Population structure and the impact of regional and local habitat isolation upon levels of genetic diversity of the endangered damselfly *Coenagrion mercuriale* (Odonata: Zygoptera)

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**SUMMARY**

1. *Coenagrion mercuriale* is one of Europe’s most threatened damselflies. There is concern for the long-term persistence of many of its U.K. colonies because adult lifetime movement is limited, making isolated populations susceptible to extinction.
2. Using 14 microsatellite loci we characterised levels of genetic diversity, evidence for a recent decline and the spatial genetic structure for *C. mercuriale* population in Wales, U.K.
3. Spatial isolation is not an absolute predictor of low genetic diversity at either local or regional scales.
4. One population inhabiting a remote, edge of range site is genetically impoverished with levels of variability (at microsatellite loci) among the lowest reported for any insect species.
5. Agricultural land and high ground are physical barriers to dispersal by adults.
6. Consistent with work from elsewhere, movement by mature *C. mercuriale* in Pembrokeshire is sufficient to prevent significant genetic differentiation throughout a habitat matrix of some 3–4 km if the suitable habitat sites are <2 km apart and lack barriers to movement. Even within a good habitat matrix, however, genetic isolation by distance develops within 10 km.

**Keywords:** biodiversity, bottleneck, conservation, dispersal, population structure

**Introduction**

Destruction and fragmentation of habitat are major causes of biodiversity loss [World Conservation Monitoring Centre (WCMC), 1992]. Organisms sensitive to the effects of habitat fragmentation are likely to have some combination of low natural abundance/high area requirement, large population fluctuations, low intrinsic growth rate, specialised habitat requirements and/or poor dispersal capability (Henle *et al.*, 2004). Particularly by impacting upon the capability of individuals to disperse, habitat fragmentation can lead to a reduction in population size that will heighten extinction risk by increasing vulnerability to stochastic demographic changes or reducing genetic diversity (Frankham, 1995; Frankham, Ballou & Briscoe, 2002). Maintaining genetic diversity is important as it is associated with population viability (Saccheri *et al.*, 1998; Madsen *et al.*, 1999; Spielman *et al.*, 2004a; Spielman, Brook & Frankham, 2004b) and the evolutionary potential of a species to respond to environmental change (Frankham *et al.*, 2002; Reed & Frankham, 2003; Schmitt & Hewitt, 2004). European wetlands, which are inherently discontinuous, are becoming increasingly patchy, largely because of pollution, eutrophication or changes in land use. A decline in movement among habitat patches may be expected for many freshwater species, with the expected consequences of genetic erosion and population decline.

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As successful conservation of insect taxa is largely achieved by maintaining areas of suitable habitat it is imperative that species’ dispersal capabilities are characterised so that an appropriate level of connectivity within a habitat network can be established in order to ameliorate the detrimental effects of fragmentation. Characterising dispersal can be problematic for freshwater insects as many can disperse as aquatic larvae and/or terrestrial adults. Nevertheless, a number of studies have directly tracked dispersal of aquatic insects (e.g. Miller, Blinn & Keim, 2002; Elliott, 2003; Petersen et al., 2004; Watts et al., 2004a). Such direct observations, however, are likely to overlook rare, long-distance movement events and, importantly, fail to inform on the successful transfer of gametes that affect levels of diversity within populations (Slatkin, 1985). Many studies, therefore, have used the spatial distribution of neutral genetic markers to make indirect appraisals of the dispersal characteristics of a variety of freshwater insect taxa (e.g. Geenen et al., 2000; Smith & Collier, 2001; Kelly, Rundle & Bilton, 2002; Miller et al., 2002; Hughes, Hillyer & Bunn, 2003a; Hughes et al., 2003b; Wilcock, Nichols & Hildrew, 2003; Wishart & Hughes, 2003; Watts et al., 2004a).

Odonates (damselsfies and dragonflies) are a key component of many freshwater ecosystems that are generally perceived to be good fliers, potentially capable of wide dispersal and possibly less sensitive to habitat fragmentation than other freshwater taxa. Numerous odonate species are declining, however, with nearly 40% of indigenous European odonates classified as endangered, vulnerable or rare under IUCN red book categories (Van Tol & Verdonk, 1988). The axiom of wide dispersal may hold for many anisopterans (Corbet, 1999; Freeland et al., 2003) but zygopterans are weaker fliers, with many species not dispersing beyond several kilometres (Conrad et al., 1999; Geenen et al., 2000; Purse et al., 2003; Watts et al., 2004a). Thus, it is somewhat surprising that genetic differentiation has been reported among zygopteran populations separated by up to 100 km (Andrés, Sanchez-Guillén & Cordero Rivera, 2000, 2002) but not shorter distances (Geenen et al., 2000; Wong, Smith & Forbes, 2003; but cf. Watts et al., 2004a).

