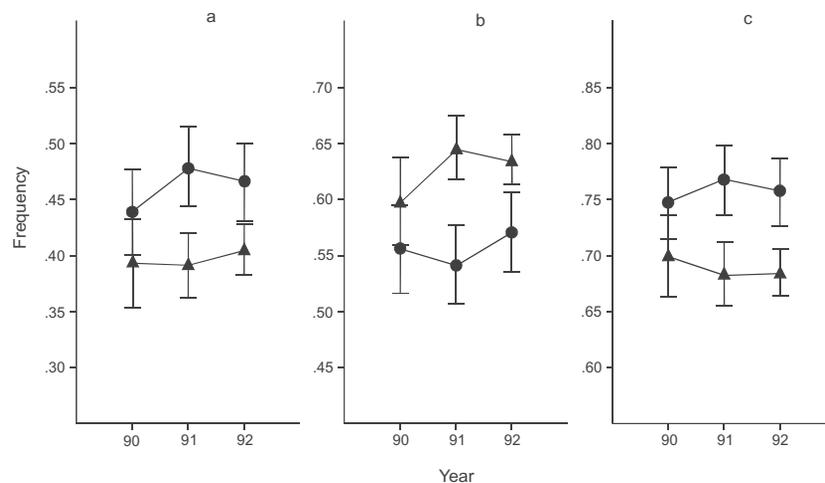
| Locus        | White Horse Spinney |        |        | Knott Wood |        |        | Manor Wood |       |       | Geescroft Wilderness |       |       | COLT  | TREG  | OXF   |        |       |
|--------------|---------------------|--------|--------|------------|--------|--------|------------|-------|-------|----------------------|-------|-------|-------|-------|-------|--------|-------|
|              | 1990                | 1991   | 1992   | 1990       | 1991   | 1992   | 1990       | 1991  | 1992  | 1990                 | 1991  | 1992  |       |       |       | FLIT   | POT   |
| <i>Got-f</i> |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| (N)          | 118                 | 117    | 96     | 298        | 567    | 936    | 223        | 179   | 108   | 334                  | 372   | 397   | 128   | 59    | 75    | 111    | 19    |
| a            | 0.432               | 0.393  | 0.365  | 0.393      | 0.391  | 0.407  | 0.410      | 0.385 | 0.477 | 0.439                | 0.478 | 0.463 | 0.320 | 0.339 | 0.467 | 0.414  | 0.289 |
| b            | 0.195               | 0.218  | 0.260  | 0.232      | 0.242  | 0.202  | 0.195      | 0.179 | 0.153 | 0.232                | 0.176 | 0.181 | 0.242 | 0.263 | 0.153 | 0.234  | 0.184 |
| c            | 0.373               | 0.389  | 0.375  | 0.376      | 0.368  | 0.390  | 0.395      | 0.436 | 0.370 | 0.328                | 0.345 | 0.355 | 0.438 | 0.398 | 0.380 | 0.351  | 0.526 |
| d            |                     |        |        |            |        |        |            |       |       | 0.001                |       |       |       |       |       |        |       |
| Het:         |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| Obs          | 0.517               | 0.632* | 0.688* | 0.708      | 0.600* | 0.658  | 0.646      | 0.542 | 0.630 | 0.632                | 0.640 | 0.632 | 0.609 | 0.593 | 0.627 | 0.514* | 0.474 |
| Exp          | 0.636               | 0.647  | 0.659  | 0.651      | 0.654  | 0.641  | 0.638      | 0.630 | 0.612 | 0.646                | 0.621 | 0.626 | 0.647 | 0.657 | 0.614 | 0.650  | 0.605 |
| <i>Pgi</i>   |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| (N)          | 123                 | 119    | 111    | 312        | 587    | 941    | 223        | 180   | 110   | 337                  | 396   | 397   | 128   | 59    | 79    | 113    | 19    |
| a            | 0.008               | 0.021  | 0.005  | 0.003      | 0.007  | 0.001  | 0.001      | 0.001 | 0.001 | 0.001                | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001  | 0.001 |
| b            | 0.093               | 0.109  | 0.077  | 0.125      | 0.106  | 0.130  | 0.143      | 0.114 | 0.114 | 0.139                | 0.146 | 0.147 | 0.109 | 0.161 | 0.209 | 0.199  | 0.105 |
| c            | 0.211               | 0.197  | 0.234  | 0.244      | 0.218  | 0.198  | 0.204      | 0.222 | 0.255 | 0.260                | 0.273 | 0.249 | 0.219 | 0.212 | 0.152 | 0.243  | 0.053 |
| d            | 0.663               | 0.643  | 0.658  | 0.598      | 0.647  | 0.636  | 0.621      | 0.631 | 0.595 | 0.555                | 0.543 | 0.568 | 0.633 | 0.593 | 0.563 | 0.535  | 0.789 |
| e            | 0.033               | 0.021  | 0.027  | 0.030      | 0.028  | 0.035  | 0.031      | 0.033 | 0.036 | 0.046                | 0.038 | 0.035 | 0.039 | 0.034 | 0.076 | 0.022  | 0.053 |
