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Genetic diversity of island populations of the common shrew *Sorex araneus*

Thomas A. White & Jeremy B. Searle*

ABSTRACT. Populations of many species are currently being fragmented and reduced by human interactions. These processes will tend to reduce genetic diversity within populations due to genetic drift, inbreeding and reduced migration. Conservation biologists need to know the effect of population size on genetic diversity as this is likely to influence a population's ability to persist. Island populations represent an ideal natural experiment with which to study this problem. In a study of common shrews (*Sorex araneus*) on offshore Scottish islands, 147 individuals from 6 islands of different sizes and 2 mainland sites were trapped and genotyped at 10 microsatellite loci. Pairwise F_{ST} values (between 0.06 and 0.56) showed that all the island populations were significantly genetically divergent from one another. All island populations exhibited lower allelic diversity and heterozygosity than the mainland populations, and these measures of genetic diversity were positively correlated with log island size.

KEY WORDS: genetic diversity, island populations, *Sorex araneus*.

Thomas A. White [tawhite201@hotmail.com], Department of Biology, University of York, PO Box 373, York YO10 5YW, United Kingdom; Jeremy B. Searle [jbs3@york.ac.uk], Department of Biology, University of York, PO Box 373, York YO10 5YW, United Kingdom.

Генетическое разнообразие островных популяций обыкновенной бурозубки *Sorex araneus*

Т.А. Уайт, Дж. Б. Сирл

РЕЗЮМЕ. Ареалы многих видов могут редуцироваться или фрагментироваться под воздействием хозяйственной деятельности человека. Обычно такие процессы ведут к снижению уровня генетического разнообразия вида за счет дрейфа генов, инбридинга и сокращения миграции. Специалистам, занятым природоохранными мероприятиями, необходимо учитывать взаимосвязь между размером популяции и уровнем ее генетического разнообразия, что, как принято считать, связано с жизнеспособностью этой популяции. Островные популяции представляют собой идеальную природную модель для изучения такой взаимосвязи. В ходе исследования обыкновенной бурозубки (*Sorex araneus*) генотипирование по 10 микросателлитным локусам было проведено для 147 индивидуумов, представляющих популяции шести удаленных от берега шотландских островов различного размера и, кроме того, 2 материковые популяции. Оценка генетической подразделенности (F_{ST} от 0.06 до 0.56) показала, что все островные популяции существенно дивергировали друг от друга, причем размах изменчивости в них оказался уже по сравнению с материковыми популяциями. Корреляционный анализ позволил выявить позитивную связь между размерами островов и уровнем аллельного разнообразия и гетерозиготности населяющих эти острова популяций.

КЛЮЧЕВЫЕ СЛОВА: генетическое разнообразие, островные популяции, *Sorex araneus*.

Introduction

According to traditional population genetics theory, small isolated populations such as those existing on islands are expected to lose genetic diversity through random genetic drift and inbreeding (Frankham, 1996; Frankham *et al.*, 2002). Loss of genetic diversity is expected to lead to reduced evolutionary potential, inbreeding depression and increased probability of extinction. This has been shown by many studies (Eldridge *et al.*, 1998; Hinten *et al.*, 2003), but not all (Frankham, 1997). Many habitats are now becoming fragmented due to human actions, and populations in these fragments exhibit many of the same properties as island populations. From a conservation and species management point of view it would be very useful to find out at what rate populations of different sizes lose genetic diversity. Island populations represent ideal natural experiments for this kind of study.

The fieldwork for this project was carried out in the Inner Hebrides off the west coast of Scotland, where there are a range of islands of different sizes but with similar habitat types and climatic conditions. It is thought that these islands were colonised at the end of the last

* Corresponding author

glaciation, approximately 10,000 years ago (Yalden, 1982, 1999). Here we report our first results and the patterns of genetic variation in common shrews on these islands.

Material and methods

Collection of samples. Trapping of common shrews was carried out in the summer of 2004 on the west-coast Scottish islands of Raasay, Skye, Lismore, Gigha, Sanda and Islay. Mainland samples were also collected from Appin and Kintyre. Trapping locations are shown in Fig. 1. As far as possible, trapping sites were chosen to ensure a representative sample of the whole island or region. Specimens were collected for deposit at the National Museums of Scotland (Edinburgh, UK) and will be used for morphological and genetical analysis. The first molecular studies are described here. The specimens were stored in absolute ethanol.

