HERPETOLOGICAL JOURNAL, Vol. 11, pp. 23-27 (2001)

A GENETIC ASSESSMENT OF BRITISH POPULATIONS OF THE SAND LIZARD (*LACERTA AGILIS*)

TREVOR J. C. BEEBEE AND GRAHAM ROWE

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG

We investigated sand lizard (*Lacerta agilis*) populations in Britain by genetic analysis across eight polymorphic microsatellite loci. Genetic diversity as determined by mean expected heterozygosity was high in all three distinct regions where the species occurs (Dorset, Surrey and Merseyside), though allelic diversity was lower on Merseyside than in Surrey or Dorset. There was significant genetic differentiation between populations in all three of these widely separated zones, as judged both by Fst and Rst estimators. A genetic test for population bottlenecks confirmed that in at least two of the areas currently inhabited, Surrey and Merseyside, *L. agilis* has undergone substantial recent declines. The significance of these findings for sand lizard conservation is discussed.

Key words: sand lizards, *Lacerta*, conservation, genetics, microsatellites

INTRODUCTION

The sand lizard *Lacerta agilis* is one of only six reptiles native to Britain. It is also one of the two rarest, confined within recent historical times to three small areas of suitable habitat (Smith, 1951): the lowland heaths of Dorset and south-west Hampshire, heathland in the Surrey Weald, and the coastal dunes of Merseyside and North Wales. A major decline in the distribution and abundance of *L. agilis* has followed widespread losses of these critical habitats (e.g. Moore, 1962; Jackson, 1979; Corbett 1988; Webb 1990). Sand lizard populations have also become increasingly fragmented within the three distribution zones. These conditions can lead to genetic depauperization and inbreeding depression, issues that have caused concern among conservation biologists in relation to a wide range of endangered species, including sand lizards (e.g. Frankham, 1996; Olsson, Gullberg & Tegelstrom, 1996; Gullberg, Olsson & Tegelstrom, 1999). Although successful management practices have been developed for *L. agilis* in Britain and the species is given maximum protection under the law (Corbett & Tamarind 1979; Moulton & Corbett, 1999), neither of these important developments will necessarily alleviate any consequences of genetic impoverishment.

Polymorphic microsatellite loci are widely used markers for the study of population genetics in the context of relatively short (ecological) time periods (e.g. Jarne & Lagoda, 1996; Sunnucks, 2000). A suite of *L. agilis* microsatellites was recently developed by Gullberg, Tegelstrom & Olsson (1997) and used to investigate the structure of Swedish sand lizard populations (Gullberg, Olsson & Tegelstrom, 1998). In this paper we describe a study of British sand lizards, using those microsatellite markers to investigate genetic diversity and differentiation among animals living in the three areas where the species currently exists in Britain.

Correspondence: T. J. C. Beebee, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. E-mail: t.j.c.beebee@sussex.ac.uk

MATERIALS AND METHODS

SAMPLING

Terminal digits from toes were obtained, under licence, from lizards in all three areas of the British distribution (Fig. 1). Toe clipping causes no significant damage to the animal and for many years has been used as a marking procedure for *L. agilis*. Nevertheless, because the species is both rare and endangered the sample sizes were small. Seven Merseyside lizards, eight Dorset lizards and eleven Surrey lizards were toe-clipped during 1999 and the toes were stored in ethanol prior to DNA extraction. The Merseyside lizards and some of the Surrey lizards were maintained in vivaria for captive breeding purposes at the time of sampling, but all were wild-caught animals. None were from sites where there had been previous releases of translocated lizards. The Dorset animals came from two separate heathland sites (four from each). All the lizards were released immediately after sampling, either at the site of capture or back into vivaria.