| f            |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| Het:         |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| Obs          | 0.488               | 0.504  | 0.477  | 0.554      | 0.514  | 0.520* | 0.516      | 0.517 | 0.566 | 0.582                | 0.611 | 0.597 | 0.578 | 0.525 | 0.582 | 0.611  | 0.421 |
| Exp          | 0.506               | 0.535  | 0.506  | 0.567      | 0.521  | 0.537  | 0.551      | 0.539 | 0.573 | 0.603                | 0.608 | 0.592 | 0.538 | 0.576 | 0.610 | 0.614  | 0.360 |
| <i>Pgm</i>   |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| (N)          | 119                 | 120    | 109    | 310        | 559    | 939    | 222        | 179   | 110   | 330                  | 378   | 397   | 127   | 59    | 78    | 113    | 19    |
| a            | 0.013               | 0.004  | 0.005  | 0.008      | 0.007  | 0.011  | 0.007      | 0.007 | 0.005 | 0.007                | 0.004 | 0.008 | 0.008 | 0.008 | 0.008 | 0.009  | 0.009 |
| b            | 0.660               | 0.767  | 0.720  | 0.700      | 0.683  | 0.684  | 0.709      | 0.712 | 0.718 | 0.747                | 0.769 | 0.758 | 0.689 | 0.686 | 0.744 | 0.633  | 0.789 |
| c            | 0.328               | 0.229  | 0.275  | 0.292      | 0.309  | 0.306  | 0.284      | 0.288 | 0.277 | 0.253                | 0.228 | 0.234 | 0.311 | 0.305 | 0.256 | 0.358  | 0.211 |
| Het:         |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| Obs          | 0.429               | 0.350  | 0.358  | 0.426      | 0.413  | 0.419* | 0.392      | 0.464 | 0.391 | 0.373                | 0.320 | 0.388 | 0.402 | 0.390 | 0.359 | 0.451  | 0.421 |
| Exp          | 0.457               | 0.360  | 0.406  | 0.425      | 0.437  | 0.439  | 0.416      | 0.410 | 0.407 | 0.378                | 0.358 | 0.370 | 0.429 | 0.436 | 0.381 | 0.471  | 0.332 |
| <i>G6pd</i>  |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| (N)          | 114                 | 100    | 24     | 284        | 521    | 782    | 197        | 172   | 29    | 309                  | 279   | 251   | 128   | 58    | 64    | 106    | 19    |
| a            | 0.013               | 0.020  |        | 0.018      | 0.025  | 0.036  | 0.015      | 0.032 |       | 0.032                | 0.025 | 0.022 | 0.035 | 0.026 | 0.023 | 0.033  | 0.079 |
| b            | 0.978               | 0.980  | 1.000  | 0.982      | 0.974  | 0.957  | 0.982      | 0.965 | 1.000 | 0.966                | 0.971 | 0.978 | 0.965 | 0.974 | 0.977 | 0.967  | 0.921 |
| c            | 0.009               |        |        | 0.001      | 0.001  | 0.007  | 0.003      | 0.003 |       | 0.002                | 0.004 |       |       |       |       |        |       |
| Het:         |                     |        |        |            |        |        |            |       |       |                      |       |       |       |       |       |        |       |
| Obs          | 0.044               | 0.040  | 0.000  | 0.028      | 0.052  | 0.084  | 0.036      | 0.070 | 0.000 | 0.061                | 0.057 | 0.036 | 0.070 | 0.052 | 0.047 | 0.066  | 0.158 |
| Exp          | 0.043               | 0.039  | 0.000  | 0.035      | 0.051  | 0.084  | 0.035      | 0.068 | 0.000 | 0.066                | 0.056 | 0.043 | 0.068 | 0.050 | 0.046 | 0.064  | 0.145 |