This paper presents an analysis of genetic variability and population structure of an endangered damselfly, Coenagrion mercuriale (Charpentier, 1840) (Odonata: Zygoptera). Coenagrion mercuriale, restricted to the south and west of Europe, is considered extinct or on the edge of extinction in seven European countries (Grand, 1996) and is listed in Appendix II of the Berne Convention of 1979 (Grand, 1996), in Appendix II of the European Community Habitat and Species Directive of 1992 (Jackson & McLeod, 2000) and as rare in the British Red Data Book. This species is protected by the Wildife and Countryside Act (TSO, 1981) within the U.K. where, largely because of decline/fragmentation of suitable habitat, it has suffered a 30% reduction in distribution during the last 100 years (Thompson, Rouquette & Purse, 2003). Coenagrion mercuriale is now limited to a few populations in southern England and Wales, with core sites in the New Forest and along the River Itchen (both in England) and in Pembrokeshire (Wales). There is concern for the long-term persistence of C. mercuriale because adult lifetime movement rarely exceeds 2 km and is typically <100 m (Hunger & Röske, 2001; Purse et al., 2003; Watts et al., 2004a), restricting (re-) colonisation of suitable habitat to localities in close proximity to extant sites. A previous study in the Itchen Valley demonstrated that limited movement by C. mercuriale leads to significant, local spatial genetic structuring (Watts et al., 2004a). Here, we extend our investigation into the population structure of C. mercuriale to include all remaining population centres in Wales (U.K.) that contrast with sites along the Itchen Valley by exhibiting a greater level of spatial isolation. In addition to exploring the pattern of spatial genetic structure, we ask:

(i) What is the effect of habitat isolation upon genetic diversity at local and regional scales? and

(ii) Do populations of C. mercuriale show a genetic signature of demographic decline?

These questions are discussed with respect to the general conservation of this species.

**Methods**

**Description of study sites**

We studied the remaining regions in Wales (Pembrokeshire, Gower and Anglesey) that host populations of C. mercuriale. This species does not inhabit areas above 300 m in the U.K. (Purse, 2001) but the Pembrokeshire populations (situated >150 m above sea level) are at higher altitudes than other sites in Wales (60–70 m; S. Coker, personal communication).
In Pembrokeshire, populations distributed in springs on the Preseli Hills (Fig. 1) are undergoing a reduction in size and distribution but still thought to be large and extensive enough to be self-sustaining. Within this area we collected samples from nine sites (Fig. 1) that represent four of the five population centres that have some separation by improved agricultural land; northern [Brynerian (BRY), Clun Maen (CLM)], eastern (not sampled), southern [Cors Tewygl (COT), Dolau Isaf (DOI), Gors Fawr (GOF), Pantithel (PAN), Waun Isaf North (WAN)] and two colonies at Waun Maes (WAM) and Waun Fawr (WAF). The latter two sites are separated from the core areas by relatively large distances (approximately 3 and 6 km, respectively) and high (>300 m) ground. At 290 m in altitude, WAM is 80–90 m higher than the other Pembrokeshire sites and at the environmental limit of this species (Purse, 2001). Although presently vigorous, the WAF population is believed to be susceptible to even subtle environmental changes because it is too remote to receive migrants (Coker, 2001). All colonies (except DOI and PAN which are on private land) in Pembrokeshire are on common grazing land. Coenagrión mercuriale was presumably more widespread on Gower but is now found at only two sites (Cefn Bryn and Rhossili) that are separated by 7.5 km of farmland. At the extreme north of C. mercuriale’s U.K. (and European) distribution and 150 km from the nearest populations in Pembrokeshire, Nant Isaf on the island of Anglesey represents an extremely remote site (Fig. 1). There is concern for the small C. mercuriale population at Anglesey because changes in the grazing regime on this privately owned farmland might have reduced habitat quality.