MICROSATELLITE ANALYSIS

DNA was obtained from each toe by a standard proteinase K digestion, phenol-chloroform extraction and ethanol precipitation procedure. 50-100 ng DNA were used in PCR assays of final volume 20 µl, otherwise as described by Gullberg *et al.* (1997), using α^{33}P- dATP as a radioactive label and with separate primers for each of the microsatellite loci. The PCR products were electrophoresed through 6% polyacrylamide gels, subjected to autoradiography and alleles were scored by reference to M13 sequence markers all as described elsewhere (Rowe, Beebee & Burke, 1997).

DATA ANALYSIS

Conformance to Hardy-Weinberg equilibrium and linkage equilibrium between loci were tested using the computer programs BIOSYS-1 and GENEPOP 3.1

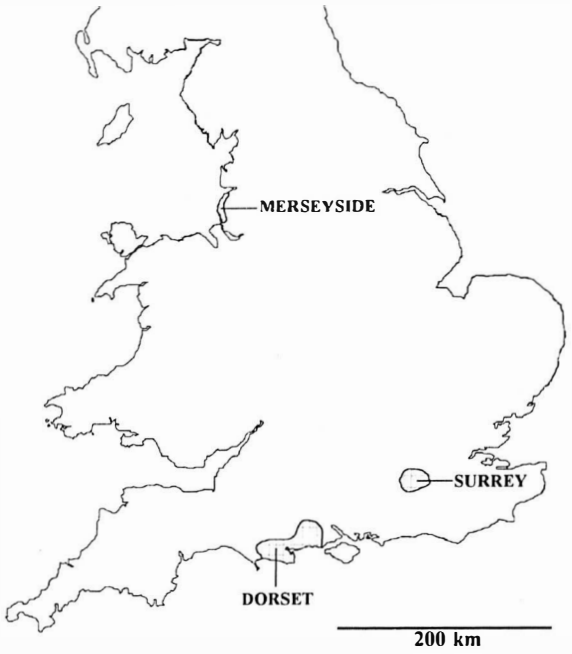

FIG. 1. Distribution of *L. agilis* in Britain showing sampling sites.

(Swofford & Selander, 1981; Raymond & Rousset, 1995). Genetic diversity indices, notably mean number of alleles per locus, percentage of loci polymorphic at the 95% criterion (P^{95}), observed and expected (unbiased) heterozygosities (H_o and H_e respectively) and Cavalli-Sforza chord (D_c) genetic distances (Cavalli-Sforza & Edwards, 1967) were also estimated using BIOSYS-1. Genetic differentiation between populations was measured by pairwise Fst (Weir & Cockerham, 1984) and Rst (Slatkin, 1995) using the programs FSTAT 1.2 and RSTCALC 2.1 respectively (Goudet, 1999; Goodman, 1997). Recent population trends were investigated with the BOTTLENECK program (Piry, Luikart & Cornuet, 1999). Randomization tests were carried out using the program RT version 2.1 (Manly 1997) using 5000 randomizations in two-sample comparisons. Other statistical analyses were performed using the STATISTIX computer package after testing data for normality as appropriate.

RESULTS

Of the 10 microsatellite loci available for study, eight (*La-1, -2, -3, -4, -5, -8, -9* and *-10*) demonstrated consistent polymorphic banding patterns in British *L.*