**Table 2.** Estimates of the standardized gene frequency variance ( $F_{st}$ ) and exact tests for heterogeneity ( $P$ ), among four *Epirrita dilutata* populations at Rothamsted, 1990–92

| Locus        | 1990     |        | 1991     |          | 1992     |        |
|--------------|----------|--------|----------|----------|----------|--------|
|              | $F_{st}$ | $P$    | $F_{st}$ | $P$      | $F_{st}$ | $P$    |
| <i>Got-f</i> | 0.0009   | 0.2186 | 0.0061   | 0.0005   | 0.0031   | 0.0072 |
| <i>Pgi</i>   | 0.0029   | 0.1602 | 0.0071   | <<0.0001 | 0.0045   | 0.0105 |
| <i>Pgm</i>   | 0.0024   | 0.0261 | 0.0092   | 0.0010   | 0.0065   | 0.0102 |
| All          | 0.0020   | 0.0296 | 0.0072   | <<0.0001 | 0.0045   | 0.0001 |

**Table 3.** Pairwise locus  $\times$  locus comparisons of four Rothamsted populations of *Epirrita dilutata* in years 1990–92. Only comparisons in which at least one locus displayed significant heterogeneity ( $P < 0.05$ ) are shown

|      |    |     | <i>Got-f</i> | <i>Pgi</i> | <i>Pgm</i> | All      |
|------|----|-----|--------------|------------|------------|----------|
|      |    |     |              |            |            |          |
| 1990 | GW | WHS | 0.4953       | 0.0244     | 0.0002     | 0.0003   |
|      | GW | KW  | 0.1783       | 0.2039     | 0.0125     | 0.0174   |
|      | GW | MW  | 0.0777       | 0.0695     | 0.0467     | 0.0110   |
| 1991 | GW | WHS | 0.0637       | <0.0001    | 1.0000     | 0.0003   |
|      | GW | KW  | <<0.0001     | <0.0001    | <0.0001    | <<0.0001 |
|      | GW | MW  | 0.0053       | 0.0444     | 0.0645     | 0.0011   |
|      | KW | MW  | 0.0233       | 0.8769     | 0.2199     | 0.0945   |
| 1992 | KW | WHS | 0.7040       | 0.0001     | 0.0354     | 0.0003   |
|      | GW | WHS | 0.0095       | 0.0078     | 0.4455     | 0.0021   |
|      | GW | KW  | 0.0350       | 0.0109     | 0.0005     | <0.0001  |
|      | MW | WHS | 0.0131       | 0.4422     | 1.0000     | 0.1124   |

**Figure 3.** Frequencies of the most common alleles of three enzyme loci (a: *Got-f*<sub>a</sub>; b: *Pgi*<sub>e</sub>; c: *Pgm*<sub>b</sub>) for *Epirrita dilutata* at two woods at Rothamsted (Knott Wood, triangle; and Geescroft Wilderness, circle) separated by 1.5 km. Error bars are 95% confidence intervals.

