

Molecular and ecological evidence for small-scale isolation by distance in an endangered damselfly, *Coenagrion mercuriale*

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Abstract

Coenagrion mercuriale (Charpentier) (Odonata: Zygoptera) is one of Europe's most threatened damselflies and is listed in the European Habitats directive. We combined an intensive mark-release-recapture (MRR) study with a microsatellite-based genetic analysis for *C. mercuriale* from the Itchen Valley, UK, as part of an effort to understand the dispersal characteristics of this protected species. MRR data indicate that adult damselflies are highly sedentary, with only a low frequency of interpatch movement that is predominantly to neighbouring sites. This restricted dispersal leads to significant genetic differentiation throughout most of the Itchen Valley, except between areas of continuous habitat, and isolation by distance (IBD), even though the core populations are separated by less than 10 km. An urban area separating some sites had a strong effect on the spatial genetic structure. Average pairwise relatedness between individual damselflies is positive at short distances, reflecting fine-scale genetic clustering and IBD both within- and between-habitat patches. Damselflies from a fragmented habitat have higher average kinship than those from a large continuous population, probably because of poorer dispersal and localized breeding in the former. Although indirect estimates of gene flow must be interpreted with caution, it is encouraging that our results indicate that the spatial pattern of genetic variation matches closely with that expected from direct observations of movement. These data are further discussed with respect to possible barriers to dispersal within the study site and the ecology and conservation of *C. mercuriale*. To our knowledge, this is the first report of fine-scale genetic structuring in any zygopteran species.

Keywords: *Coenagrion mercuriale*, conservation, dispersal, isolation by distance, landscape genetics, microsatellite

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Introduction

Dispersal is a key life-history trait that determines the abundance, dynamics and the persistence of populations (Dieckmann *et al.* 1999), and from an evolutionary perspective establishes the level of gene flow and effective population size, affecting processes such as genetic drift, local adaptation and speciation. Estimating migration rates in the natural environment is therefore fundamental to our understanding of species' dynamics (Clobert *et al.* 2001). Directly surveying the movements of organisms

(or their gametes), however, is frequently problematic because logistical and financial constraints limit the spatial and temporal scope of field studies and bias against the detection of long-distance and/or rare movements (Slatkin 1985). Indirect estimates of migration inferred from the spatial variation of neutral genetic markers are, by contrast, relatively easy to attain and offer a 'time-averaged' perspective of dispersal (Slatkin 1985). The interpretation of migration rates from genetic markers is not necessarily straightforward (Thompson & Goodman 1997; Bossart & Prowell 1998; Whitlock & McCauley 1999; Rousset 2001) and it has been suggested that genetic and ecological estimates of dispersal might not be comparable (Koenig *et al.* 1996). Other authors have argued that

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indirect estimates of dispersal are rarely so biased as to be rendered uninformative (Bohonak *et al.* 1998) and, more recently, Berry *et al.* (2004) and Wilson & Rannala (2003) both reported that individual-based assignment tests can provide reliable estimates of migration rates. Where possible, therefore, it can be informative to make direct observations on movement as well as describing the spatial pattern of genetic variation, with the expectation that studies at local geographical scales will be less susceptible to certain types of demographic instability and more likely to yield comparable estimates of dispersal (Rousset 2001).

The pattern and level of gene flow is not simply a species' property because dispersal can be strongly influenced by the landscape structure (Piertney *et al.* 1998; Dieckman *et al.* 1999; van Dyck & Matthysen 1999; Wiens 2001). This is particularly relevant for species inhabiting fragmented biotopes because successful movement between isolated patches of suitable habitat can be substantially altered by the inhospitable matrix environment. Small populations that become isolated will lose genetic variation through genetic drift. Limited gene flow may facilitate local adaptation (Lenormand 2002) but there is concern that reduced genetic variation will be detrimental, for example, as a loss of evolutionary potential (Frankham 1995) or a manifest reduction in viability (Saccheri *et al.* 1998). There is no general consensus to the costs and benefits of gene flow (Stockwell *et al.* 2003) but the habitats of many species are becoming artificially modified at an alarming rate. Thus, understanding the ecological and evolutionary effects of habitat fragmentation is not only a challenging scientific topic but is important from a conservation perspective.

Damselflies are relatively large, highly conspicuous insects and represent excellent model organisms for directly observing dispersal. We are interested in *Coenagrion mercuriale* (Charpentier) (Odonata: Zygoptera) because it is one of Europe's most threatened damselflies. It is listed on Annex II of the EC Habitats Directive and Appendix II of the Bern Convention and protected within Europe as a whole and by specific legislation in several countries. This species is one of two British resident odonates to be listed in the European Habitats directive that requires member states to designate Special Areas of Conservation for its protection: it therefore has a high conservation profile. The population centres are south-western Europe (the Iberian peninsula, France and Italy) and North Africa. *C. mercuriale* has either disappeared or is on the edge of extinction in Belgium, the Netherlands, Luxembourg, Slovenia, Romania, Poland and Austria (Grand 1996) and is declining in other countries on the northern edge of its range, such as Germany and the UK. It is estimated that it has suffered a 30% decline in the UK since 1960, largely due to anthropogenic changes in land use (Thompson *et al.* 2004). In the UK, *C. mercuriale* has a patchy distribution that is determined by the availability of specific breeding habitat, either small

lowland heathland streams emanating from base-rich substrate or in ditches on water-meadow systems on chalk streams. Within these biotopes *C. mercuriale* is confined to shallow, unshaded and permanently flowing small watercourses with perennial herbaceous aquatic vegetation.

Previous mark-release-recapture (MRR) data have suggested that most adults rarely travel over 100 m, although movement over 1 km has been recorded (Hunger & Röske 2001; Purse *et al.* 2003) and there is therefore strong concern about the susceptibility of this species to the effects of habitat reduction and population isolation. In particular, the geographic pattern of restricted movement displayed by *C. mercuriale* is expected to lead to isolation by distance (IBD) (Wright 1943) and reinforce genetic divergence between populations. The relatively strong population of *C. mercuriale* from the Itchen Valley, Hampshire, UK offered an ideal opportunity to study dispersal and population-genetic structure in this species because a contrast can be made between two regions that are (1) separated by a large urban area and (2) have an intrinsically different habitat matrix. The water meadow system occupied by *C. mercuriale* in the south has been actively managed for conservation since 1987 and now represents a large area of continuous habitat, although a railway line and a motorway that traverse parts of this southern population could act as barriers to movement. To the north, the trout fishery and the presence of grazing areas or recreational land combine to leave only infrequent pockets of habitat that are suitable for *C. mercuriale*.

This work forms part of an effort to formulate a management plan for the long-term persistence of this species in the Itchen Valley. The general aims of the present study are to investigate the genetic structure of *C. mercuriale* with respect to direct observations on dispersal as well as geographical distance and the landscape, but we specifically address the following questions:

- 1 What are the dispersal characteristics of adult *C. mercuriale* in the Itchen Valley?
- 2 How do direct observations of movement compare with population-genetic structure?
- 3 Over what scale, if at all, does spatial genetic structure (IBD) develop?
- 4 What are the effects of barriers and habitat fragmentation upon movement and genetic structure and how has habitat management affected *C. mercuriale* in the Itchen Valley?

