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Spatial genetic structuring in a vagile species, the European wood mouse

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population structure; dispersal; isolation by distance; habitat fragmentation; European wood mouse; small mammals; *Apodemus sylvaticus*.

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Abstract

We examined the genetic structure of natural populations of the European wood mouse *Apodemus sylvaticus* at the microgeographic (<3 km) and macrogeographic (>30 km) scales. Ecological and behavioural studies indicate that this species exhibits considerable dispersal relative to its home-range size. Thus, there is potential for high gene flow over larger geographic areas. As levels of population genetic structure are related to gene flow, we hypothesized that population genetic structuring at the microgeographic level should be negligible, increasing only with geographic distance. To test this, four sites were sampled within a microgeographic scale with two additional samples at the macrogeographic level. Individuals ($n = 415$) were screened and analysed for seven polymorphic microsatellite loci. Contrary to our hypothesis, significant levels of population structuring were detected at both scales. Comparing genetic differentiation with geographic distance suggests increasing genetic isolation with distance. However, this distance effect was non-significant being confounded by surprisingly high levels of differentiation among microgeographic samples. We attribute this pattern of genetic differentiation to the effect of habitat fragmentation, splitting large populations into components with small effective population sizes resulting in enhanced genetic drift. Our results indicate that it is incorrect to assume genetic homogeneity among populations even where there is no evidence of physical barriers and dispersal can occur freely. In the case of *A. sylvaticus*, it is not clear whether dispersal does not occur across habitat barriers or behavioural dispersal occurs without consequent gene flow.

Introduction

Most mammal species exhibit distinct levels of population subdivision associated with small effective population sizes and low dispersal rates, which are a consequence of both social and mating behaviour (Chepko-Sade & Halpin, 1987). However, unlike larger mammals, the frequency and distance of dispersal in small rodents, relative to their home-range size, are significant (Gaines & McClenaghan, 1980; Gliwicz, 1988, 1992; Krebs, 1991; Zhang & Usher, 1991; Halle, 1993). Assuming such dispersal results in gene flow, species with a high propensity for dispersal should display low levels of population genetic substructuring (i.e. close to panmixia) due to the homogenizing effect of gene flow on gene frequencies, which opposes the diversifying effects of genetic drift and natural selection (Slatkin, 1987). Dispersal in small mammal species, however, may be limited by geographic barriers that would not influence larger species, including wide stretches of open land, roads, water bodies or sparse hedgerows (Clarke & Johnson, 1990; Gerlach & Musolf, 2000; Arthur, Pech & Dickman, 2004; Berthier *et al.*, 2005).

The degree of genetic differences among populations is considerably influenced by recurrent levels of gene flow and, hence, geographic distance. Several studies on small rodent species demonstrated population genetic differentiation despite of the potential for dispersal (Calhoun & Greenbaum, 1991; Mossman & Waser, 2001; Berthier *et al.*, 2005). However, inconsistencies observed between species indicate that patterns of population subdivision are highly heterogeneous, and assumptions of dispersal and population structure, at present, cannot be easily generalized. Species with low rates of gene flow may also consist of many small subpopulations, each following more or less independent evolutionary pathways mediated by genetic drift and/or selection (Dallas *et al.*, 1995). Small isolated populations are potentially more susceptible to genetic drift and inbreeding, which can increase genetic erosion and thus reduce their evolutionary potential to changing environments.

The European wood mouse *Apodemus sylvaticus* is widely distributed in the western Palaearctic, and is common across a variety of habitats from arable land to

hedgerows and woodland (Corbet & Harris, 1991). Population dynamics are similar across these habitats (Flowerdew, 1985, 1991; Montgomery, 1989*a*; Montgomery & Dowie, 1993). Despite considerable ecological and behavioural data accumulated through decades of intensive study of natural populations (Montgomery & Gurnell, 1985; Wolton, 1985; Wilson, Montgomery & Elwood, 1992; Baker, Makova & Chesser, 1999), little is still known about the scale at which populations of *A. sylvaticus* are subdivided into smaller and potentially independent evolutionary units. Enclosure studies offer tantalizing glimpses on factors such as multiple paternity and inbreeding avoidance, but suffer from constraint of movements, and the unavoidable bias from non-natural environments (e.g. Bartmann & Gerlach, 2001). Montgomery & Montgomery (1989), based on the analyses of parasite burden, suggested that the movement of individual *A. sylvaticus* in the wild is limited to a single habitat. Tattersall *et al.* (2004), however, observed the movement of *A. sylvaticus* between different habitat patches at low rates that varied between habitats. In contrast, a combined radiotracking and trapping study indicated frequent dispersal within a single habitat in both sexes (O'Neill, 2001). In this latter study, 56% of males and 45% of females with known histories were found to disperse. The mean minimum linear distance moved was 237.4 ± 20.0 m (maximum 2065 m), and 205.5 ± 21.2 m (maximum 1114 m) for males and females, respectively (O'Neill, 2001). Hence, there is evidence that although dispersal is common and distances covered are great, habitat boundaries could play some role in limiting gene flow. This interaction of geographical distance and genetic differentiation in the context of a heterogeneous landscape is evident in a recent molecular genetics study examining the effect of environmental pollution on genetic diversity in *A. sylvaticus* (Berckmoes *et al.*, 2005). The authors demonstrated that population differentiation increased with geographic distance among sites of comparable history, habitat type and composition along a heavy metal pollution gradient. However, although genetic diversity was not affected by heavy metal exposure, it was unclear as to whether population genetic structure was under environmental influence.