**Table 4.** Exact tests for heterogeneity of gene frequencies among years (1990, 1991 and 1992) in four *Epirrita dilutata* populations at Rothamsted (White Horse Spinney (WHS), Knott Wood (KW), Manor Wood (MW) and Geescroft Wilderness (GW))

| Locus        | WHS     | KW      | MW      | GW      |
|--------------|---------|---------|---------|---------|
| <i>Got-f</i> | 0.49583 | 0.11009 | 0.21501 | 0.10222 |
| <i>Pgi</i>   | 0.34085 | 0.05886 | 0.70280 | 0.83170 |
| <i>Pgm</i>   | 0.08354 | 0.80972 | 0.67287 | 0.16028 |
| All          | 0.2024  | 0.1051  | 0.5998  | 0.1979  |

Rothamsted (1991 data), indicating little more differentiation at this scale. (Values of  $F_{st}$  for the individual loci ranged from 0.0035 to 0.0119 for *Got-f*, 0.0096–0.0100 for *Pgi* and 0.0013–0.0135 for *Pgm*).

#### VARIATION IN *E. CHRISTYI*

For those sites with two sample sets (Knott Wood and Manor Wood at Rothamsted) tests indicated no heterogeneity and the data were combined. Of the 30 (population  $\times$  locus) tests for deviation from H–W expectations only one deviated significantly.

Allele frequencies for the three polymorphic loci, *Got-f*, *Pgi* and *Pgm*, were found to be broadly similar for all populations of *E. christyi* sampled (Table 5). Tests for heterogeneity in allele frequency revealed no significant differences, at any locus, between the two populations at Rothamsted (Knott Wood and Manor Wood) in 1990 and 1991. However significant heterogeneity was found among populations sampled across Britain at all three loci ( $P < 0.01$ ) and overall ( $P \ll 0.0001$ ) when the data for Knott Wood (1990) were used to represent Rothamsted and the data for 1992 used to represent Potton Wood. Among British populations of *E. christyi*  $F_{st}$  was 0.0226 (values for individual loci were 0.0149 for *Got-f*, 0.0274 for *Pgi* and 0.0378 for *Pgm*).

For the three populations sampled in more than one year temporal heterogeneity was detected at the *Pgi* and *Pgm* loci in Knott Wood and Potton Wood, respectively. However, overall the results indicate little temporal variation over the period studied.

## DISCUSSION

### SPECIES DIFFERENCES

The low genetic distance ( $0.156 \pm 0.006$ ) found between *E. dilutata* and *E. christyi* accords with the morphological similarity between the species, and is an indication of their close relationship. Compared with other Lepidoptera, this value is lower than the

genetic distance among species of *Speyeria* spp. (0.182) (Brittnacher, Sims & Ayala, 1978) and *Heliothis* spp. (0.340) (Daly & Gregg, 1985); (0.870) (Sluss *et al.*, 1978), but higher than that found between two *Xestia* species (0.104) (Hudson & Lefkovich, 1982).

The taxonomy of the genus *Epirrita* has, in the past, proved difficult due to the morphological variability found within each species. *Epirrita christyi* was originally described as a form of *E. dilutata* Prout but even after it was assigned specific status (Allen, 1906), some authorities (e.g. Harrison, 1920) argued that *E. dilutata* and *E. christyi* were not true 'Linnaean' species. In the present study, no fixed allele differences were found for the 12 enzyme loci that could be resolved. However, marked allele frequency differences between the species were consistent across different sites (Rothamsted, Potton Wood and Tregaron) where both species were present. The largest difference occurred with *G6pd*, which was fixed for allele *G6pd<sub>a</sub>* in *E. christyi* and nearly fixed for allele *G6pd<sub>b</sub>* in *E. dilutata* (with *G6pd<sub>a</sub>* and *G6pd<sub>c</sub>* occurring at low frequency). This difference together with the absence, in *E. christyi*, of several alleles (at *Got-f* and *Pgi*) found in *E. dilutata*, strongly suggest that interspecies gene flow, from *E. dilutata* to *E. christyi*, is not occurring. It is possible the reverse occasionally occurs (i.e. gene flow from *E. christyi* to *E. dilutata*) because *E. christyi* does not possess any alleles additional to those found in *E. dilutata*. However this must be rare because of the strong frequency differences at *G6pd*. Overall therefore, the electrophoretic evidence strongly supports the specific status of the two species.