**DNA extraction and polymerase chain reaction**

Genomic DNA was extracted using a high salt protocol (Sunnucks & Hales, 1996) from a tibia (stored in a 1.5 mL tube containing 100% ethanol) for about 50 damselflies per site (Appendix). Loss of a leg does not measurably affect fitness in damselflies (Fincke & Hadrys, 2001) and we observed no significant effect of sampling upon recapture rate elsewhere (D.J. Thompson, unpublished data). We examined allelic variation in 14 microsatellite loci (Appendix) characterised by Watts, Thompson & Kemp (2004b) and Watts et al. (2004c). Approximately 5 ng of DNA was used for a 10 μL polymerase chain reaction (PCR) containing 75 mM Tris-HCl pH 8.9, 20 mM (NH₄)₂SO₄, 0.01% v/v Tween-20, 0.2 mM each dNTP, 3.0 mM MgCl₂, 20 pmol forward primer, 30 pmol reverse primer and 0.25 U Taq polymerase (ABgene, Epsom, U.K.). Thermal cycling conditions are described by Watts et al. (2004b,c). PCR products were pooled into one of two genotyping pools, determined by allelic size range and the 5’ fluorescent dye, along with a GeneScan-500 LIZ size standard (Applied Biosystems, Inc.)
Foster City, CA, U.S.A.) and separated by capillary electrophoresis through a denaturing acrylamide gel on an ABI3100 automated sequencer (Applied Biosystems). Alleles were sized using the cubic model of analysis in the GeneMapper analysis software (Applied Biosystems).

Data analysis

Genetic variability. Genotypic linkage equilibrium among all locus-pair combinations was assessed for each population using the Fisher’s exact test implemented by the online version (3.1c, http://wbiomed.curtin.edu.au/genepop/) of GenePop (Raymond & Rousset, 1995). Genetic diversity within each sample was measured by the allelic richness ($A_R$), expected heterozygosity ($H_e$) and Wright’s (1951) inbreeding coefficient ($f$) using FSTAT v.2.9.3 (Goudet, 1995); $A_R$ was standardised at 31 individuals to account for missing genotypes at one locus-sample combination (LIST4-002, CLM). We used FSTAT to test the significance of any differences in $A_R$, $H_e$ and $f$ among sites at Pembrokeshire, Gower and Anglesey (the latter site was randomly divided into two) by a permutation procedure (5000 permutations of samples among groups). The significance of any deviation from expected Hardy–Weinberg equilibrium (HWE) conditions was estimated by making 5000 permutations of alleles among individuals within samples using FSTAT.

Population bottleneck. We examined each sample for evidence of a population decline (Thompson et al., 2003) using two approaches. First, we used BOTTLENECK v.1.2.02 software (Piry, Luikart & Cornuet, 1999) to estimate (assuming populations are in mutation-drift equilibrium) the expected distribution of heterozygosity from the observed number of alleles under the infinite allele (IAM) and stepwise mutation (SMM) models of mutation. A Wilcoxon signed-rank test was used to test whether there was a significant heterozygote excess (compared with that expected given the sample’s allelic diversity) that is characteristic of a population bottleneck (Cornuet & Luikart, 1996; Luikart & Cornuet, 1998). Second, for each sample we calculated the average (over all loci) ratio ($M$) of allelic size range (number of microsatellite repeats) against the total number of alleles; values of $M$ above 0.82 indicate a stable population (Garza & Williamson, 2001).

Population structure. We tested for heterogeneity of genotype frequencies among all pairs of populations using the exact test employed by FSTAT v.2.9.3 (Goudet, 1995); HWE within samples was not assumed and genotypes were permuted 5000 times among samples. Genetic differentiation between all pairs of samples was also determined by calculating Weir & Cockerham’s (1984) estimator of Wright’s (1951) $F_{ST}$ ($\theta$ in Weir & Cockerham’s terminology) using ARLEQUIN v.2.001 (Schneider, Roessli & Excoffier, 2000). The significance of the estimates of $\theta$ from zero was assessed by making 10 000 permutations of genotypes between populations. Hierarchical analysis of molecular variance (AMOVA; Schneider et al., 2000) was used to partition the contribution to genetic diversity arising from differences (i) between regions (Pembrokeshire, Gower and Anglesey), (ii) among sample sites within regions and (iii) among individuals within sites. The significance of the fixation indices was tested using 10 000 permutations.

Isolation by distance genetic structure (Wright, 1943) was examined for (i) all populations and (ii) the Pembrokeshire samples separately by a regression of pairwise estimates of genetic differentiation $[\theta/(1 – \theta)]$ against the corresponding natural logarithm of the geographic distance (m) separating the populations (Rousset, 1997). For simplicity we calculated the Euclidian distance among pairs of samples. Some sites in Pembrokeshire are separated by high ground that may limit dispersal more than expected from their linear separation (Fig. 1); therefore, we also measured the shortest routes that were restricted to terrain <300 m high (i.e. that circumvented the central ridge) between pairs of Pembrokeshire sites. A Mantel test (1000 permutations of population locations among all locations) was used to assess the significance of any correlation between pairwise genetic differentiation and geographic distance using the online version (3.1c) of GenePop (Raymond & Rousset, 1995). Mantel tests were applied to all pairs of samples as well as the colonies from Pembrokeshire only; for the latter analysis we considered both Euclidian distance among samples as well as the routes that avoid high ground.