Materials and methods

Description of study site

There are four principal population centres in the Itchen Valley (Strange 1999), the Lower Itchen Complex (LIC), Highbridge, Twyford Moors and Mariner's Meadow (Fig. 1),

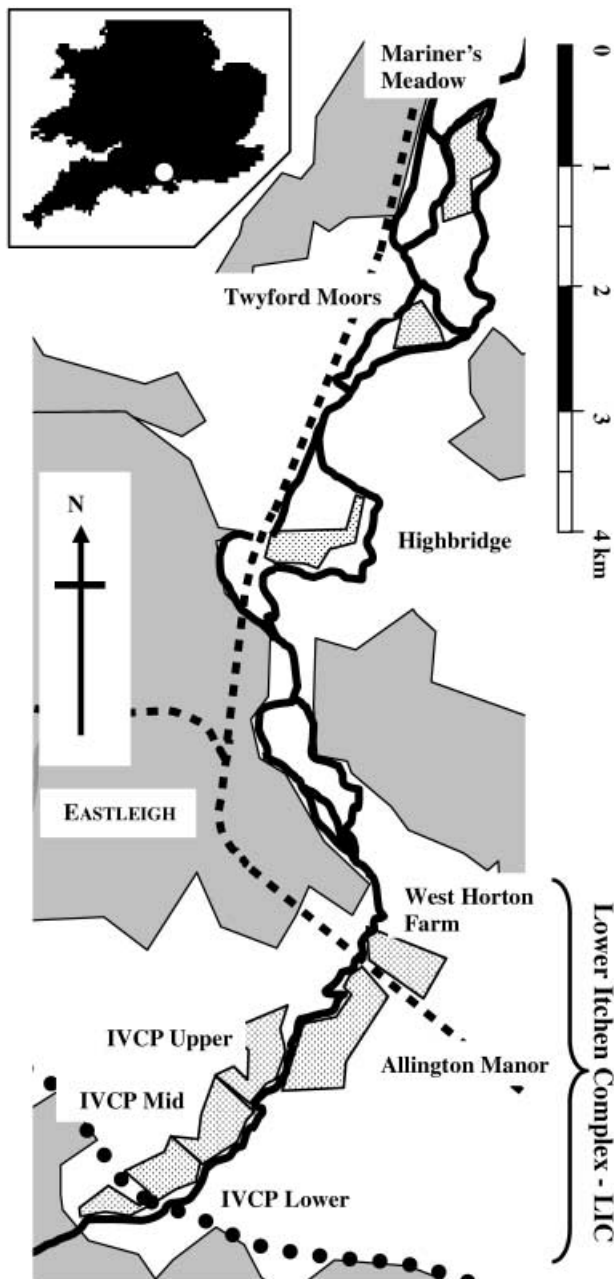


Fig. 1 Map of the eight sampling locations (stippled areas) and the main landscape features of the Itchen Valley study area. Urban areas are indicated by grey shading; the thick black line represents the course of the River Itchen; dashed line marks the position of the railway; dotted line shows the motorway that bisects Itchen Valley Country Park (IVCP)–Lower site. The white dot in the insert indicates the location of the Itchen Valley in England.

which mainly consist of small watercourses. We divided the LIC (the largest population) into five subsections of approximately equal length to ensure comparable sampling intensity for MRR. Moving northwards, the LIC is represented by three sites (Lower, Middle and Upper) in Itchen Valley Country Park (IVCP), Allington Manor and

West Horton (Fig. 1). The three remaining sites (Highbridge, Twyford Moors and Mariner's Meadow) are separated from the LIC by some 3 km of suboptimal habitat largely because of the town of Eastleigh. The railway line separating Allington Manor from West Horton and the motorway bisecting the IVCP–Lower site (Fig. 1) were thought to be potential barriers to movement. Despite the presence of the railway and motorway, the LIC is managed for *C. mercuriale* conservation and we considered this area as a continuous habitat patch. Most of the land surrounding Highbridge, Twyford Moors and Mariner's Meadow is used either for grazing or recreation and the main River Itchen itself is managed as a trout fishery, which creates fast-flowing water essentially unsuitable for *C. mercuriale* larvae (although they do occur sporadically), leaving only infrequent pockets of water meadows. We treated these three sites as representing a matrix of three separate habitat patches (but as continuous within each sample area).

Field work

Coenagrion mercuriale emerge from the water between May and July and the flight season lasts until mid-August (Brooks & Lewington 2002). Adults may live for 1–2 weeks, but typically suffer heavy mortality within a few days (Brooks & Lewington 2002), during which period they mate and lay eggs in aquatic vegetation. Adults were sampled between 09.30 hours and 16.00 hours every day for 5 weeks from 12 June, 2001, except during bad weather when mature adult damselflies do not visit breeding sites (Banks & Thompson 1985). No MRR was undertaken at the Twyford Moors site because it was close to a trout fishery and we were not granted full access. All unmarked mature adult damselflies were caught using a kite net, marked with a small dab of paint on the dorsum of the thorax and numbered on the left forewing using waterproof ink. This technique has been used repeatedly in long-term field studies of damselflies and is harmless to marked specimens. When marked animals were encountered, their numbers were either read using close-focusing binoculars or they were recaptured if there was any doubt as to their number. The position of every encounter was noted on a global positioning system (GPS), calibrated to the Ordnance Survey.

DNA extraction and polymerase chain reaction (PCR)

Because *C. mercuriale* is protected we employed a non-destructive sampling regime to obtain DNA by removing the hind legs (which were stored in individual 1.5 mL microcentrifuge tubes containing 100% ethanol) from up to 50 animals from each site. Removal of single damselfly legs does not measurably affect fitness (Fincke & Hadrys 2001) and there was no significant effect of leg removal upon recapture rate in this study (D. J. Thompson, unpublished).

Genomic DNA was extracted from the leg samples using a high salt protocol (Sunnucks & Hales 1996). We examined allelic variation in 16 microsatellite loci (LIST4-002, LIST4-023, LIST4-024, LIST4-030, LIST4-031, LIST4-034, LIST4-035, LIST4-037, LIST4-042, LIST4-053, LIST4-060, LIST4-062, LIST4-063, LIST4-066, LIST4-067 and LIST4-071) described by Watts *et al.* (2004a,b). Approximately 5 ng of DNA was used for PCR in a 10 μ L final reaction volume containing 75 mM Tris-HCl pH 8.9, 20 mM $(\text{NH}_4)_2\text{SO}_4$, 0.01% v/v Tween-20, 0.2 mM each dNTP, 3.0 mM MgCl_2 , 20 pmol forward primer, 30 pmol reverse primer and 0.25 U *Taq* polymerase (ABgene, ABgene® House, Surrey, UK). Thermal cycling conditions for the primers are described elsewhere (Watts *et al.* 2004a,b). The PCR products were pooled into one of three genotyping pools, determined by allelic size range and the 5' fluorescent dye, along with a 400 bp (D1) size standard (Beckman Coulter (UK) Limited, High Wycombe, UK) and separated by capillary electrophoresis through a denaturing acrylamide gel on a Ceq8000XL automated sequencer (Beckman Coulter). Alleles were sized using the cubic model of analysis in the Ceq8000XL fragment analysis software.