The levels of genetic diversity and patterns of population structure of *A. sylvaticus* populations are examined in the present study. We test the hypothesis that gene flow in natural populations of *A. sylvaticus* is high (i.e. positively correlated with dispersal potential) and, hence, genetic structuring is negligible on a microgeographic scale. We define the microgeographical scale as <3 km on the basis that routine movements of individual *A. sylvaticus* can be in excess of 400 m per night in radiotelemetry studies, or over 1 km for longer durations (Wolton & Flowerdew, 1985; O'Neill, 2001; Tattersall *et al.*, 2004). To test this hypothesis we assess population genetic structuring at the microgeographic level, examining population samples separated by as little as 850 m through to *c.* 3 km, and at macrogeographic scale, comparing samples separated by up to 30 km.

Materials and methods

Study area

Sampling took place during 2001 and 2002 over a large geographic area (50×30 km^2) comprising three major sites (Fig. 1). Tollymore Forest Park is situated *c.* 45 km south of Belfast, Northern Ireland. The Park covers an area of 7 km^2 mixed deciduous and coniferous plantations generally over 50 years old, and interspersed with older stands of hardwood species, fields and recreational areas (Montgomery, Wilson & Elwood, 1997). Moira, in contrast, represents a relatively small site (0.01 km^2) situated *c.* 25 km north-west of Belfast. This site is comprised mainly of deciduous scrubs, with a primary tree cover consisting of oak (*Quercus* spp.) and beech *Fagus sylvatica*, which are found sparsely along the remnants of field boundaries. Craigantlet covers an area of *c.* 3 km^2 and consists of both pastoral farmland and

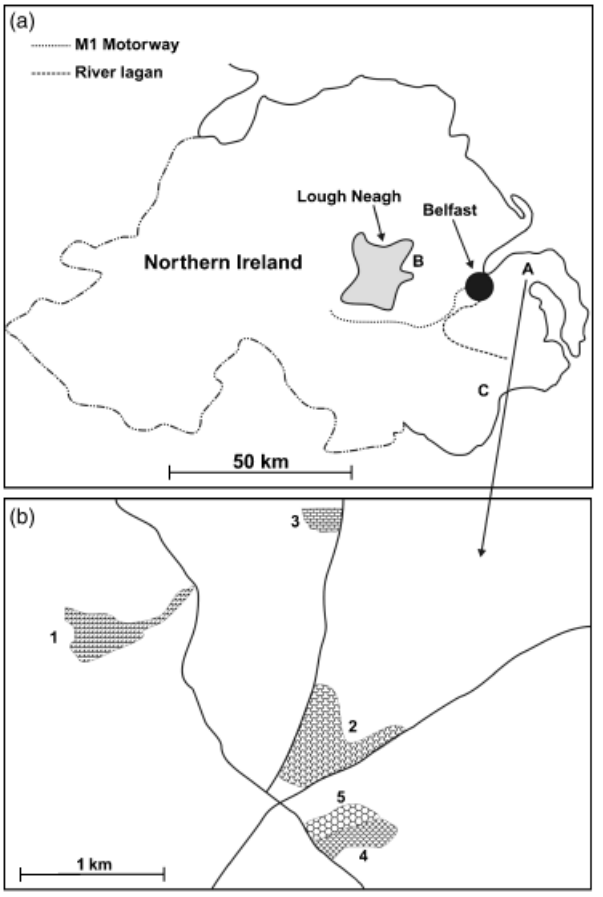

Figure 1 Macrogeographic (a) and microgeographic (b) sampling locations of wood mice *Apodemus sylvaticus*. Macrogeographic sample – A, Craigantlet; B, Moira; C, Tollymore Forest Park. Microgeographic sample – 1, Creighton's Green; 2, Cairn Wood, 3, Hedgerow; 4, Quarry Forest. Location 5 shows position of the quarry, which separate the Quarry Forest sample from other geographically close samples. Major geographical barriers/landmarks are also displayed. Full lines in (b) represent local roads.

managed forests located *c.* 15 km north-east of Belfast. Within this area, four subsites were sampled to allow for the investigation of population genetic structure at the microgeographic scale: (1) Creighton's Green was planted between 1958 and 1960 with a total area of 0.15 km^2 site, consisting primarily of deciduous stands interspersed by blocks of conifers with an understorey of deciduous shrubs; (2) Cairn Wood was planted in 1955 and covers an area of 0.23 km^2. Again this has both deciduous and coniferous stands and a ground cover of mixed shrubs; (3) Hedgerow comprising deciduous species delineating the margins of three fields. This area was primarily used for dairy cattle farming covering an area of 0.075 km^2; (4) Quarry Forest was planted in 1959, with a total area of 0.096 km^2 comprising mainly mixed coniferous blocks with a sparse understorey of mixed shrubs.