Multiple testing. Where appropriate a sequential Bonferroni correction (Rice, 1989) was applied to adjust the significance of ($k$) multiple tests.

Results

Genetic diversity

Because only two loci demonstrated significant linkage disequilibrium within any sample (LIST4-035–LIST4-066 in GOF, \( P < 0.05, k = 91 \)) all loci were retained for subsequent genetic analyses. Individual sample-locus results for measures of genetic diversity are presented in Appendix. Almost all samples met expected HWE conditions with four and one sample-locus combination(s) having a significant \( (P < 0.05, k = 14) \) heterozygote deficit or excess respectively (Appendix).

Raw values of allelic richness \( (A_R) \), expected heterozygosity \( (H_e) \) and Wright’s (1951) inbreeding coefficient \( (f) \) are provided in Appendix. Particularly notable is that five loci (36%) were monomorphic at Anglesey, while only one locus (LIST4-037) was invariable in two of the samples elsewhere (WAM & WAF). Average levels of genetic diversity are generally similar among samples from Pembrokeshire and Gower, but substantially lower in Anglesey (Fig. 2a–c). Permutation analyses demonstrated that \( A_R, H_e \) or \( f \) did not significantly differ between groups of samples from Pembrokeshire and Gower \( (P = 0.234, 0.954 \) and 0.108, respectively), although when Anglesey was included there were significant differences in \( A_R (P = 0.001) \) and \( H_e (P = 0.001) \) but not \( f \) \( (P = 0.396) \) among groups.

Bottleneck analysis

Only C. mercuriale from Gower and Pembrokeshire (five samples) demonstrated a significant \( (P < 0.05) \) heterozygote excess from that expected under an IAM of mutation [but none did under the SMM – see Luikart & Cornuet (1998) for discussion on mutational models]. This heterozygote excess remained significant \( (P < 0.05, k = 12) \) after a sequential Bonferroni correction (Rice, 1989) for CEB and RHO only. The ratio \( M \) was 0.72 or less for all samples, with the lowest value \( (M = 0.61) \) at RHO (Table 1); \( M \) was 0.753 (variability 0.052) for the combined Pembrokeshire population.

Genetic differentiation between samples

Sites at Anglesey and Gower had significantly different \( (P < 0.05, k = 45) \) genotype frequencies from all

Fig. 2 Variation (mean ± 95% CI) in (a) allelic richness \( (A_R) \) standardised for a sample size of 31 individuals (b) expected heterozygosity \( (H_e) \) and (c) Wright’s (1951) inbreeding coefficient \( (f) \) among samples of C. mercuriale from the Pembrokeshire (PAN-WAF), Gower and Anglesey. See Methods and Fig. 1 for further description of site names and locations.
Table 1 Results of bottleneck analysis for samples of C. mercuriale from Pembrokeshire, Gower and Anglesey (see Methods and Fig. 1 for full sample site names and their location).

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>Bottleneck</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IAM</td>
<td>SMM</td>
</tr>
<tr>
<td>Pembrokeshire</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAN</td>
<td>0.2508</td>
<td>0.9406</td>
<td>0.6549</td>
</tr>
<tr>
<td>GOF</td>
<td>0.0209</td>
<td>0.8794</td>
<td>0.6760</td>
</tr>
<tr>
<td>COT</td>
<td>0.0338</td>
<td>0.4758</td>
<td>0.6694</td>
</tr>
<tr>
<td>WAN</td>
<td>0.0595</td>
<td>0.8917</td>
<td>0.6542</td>
</tr>
<tr>
<td>DOI</td>
<td>0.0863</td>
<td>0.9031</td>
<td>0.6404</td>
</tr>
<tr>
<td>CLM</td>
<td>0.0101</td>
<td>0.5961</td>
<td>0.6695</td>
</tr>
<tr>
<td>BRY</td>
<td>0.0209</td>
<td>0.7869</td>
<td>0.6328</td>
</tr>
<tr>
<td>WAM</td>
<td>0.0090</td>
<td>0.2367</td>
<td>0.6182</td>
</tr>
<tr>
<td>WAF</td>
<td>0.1082</td>
<td>0.4197</td>
<td>0.6378</td>
</tr>
<tr>
<td>Gower</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefn Bryn</td>
<td>0.0003*</td>
<td>0.1955</td>
<td>0.7245</td>
</tr>
<tr>
<td>Rhossili</td>
<td>0.0017*</td>
<td>0.3129</td>
<td>0.6120</td>
</tr>
<tr>
<td>Anglesey</td>
<td>0.0645</td>
<td>0.1250</td>
<td>0.7000</td>
</tr>
</tbody>
</table>

Bottleneck: * indicates a significant \( P < 0.05, k = 12 \) heterozygote excess for microsatellite loci under infinite allele (IAM) and stepwise (SMM) models of mutation. M: ratio of allelic size range (number of microsatellite repeats) against the total number of alleles.