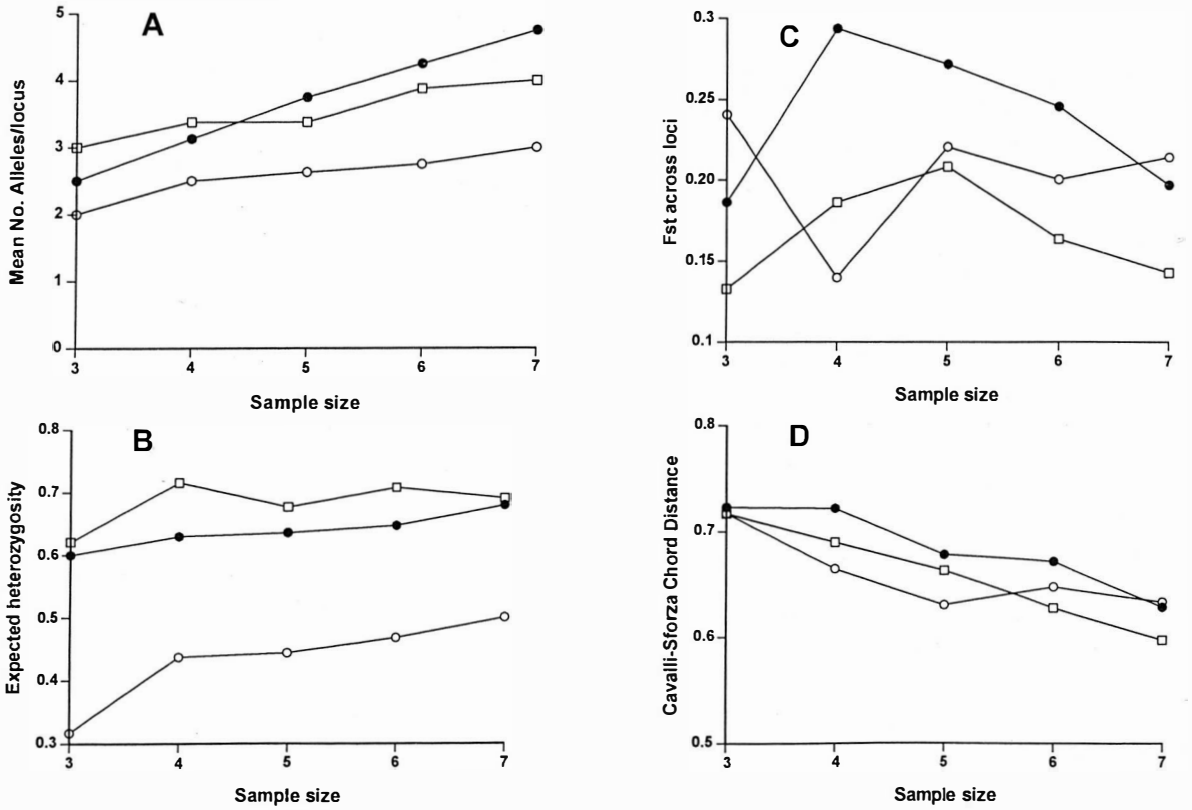

FIG. 2. Sample size dependency of genetic estimators. A: Allele number per locus. Data are averages of five randomly selected groups of each sample size except 7 (for which there was only one possible group in Merseyside). B: Mean expected heterozygosity, H_e. Data are averages of five randomly selected groups of each sample size except 7 (for which there was only one possible group in Merseyside). C: Mean Fst. Data are averages of five random comparisons between some of the randomly chosen groups used in A and B, excepting 7 where there was just one possible comparison. D: Cavalli-Sforza Chord Distance (D_c). Data are averages of five random comparisons between some of the randomly chosen groups used in A and B, excepting 7 where there was just one possible comparison. Solid circles: Dorset population (A & B) or Dorset x Merseyside comparison (C & D); Open circles: Merseyside population (A & B) or Merseyside x Surrey comparison (C & D); Open squares: Surrey population (A & B) or Dorset x Surrey comparison (C & D).

TABLE 1. Genetic variation among *L.agilis* populations. P^{95}, percentage of polymorphic loci at the 95% criterion; H_o, mean observed heterozygosity; H_e, mean expected heterozygosity; SD, standard deviation.

Location	Total alleles	Mean no. alleles/ locus±SD	P^{95}	H_o	H_e
Dorset	39	4.88±0.74	100	0.570	0.678
Surrey	37	4.63±0.68	100	0.549	0.691
Merseyside	24	3.00±0.57	75	0.423	0.500

agilis. *La-6* yielded no PCR products from British sand lizards while *La-7* produced no products from Swedish lizards (Gullberg *et al.* 1997) or from British ones. Dorset and Surrey lizards were polymorphic at all eight loci whereas Merseyside lizards were monomorphic at *La-3* and *La-10*. Only *La-10* in Surrey lizards failed to conform with Hardy-Weinberg expectations and no sets of loci showed significant linkage disequilibrium in any population. The markers were therefore considered appropriate for investigating genetic diversity in the British sand lizard populations.