Locations were chosen within each site for sampling, ensuring that the whole trapping area was covered evenly. Mice were caught using Longworth live traps. Each sampling site was trapped for three consecutive nights with traps checked daily between 7 and 9 AM throughout the trapping period. All individuals were sexed, checked for reproductive condition and weighed. Biopsy tissues comprising 1 cm of tail skin were removed from each individual. Individuals were released immediately after biopsy sampling at the exact location of capture. Individuals were regularly re-trapped over a period of 3 days to 5 months, suggesting that the sampling technique did not adversely affect individual survival. Tail skin biopsies were stored in 99% molecular grade ethanol at 4 °C until DNA extraction. Tissue samples were taken from 415 individuals.

Microsatellite genotyping

Total genomic DNA was extracted from tissues following phenol–chloroform methodology described in Taggart *et al.* (1992). DNA concentration was determined using a spectrophotometer (μ-Quant Bio-Tek Instruments Inc., Winooski, VT, USA). Samples were standardized to a final concentration of 50 ng μL^{-1} and checked for quality on 0.8% 0.5 × TBE agarose gels containing ethidium bromide [10 mg μL^{-1} stock–8 μL gel^{-1} (80 μg)]. All individuals were screened for seven polymorphic microsatellite marker loci as follows: *As*-7, *As*-20, *As*-34 (Harr, Musolf & Gerlach, 2000); GCATD7S and TNF(CA) (Makova *et al.*, 1998); MSAF-8 (Gockel *et al.*, 1997); and WM2 (Barker, 2002). Initial polymerase chain reaction (PCR) conditions for these microsatellites followed those described by the respective authors. PCR products were separated by electrophoresis on 6% polyacrylamide gels run on a LI-COR 4200 automated sequencer. Infrared-labelled size standards (MicroStep-13b and 20a from Microzone™, West Sussex, UK) were run every 15 samples to assist the sizing of allelic fragments. At least one control sample (i.e. a sample of known genotype) was included in each run to ensure accuracy and consistency of typing among different gels. The Geneprofiler (v3.46) software (Scanalytics) was used to collect genotypic data from the LI-COR system.

Genetic data analysis

All samples were analysed together, with results interpreted taking in consideration geographical distance among samples (i.e. macro- and microgeographic scales). Summary population statistics (allelic diversity and frequency, expected and observed heterozygosity) and tests for departure from Hardy–Weinberg equilibrium (HWE) (Guo & Thompson, 1992) were calculated using the Genepop 3.4 software (Raymond & Rousset, 1995).

The level of population structuring and associated levels of genetic differentiation was assessed among samples from discrete sites using a number of independent approaches. Genetic differentiation between all pairs of samples was tested by means of exact tests for allelic (genic) frequency distribution as implemented in Genepop 3.4 (Raymond & Rousset, 1995). The Markov Chain parameters were set to 2000 dememorizations, 200 batches and 2000 iterations per batch.

Hedrick's (2005) standardized population differentiation statistic based on Weir & Cockerham's (1984) estimator (θ) of Wright's F_{ST} was calculated for both overall and pairwise comparisons. These statistics were obtained using RecodeData v.0.1 (Meirmans, 2006) and F_{STAT} v2.9.3.2 (Goudet, 2005) following procedure described by Meirmans (2006). Significance of F_{ST} values were assessed by permutation (1000 permutations over population samples) as implemented in F_{STAT}. This particular test was based on the original values of Weir & Cockerham's (1984) estimator (θ) of Wright's F_{ST}. In order to assess the patterns of genetic relationships between/among the six population samples, factorial correspondence analysis (FCA) based on allele frequency was used. A three-dimensional FCA plot was generated using the program Genetix 4.03 (Belkhir, 2002).

To test for genetic isolation by distance, pairwise standardized F_{ST} values calculated between sample sites were plotted against geographical distance at both the micro- and macrogeographic level using a Mantel test [measured by $F_{ST}/1-F_{ST}$ vs. log (geographic distance)]. The significance levels of observed associations were evaluated by permutation. This analysis was performed using Mantel v2 (Liedloff, 1999) using a total of 10 000 permutations. Straight line distances were estimated between all sample sites without considering whether the surrounding habitat facilitated movement of *A. sylvaticus*. Given the highly heterogeneous nature and scale of the area sampled this level of analysis could not have been conducted in a fashion likely to have led to interpretable results.