Other samples (Table 1). Among populations from Pembrokeshire, there was significant \( P < 0.05, k = 66 \) heterogeneity in genotype frequency between samples separated by the central hill ridge (except between GOF and CLM) and also among all four northern sites. The southern Pembrokeshire samples were more similar, with significant \( P < 0.05, k = 66 \) genotype differences between PAN and GOF, and PAN and WAN only (Table 1).

All pairwise estimates of genetic differentiation \( (\theta) \) between either Anglesey and the Gower sites and all other samples were significantly \( P < 0.05, k = 66 \) different from zero, with comparisons involving Anglesey notably high \( (\theta = 0.35–0.44) \). Genetic differentiation among the southern Pembrokeshire samples was either low \( \theta = 0.016 \) and 0.018, PAN-COT and PAN-WAN, respectively, \( P < 0.05, k = 66 \) or non-significant \( P > 0.05, k = 66 \). With the exception of two comparisons involving CLM, all northern Pembrokeshire samples were significantly dissimilar to all other samples from Pembrokeshire irrespective of their geographic location. Estimates of \( \theta \) between WAM and other samples from Pembrokeshire are relatively large compared with \( \theta \) between other pairs of samples from this area. Likewise, \( \theta \) between the Gower colonies is also greater than that generally observed between similarly spaced sites in Pembrokeshire (Table 2). Hierarchical AMOVA revealed significant \( P < 0.05 \) genetic structure at all levels but with almost all variance (>97%) attributed to differences within samples and among regions (Table 3).

There is a significant pattern of genetic isolation by distance at a broad scale between all of the samples \( (y = 0.120x - 0.974, P = 0.0002, r^2 = 0.63) \) and also locally among the Pembrokeshire samples (Fig. 3). For the latter samples, although there is a positive and significant regression between genetic differentiation \( [\theta/(1-\theta)] \) and Euclidian distance \( (\text{in m}) \) between samples \( (y = 0.013x - 0.077, P = 0.016, r^2 = 0.24) \) the fit of this relationship \( (y = 0.014x - 0.087, P = 0.003, \text{M}) \).

Table 2 Genetic differentiation among samples of C. mercuriale from Pembrokeshire, Gower and Anglesey; see Methods and Fig. 1 for full sample site names and their location. Exact probability of genotypic differentiation between samples (below diagonal) and pairwise estimates of genetic differentiation \( (\theta) \) (above diagonal).

<table>
<thead>
<tr>
<th>Pembrokeshire</th>
<th>Gower</th>
<th>Anglesey</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN ( 0.0112 )</td>
<td>0.0158*</td>
<td>0.0178*</td>
</tr>
<tr>
<td>GOF ( 0.0031 )</td>
<td>0.0022</td>
<td>0.0070</td>
</tr>
<tr>
<td>COT ( 0.00379 )</td>
<td>0.18485</td>
<td>0.0076</td>
</tr>
<tr>
<td>WAN ( 0.00152 )</td>
<td>0.02121</td>
<td>0.02348</td>
</tr>
<tr>
<td>DOI ( 0.01136 )</td>
<td>0.02500</td>
<td>0.02803</td>
</tr>
<tr>
<td>CLM ( 0.00076 )</td>
<td>0.00152</td>
<td>0.00076</td>
</tr>
<tr>
<td>BRY ( 0.00076 )</td>
<td>0.00076</td>
<td>0.00076</td>
</tr>
<tr>
<td>WAM ( 0.00076 )</td>
<td>0.00076</td>
<td>0.00076</td>
</tr>
<tr>
<td>WAF ( 0.00076 )</td>
<td>0.00076</td>
<td>0.00076</td>
</tr>
</tbody>
</table>

* Indicates a significant \( P < 0.05, k = 45 \) genetic difference between populations.

Table 3 Hierarchical analysis of molecular variance (AMOVA)
of microsatellite loci among samples of C. mercuriale from three
regions in Wales, U.K.: Pembrokeshire, Gower and Anglesey

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Fixation index</th>
<th>P-value</th>
<th>Percentage variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among regions</td>
<td>0.2343</td>
<td>0.0012</td>
<td>20.96</td>
</tr>
<tr>
<td>Among samples within regions</td>
<td>0.0312</td>
<td>0.0000</td>
<td>2.47</td>
</tr>
<tr>
<td>Within samples</td>
<td>0.2097</td>
<td>0.0000</td>
<td>76.57</td>
</tr>
</tbody>
</table>

P = probability of a more extreme variance component than that observed.