Because the sample sizes were so small it was important to test the effects of this factor on the various genetic estimators. To do this, random samples of 3-7 individuals were selected from each population and a range of genetic parameters estimated as a function of sample size (Fig. 2). Mean allele numbers per locus and genetic distance (D_c) both showed linear sample-size effects within the range available for analysis. The absolute values of these parameters were therefore meaningless in the present study, but relative comparisons were nevertheless informative. Thus Merseyside allele numbers were consistently lower than those of Dorset and Surrey, whereas genetic distances were consistently similar between all three localities. Randomization tests indicated that Merseyside allele numbers were significantly lower than those in Surrey (P=0.030) or in Dorset (P=0.025) but that there were no differences in this parameter between Surrey and Dorset (P=0.356). By contrast, sample-size dependent trends were weak or non-existent for heterozygosity and differentiation estimators (H_e and Fst respectively), a situation which also held for Rst (data not shown). Estimates of the partitioning of genetic variation (pooling across all loci) indicated that inter regional differentiation (mean Fst=0.191) somewhat exceeded variation within regions (mean Fis=0.116).

TABLE 2. Genetic differentiation of *L. agilis* populations. The probability (P) that Fst or Rst is not significantly different from 0.

Comparison	Fst (P)	Rst (P)
Dorset x Surrey	0.133 (<0.001)	0.165 (0.02)
Dorset x Merseyside	0.186 (<0.001)	0.295 (0.009)
Surrey x Merseyside	0.241 (<0.001)	0.288 (0.001)

Estimates of the sample-size independent parameters for the full data set are shown in Tables 1 and 2. Taken together the data imply that genetic diversity in the Merseyside sand lizards was lower than in Surrey and Dorset lizards, which were broadly similar, though differences in H_e were not statistically significant (Kruskal-Wallis statistic = 2.1359, P=0.3437). Randomization tests of heterozygosity also failed to show any significant differences between regions. Genetic differentiation, however, was substantial between all three regions with both Fst and Rst values significantly different from zero in all pairwise comparisons.

Application of a bottleneck test based on excess heterozygosity relative to allele numbers (Cornuet & Luikart, 1996) supported the inference that there have been substantial recent declines in sand lizard numbers. Despite the fact that sample size was lower than that recommended for adequate statistical power in this test, two of the three areas (Merseyside and Surrey) demonstrated significant heterozygote excess – indicative of bottlenecking – with P=0.023 and P=0.027 respectively.

DISCUSSION

Although sample sizes were small, microsatellite analysis across eight loci has provided useful insights into the British sand lizard populations. Larger samples would have permitted statistically more robust analyses, but in our estimation these would probably not have altered the main conclusions. Genetic diversity was related to population size in the expected way, with the smallest and most isolated population, in Merseyside, demonstrating the lowest diversity of the three British regions. Recent estimates of adult sand lizard population sizes in Merseyside, Surrey and Dorset are in the region of 200-500, <1000 and 6000-8000 adults respectively (Corbett, 1994; Wheeler, Simpson & Houston, 1993). This relationship was unlike the situation in Sweden where, surprisingly, no such correlation between genetic diversity at microsatellite loci and population size was evident (Madsen *et al.*, 2000). Reasons for this difference are unknown, but the Swedish study was at a finer level of scale than ours and there is clearly scope for more detailed analysis of British sand lizard genetics within each region. One consequence of heathland fragmentation, in particular, may be a reduction in the genetic diversity of sand lizards at a local level. However, assuming our data are representative of regional patterns, lizards in all three areas maintained substantial diversity at microsatellite loci. Indeed, British *L. agilis* compare favourably in terms of heterozygosity with Hungarian animals tested across the same loci (mean H_e=0.70) and proved substantially more diverse than Swedish sand lizards with a mean H_e of 0.45 (Gullberg, Olsson & Tegelstrom, 1998).