The program STRUCTURE v2.2 (Pritchard, Stephens & Donnelly, 2000) was used to identify the minimum number of populations that best explained the data and to subsequently assign individuals to these populations. This Bayesian clustering approach capitalises on the high level of polymorphism observed at microsatellite markers with the advantage that no *a priori* initial hypothesis of population structure is required. STRUCTURE analysis was carried out using the admixture model with correlated allelic frequencies. Simulations were run for 10 000 interactions

following a burn-in of length of 20 000. The following parameters were used: USEPOPINFO = 0, K (number of populations) ranged from 1 to 10. For each value of K, 20 runs were carried out to check concordance of the data. To identify the most likely value of K explaining the data, the approach described by Evanno, Regnaut & Goudet (2005), delta K, was used.

Results

Population statistics

Unambiguous genotypes at eight microsatellite loci were determined for a total of 415 individuals *A. sylvaticus* (mean = 408.3 individuals per locus) over six locations representing varying geographic distances. Full genotypic data were available for 98.4% of the specimens screened with no bias observed among loci. Summary population sample statistics are presented in Table 1. Allelic diversity varied considerably among loci and samples. Mean observed heterozygosity across loci per sample ranged from 25.6% (WM2) to 100% (*As*-20) with a mean value of 77% across all sites per loci. Observed heterozygosity ranged from 74.6% (Quarry Forest) to 79.9% (Creighton's Green).

The highest allelic diversity among samples was observed at Tollymore Forest Park (mean = 15.4 alleles locus^{-1}) and the lowest level at the Quarry Forest (mean-8.9 alleles locus^{-1}). Within the microgeographic samples, Creighton's Green had the highest allelic diversity (mean-11.1 alleles locus^{-1}) (Table 1). In order to determine whether these differences represent a real phenomenon or result from unequal sample sizes the rarefaction method of Petit, El-Mousadik & Pons (1998), which estimates the number of alleles that would be present in smaller sample sizes based upon the allelic frequency distribution, was adopted to standardize estimates of allelic richness based on the smallest sample (Table 1). Following this approach, the highest allelic richness was again observed at the Tollymore Forest Park, with a mean of 10.6 alleles locus^{-1}, whereas the lowest allelic richness was observed in the Moira sample, with a mean of 7.6 alleles locus^{-1}. Within the microgeographic samples, Creighton's Green, with a mean of 9.7 alleles locus^{-1}, was the site with the highest allelic richness, whereas the Quarry Forest, with a mean of 8.6 alleles locus^{-1} was the lowest. Following Bonferroni corrections for multiple tests, only one population sample deviated from HWE (Tollymore Forest Park; Table 1).

Population differentiation

All pairwise population sample genetic comparisons (i.e. heterogeneity tests for allelic frequency distribution), regardless of scale, were found to be highly significant (Table 2). The seven loci used in this study proved to be equally discriminatory with population samples differing significantly at two to seven loci in each case.

The overall standardized F_{ST} value for the six samples was estimated to be 0.185 ($P < 0.001$), confirming a considerable degree of genetic differentiation among populations. Excluding the population samples from Moira and Tollymore, the overall standardized F_{ST} value for the four samples from the Craigantlet region was also significant 0.179 ($P < 0.001$). All pairwise population sample comparisons based in standardized F_{ST} were found to be significant with values ranging from 0.086 to 0.304 (Table 3). Non-standardized F_{ST} values are several fold lower as expected from given bias associated with this statistics and microsatellite data (Hedrick, 2005; Jost, 2008). The greatest level of differentiation was observed between the Craigantlet region and Moira, with the Hedgerow sample, in particular proving most distinct ($F_{ST} = 0.304$; $P < 0.001$). The lowest levels of differentiation were observed at the microgeographic scale and involved pairwise genetic comparisons of samples from Cairn Wood, Creighton's Green and the Hedgerow system. However, the Quarry Forest sample, also located within the microgeographic area, displayed a considerable degree of genetic divergence in comparison to the three other samples.

FCA (Fig. 2a and b) substantiates the observed genetic structuring among samples by revealing a clear separation between all sample sites with surprisingly little overlap. Within the Craigantlet area, samples had a greater propensity to cluster with those within the same microgeographic region and were well separated from both Tollymore and Moira (Fig. 2a). However, further examination within this microgeographic area suggests a surprising degree of genetic divergence among population samples (Fig. 2b). Analysis of isolation by distance (data not shown) suggested a weak, positive association in the regression of genetic distance (standardized $F_{ST}/1-F_{ST}$) against geographic distance at both the micro- and macrogeographic scale. Results from the Mantel test, however, indicated that the observed associations at both levels were not significant ($P = 0.61$ and 0.69, respectively).

To account for the varying degrees of genetic divergence observed at both the micro- and macrogeographic scales, STRUCTURE runs were carried out for the full dataset and for samples from the Craigantlet area only. For the full dataset, the highest probability of the data was observed at $K = 3$. The Tollymore Forest Park and Moira samples anchor two of the clusters, while the third cluster was mostly comprised of samples from the Craigantlet area (Fig. 3a). The results of the STRUCTURE analysis based on samples from the Craigantlet area only, while not as clear as for the full dataset, confirm the existence of genetic substructuring also at the microgeographic scale. Thus, the probability of the data increases to reach a maximum value at $K = 4$. The best-defined cluster is anchored by the Quary Forest sample, with the three additional clusters more or less anchored by the individuals belonging to Cairn, Creighton's Green and Hedgerow samples, respectively (Fig. 3b).