The lower heterozygosity and fewer alleles per locus in *E. christyi* accord with a lower average population size compared with *E. dilutata* (indicating that the former may have gone through a population bottleneck either during or since speciation). A preliminary investigation has shown that *E. autumnata* (Borkhausen) (which has fixed allele differences at two PEP loci with respect to *E. dilutata/christyi*) shares some of the alleles found in *E. dilutata* (*Got-f<sub>a</sub>* and *Pgi<sub>c</sub>*) that are absent from *E. christyi* (I.R. Wynne, unpubl. data). If identical by descent, then these alleles would appear to have been lost by *E. christyi* during or after speciation with *E. dilutata*.

### GENETIC STRUCTURE OF *E. DILUTATA* AND *E. CHRISTYI* POPULATIONS

Allele frequencies for the variable loci ( $\leq 95\%$  criterion), *Got-f*, *Pgi* and *Pgm*, were similar in the four woodland populations of *E. dilutata* sampled on the Rothamsted farm estate. Estimates of  $F_{st}$  were low (Table 3), indicating low levels of differentiation between populations. However, although frequency differences were slight, significant heterogeneity was

**Table 5.** Allele frequencies at three polymorphic and one discriminatory enzyme loci in British populations of *Epirrita christyi*: Knott Wood (KW); Manor Wood (MW); Potton Wood (POT); Tregaron (TREG); White Parish Common (WPC); Bently Wood (BENT). Also provided are observed (Obs.) and expected (Exp.) heterozygosity (Het.) frequencies. (\* indicates a significant deviation from Hardy–Weinberg equilibrium at  $P < 0.05$ ). Allelic nomenclature is the same as for *E. dilutata*

| Locus        | KW<br>1990 | KW<br>1991 | KW<br>1992 | MW<br>1990 | MW<br>1991 | POT<br>1992 | POT<br>1993 | TREG  | WPC   | BENT   |
|--------------|------------|------------|------------|------------|------------|-------------|-------------|-------|-------|--------|
| <i>Got-f</i> |            |            |            |            |            |             |             |       |       |        |
| (N)          | 326        | 535        | 481        | 42         | 33         | 144         | 50          | 62    | 22    | 33     |
| a            |            |            |            |            |            |             |             |       |       |        |
| b            | 0.695      | 0.685      | 0.678      | 0.726      | 0.652      | 0.781       | 0.830       | 0.806 | 0.659 | 0.803  |
| c            | 0.305      | 0.315      | 0.322      | 0.274      | 0.348      | 0.219       | 0.170       | 0.194 | 0.341 | 0.197  |
| d            |            |            |            |            |            |             |             |       |       |        |
| Het:         |            |            |            |            |            |             |             |       |       |        |
| Obs          | 0.439      | 0.398      | 0.412      | 0.452      | 0.576      | 0.368       | 0.260       | 0.387 | 0.409 | 0.333  |
| Exp          | 0.424      | 0.432      | 0.437      | 0.398      | 0.454      | 0.342       | 0.282       | 0.312 | 0.449 | 0.316  |
| <i>Pgi</i>   |            |            |            |            |            |             |             |       |       |        |
| (N)          | 350        | 536        | 486        | 42         | 31         | 144         | 59          | 62    | 23    | 33     |
| a            |            |            |            |            |            |             |             |       |       |        |
| b            |            |            |            |            |            |             |             |       |       |        |
| c            |            |            |            |            |            |             |             |       |       |        |
| d            | 0.913      | 0.893      | 0.876      | 0.833      | 0.871      | 0.802       | 0.847       | 0.871 | 0.891 | 0.909  |
| e            | 0.087      | 0.107      | 0.124      | 0.155      | 0.129      | 0.198       | 0.153       | 0.129 | 0.109 | 0.091  |
| f            |            |            |            | 0.012      |            |             |             |       |       |        |
| Het:         |            |            |            |            |            |             |             |       |       |        |
| Obs          | 0.151      | 0.192      | 0.208      | 0.333      | 0.194      | 0.313       | 0.271       | 0.161 | 0.217 | 0.121  |
| Exp          | 0.159      | 0.192      | 0.218      | 0.281      | 0.225      | 0.317       | 0.259       | 0.225 | 0.194 | 0.165  |
| <i>Pgm</i>   |            |            |            |            |            |             |             |       |       |        |
| (N)          | 352        | 537        | 486        | 41         | 33         | 144         | 59          | 62    | 23    | 33     |
| a            | 0.014      | 0.012      | 0.006      | 0.024      | 0.030      | 0.007       | 0.017       | 0.016 | 0.022 | 0.045  |
| b            | 0.911      | 0.914      | 0.925      | 0.951      | 0.955      | 0.993       | 0.949       | 0.984 | 0.891 | 0.848  |
| c            | 0.075      | 0.074      | 0.069      | 0.024      | 0.015      |             | 0.034       |       | 0.087 | 0.106  |
| Het:         |            |            |            |            |            |             |             |       |       |        |
| Obs          | 0.165      | 0.151      | 0.142      | 0.098      | 0.091      | 0.014       | 0.068       | 0.032 | 0.217 | 0.182* |
| Exp          | 0.165      | 0.158      | 0.140      | 0.094      | 0.088      | 0.014       | 0.098       | 0.032 | 0.198 | 0.267  |
| <i>G6pd</i>  |            |            |            |            |            |             |             |       |       |        |
| (N)          | 341        | 491        | 413        | 42         | 33         | 140         | 56          | 60    | 23    | 31     |
| a            | 1.000      | 1.000      | 1.000      | 1.000      | 1.000      | 1.000       | 1.000       | 1.000 | 1.000 | 1.000  |
| b            |            |            |            |            |            |             |             |       |       |        |
| c            |            |            |            |            |            |             |             |       |       |        |
| Het:         |            |            |            |            |            |             |             |       |       |        |
| Obs          | 0.000      | 0.000      | 0.000      | 0.000      | 0.000      | 0.000       | 0.000       | 0.000 | 0.000 | 0.000  |
| Exp          | 0.000      | 0.000      | 0.000      | 0.000      | 0.000      | 0.000       | 0.000       | 0.000 | 0.000 | 0.000  |