Sand lizards also make an interesting comparison with natterjack toads (*Bufo calamita*), a species with a

similarly restricted distribution in Britain (Beebee, 1977). Natterjacks on the Merseyside coast were also assayed across eight microsatellite loci and exhibited lower genetic variation than sand lizards in the same area, despite the fact that the current census population size of natterjacks is at least tenfold larger than that of sand lizards (Corbett, 1994; Rowe, Beebee & Burke, 1998; 1999). Thus Merseyside natterjacks (with a sample size of 200) exhibited a mean of only 2.35 alleles per locus, a P^{95} of 62.5% and a mean H_e of 0.295. These differences may stem from the very different population dynamics of lizards and toads, with the latter undergoing larger population fluctuations over short time periods (Beebee, Denton & Buckley, 1996). Unlike lizards, toads have a breeding system in which many individuals in any particular generation probably fail to reproduce successfully (Scribner, Arntzen & Burke, 1997). Effective population sizes (i.e. numbers of animals reproducing successfully averaged over multiple generations) are therefore likely to be much smaller in these amphibians, relative to census sizes, than is the case with lizards. Both of these features are likely to impact on genetic diversity, although other reasons (such as different mutation rates in toads and lizards) could also account for the interspecific differences observed.

The estimators of genetic differentiation in sand lizards revealed significant differences between all three sample areas that are consistent with separation of the three regions at roughly the same time, presumably soon after postglacial colonization when forest development eliminated intervening open habitats (Vincent, 1990). These results also suggest that for conservation purposes populations in the three regions should be considered as distinct clades worthy of protection in their own right.

The genetic bottleneck tests indicated that there have been substantial recent declines of sand lizard effective population sizes in at least two of the three geographical regions. This independent genetic assessment of the fate of British sand lizards accords with conclusions derived from direct field survey (Corbett, 1994; Moulton & Corbett, 1999). There can be little doubt that this species has responded dramatically to the extensive losses of, and damage to, its sensitive heathland and dune habitats, and that conservation measures for it are fully justified.

ACKNOWLEDGEMENTS

We thank the Herpetological Conservation Trust, especially Chris Davies, Nick Moulton and Mike Preston for assistance with sampling, Madryn Lake and Inga Zeisset for assistance with the microsatellite analyses, Bryan Manly for advice about randomization tests, and Tony Gent, Peter Rothery and an anonymous referee for helpful suggestions. The work was carried out under Home Office licence PPL 70/3950.

REFERENCES

Beebee, T. J. C. (1977). Environmental change as a cause of natterjack toad (*Bufo calamita*) declines in Britain. *Biological Conservation* **11**, 87-102.

Beebee, T. J. C., Denton, J. S. & Buckley, J. (1996). Factors affecting population densities of adult natterjack toads *Bufo calamita* in Britain. *Journal of Applied Ecology* **33**, 263-268.

Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. *Evolution* **21**, 550-570.

Corbett, K. F. (1988). Distribution and status of the sand lizard *Lacerta agilis agilis* in Britain. *Mertensiella* **1**, 92-100.

Corbett, K. F. (1994). *Pilot study for sand lizard UK recovery programme*. English Nature, Peterborough.

Corbett, K. F. & Tamarind, D.L. (1979) Conservation of the sand lizard, *Lacerta agilis*, by habitat management. *British Journal of Herpetology* **5**, 799-823.

Cornuet, J. M. & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.

Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**, 1500-1508.

Goodman, S. J. (1997). RSTCALC: A collection of computer programs for calculating unbiased estimates of genetic differentiation and gene flow from microsatellite data and determining their significance. *Molecular Ecology* **6**, 881-885.