Discussion

The present results indicate that there is a significant degree of population differentiation between all sample sites, not

Table 1 Summary statistics for *Apodemus sylvaticus* samples screened for seven microsatellite loci

Sample/microsatellite locus	*GCATD7S*	*WM2*	*TNF* (CA)	*MSAF*-8	*As*-7	*As*-20	*As*-34	Average
Creighton's Green								
n	41	40	40	38	41	38	41	39.9
A	9	4	14	19	10	14	11	11.6
H_O	0.756	0.300	0.850	0.974	0.854	1.000	0.829	0.795
H_E	0.827	0.343	0.859	0.926	0.863	0.909	0.854	0.797
A_R	7.79	3.51	11.76	15.77	9.10	12.79	9.84	10.1
HWE	0.551	0.149	0.844	0.288	0.023	0.504	0.04	0.054
Cairn Wood								
n	49	49	49	48	50	50	50	49
A	8	3	12	18	11	15	10	11.0
H_O	0.735	0.429	0.816	0.958	0.860	0.940	0.800	0.791
H_E	0.771	0.429	0.862	0.930	0.862	0.877	0.842	0.796
A_R	6.05	3.00	10.15	15.39	9.81	11.79	8.67	9.3
HWE	0.161	0.862	0.223	0.106	0.024	0.092	0.056	0.015
Hedgerow								
n	42	43	43	43	44	40	42	42.4
A	7	3	11	19	9	12	13	10.6
H_O	0.810	0.256	0.767	0.930	0.932	0.925	0.833	0.779
H_E	0.773	0.323	0.817	0.930	0.828	0.876	0.892	0.777
A_R	6.05	2.99	9.05	16.23	7.96	10.73	11.71	9.2
HWE	0.896	0.079	0.432	0.018	0.451	0.928	0.326	0.206
Quarry Forest								
n	24	25	22	25	25	25	25	24.4
A	6	4	11	15	11	9	8	9.1
H_O	0.708	0.520	0.818	0.960	0.880	0.960	0.520	0.767
H_E	0.736	0.460	0.869	0.909	0.784	0.840	0.575	0.739
A_R	5.91	3.87	11.00	14.45	10.37	8.75	7.63	8.9
HWE	0.427	1	0.156	0.484	0.092	0.354	0.171	0.249
Moira								
n	67	66	66	67	65	62	67	65.7
A	7	4	11	15	10	9	9	9.3
H_O	0.687	0.470	0.864	0.940	0.831	0.903	0.836	0.790
H_E	0.653	0.493	0.814	0.908	0.851	0.800	0.756	0.754
A_R	5.23	3.98	9.21	13.41	8.65	7.35	6.62	7.8
HWE	0.916	0.088	0.681	0.321	0.487	0.031	0.054	0.082
Tollymore Forest								
n	188	188	188	188	188	187	188	187.9
A	15	6	17	28	13	17	18	16.3
H_O	0.798	0.298	0.894	0.920	0.856	0.818	0.777	0.766
H_E	0.819	0.310	0.877	0.947	0.825	0.889	0.848	0.788
A_R	8.89	3.98	11.04	18.82	10.62	12.53	10.61	10.9
HWE	0.602	0.297	**0.001**	0.019	0.119	**<0.0001**	**0.005**	**<0.0001**

Significant values (after Bonferroni correction for multiple tests) are given in bold. Averages values per population sample are also provided.
A, number of alleles; A_R, allelic richness; H_E, expected heterozygosity (Nei, 1987); HWE, *P*-values of exact tests for non conformance to Hardy–Weinberg expectations (Guo & Thompson, 1992); H_O, observed heterozygosity; *n*, number of individuals screened per sample; HWE, Hardy–Weinberg equilibrium.

only at the macrogeographic scale where samples were separated by up to 30 km, but surprisingly also at the microgeographic scale within which samples were separated by as little as 850 m. Furthermore, there was only weak evidence that similarity in the genetic composition of this structured population decreased with increasing geographical distance. Given the lack of obvious geographic barriers to dispersal at the microgeographic level and the dispersal potential exhibited by wood mouse, it was presumed that extensive gene flow would occur among adjacent populations. The highly significant pairwise comparisons at both local and regional scale, however, clearly indicate that this is not the case. Hence, each location sampled seems to represent a discrete population unit with an effective population size sufficiently small for genetic drift to influence genetic differentiation (Berckmoes *et al.*, 2005). This result is unexpected given previous ecological and behavioural studies on *Apodemus* spp. (Wolton & Flowerdew, 1985;

Table 2 *P*-values for pairwise comparisons resulting from heterogeneity tests of allelic frequency distributions between *Apodemus sylvaticus* population samples