Data analysis

Genotypic linkage equilibrium among all locus-pair combinations was assessed for the pooled data set using the Fisher's exact test implemented by GENEPOP version 3.3 (Raymond & Rousset 1995). ARLEQUIN version 2.001 (Schneider *et al.* 2000) software was used to calculate the number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E) and Wright's (1951) inbreeding coefficient (f); f was estimated using the method of Weir & Cockerham (1984). The observed genotype frequency at each locus and for every population was then tested for departure from expected Hardy-Weinberg equilibrium conditions (HWE) using ARLEQUIN version 2.001.

Genetic differentiation between samples was determined by calculating Weir & Cockerham's (1984) estimator of Wright's (1951) F_{ST} (θ in Weir & Cockerham's terminology) using the analysis of molecular variance approach in ARLEQUIN version 2.001 (Schneider *et al.* 2000). We calculated θ between subsamples collected either side of the motorway within the IVCP-Lower sample ($n = 24$ for each subsample) and also for all pairwise comparisons between the eight sample sites (with the two IVCP-Lower subsamples pooled as a single sample). The significance of the estimates of θ from zero was assessed by making 1000 permutations of genotypes between populations. Genetic differentiation under IBD is expected to increase (or decrease for relatedness) linearly with spatial distance in a one-dimensional habitat and linearly with the logarithm of spatial separation in two-dimensional space (Rousset 1997; Hardy & Vekemans 1999). Isolation by distance between

samples was examined by a regression of pairwise estimates of genetic distance, as defined by $\theta/(1 - \theta)$, against the corresponding logarithms of geographical distance (ln-metres) separating the populations (Rousset 1997). A Mantel test (1000 permutations of population locations among all locations) was used to assess the significance of any correlation between genetic distance and geographical distance using SPAGED1 (Hardy & Vekemans 2002).

Principal component analysis (PCA) aims to explain multivariate data by a few linear combinations of the original variables while still retaining nearly as much of the total variation between samples (Johnson & Wichern 1992). A plot of the sample scores (eigenvectors) of significant principal components offers a convenient representation of the overall spatial variation in data as long as the principal components still account for a significant amount of the total between-sample variation. Rather than attempt to describe the spatial pattern of individual allele frequencies (81 variables) we summarized the multiallelic variation between damselfly samples to two-dimensions by a PCA of the sample allele frequencies using PCA-GEN version 1.2.1 (Goudet 1999). The significance of each principal component was assessed from 5000 randomizations of genotypes.

The spatial pattern of individual genetic variation was investigated by spatial autocorrelation (Sokal & Oden 1978) since it yields information about both the pattern and scale of spatial substructure and is sensitive at recovering fine-scale genetic structure (Sokal *et al.* 1997; Peakall *et al.* 2003; Vekemans & Hardy 2004). Because of the dichotomy in the location of the samples, we divided the samples into two groups according to their position relative to the urban area of Eastleigh (Fig. 1): (1) the three northern samples (Mariner's Meadow, Twyford Moors and Highbridge) and (2) the five samples within the LIC as separate groups. For a separate analysis, we (3) restricted pairwise comparisons to either within or between continuous habitat patches (with each patch defined as Mariner's Meadow, Twyford Moors, Highbridge and LIC). For each of the three data treatments described above we calculated the correlation in average kinship (F_{ij} ; Ritland 1996) relative to the whole data set between pairs of *C. mercuriale* separated by a range of increasing spatial scales. Although our results are essentially the same with other measures more frequently used in spatial autocorrelation (such as Moran's I , see also Hardy & Vekemans 1999), Ritlands (1996) F_{ij} tends to be more powerful at detecting spatial genetic structure than other estimators of relatedness when using highly polymorphic markers (Vekemans & Hardy 2004). To avoid a bias in the correlation coefficient arising because of unequal sample sizes within each spatial category we allowed SPAGED1 to assign distance categories that contained a similar number of pairwise comparisons (for further details see Hardy & Vekemans 2002). The average correlogram over all loci is presented as this avoids variation in

correlogram profiles based on the frequencies of individual alleles that are subject to stochastic processes (Hardy & Vekemans 1999; Smouse & Peakall 1999). Confidence intervals for average kinship coefficients at each distance class were generated from twice the standard error estimates, as calculated by jackknifing over all 14 loci retained for genetic analysis (see Results). Under certain circumstances distances based upon landscape connectivity rather than Euclidian space may improve the relationship between genetic and geographical distance (Arnaud 2003), however, we could not address this issue because of problems with land access (to establish a matrix habitat of connectivity). For all spatial statistics, Euclidian geographical distances between individuals and population centres were calculated from the GPS x and y coordinates using SPAGEDI (Hardy & Vekemans 2002).

Results

Mark-release-recapture

The pattern of recaptures that emerges during this study for *C. mercuriale*, which will be described in more detail elsewhere (J. R. Rouquette & D. J. Thompson unpublished), is typical of most damselfly species studied to date (Corbet 1999). That most animals that are captured were not seen again (Table 1) is not a reflection of any damage incurred during handling, but rather that the mature adult lifespan of damselflies is relatively short (with a mean time of around 5–6 d). Movement between sites was restricted, involving only 85 individuals (3.4% of all marked individuals). Two individuals transferred between sites twice to give a total of 87 intersite movement events (Table 1). Movement was not observed between Mariner's Meadow or Highbridge and any other site, but was recorded to and from the remaining sites. Movement was, in almost all

cases, between immediately adjacent sites. Although a small distance of unsuitable habitat lay between some sites within the LIC (e.g. the main river between Allington Manor and IVCP–Upper) this did not obstruct dispersal (Table 1).

Net lifetime movement is defined as the distance from where the animal was first marked to the place where it was last recaptured. The pattern of movement, which was similar at each of the seven sites used in the MRR study, is illustrated for Mariner's Meadow and IVCP–Middle which represent the extremes in terms of maximum distance moved (Fig. 2). The majority of individuals moved only a short distance in their mature adult lifetimes, but a few individuals are capable of longer distances. At Mariner's Meadow 78% of marked animals moved less than 50 m, with one individual moving 554 m (Fig. 2a). In the middle section of IVCP 68% of marked animals moved less than 50 m, but a few individuals moved much further, including one male that moved 1790 m (Fig. 2b). Variation in the average net lifetime movement of recaptured damselflies (Fig. 3) between sites was significant ($F = 23.59$, $df = 6$, $P < 0.001$, one-way ANOVA on $\log + 1$ transformed data) with a trend of greater average movement within the LIC (Fig. 3). Damselflies from Mariner's Meadow are significantly more sedentary than those from Highbridge, Allington Manor, IVCP–Middle and IVCP–Upper ($P < 0.05$, least significant difference test).