PCR amplification. Twenty individuals from each island were genotyped at 10 microsatellite loci, except for Sanda where 7 individuals were used. For each individual, DNA was extracted using one hind foot with the Dneasy® kit (Qiagen). DNA was PCR amplified for 10 autosomal microsatellite loci (L2, L33, L68, L97, L14, L69, L9, L62, L57, L67) (Wyttenbach *et al.*, 1997; Balloux *et al.*, 1998; Borodin, 2002).

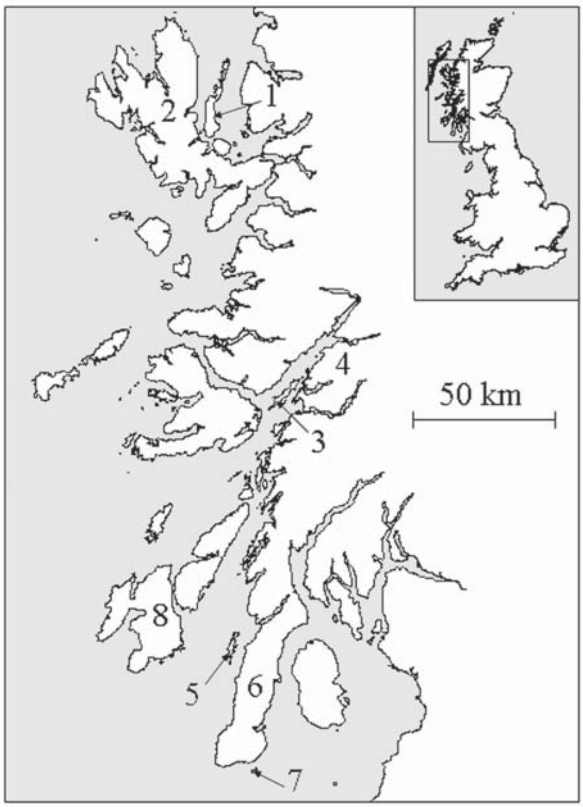

Figure 1. Outline map showing locations of island and mainland trapping sites in western Scotland: 1 — Raasay, 2 — Skye, 3 — Lismore, 4 — Appin, 5 — Gigha, 6 — Kintyre, 7 — Sanda, 8 — Islay.

Amplification was carried out in 25ml reactions containing 2ml template DNA (approximate concentration 50ng/ml), 2.5ml PCR buffer (Bioline), 0.8mM dNTP, 0.2mM of each primer and 5U of *Taq* polymerase (Bioline). $MgCl_2$ concentrations used were 4mM for L33, L97, L57 and L67, 3mM for L2 and 6mM for all other loci.

PCR comprised initial denaturation for 4 min at 94°C, followed by 37 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 60°C (for loci L14, L69, L9 and L62) or 55°C (all other loci) and elongation for 45 s at 72°C, and terminated with a final elongation step of 7 min at 72°C. Allele lengths were scored using GeneScan® 3.1.2.

Intra-population data analysis. The number of microsatellite alleles, observed and expected levels of heterozygosity and inbreeding coefficients for each locus in each population were calculated using the program FSTAT 2.3.9.2 (Goudet, 1995). Significance levels of F_{IS} were calculated using exact tests in FSTAT. FSTAT was also used to assess linkage disequilibrium between all pairs of loci within each population.

The program STRUCTURE 1.0 (Pritchard *et al.*, 2000) was used to confirm that there was no internal structure within the island populations. The program was run for 10^6 iterations after a burn-in period of 30000 iterations. Replicate runs were carried out to ensure that consistent results were produced with these parameters.

Each population was tested for heterozygote excess in order to detect recent population bottlenecks. The program BOTTLENECK (Cornuet & Luikart, 1996; Piry *et al.*, 1999) was run under the two-phase model of microsatellite evolution (Di Rienzo *et al.*, 1994) with 10% of the infinite allele model and 90% of the stepwise mutation model.