$r^2 = 0.36$) is improved if the distance measured between sites is restricted to a route below 300 m altitude.

Discussion

Molecular genetic studies have provided detailed insights into factors affecting the spatial genetic structure of aquatic insects (e.g. Geenen et al., 2000; Smith & Collier, 2001; Kelly et al., 2002; Miller et al., 2002; Hughes et al., 2003a,b; Wilcock et al., 2003; Wishart & Hughes, 2003; Watts et al., 2004a). However, most studies focus on dispersal rather than processes affecting levels of genetic variability, despite an increasing recognition that conservation strategies should prevent or minimise genetic erosion and if possible restore background levels of variability (Couvet, 2002; Frankham et al., 2002). In this study the large Pembrokeshire populations are the most genetically diverse, although somewhat surprisingly the smaller, separate Gower colonies have resisted substantial genetic erosion. The contrast in levels of diversity at these sites with that observed at Anglesey (Fig. 2a,b) may be attributed to one or a combination of several causes.

Impact of habitat isolation and population decline

Anglesey, some 150 km north from Pembrokeshire (Fig. 1) and probably a remnant from when C. mercuriale extended along the lowland coastal areas of Wales, meets several (not mutually exclusive) criteria associated with genetically depauperate insect populations: isolated (Keller & Largiader, 2003; Williams, Brawn & Paige, 2003; Krauss et al., 2004), small size (Schmitt & Seitz, 2002; Harper, MacLean & Goulson, 2003; Joyce & Pullin, 2003) and/or at the edge of a species’ range (Schmitt & Hewitt, 2004). The contrast in diversity at the isolated sites of Anglesey and Gower underlines the point that genetic impoverishment is not simply an unavoidable consequence of habitat fragmentation but rather reflects the effect of population size upon the rate of genetic drift (see Frankham, 1996; Frankham et al., 2002). For example, using the relationship:

$$H_i = H_e(1 - 1/2N_e)^r$$

Fig. 3 Relationship between geographic distance (ln m) separating samples of C. mercuriale from Pembrokeshire, Gower and Anglesey and the corresponding estimate of pairwise genetic differentiation as defined by $\theta/(1-\theta)$. Expanded plot of the Pembrokeshire samples in the dashed frame highlights the isolation by distance relationship at this site.

where \( H_t = \) expected heterozygosity after \( t \) generations, \( H_0 = \) initial expected heterozygosity and \( N_e = \) effective population size (Crow & Kimura, 1970), the present level of expected heterozygosity at Anglesey (from an initial \( H_t = 0.5 \)) is equivalent to \( N_e = 10 \) or 1000 for about 20 or 1900 generations, respectively. It may seem remarkable that there is no evidence for a bottleneck at Anglesey but this is consistent with the poor statistical power of (i) the ratio test when the starting population size is small and (ii) both tests with increasing time following the bottleneck (Luikart & Cornuet, 1998; Garza & Williamson, 2001). A useful feature of the ratio test, however, is that allelic diversity remains low in permanently reduced populations, although the value of \( M \) partially recovers (Garza & Williamson, 2001). The Anglesey colony evidently suffered a severe reduction in size. While the colony at Anglesey may have been founded by one/few gravid females (see e.g. Joyce & Pullin, 2003 for genetic diversity in an introduced, isolated population of *Euphydryas aurinia* Rott.) this is unlikely given this species’ poor dispersal capability (Hunger & Röске, 2001; Purse et al., 2003; Watts et al., 2004a).

Adaptation to an extreme, edge of range environment may further contribute to the low genetic diversity at Anglesey, but the consistency across loci suggests that genome-wide drift is the principal cause.

Also in contrast to expectations were the similar levels of genetic diversity at Pembrokeshire and Gower. Similarly, Monaghan et al. (2002) found no effect of fragmentation on the genetic diversity of stream insects because population sizes were large enough to minimise the rate of genetic drift and/or there is gene flow among populations. The Gower sites, 50 km south of the Pembrokeshire colonies, are likely to have been isolated for a shorter duration than Anglesey and may have experienced more recent immigration (contemporary gene flow to the Gower is unlikely, see below) and/or historically maintained larger effective population sizes. The genetic signature of recent population decline corresponds with qualitative observations of a reduction in distribution of *C. mercuriale* at Gower (D.J. Thompson, personal observations) that will accelerate a reduction in gene diversity.