Damselflies moved either side of the motorway and the railway. There were 21 movements across the railway line; 13 movements were from West Horton to Allington Manor and eight were in the other direction (Table 1). The motorway was traversed nine times in each direction by 16 individuals (two individuals crossed twice). There was no apparent reduction in the mean movement of damselflies captured close to the motorway though the small sample sizes exclude the possibility of a rigorous statistical examination.

Table 1 Number of adult *Coenagrion mercuriale* marked and subsequently recaptured from eight sites in the Itchen Valley and a matrix of the number of times individuals moved between sites during mark-release-recapture (MRR)

	Mark	Recaptured		Number of <i>C. mercuriale</i> arriving at site						
				MM	HB	WH	AM	IVCP		
								Upper	Mid	Lower
MM	1144	480	No. <i>C. mercuriale</i>		0	0	0	0	0	0
HB	779	296	departing site	0		0	0	0	0	0
WH	265	71		0	0		13	0	0	0
AM	692	229		0	0	8		2	0	0
Upper	2484	690		0	0	0	6		7	1
Mid	2270	466		0	0	0	3	5		25
Lower	1072	291		0	0	0	0	0	17	

MM, Mariner's Meadow; HB, Highbridge; WH, West Horton; AM, Allington Manor; IVCP, Itchen Valley Country Park; Upper, Middle and Lower, IVCP Upper, Middle and Lower sites. Note: MRR could not be undertaken at Twyford Moors.

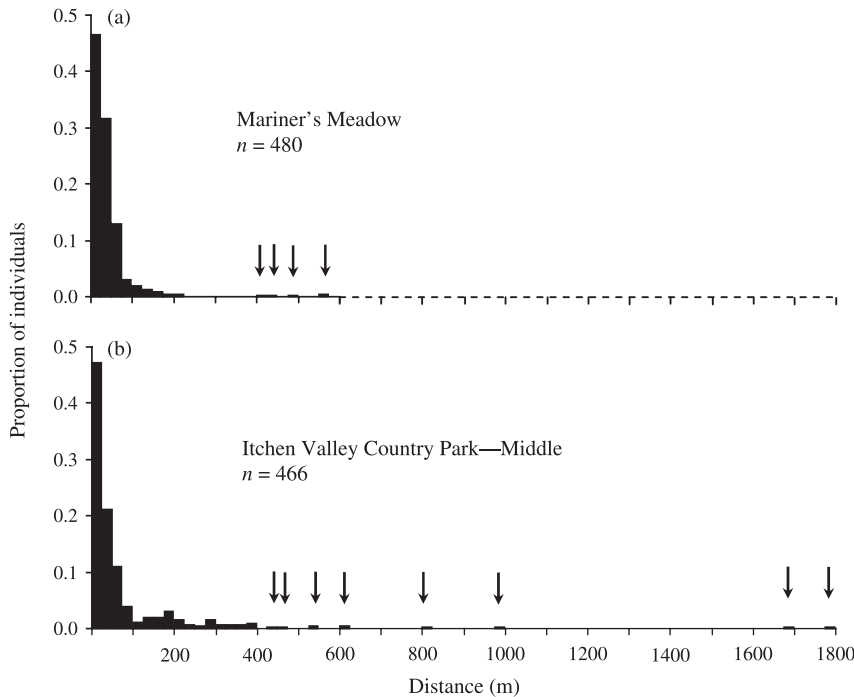


Fig. 2 Frequency of net lifetime movement of adult *Coenagrion mercuriale* in 25 m distance categories for (a) Mariner's Meadow and (b) Itchen Valley Country Park (IVCP)—Middle, both to the same scale; n , number of recaptured individuals. Arrows highlight infrequent ($n = 1-2$) movement events.

Genetic diversity

Three pairs of loci (LIST4-053 and LIST4-060, LIST4-023 and LIST4-071, and LIST4-035 and LIST4-053) showed significant ($P < 0.05$) linkage disequilibrium after a sequential Bonferroni correction (Rice 1989) for multiple tests at each locus ($k = 15$). LIST4-053 and LIST4-071 were dropped from the analyses. Most samples were in expected HWE conditions with only four comparisons showing a significant ($P < 0.05$) heterozygote deficit. LIST4-060 had the majority (three) of significant heterozygote deficits and the largest inbreeding coefficients (Appendix 1), which may be due to null alleles (four individuals showed drop-outs, but none from Mariner's Meadow). This locus was retained to maintain precision and testing power since a low frequency of null alleles has little effect on estimates of θ or F_{ij} (see Hardy 2000 cited in Kelly *et al.* 2004). Genetic variability at the microsatellite loci was moderate with between 1 and 12 alleles per sample and up to 17 alleles over all sites. There were differences in genetic variability between sites with a trend of more alleles and increased heterozygosity in the south (Appendix 1). The inbreeding coefficient varied between loci with all samples showing positive and negative values at one or more loci. The highest inbreeding coefficients were observed at Mariner's Meadow and Allington Manor (Appendix 1).

Genetic differentiation: F-statistics

Genetic differentiation between samples collected from either side of the motorway was not significant ($\theta =$

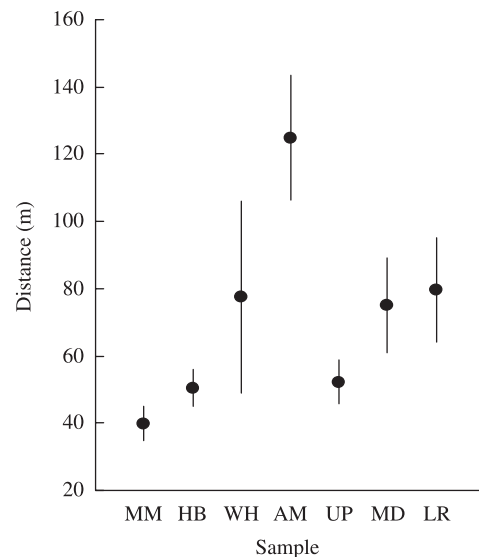


Fig. 3 Mean net distance moved ($\pm 95\%$ CI) by adult *Coenagrion mercuriale* at each of the sites used in the mark-release-recapture (MRR) study. MM, Mariner's Meadow; TM, Twyford Moors; HB, Highbridge; WH, West Horton; AM, Allington Manor; UP, MD and LR, Itchen Valley Country Part Upper, Middle and Lower sites, respectively.

-0.00305 , $P = 0.739$) and (as mentioned above) individuals from IVCP—Lower were pooled for all sample-based analyses. Pairwise estimates of θ between samples varied from 0.0018 up to 0.139. The majority of the estimates of θ

Table 2 Pairwise estimates of genetic differentiation (θ) between samples of *Coenagrion mercuriale* from the Itchen Valley

	MM	TM	HB	WH	AM	IVCP Upper	Middle
TM	0.1010*						
HB	0.1194*	0.0314*					
WH	0.0760*	0.0610*	0.0598*				
AM	0.1016*	0.0599*	0.0619*	0.0018			
Upper	0.1115*	0.0664*	0.0544*	0.0175*	0.0177*		
Middle	0.1392*	0.0652*	0.0622*	0.0285*	0.0230*	0.0030	
Lower	0.0963*	0.0496*	0.0549*	0.0062	0.0060	0.0042	0.0103

MM, Mariner's Meadow; TM, Twyford Moors; HB, Highbridge; WH, West Horton; AM, Allington Manor; IVCP, Itchen Valley Country Park; Upper, Middle and Lower, IVCP Upper, Middle and Lower sites.