Inter-population data analysis. The degree of microsatellite genetic differentiation between pairs of populations was determined by calculating F_{ST} (Weir & Cockerham, 1984) and R_{ST} (Slatkin, 1995) using the programs FSTAT and GENEPOP 3.4 (Raymond & Rousset, 1995). The significance of the genetic differentiation was calculated using exact tests carried out in FSTAT.

Island size and genetic diversity. Two measures of genetic diversity, mean number of alleles per locus and expected heterozygosity were calculated for each island population and each measure was regressed onto log island size.

Results

Intra-population. Tests for linkage disequilibrium revealed that all loci were segregating independently.

Mean numbers of alleles per locus, mean expected heterozygosity, and mean F_{IS} for each population are shown in Tab. 1. It can be seen that the mainland sites Kintyre and Appin have large numbers of alleles and high heterozygosity levels. Skye, which is the largest

Table 1. Summary statistics for each of the populations in the study (* $p<0.05$).

Population	Island size (Hectares)	Mean expected heterozygosity	Mean number of alleles per locus	Mean F_{IS}
Kintyre	not applicable	0.78	10.2	0.05
Appin	not applicable	0.81	10.4	0.15*
Skye	164215	0.80	7.9	0.22*
Islay	60427	0.52	4.7	0.08
Raasay	6140	0.57	4.6	0.12
Lismore	1972	0.48	4.0	–0.06
Gigha	1267	0.39	3.5	0.12
Sanda	55	0.35	2.2	0.35*

island and the one most connected to the mainland, is also characterized by high genetic diversity. The other islands clearly have reduced genetic diversity compared to this background level.

F_{IS} levels show some inbreeding in some populations (Tab. 1). However, results from the program STRUCTURE (not presented here) show that each island is clearly acting as one population and there is no internal population structure on any of the islands.

Using the BOTTLENECK test for heterozygote excess none of the populations show any evidence of recent bottlenecking except for Raasay (Wilcoxon test, $p<0.01$).

Inter-population. Significant allele divergences were found for all populations ($p<0.001$). Genetic divergence levels as measured by pairwise F_{ST} and R_{ST} are given in Tab. 2. The two mainland areas, Appin and Kintyre, are the least divergent ($F_{ST}=0.06$, $R_{ST}=0.24$) and Skye is also comparatively genetically similar to these mainland populations. As Skye is a very large island this may be caused by retention of ancestral lineages rather than recurrent migration. All of the other island populations are highly divergent from one another. Islay in particular is very highly diverged from all the other populations. However, in each case island populations are most closely related to their nearest mainland population.

Relationship between island size and genetic diversity. Both measures of genetic diversity increased with log island size (Figs 2 and 3). Significant regressions were found for both expected heterozygosity ($F_{1,5}=8.243$, $p=0.045$, adjusted $r^2=0.592$) and mean number of alleles per locus ($F_{1,5}=12.281$, $p=0.025$, adjusted $r^2=0.693$).

Discussion

All the island populations in this study showed reduced levels of genetic diversity when compared to adjacent mainland populations. The island populations showed no internal structure, indicating that common shrews are able to disperse widely within islands. However, we found high levels of genetic differentiation between populations, indicating that marine channels are strong barriers to dispersal. As genetic differentiation is so strong we expect that migration between island and mainland populations is extremely rare.

There is a significant positive relationship between island size and genetic diversity. If island size is a good correlate of population size then these data support the theory that small populations lose diversity more quickly through genetic drift and inbreeding than large populations. Different habitat types support different numbers of common shrews, so island size may not be directly proportional to population size, depending on

Table 2. Matrix of genetic distances between pairs of populations. F_{ST} values are shown above right of the diagonal and R_{ST} values are shown below left.