Pairwise comparison/locus	*GCATD7S*	*WM2*	*TNF* (CA)	*MSAF8*	*As-7*	*As-20*	*As-34*	All
Cairn & Creighton's	0.037	0.288	0.027	***	***	0.025	0.102	***
Cairn & Hedgerow	0.020	0.427	***	***	***	***	0.008	***
Cairn & Quarry	***	0.019	0.014	***	***	0.033	***	***
Cairn & Moira	***	***	***	***	***	***	***	***
Cairn & Tollymore	***	***	***	***	***	***	***	***
Creighton's & Hedgerow	0.325	0.809	***	***	***	**	0.054	***
Creighton's & Quarry	*	**	*	***	***	0.008	**	***
Creighton's & Moira	***	***	***	***	***	***	***	***
Creighton's & Tollymore	***	**	**	***	***	***	*	***
Hedgerow & Quarry	**	**	***	***	***	*	***	***
Hedgerow & Moira	***	***	***	***	***	***	***	***
Hedgerow & Tollymore	***	**	***	***	***	***	***	***
Quarry & Moira	***	***	***	***	***	***	***	***
Quarry & Tollymore	0.203	***	***	***	***	***	***	***
Moira & Tollymore	***	***	***	***	***	***	***	***
Number of sign comparisons	11	11	13	15	15	12	12	15

Values were estimated in Genepop (Raymond & Rousset, 1995). Values are given for individual loci and overall. Significant values (after Bonferroni correction for multiple tests) are represented as
$^{*}P<0.05$; $^{**}P<0.01$ and $^{***}P<0.001$.

Table 3 Population pairwise standardized F_{ST} values (below diagonal) and significant level ($P<0.05$) above diagonal

Samples	1	2	3	4	5
(1) Tollymore Forest	–	*	*	*	*
(2) Moira	0.224 (0.051)	–	*	*	*
(3) Creighton's Green	0.130 (0.027)	0.239 (0.055)	–	*	*
(4) Cairn Wood	0.154 (0.032)	0.292 (0.067)	0.860 (0.018)	–	*
(5) Hedgerow	0.144 (0.031)	0.305 (0.072)	0.120 (0.026)	0.124 (0.027)	–
(6) Quarry Forest	0.173 (0.040)	0.268 (0.068)	0.177 (0.041)	0.193 (0.044)	0.206 (0.050)

Values in parentheses represent Weir & Cockerham's (1984) 'non-standardized' estimator (θ) of Wright's F_{ST}, which are displayed for comparison.
$^{*}P<0.05$

Montgomery, 1989*b*) and, in particular, the evidence from the Craigantlet region that both male and female mice disperse frequently over considerable distances (O'Neill, 2001). The overall levels of heterozygosity and allelic diversity observed among the *A. sylvaticus* populations samples were comparable to or greater than that reported in other investigations based on microsatellite loci for this species (Gockel *et al.*, 1997; Makova *et al.*, 1998; Harr *et al.*, 2000) suggesting that the present results are characteristic of populations of this species.

Only the Tollymore Forest Park sample deviated from HWE due to a significant deficit of heterozygotes at several loci. The characteristic annual cycles of abundance in *A. sylvaticus* populations, where numbers can fluctuate by up to two orders of magnitude (Flowerdew, 1985, 1991; Montgomery, 1989*a*,*b*; Booth, 2005), may lead to substructuring within a population sample containing both over-wintered adults and young born that year. Sample collection within Tollymore Forest Park spanned the period of May–October. Therefore, this population sample contained both over-wintered adults and young born that year that by default do not interbreed. This mixture can potentially result in an admixture effect (i.e. 'Wahlund' effect) that could account for the observed discrepancies. When samples were split into their representative cohorts, however, deviations from HWE were still observed (data not shown). Thus, based on the results presented in here, we suspect that deviation within the Tollymore Forest sample arose from the existence of a certain degree of population admixture. That is, the sample of Tollymore is likely to be comprised of individuals belonging to distinct Mendelian populations.

Enclosure studies support the idea that populations of *A. sylvaticus* are structured on the basis of spatial associations among related individuals (Bartmann & Gerlach, 2001). It is important to note that the comparison of temporally disparate samples from the same area indicates that allelic frequencies change little over a 20-year period (Booth, 2005). This indicates that the spatial genetic structuring observed in the present study is not ephemeral resulting simply from the replacement of individuals from one generation to the next and the association of related individuals, but is a permanent feature of a population of a