*Significant ($P < 0.05$) genetic differentiation after a sequential Bonferroni correction (Rice 1989). Other abbreviations provided in Table 1.

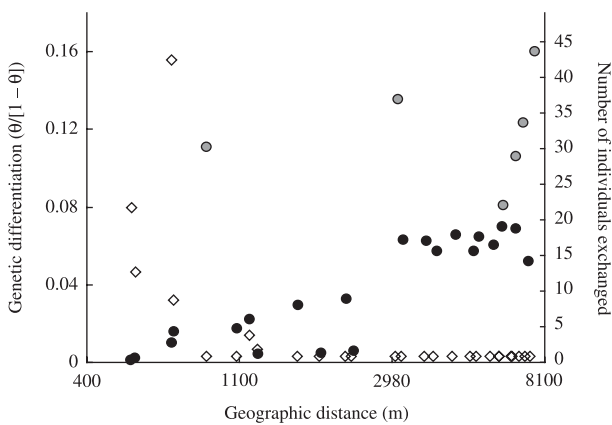


Fig. 4 Relationship between geographical distance separating samples of *Coenagrion mercuriale* in the Itchen Valley (ln-metres) and the corresponding estimate of pairwise genetic differentiation (circles) as defined by $\theta/[1 - \theta]$. Grey circles specify pairwise comparisons involving Mariner's Meadow and black circles denote all other intersample comparisons. Also shown are the numbers of damselflies that moved between sites (open diamonds).

were significantly different from zero with a general trend of lower genetic differentiation between the five sites within the LIC, compared with that between the three northern populations. None of the sites within the IVCP was significantly different from the other. Mariner's Meadow is the most genetically different site (Table 2).

There is a significant ($r^2 = 0.454$, $P < 0.001$) pattern of IBD between samples (Fig. 4) with the linear relationship between genetic distance (y , $\theta/[1 - \theta]$) and geographical distance (x , ln-metres) described by $y = 0.033x - 0.205$. The increase in genetic differentiation with distance is mirrored by a reduction in movement between sites (Fig. 4). Pairwise estimates of θ involving Mariner's Meadow are higher than those between other samples at comparable spatial scales, particularly for the comparisons with Twyford Moors and Highbridge (Table 2 and Fig. 4). When the Mariner's Meadow comparisons are removed from the

regression then the fit of the relationship between gene flow and distance is improved ($r^2 = 0.783$, $P = 0.015$) and described by $y = 0.027x - 0.172$.

Genetic differentiation: principal component analysis

Only the first two principal component axes accounted for a significant ($P < 0.05$) amount of the variation in allele frequencies between samples, and together accounted for three-quarters of the total θ between samples (Table 3). Differences between populations for their first two principal component scores revealed three groups, (1) a tight cluster containing all samples within the LIC, (2) Twyford Moors and Highbridge and (3) Mariner's Meadow, which approximately reflects their relative geographical positions, especially the separation of samples by the urban area (Fig. 5). Mariner's Meadow is separated from the other sites at the first principal component (with slightly larger divergence between Mariner's Meadow and the LIC) and Highbridge and Twyford Moors are mainly separated from the LIC (and also from Mariner's Meadow) by the second principal component. Inspection of the principal component scores reveals that in no instance are the differences between clusters the result of allelic variation at a single locus and only two out of the 14 loci (LIST4-030 and LIST4-037) make little overall contribution to either principal component (data not shown). Briefly, variation in the principal component scores between Mariner's Meadow and both other clusters at the first axis mirror the different frequency of alleles from five loci (LIST4-024, LIST4-035, LIST4-060, LIST4-062 and LIST4-067), with alleles from another 5 loci (LIST4-002, LIST4-031, LIST4-034, LIST4-042, LIST4-063) making contributions to variation depending upon the site. Separation of the Highbridge and Twyford Moors from the LIC on the second axis largely reflects differences at LIST4-024, LIST4-060, LIST4-063 and LIST4-067 (and LIST4-002 between LIC and Highbridge).

Table 3 Summary statistics for principal component analysis of allele frequency variation between eight samples of *Coenagrion mercuriale* from the Itchen Valley

	Principal component axis						
	1	2	3	4	5	6	7
Per axis inertia (eigenvalue)	0.184	0.119	0.038	0.033	0.011	0.009	0.007
Cumulative percentage inertia	45.80	75.36	84.73	93.04	95.83	98.14	100.00
θ	0.0254	0.0164	0.0052	0.0046	0.0016	0.0013	0.0010
P	0.0006	0.0002	1.0000	—	—	—	—

θ , estimate of genetic differentiation for principal component axis; P , probability that θ differs from 0.

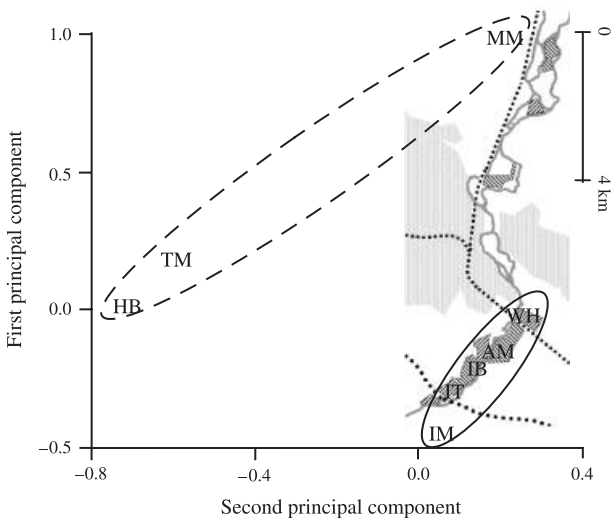


Fig. 5 Scatterplot of the first and second principal component scores (based on variation in allele frequency) for samples of *Coenagrion mercuriale* from the Itchen Valley superimposed on a map of the sample area (see Fig. 1). Solid and dashed lines enclose samples taken from continuous area or matrix of habitats, respectively. Both principal components account for statistically significant ($P < 0.001$) variation in genetic differences between sites.