Goudet, J. (1999). *FSTAT, a program for IBM PC compatibles to calculate Weir & Cockerham's (1984) estimators of F-statistics (version 1.2)*. Institute of Zoology and Animal Ecology, Lausanne University, Switzerland.

Gullberg, A., Olsson, M. & Tegelstrom, H. (1998). Colonisation, genetic diversity, and evolution in the Swedish sand lizard, *Lacerta agilis* (Reptilia, Squamata). *Biological Journal of the Linnean Society* **65**, 257-277.

Gullberg, A., Olsson, M. & Tegelstrom, H. (1999). Evolution in populations of Swedish sand lizards: genetic differentiation and loss of variability revealed by multilocus DNA fingerprinting. *Journal of Evolutionary Biology* **12**, 17-26.

Gullberg, A., Tegelstrom, H. & Olsson, M. (1997). Microsatellites in the sand lizard (*Lacerta agilis*): description, variation, inheritance, and applicabilty. *Biochemical Genetics* **35**, 281-295.

Jackson, H. (1979). The decline of the sand lizard *Lacerta agilis* L. population on the sand dunes of the Merseyside coast, England. *Biological Conservation* **16**, 177-193.

Jarne, P. & Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution* **11**, 424-429.

Madsen, T., Olsson, M., Wittzell, H., Stille, B., Gullberg, A., Shine, R., Andersson, S. & Tegelstrom, H. (2000). Population size and genetic diversity in sand lizards (*Lacerta agilis*) and adders (*Vipera berus*). *Biological Conservation* **94**, 257-262.

Manly, B. F. J. (1997). RT: A program for randomisation testing. Version 2.1. Western Ecosystem Technology Inc., Wyoming, USA.

Moore, N. W. (1962) The heaths of Dorset and their conservation. *Journal of Ecology* **60**, 369-391.

Moulton, N. & Corbett, K. F. (1999) *The Sand Lizard Conservation Handbook*. English Nature, Peterborough.

Olsson, M., Gullberg, A. & Tegelstrom, H. (1996). Malformed offspring, sibling matings, and selection against inbreeding in the sand lizard (*Lacerta agilis*). *Journal of Evolutionary Biology* **9**, 229-242.

Piry, S., Luikart, G. & Cornuet, J. M. (1999). BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**, 502-503.

Raymond, M. & Rousset, F. (1995). GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Rowe, G., Beebee, T. J. C. & Burke, T. (1997) PCR primers for polymorphic microsatellite loci in the anuran amphibian *Bufo calamita*. *Molecular Ecology* **6**, 401-402

Rowe, G., Beebee, T. J. C. & Burke, T. (1998). Phylogeography of the natterjack toad *Bufo calamita* in Britain: genetic differentiation of native and translocated populations. *Molecular Ecology* **7**, 751-760.

Rowe, G., Beebee, T. J. C. & Burke, T. (1999). Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. *Animal Conservation* **2**, 85-92.

Scribner, K. T., Arntzen, J. W. & Burke, T. (1997). Effective number of breeding adults in *Bufo bufo* estimated from age-specific variation at minisatellite loci. *Molecular Ecology* **6**, 701-712.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457-462.

Smith, M. A. (1951). *The British Amphibians and Reptiles*. Collins, London.

Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology & Evolution* **15**, 199-203.

Swofford, D. L. & Selander, R. B. (1981). BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**, 281-283.

Vincent, P. (1990). *The Biogeography of the British Isles*. Routledge, London.

Webb, N. R. (1990) Changes on the heathlands of Dorset, England, between 1978 and 1987. *Biological Conservation* **51**, 273-286.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Wheeler, D. J., Simpson, D. E. & Houston, J. A. (1993). In: *The Sand Dunes of the Sefton Coast*. Ed. D. Atkinson & J. Houston. National Museums and Galleries, Merseyside.

Accepted: 12.1.01