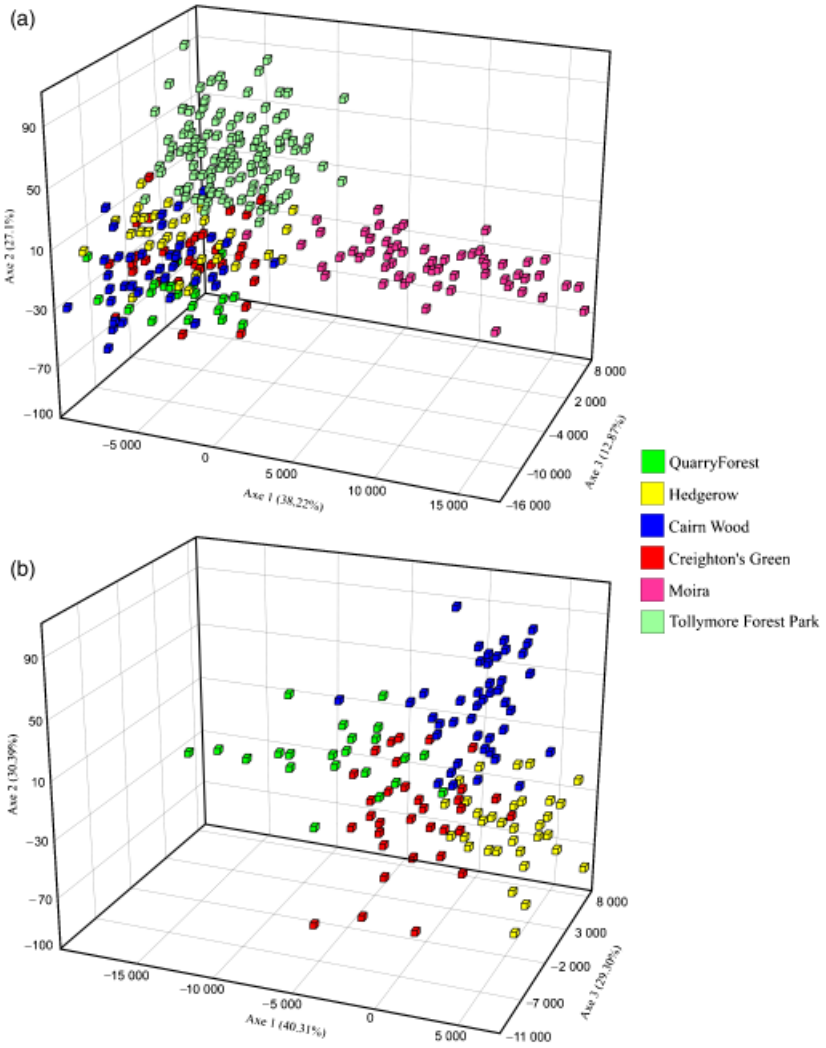

Figure 2 Genetic differentiation among individual wood mice *Apodemus sylvaticus* from six sampled locations based on factorial correspondence analysis of allelic frequency at seven microsatellite loci. (a) All samples considered together (i.e. representing micro- and macrogeographic scales); (b) only samples from geographically close areas (i.e. microgeographic scale).

species capable of using a range of habitats in a highly heterogeneous and fragmented landscape. Evidence for at least some degree population admixture is provided from the results of the STRUCTURE analysis involving Craigantlet area. This analysis not only confirms the existence of genetic substructuring at the microgeographic level, but also suggests that some of the samples, which were assumed to represent Mendelian populations, are most likely comprised of individuals from distinct breeding units. While results are not conclusive, STRUCTURE analyses involving the sample from Tollymore (data not shown), also indicate the existence of further substructuring within this sample as suggested above. Additional sampling of the area, and the use of additional microsatellite marker loci would have to be carried out to confirm this hypothesis.

There was no major association between genetic and geographic distance. Within the Craigantlet area, the Quarry Forest mice exhibited the lowest level of observed heterozygosity and the lowest allelic richness. This latter sample also exhibited the higher degree of genetic integrity or differentiation. Similar trends have been observed in populations of small mammal species, which are considered isolated (Stewart & Baker, 1992; Wauters *et al*., 1994). An obvious physical barrier that may contribute to such isolation is the active, heavily used quarry, which separates the Quarry Forest from Cairn Wood (Fig. 1). This quarry predates the commercial afforestation in the Craigantlet area, and represents a potentially hostile barrier to small mammal dispersal, featuring open terrain and thus lacking cover and dispersal corridors. Feral cats, foxes and raptors were commonly observed close to the quarry site. Experimental and observational evidence suggests that rodents suffer greater predation pressure from avian predators in open habitats compared with structurally more complex microhabitats (Simonetti, 1989). Furthermore, habitat selection studies suggest *A. sylvaticus* avoid areas without ground cover (Hoffmeyer, 1973; Abramsky, 1981; Montgomery, 1985). Isolation is further enhanced by the sparse hedgerow system bordering the Quarry forest, offering few routes that could be considered attractive as dispersal pathways by