Genetic differentiation: spatial autocorrelation

Positive kinship coefficients (significantly greater than zero) were observed for both (continuous and noncontinuous) groups of samples up to 800 m, with average kinship between damselflies from Mariner's Meadow, Twyford Moors and Highbridge significantly greater (nonoverlapping 95% confidence intervals) than between individuals from the LIC at equivalent distance classes (Fig. 6). Both correlograms show a similar pattern of positive kinship at the smallest distances followed by an approximately linear decline towards zero and subsequent fluctuation about zero. The decline in spatial autocorrelation, indicative of IBD (Sokal & Wartenberg 1983; Sokal *et al.* 1997), occurs rapidly over short distances of 500–800 m for Mariner's

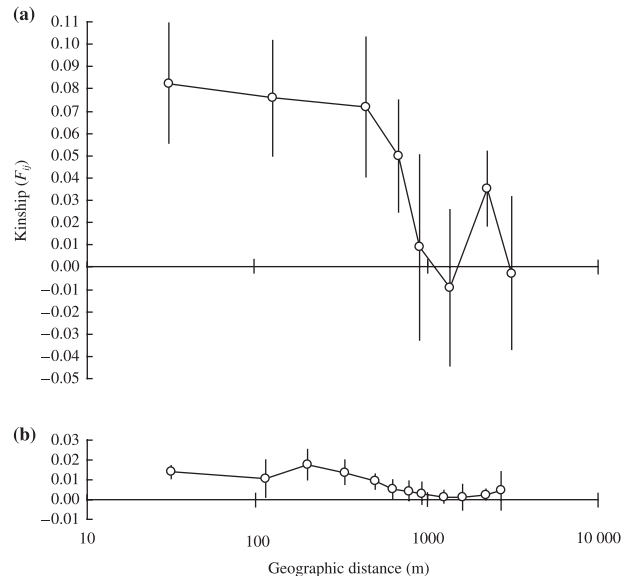


Fig. 6 Correlograms showing the combined genetic correlation in kinship (F_{ij}) (Ritland 1996) as a function of average spatial separation for pairs of *Coenagrion mercuriale* from (a) Mariner's Meadow, Twyford Moors and Highbridge and (b) the Lower Itchen Complex. Confidence intervals are twice the standard error, obtained by jackknifing over 14 microsatellite loci.

Meadow, Twyford Moors and Highbridge (Fig. 6a) and more gradually between about 300–800 m for the LIC (Fig. 6b).

When all *C. mercuriale* from within a continuous habitat patch are combined we again observe positive values of average kinship (significantly greater than zero) at small distances that decline with increasing spatial separation, reaching zero at 800–1000 m (Fig. 7). When restricting the analysis to all pairs of individuals from different patches, values for average kinship are negative with the exception of the first distance class (mean value 2060 m) where the confidence intervals overlap with that of the average kinship values within patches (Fig. 7).

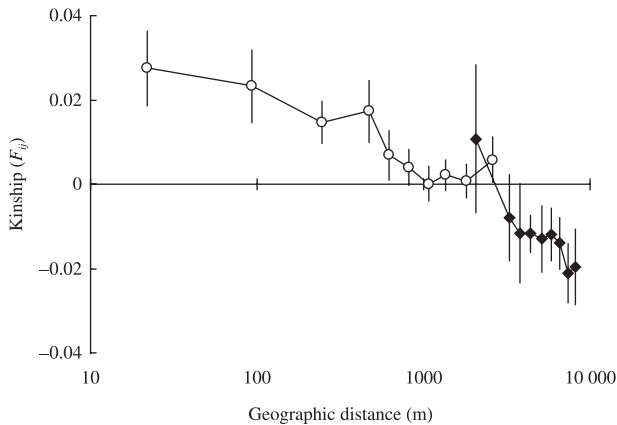


Fig. 7 Correlogram showing the combined genetic correlation in kinship (F_{ij}) (Ritland 1996) as a function of average spatial separation for pairs of *Coenagrion mercuriale* that were found either within (circles) or between (diamonds) a continuous habitat patch (habitat patches are defined as Mariner's Meadow, Twyford Moors, Highbridge and Lower Itchen Complex). Confidence intervals are twice the standard error, obtained by jackknifing over 14 microsatellite loci.

Discussion

Direct observations of adult dispersal

We combined an intensive MRR data set with a genetic analysis of nearly 400 *C. mercuriale* from the Itchen Valley as part of an effort to understand the dispersal characteristics and population-genetic structure of this protected species in a fragmented landscape. *Coenagrion mercuriale* do not freely disperse throughout the Itchen Valley, only rarely flying more than several hundred metres. This might appear surprising given that the study site is less than 10 km long (and some species of damselfly can fly at speeds of up to 10 km/h) but our data are comparable with direct estimates of dispersal from elsewhere in the UK (Purse *et al.* 2003) and Europe (Hunger & Röske 2001). *Coenagrion mercuriale* is probably a weak flier (Brooks & Lewington 2002) because of physical constraints and its behaviour. For example, it is one of the smallest of the blue damselflies, and body size has been correlated with dispersal capability in some odonates (Conrad *et al.* 1999; Angelibert & Giani 2003). Furthermore, *C. mercuriale* is not territorial (i.e. does not need to disperse to obtain territories), tends to remain close to vegetation and does not fly in windy conditions. The consequence of this is that *C. mercuriale* moves more frequently within than between sites and that between-site movement is predominantly to neighbouring areas (Table 1; Purse *et al.* 2003).

While the dispersal of adult damselflies is limited we did not explore the opportunity of movement during other life-history stages. Since larvae of *C. mercuriale* specifically require shallow, slow-moving watercourses with plenty of

vegetation they are unlikely to move into the main river and be dispersed by the current. Some odonates, including the congener *Coenagrion puella* (Banks & Thompson 1985; Angelibert & Giani 2003), disperse during their maiden flight as immature adults (teneral). Teneral dispersal has not been observed in *C. mercuriale* (D. J. Thompson & J. R. Rouquette personal observations) and we did not mark tenerals because they are delicate and we could not risk increasing the mortality rate of this species. Our belief that movements by larvae or tenerals of *C. mercuriale* are generally limited, however, is consistent with the ecology and behaviour of this species and also its population-genetic structure.

Population-genetic structure and isolation by distance

Significant genetic differentiation between many sites (Table 2) reinforces the conclusion made from direct observations on movement that *C. mercuriale* does not constitute a panmictic population within the Itchen Valley. The relationship between direct movement and genetic is only qualitatively made (there were too few zero movements between patches for a meaningful treatment of the data) in Fig. 4, where a pronounced decrease in migration leads to rapid IBD that is consistent with the pattern of direct movement among sites (Table 1).

Simulation (Sokal & Wartenberg 1983; Sokal *et al.* 1997) and natural (Arnaud *et al.* 2001; Peakall *et al.* 2003) studies have demonstrated that short-range gene flow is characterized by an initial positive autocorrelation that declines to zero then becomes negative and is sometimes followed by an oscillation of positive and negative values (see below). Although it can sometimes be difficult to unambiguously determine whether restricted dispersal or other processes that lead to strong genetic structure (Bossart & Prowell 1998) we observed a low frequency of movement beyond 200–500 m that leads to IBD developing within continuous habitat patches (i.e. less than 1000 m; Figs 6b and 7). The initial 'plateau' of positive kinship coefficients (Fig. 6) likely occurs because of restricted movement and localized breeding at this small spatial scale. Overall, we regard fine-scale genetic IBD brought about by restricted dispersal to be a salient characteristic of this species. An interesting feature is the rise in average kinship (albeit with wide confidence intervals) at just over 2 km (Figs 6 and 7) which could reflect a residual signature of nonequilibrium gene flow or simply a stochastic effect driven by genetic drift.