Within Pembrokeshire, localised differences in genetic variability are indicative of fine-scale variation in demography that is typical of many insect populations (e.g. Schmitt & Seitz, 2002; Wynne et al., 2003). Of note is the likely recent decline in size at WAM (cf. Table 1) and concurrent reduction in \( A_g \) (it is possible that low diversity reflects a selective response to altitude); any loss of variability at WAM will be sustained by restricted immigration (see below). By contrast, the level of variability at WAF further highlights the point that the rate of genetic drift in large populations is generally low, such that substantial levels of diversity may be retained even when a population is isolated (see also Keller, Nentwig & Largiadèr, 2004). Elsewhere in Pembrokeshire, connectivity between the southern, eastern and northern populations may buffer individual colonies against genetic erosion associated with demographic fluctuations.

**Population genetic structure and movement among sites**

The substantial level of genetic differentiation that may be attributed to differences among regions (Table 3; Fig. 3) undoubtedly corresponds to breaks in distribution that far outreach *C. mercuriale*’s dispersal capability (Hunger & Röске, 2001; Purse et al., 2003; Watts et al., 2004a). The pattern of genetic differentiation among populations with distance, however, provides more insight into processes affecting population structure (Miller et al., 2002). Weak and/or variable regional genetic differences and absence of isolation by distance that have been reported for some aquatic insects are indicative of, for example, wide dispersal, genetic disequilibrium, balancing selection or poor marker resolution (e.g. Smith & Collier, 2001; Miller et al., 2002; Monaghan et al., 2002; Hughes et al., 2003b). In contrast, an apparent pattern of isolation by distance (Fig. 3) is a feature of restricted dispersal and relatively small populations that facilitates rapid population-differentiation through genetic drift (see also Miller et al., 2002; Wilcock et al., 2003).

At a local scale, genetic differentiation between the Gower sites is greater than that observed between similarly spaced sites in continuously distributed *C. mercuriale* populations (Table 2; Watts et al., 2004a) indicating that dispersal is disrupted by agricultural improvement. Indeed, restricted gene flow has been associated with anthropogenic changes to habitat for several terrestrial (Keller & Largiadèr, 2003; Williams et al., 2003; Keller et al., 2004) and aquatic insects (Smith & Collier, 2001; Watts et al., 2004a). Similarly, the improved fit of the regression based on movement among sites within Pembrokeshire that avoids high ground corresponds
with observations that, like many freshwater insects (e.g. Petersen et al., 2004), adult *C. mercuriale* move exclusively between areas of suitable habitat (Purse et al., 2003); the hill ridge is a natural barrier to dispersal (see also Wishart & Hughes, 2003). Low or non-significant genetic differences among adjacent areas in Pembrokeshire (Table 2) indicate dispersal by *C. mercuriale* over 1–2 km, although this does not prevent genetic isolation by distance (see Fig. 3, insert). Increased genetic differentiation at WAM (Table 2) is a likely consequence of isolation by high ground, but directional selection or drift during the recent demographic reduction may also accentuate genetic differences between this site and elsewhere (see Hedrick, Gutierrez-Espeleta & Lee, 2001). Variable but often low genetic differences between the central sites and WAF do not indicate connectivity, but reflect a historical genetic signature with the large WAF population not yet achieving migration-drift equilibrium since its isolation. Neither Geenen et al. (2000) or Wong et al. (2003) identified fine-scale genetic structure in other species of zygopteran but the pattern and magnitude of local genetic differences in Pembrokeshire parallels that observed for this species in the Itchen Valley (Watts et al., 2004a).

**Implications for conservation**

Population size is an important correlate of extinction risk (O’Grady et al., 2004) and, accordingly, the current U.K. strategy for *C. mercuriale* conservation is based upon habitat protection to maintain large populations. However, even apparently healthy populations can be at long-term risk if they lack genetic diversity (Saccheri et al., 1998; Frankham et al., 2002; Keller & Waller, 2002; Reed & Frankham, 2003; Schmitt & Hewitt, 2004; Spielman et al., 2004a,b). Improving connectivity among fragmented populations will help maintain/restore genetic diversity and preserve evolutionary patterns and processes (Moritz, 1999): such a strategy is not possible between regions but is generally appropriate within each area, especially the Gower sites. Somewhat ironically, the historical lack of management (agricultural improvement) imposed by its common land status has helped limit genetic erosion in the Pembrokeshire, although a recent decline in sheep grazing has unfortunately reduced habitat quality. It is relevant, therefore, that $M < 0.68$, characteristic of each Pembrokeshire sample, is symptomatic of a recent reduction in population size (Garza & Williamson, 2001); even when genotype data for all Pembrokeshire sites are combined to constitute a single population the calculated value of $M = 0.75$ is still below that observed in demographically stable populations ($M = 0.82$, Garza & Williamson, 2001). Data on levels of genetic variability in *C. mercuriale* populations towards the centre of its range would provide further context to the variation observed in Pembrokeshire and Gower.