found among the four sites indicating that the total population at Rothamsted is subdivided. For all three years, most of the heterogeneity observed could be attributed to the differences between Geescroft Wilderness (GW) and the other three sites. Very few significant differences occurred between White Horse Spinney (WHS), Knott Wood (KW) and Manor Wood (MW). WHS and KW are connected by a hedgerow and a well-wooded disused railway line, which may function as 'corridors' (as well as breeding sites) by which individuals may be exchanged. MW is separated from

KW by only 300 m and historically (before the 1930s) these two woods were continuous (Anon, 1946; Salter & Tinsley, 1993). However, GW is separated from MW (the nearest wood to GW) by some 700 m with no suitable corridors between them. The higher level of differentiation between the more isolated population at GW and the other three sites may therefore reflect a restriction of gene flow. Only two sites at Rothamsted provided enough individuals to examine population structure of *E. christyi* at the local level (KW and MW) and no heterogeneity was detected between them.

Although significant allele frequency heterogeneity was observed among and between geographically distant populations, the level of differentiation was low in both *E. dilutata* and *E. christyi*. Estimates of  $F_{st}$  were low in both species, although greater for *E. christyi* (0.0226) than for *E. dilutata* (0.0051–0.0114), indicating little geographical substructure.  $F$ -statistics have been used for many species to assess the geographical structure of populations and estimate the level of gene flow between them (e.g. Pashley, Johnson & Sparks, 1985; Daly, 1989). Species of Lepidoptera, believed to be highly mobile, for which  $F_{st}$  has been estimated include *Plutella xylostella* (L.) (0.028–0.038) (Caprio & Tabashnik, 1992), *Heliothis armigera* (Hübner) (0.023) (Daly & Gregg, 1985), *Heliothis virescens* (F.) (0.002) (Korman *et al.*, 1993), *Anticarsia gemmatalis* (Hübner) (0.021) and *Pieris rapae* (L.) (0.014) (Pashley *et al.*, 1985). Fewer  $F_{st}$  estimates are available for Lepidoptera species where lower levels of gene flow are inferred, but include *Euphydryas editha* (Boisduval) (0.118) (Pashley *et al.*, 1985), *Euphydryas gillettii* (Barnes) (0.325) (Debinski, 1994), *Parnassius mnemosyne* (L.) (0.135) (Napolitano & Descimon, 1994) and *Operophtera brumata* (L.) (0.171) (Van San & Sula, 1993).