Restricted adult dispersal is a feature of many zygopteran (Banks & Thompson 1985; Conrad *et al.* 1999; Geenen *et al.* 2000). This, combined with our data, could suggest that significant population-genetic differentiation would be a typical feature for this taxon. Comparative research into the genetic structure of other damselflies is essential before any general conclusions about odonate population-genetic structure can be made because (1) there is not

necessarily a direct correlation between flight capability and odonate body size (see Purse *et al.* 2003) and (2) increased dispersal is likely when larvae inhabit flowing water habitats, tenerals exhibit dispersal behaviour (Geenen *et al.* 2000) or adults experience intraspecific competition. There are few published population genetic studies on damselflies and these have reported only low or nonsignificant genetic differences at small distances (Geenen *et al.* 2000; Wong *et al.* 2003) or significant differentiation between populations separated by considerably larger geographical scales than examined here (Andrés *et al.* 2000, 2002). This is to our knowledge the first report of fine-scale genetic structuring and strong IBD in an odonate species.

Effect of barriers and habitat fragmentation

Our understanding of the evolutionary impact of habitat fragmentation has benefited from a comparison of the genetic structure of populations in patchy and continuous habitats (Cunningham & Moritz 1998; Knutsen *et al.* 2000; Williams *et al.* 2003). That urbanization within the Itchen Valley is affecting the population structure of *C. mercuriale* is evident from an absence of movement between sites (Table 1) and the genetic discontinuity between samples either side of Eastleigh that is best visualized by PCA (Fig. 5). Pierny *et al.* (1998) also used PCA to correlate landscape features with genetic differentiation. It still appears to be under-utilized for ecological genetics but would appear to be worth pursuing since this technique conveniently summarizes overall variation in allele frequencies. The PCA scores revealed that genetic differences resulted from variation at alleles across several loci and are thus likely to represent neutral evolutionary processes rather than selection at a single locus.

Although *C. mercuriale* is a poor disperser, the low/nonsignificant genetic differentiation throughout the LIC indicates that the active maintenance of large areas of continuous habitat has apparently been successful in promoting movement and subsequent gene flow. That neither the railway nor the motorway appeared to inhibit adult flight was reflected by an absence of significant genetic differentiation between samples either side of these potential barriers. The presence of small watercourses underneath the railway and motorway probably facilitate adult movement. This is important from a conservation standpoint as it indicates that the maintenance of even small (*c.* 2 m wide) watercourses facilitates movement in this species.

That the amount of movement (Table 1) and genetic differentiation (Table 2) differed between groups of samples corresponds to a contrast in habitat continuity between these groups despite the similar distance spanning them. One relevant issue here is that the very restricted adult flight by *C. mercuriale* makes it difficult to be certain that we are observing an effect of barrier/habitat fragmentation

because at the scale separating Mariner's Meadow, Twyford Moors and Highbridge strong genetic differences could simply be a function of IBD. If habitat fragmentation has a strong effect in reducing dispersal we would expect lower average kinship between damselflies within a continuous habitat patch than between individuals from different patches. Unfortunately, the spatial distribution and the size of the habitat patches limits this analysis but the absence of a significant difference in average kinship at shortest overlapping distance class suggests that IBD itself can be as effective a barrier as poor habitat for *C. mercuriale* (Fig. 7). We did observe significantly higher average kinship values at similar spatial scales (Fig. 6), generally greater estimates of θ (Table 2) and generally fewer alleles for individuals at the northern sites compared with those from LIC. It is likely increased movement throughout the LIC, perhaps combined with larger population sizes (the number of marked individuals approximates relative population sizes, but note that capture was facilitated at the compact Mariner's Meadow site; Table 1), have reduced levels of inbreeding within the LIC compared with individuals from elsewhere in the Itchen Valley.

Mariner's Meadow is historically the most isolated site and has low genetic variability, a relatively high inbreeding coefficient (Appendix 1) and quite different allele frequencies from other sites in the Itchen Valley (Fig. 5). We examined our data for evidence that Mariner's Meadow passed through a bottleneck using the Wilcoxon sign-rank test in BOTTLENECK software (Cornuet & Luikart 1996) but the results are ambiguous since either all (except IVCP-Upper and IVCP-Lower) or none of the populations apparently suffered from a recent bottleneck according to whether the infinite allele model or stepwise model of mutation was applied, respectively (data not shown). It is certainly interesting that damselflies from north of Eastleigh (especially Mariner's Meadow) are the most sedentary (Fig. 3) as increased habitat fragmentation may lead to selection against dispersal from isolated populations (Dieckmann *et al.* 1999). The specific response to environmental change, however, is largely unpredictable: for example, certain damselfly species possess a more mobile morphology in fragmented landscapes (Taylor & Merriam 1995), yet dragonflies (Samways & Osborn 1998) switched towards a less mobile phenotype when isolated. Although speculative, if *C. mercuriale* possesses heritable variation in flight capability then the isolation of Mariner's Meadow may have facilitated a phenotypic shift such that the population remains isolated even in the presence of neighbouring populations (more recently established from the south).

Conservation of C. mercuriale in the Itchen Valley

Many populations of *C. mercuriale* have relatively large population sizes, but given its low dispersal capability

there is concern about the long-term viability of *C. mercuriale* populations, which are prone to extinction through anthropogenic changes in habitat quality (Thompson *et al.* 2004), if even small distances separated them. Genetic information is increasingly being used to define intraspecific conservation units (Avisé & Hamrick 1996), although there is a continuing debate over defining practical criteria for establishing management units for conservation (Moritz 1994; Crandall *et al.* 2000). Diniz-Filho & Telles (2002), for example, suggest that the x -intercept (or 'patch size') of a correlogram can be used to define (and hence conserve) genetically independent units. We observed an x -intercept of 800–1000 m for both within- and between-patch comparisons that interestingly matches the scale of dispersal (cf. Fig. 2 with Figs 6 and 7). It is important to note that the use of patch size to define areas for conservation management should be treated with extreme caution because it is dependent upon the scale of sampling (Vekemans & Hardy 2004). Management of *C. mercuriale* is presently based upon maintenance of habitat quality but it is encouraging that the management and the establishment of corridors have improved connectivity (movement and gene flow).

From a conservation perspective, *C. mercuriale* is not randomly distributed throughout the Itchen Valley but rather related individuals tend to be found together, and in some areas (e.g. Mariner's Meadow) high average kinship suggests that inbreeding might be substantial. Overall, the sedentary nature of *C. mercuriale* is compatible with the hypothesis that local populations could be locally adapted, and given these circumstances any population (or genetic) augmentation would need careful consideration (Stockwell *et al.* 2003). We presently have no detailed understanding about the degree of adaptation in local populations of *C. mercuriale*, yet this topic needs to be explored as it has implications for the conservation of this species given the UK Biodiversity Action Plan aim to reintroduce *C. mercuriale* to five sites over the next 5 years.