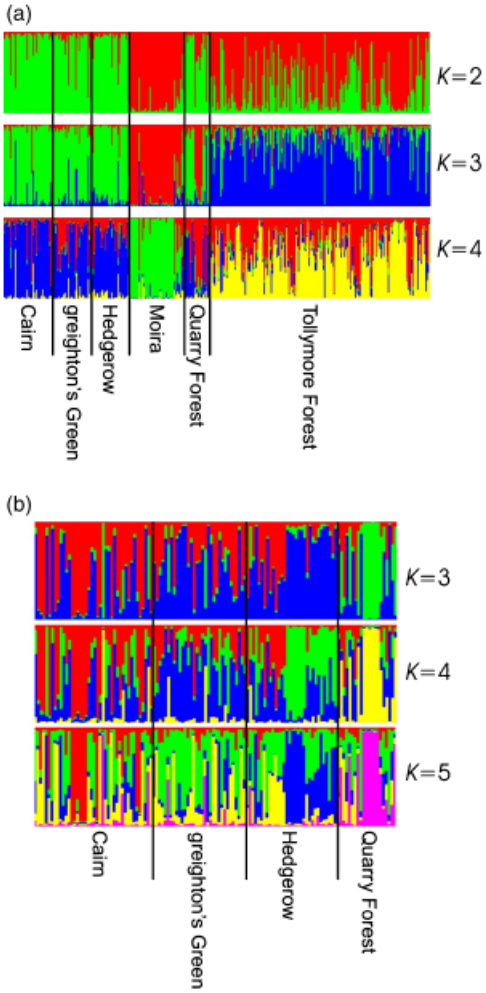

Figure 3 Estimated population structure from STRUCTURE analyses for: (a) the full dataset with *K* ranging from 2 to 4; (b) Craigantlet area on with *K* ranging from 3 to 5. Each particular individual is represented by a thin vertical line, which is divided into *K* coloured segments that represent the individual's estimated membership fractions in *K* clusters. Black thicker lines separate individuals from different samples. Individual bar plots are based on the highest-probability run (of 20) at a give value *K*. Individuals are arranged based on their sample of origin.

A. sylvaticus individuals. In contrast, the other Craigantlet sites are separated by a network of field boundaries and country roads <10 m in width, which would work towards promoting rather then preventing dispersal of small rodents (Gerlach & Musolf, 2000).

Genetic differentiation on the microgeographic scale, where individuals are highly mobile and can disperse distances well in excess of home ranges, presents a significant challenge for empirical studies of natural populations. The present results suggest a role for discrete habitat types in blocks, where there is no barrier to dispersal, but gene flow is nonetheless inhibited or limited. Thus, although individuals can freely move around a larger area, there is a tendency for breeding within natal grounds (i.e. homing behaviour). Thus, the differences in allelic frequency distribution among geographically adjacent areas are due to the enhanced effect of genetic drift acting on relatively small effective population sizes. The role of habitat blocks and homing behaviour in population structure of *A. sylvaticus* is now being investigated in a new study by the authors.

A number of man-made and natural physical barriers may inhibit the movement of individuals between more distant sites. Pairwise F_{ST} suggests that genetic differentiation at the regional level was lowest between Craigantlet and Tollymore Forest Park samples while those from Moira had a considerably higher degree of differentiation from Craigantlet region and Tollymore Forest Park. Three substantial physical barriers separate the Moira sample site from the two other macrogeographic sample areas. The River Lagan is wide enough to limit rodents swimming across and a number of recent studies have highlighted the importance of rivers as effective barriers to gene flow for mammalian species (Root *et al.*, 2003; Eriksson *et al.*, 2004). A second significant barrier to dispersal between Craigantlet and Moira is a motorway, one of the busiest commuting routes into Belfast city, separating Moira from the other two sample areas. Constructed in the 1960s, the motorway has presented a potential barrier to dispersal of mice for between 40 and 80 generations. Gerlach & Musolf (2000) noted that between 25 and 50 generations would be sufficient to result in the significant genetic differentiation observed among semi-isolated populations of bank vole *Clethrionomys glareolus*. Thus, the time-scale represented in the present study is sufficient to result in the higher level of genetic diversity detected between Moira and the other sites sampled. A third barrier to dispersal between Craigantlet and Moira that does not occur between any other sites is the city of Belfast. The city and its surrounding suburbs clearly separate *A. sylvaticus* at the two sites with no undisturbed habitat corridors linking the sampled populations. Hirota *et al.* (2004) suggested a higher level of genetic differentiation in populations of *Apodemus speciosus* separated by urban areas than between those in continuous habitat.

The present study illustrates both local and geographic population genetic structuring in a small rodent living under natural conditions. Despite considerable potential for dispersal and strong evidence of a high frequency of long distance movements in *A. sylvaticus*, there is significant population genetic structuring on a scale of *c.* 0.5–3 km. Dispersal evidently occurs but it does not necessarily translate into gene flow in this prolific and abundant rodent species. The present results support the observation by Montgomery & Montgomery (1989) that the movement of individual *A. sylvaticus* is limited to a single habitat type. Their contention was based on parasitological investigations but further research is required to determine the nature and frequency of occurrence of such interactions between mice in adjacent but different habitats. Given the significant role a number of small rodent species play in the spread of diseases, either as vectors or reservoirs for numerous diseases that can have devastating effects on both human and

livestock, it is evident from these results that a better understanding of genetic structure and evolutionary dynamics of other small rodent species is required in order to produce realistic models that will allow for prediction models of species–disease interactions. Ecology alone is not enough.

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