Due to the sensitivity of  $F_{st}$  to the number of populations sampled, sample size and heterozygote frequencies, Weir & Cockerham (1984) point out that comparisons between species should be treated with caution. Daly (1989) suggests that while the levels of mobility ( $Nm$ ) derived from  $F_{st}$  can be crudely categorized (as low, medium and high rates of gene flow), the relative rankings within each category may not be very meaningful. Interestingly, the estimates of  $F_{st}$  for *E. dilutata* and *E. christyi* are more comparable to species which exhibit high, rather than low, rates of gene flow. The population structure is not panmictic, however, as it has already here been demonstrated that population substructuring can even occur at the local level.

One of the main problems in studies of population genetic structure is the uncertainty concerning the relative contributions of selection, drift and mutation to allelic variation. One view is that the observed polymorphisms represent the equilibrium distribution of selectively neutral or slightly deleterious alleles maintained by genetic drift and mutation (e.g. Kimura & Crow, 1964; Kimura, 1968; Nei & Graur, 1984). Another view is that in a large proportion of cases the polymorphism is under the influence of some form of balancing selection (e.g. Ayala *et al.*, 1972). Slatkin (1987) points out that while genetic drift affects all loci in the same way, natural selection does not. Thus the fact that our estimates of  $F_{st}$  for each of the polymorphic loci ( $\leq 95\%$  criterion) are similar in both species means that they at least consistent with neutrality.

Another constraint of using  $F$ -statistics as an indirect measure of gene flow is the assumption that the populations under study are in equilibrium with respect to gene flow and genetic drift. For many populations this may not be the case (Slatkin, 1987; Harrison & Taylor, 1997). Where gene flow is restricted or absent between populations occupying isolated habitat patches that were formally contiguous, allele frequencies may still remain similar, so long as the long-term effective population size is large to prevent substantial genetic drift (Woiwod & Wynne, 1994). Because equilibrium is reached most quickly when population size is small and migration rate high,  $F_{st}$  amongst local populations will approach equilibrium faster than among those that are geographically distant. In such circumstances estimates of  $F_{st}$  pertain more to historical patterns of gene flow than that occurring currently between the fragmented populations. The low values of  $F_{st}$  found among geographically distant populations of *E. dilutata* may therefore reflect the historical connectivity of deciduous woodland in the past, rather than recurrent gene flow. The pattern of variation may even reflect that established during the postglacial range expansion. As these species only have one generation a year, the number of generations since the recolonization of Northern Europe is small (around 10 000). Furthermore, populations occupying even small patches of woodland can be large ( $>300 \text{ ha}^{-1}$ ), as indicated by a mark–release–recapture experiment (Wynne, 1997) and long-term monitoring by the Rothamsted Insect Survey (Taylor, Kempton & Woiwod, 1976). The level of differentiation, due to genetic drift, is therefore likely to be low even if gene flow is absent between contemporary populations. If this is the case, equilibrium between gene flow and genetic drift will not have been reached between geographically distant populations of *E. dilutata* and *E. christyi*. Indeed, populations of many insect species in northern Europe (perhaps the majority) may not be in equilibrium, particularly where fragmentation events, due to human activity, are recent. This possibility must be taken into account when genetic markers are used to assess gene flow between populations of conservation interest. If the populations have not had sufficient time since isolation, estimates of  $Nm$  based on  $F_{st}$  will always over estimate gene flow and under estimate the degree to which such species are vulnerable to local and/or regional extinction.

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