Some combination of habitat isolation, small population size and/or directional selection has lead to localised reduction in genetic variability at WAM and a striking level of genetic impoverishment at Anglesey that is more typical of threatened mammal (e.g. Hedrick et al., 2001; Whitehouse & Harley, 2001; Jones et al., 2004) than fragmented insect (e.g. Keller & Largiadèr, 2003; Williams et al., 2003; Keller et al., 2004) populations. There is particular concern for *C. mercuriale* at Anglesey which may be (i) locally adapted and/or (ii) likely to suffer as a result of no immigration (see Couvet, 2002; Keller & Waller, 2002), for example, from inbreeding depression (Saccheri et al., 1998) or increased susceptibility to disease (Spielman et al., 2004b). The former implies that there should be focus on habitat management while the latter suggests that the population may benefit from augmentation (e.g. Madsen et al., 1999). Because we have not yet addressed either scenario it would be unwise to suggest augmentation (see Moritz, 1999; Stockwell, Hendry & Kinnison, 2003). It is interesting to note that, if the decision was taken for management plans to prioritise the conservation of genetic diversity *per se*, for example to allow future evolutionary processes, rather than current adaptive novelties (see Moritz, 1999), then any effort directed towards managing the Anglesey site should perhaps be diminished and efforts focused on the larger populations in Pembrokeshire and Hampshire.

To conclude, populations of *C. mercuriale* exhibit local and regional variation in levels of genetic diversity that is associated with their population demographics. Rather than directly eliciting a loss of variation, habitat fragmentation reinforces genetic impoverishment in small populations by preventing augmentation via gene flow. Consistent with the pattern of spatial genetic structure from elsewhere in the U.K., dispersal by mature *C. mercuriale* in Pem-
brokeshire is sufficient to prevent significant genetic differentiation throughout a habitat matrix of about 3–4 km if the sites are <2 km apart and lack intervening barriers. Nevertheless, dispersal by C. mercuriale is sufficiently restricted so that isolation by distance genetic structure develops within 10 km.

Acknowledgments

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References


Genetic variation in an endangered damselfly


(Manuscript accepted 29 September 2005)

**Appendix** Genetic diversity at 14 microsatellite loci for samples of C. mercuria from the Pembrokeshire (PAN–WAF), Gower and Anglesey (see Methods and Fig. 1 for full sample site names and locations)

<table>
<thead>
<tr>
<th>Pembroke</th>
<th>Gower</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN (n = 48)</td>
<td>GOF (n = 48)</td>
</tr>
<tr>
<td>4-002</td>
<td>4-023</td>
</tr>
<tr>
<td><strong>AR</strong></td>
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</tr>
<tr>
<td><strong>Ht</strong></td>
<td>0.479</td>
</tr>
<tr>
<td><strong>f</strong></td>
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</tr>
<tr>
<td><strong>Ht</strong></td>
<td>0.555</td>
</tr>
<tr>
<td><strong>f</strong></td>
<td>0.078</td>
</tr>
</tbody>
</table>

### Appendix (Continued)

#### Pembrokeshire

<table>
<thead>
<tr>
<th></th>
<th>PAN (n = 48)</th>
<th>GOF (n = 48)</th>
<th>COT (n = 48)</th>
<th>WAN (n = 48)</th>
<th>DOI (n = 48)</th>
<th>CLM (n = 48)</th>
<th>BRY (n = 48)</th>
<th>WAM (n = 48)</th>
<th>WAF (n = 48)</th>
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<tbody>
<tr>
<td>A_g</td>
<td>3.989</td>
<td>2.447</td>
<td>2.994</td>
<td>2.972</td>
<td>2.994</td>
<td>2.987</td>
<td>2.730</td>
<td>2.992</td>
<td>2.000</td>
</tr>
<tr>
<td>f</td>
<td>0.149</td>
<td>-0.271</td>
<td>0.083</td>
<td>-0.127</td>
<td>-0.091</td>
<td>-0.098</td>
<td>0.057</td>
<td>-0.076</td>
<td>-0.259</td>
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</table>

#### Gower

<table>
<thead>
<tr>
<th></th>
<th>Cefn Bryn (n = 48)</th>
<th>Rhossili (n = 48)</th>
<th>Anglesey (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_g</td>
<td>2.000</td>
<td>2.000</td>
<td>2.000</td>
</tr>
<tr>
<td>f</td>
<td>-0.462</td>
<td>-0.112</td>
<td>0.039</td>
</tr>
</tbody>
</table>

#### Sample size (n), allelic richness (A_r), expected heterozygosity (H_e) and Wright’s (1951) inbreeding coefficient (f).

* and † indicate a significant (P < 0.05, k = 9–14) heterozygote deficit or excess, respectively – indicates monomorphic locus, so test not carried out.