	Raasay	Skye	Lismore	Appin	Gigha	Kintyre	Sanda	Islay
Raasay	–	0.21	0.29	0.17	0.37	0.18	0.37	0.32
Skye	0.43	–	0.24	0.07	0.35	0.10	0.26	0.28
Lismore	0.39	0.27	–	0.21	0.50	0.23	0.43	0.43
Appin	0.33	0.13	0.19	–	0.31	0.06	0.24	0.27
Gigha	0.84	0.70	0.77	0.49	–	0.27	0.57	0.40
Kintyre	0.69	0.43	0.57	0.24	0.38	–	0.26	0.19
Sanda	0.72	0.39	0.59	0.30	0.60	0.40	–	0.48
Islay	0.91	0.75	0.88	0.60	0.83	0.51	0.85	–

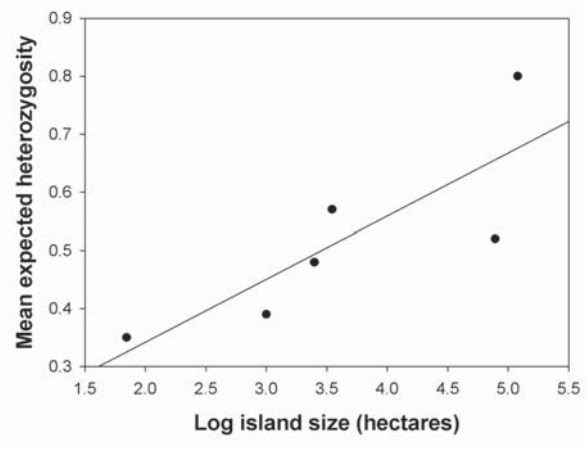

Figure 2. Relationship between expected heterozygosity and log island size. The line is fitted by least-squares regression.

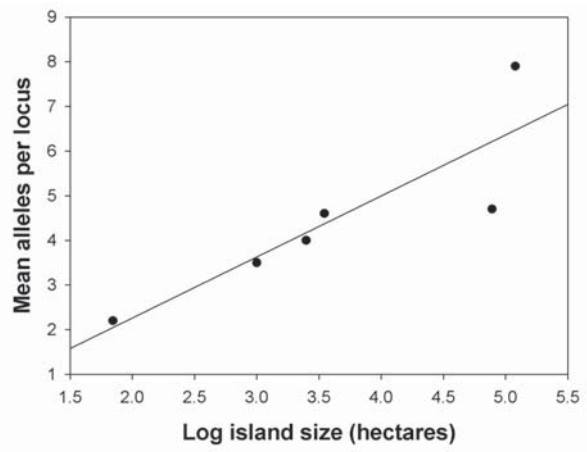

Figure 3. Relationship between the mean number of alleles per locus and log island size. The line is fitted by least-squares regression.

the habitat composition of each island. However, islands of the Inner Hebrides tend to be similar in their habitat composition so this is unlikely to be an issue.

Island size may also have been important during the initial colonisation of these islands. From the Theory of Island Biogeography (MacArthur & Wilson, 1967) colonists are more likely to reach larger islands, so these may have started out with greater levels of genetic diversity. As mentioned above, however, we expect that after the initial colonisation (by land or ice bridges), further migration was highly restricted.

In a meta-analysis Frankham (1997) found that in 165 of 202 comparisons, island populations have less allozyme variation than their mainland counterparts, the average reduction being 29%. The magnitude of these differences was found to be related to dispersal ability. The large reduction in genetic variation on islands found in the present study clearly accords with the limited marine dispersal associated with common shrews. Frankham (1996) also found significant positive correlations between genetic variation and the logarithm of island size in 16 of 19 studies involving mammals, birds, reptiles and an insect. Neutral models predict a sigmoidal relationship between heterozygosity and log population size. Frankham suggests that the linear relationship in his data set, and the one presented here, could be due to a proportion of mildly deleterious mutations or populations not having sufficient time to reach equilibrium. Microsatellites are generally considered to be neutral. Recent evidence suggests that this may not always be the case (Hammock & Young, 2005). Microsatellites may also appear to be under selection if they are closely linked to mildly deleterious alleles or if there is associative overdominance (Frankham *et al.*, 2002).

Nevertheless, the clear relationship between island size and genetic diversity found here supports the hypothesis that small populations lose genetic diversity at a higher rate than larger populations. If a reduction in genetic diversity reduces the fitness of a population and makes it more likely to go extinct, then this has important implications for conservation biology.

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