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This work forms part of a large research project into the demographic and genetic structure of *Coenagrion mercuriale* throughout the UK. Philip C. Watts is interested in the population-genetic structure and conservation biology of natural populations. James R. Rouquette is completing a PhD on the dispersal ecology of *C. mercuriale*. Ilik J. Saccheri is interested in the metapopulation dynamics and evolutionary biology of insects, Stephen J. Kemp leads the Animal Genomics Group at Liverpool University. David J. Thompson has a long-standing interest into the ecology and behaviour of damselflies and is a member of the *C. mercuriale* Biodiversity Action Plan steering group.

Appendix 1

Summary characteristics for 14 microsatellite loci in eight populations of the damselfly, *Coenagrion mercuriale*, from the River Itchen, Hampshire

	MM	TM	HB	WH	AM	IVCP			All Sites
						Upper	Mid	Lower	
LIST4-002	(48)	(30)	(48)	(48)	(46)	(47)	(48)	(48)	(363)
N_A	2	2	2	2	2	2	2	2	2
H_E	0.171	0.352	0.483	0.290	0.361	0.277	0.154	0.270	0.287
f	0.152	-0.234	-0.265	-0.005	0.116	0.004	-0.080	0.007	-0.038
LIST4-023	(47)	(30)	(48)	(47)	(46)	(48)	(46)	(48)	(360)
N_A	3	4	4	4	4	4	5	4	5
H_E	0.217	0.381	0.560	0.567	0.520	0.429	0.573	0.375	0.461*
f	0.357	0.341	-0.080	0.192	0.267	0.093	0.091	-0.048	0.152
LIST4-024	(48)	(30)	(48)	(48)	(48)	(48)	(48)	(48)	(366)
N_A	4	2	2	3	3	2	3	2	4
H_E	0.628	0.422	0.405	0.526	0.512	0.456	0.456	0.497	0.525*
f	0.138	-0.177	-0.234	-0.150	0.239	0.260	-0.144	-0.005	-0.009
LIST4-030	(48)	(30)	(48)	(48)	(48)	(45)	(47)	(48)	(362)
N_A	2	2	2	2	2	2	2	2	2
H_E	0.444	0.485	0.516	0.491	0.462	0.485	0.400	0.470	0.458
f	0.066	0.266	-0.307	-0.297	0.009	-0.100	-0.108	0.073	-0.050
LIST4-031	(48)	(30)	(48)	(48)	(44)	(45)	(48)	(48)	(361)
N_A	3	3	3	3	3	3	4	4	5
H_E	0.223	0.620	0.423	0.550	0.538	0.467	0.592	0.564	0.501*
f	0.068	-0.132	-0.084	0.202	0.090	-0.141	-0.021	0.125	0.013
LIST4-034	(48)	(30)	(48)	(48)	(47)	(48)	(48)	(47)	(364)
N_A	2	2	2	2	2	3	2	2	3
H_E	0.345	0.508	0.515	0.500	0.486	0.564	0.526	0.513	0.492
f	0.339	-0.094	0.031	0.126	-0.127	0.274	-0.115	-0.147	0.036
LIST4-035	(48)	(30)	(48)	(48)	(48)	(47)	(48)	(48)	(365)
N_A	7	6	7	7	12	8	6	8	16
H_E	0.713	0.746	0.807	0.785	0.823	0.763	0.752	0.840	0.821*
f	-0.053	-0.029	-0.072	-0.063	0.130	-0.005	0.086	-0.094	-0.013
LIST4-037	(48)	(29)	(48)	(48)	(46)	(45)	(46)	(47)	(360)
N_A	2	2	1	2	3	2	2	3	3
H_E	0.373	0.319	—	0.373	0.344	0.255	0.229	0.312	0.269
f	-0.169	-0.191	—	-0.169	0.056	0.032	0.244	-0.012	-0.030
LIST4-042	(48)	(30)	(47)	(48)	(48)	(48)	(48)	(48)	(365)
N_A	3	2	3	2	2	2	2	2	4
H_E	0.536*	0.472	0.438	0.325	0.257	0.321	0.284	0.368	0.384*
f	0.368	-0.132	0.077	-0.084	0.386	0.288	-0.017	0.153	0.130
LIST4-060	(48)	(30)	(48)	(47)	(45)	(48)	(48)	(48)	(362)
N_A	3	3	3	3	3	3	3	3	3
H_E	0.533*	0.613	0.531	0.582*	0.636	0.612*	0.612	0.656	0.632*
f	0.556	-0.048	0.178	0.376	0.315	0.547	0.230	0.182	0.292
LIST4-062	(48)	(30)	(48)	(48)	(45)	(47)	(48)	(46)	(360)
N_A	3	3	3	3	3	3	3	3	3
H_E	0.561	0.615	0.663	0.683	0.667	0.650	0.625	0.612	0.663
f	0.146	0.024	0.153	-0.027	-0.067	0.053	-0.155	-0.101	0.003
LIST4-063	(48)	(30)	(48)	(48)	(47)	(48)	(48)	(47)	(363)
N_A	4	4	3	7	5	4	4	5	7
H_E	0.521	0.590	0.646	0.498	0.501	0.370	0.377	0.540	0.524*
f	0.017	0.034	0.051	-0.076	0.030	-0.185	-0.098	0.029	-0.025

Appendix 1 *Continued*

	MM	TM	HB	WH	AM	IVCP			All Sites
						Upper	Mid	Lower	
LIST4-066	(47)	(30)	(48)	(47)	(45)	(48)	(48)	(48)	(361)
N_A	2	2	4	6	6	7	6	7	8
H_E	0.373	0.413	0.335	0.555	0.649	0.611	0.602	0.589	0.522*
f	0.262	-0.050	0.018	0.172	0.193	0.061	-0.074	0.060	0.080
LIST4-067	(48)	(30)	(48)	(48)	(45)	(48)	(47)	(47)	(361)
N_A	4	7	5	7	11	6	9	11	16
H_E	0.489	0.740	0.598	0.664	0.778	0.736	0.771	0.737	0.720*
f	0.064	0.089	-0.152	0.028	-0.002	-0.019	0.146	-0.052	0.013
All loci									
N_A	3.14	3.14	3.14	3.86	4.36	3.64	3.79	4.14	5.86
H_E	0.438	0.520	0.532	0.528	0.538	0.500	0.497	0.525	0.519
f	0.157	-0.017	-0.046	0.027	0.107	0.088	0.008	0.007	0.040

MM, Mariner's Meadow; TM, Twyford Moors; HB, Highbridge; WH, West Horton; AM, Allington Manor; IVCP, Itchen Valley Country Park; Upper, Middle and Lower, IVCP Upper, Middle and Lower sites. Sample sizes are given in brackets; N_A , number of alleles; H_E , expected heterozygosity; f , inbreeding coefficient; *, significant ($P < 0.05$ after sequential Bonferroni correction, $k = 14$) deficit of heterozygotes from expected HWE proportions; —, locus is monomorphic and no test for HWE carried out.

