Evolution in populations of Swedish sand lizards: genetic differentiation and loss of variability revealed by multilocus DNA fingerprinting

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Abstract

The Swedish sand lizard (*Lacerta agilis*) is a relict species from the period of warmth following the last glacial episode and has a fragmented distribution in central Sweden and a more continuous distribution in the southern part of the country. We used this model system of colonization–extinction for a study of genetic variability within and among Swedish populations from different parts of the distribution range using multilocus DNA fingerprinting. The results from the Swedish populations are then contrasted with those from a large Hungarian population in the centre of the species geographical distribution range, which is likely to closely resemble the ancestral founding population of Sweden. Swedish populations have a low level of genetic variability compared with the Hungarian reference population, which showed a genetic variability within the range described for outbred populations. Within the Swedish populations, the average bandsharing was 0.61, the mean heterozygosity 0.45 and the estimated number of alleles 2.7. The figures for the Hungarian population were a bandsharing of 0.19, a heterozygosity of 0.89 and an estimated number of alleles of 9.8. A population bottleneck, common to all Swedish sand lizards, is indicated by less than 20% of the alleles in the Hungarian population being retained in the Swedish populations, and higher bandsharing similarity between different Swedish populations (0.33) as opposed to the Hungarian population (0.19). The limited variability found in Swedish sand lizards is strongly subdivided between populations, with an average F_{ST} of 0.32, indicating a very limited gene flow between the isolated populations, as well as between populations in the region where the sand lizard has a more or less continuous distribution ($F_{ST} = 0.41$).

Introduction

Genetic diversity in natural populations mirrors the net outcome of forces that deplete and rejuvenate genomic variation. Therefore, a study which aims to investigate and empirically reconstruct this process in the wild can benefit from utilizing a model organism for which these parameters can be directly estimated or inferred from historical events. The Swedish sand lizard (*Lacerta agilis*) offers such a model system because it occurs in populations of various sizes and degree of isolation throughout

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the southern part of Sweden, an area for which we have considerable knowledge of the geological history. Furthermore, in recent years our group has addressed a broad range of questions in evolutionary biology on this species in, predominantly, one of these populations. We use the results from that work in the present study to discuss mechanisms that determine the dynamics of the genetic diversity in these populations.

The sand lizard (*Lacerta agilis*) has its main distribution in the centre of Europe with northern outposts in Sweden where the species is considered vulnerable, especially the isolated populations in central Sweden (Corbett, 1989). In the context of the present study, the wide distribution of sand lizards is a significant asset

because it offers an ideal opportunity for contrasting the genetic characteristics of the large continuous populations in south-eastern Europe with the corresponding genetic characteristics of the smaller more isolated Swedish populations. Since the colonization of Sweden from the European continent, there are strong reasons for assuming that the Swedish populations have declined and that they fit the description of classic bottleneck populations (*sensu* Nei *et al.*, 1975).

Our aim is to investigate the level of genetic variation within and between Swedish populations, and compare these with a central-European population (from Hungary). We do so to answer the following five questions: (i) Do the Swedish populations represent one large genetically homogenous but fragmented unit? (ii) How genetically variable are Swedish sand lizard populations, compared with those in the core area of the distribution? (iii) Can the history of individual populations reveal past population bottlenecks? (iv) Is the degree of relatedness between populations a simple effect of geographical distance and, hence, a constraint on gene flow? (v) Can the existing genetic variation be linked to population size?

Colonization history – from Pleistocene to present

About 10 000 ^{14}C years BP, the climate in northern Europe changed dramatically (Björck, 1995). In less than 100 years, the average summer temperature rose 5–6 °C (Lemdahl, 1990), which made immigration by heat-loving sand lizards possible via a land bridge that connected Scandinavia with Europe (Lemdahl, 1990). However, in probably less than 500 years this migration corridor was submerged by water from the melting glacial ice sheet (about 9200 ^{14}C years BP; Björck, 1995), whereafter sand lizards could no longer emmigrate to Sweden.

The period of post-glacial warmth had its optima between 8000 and 5000 years BP with an average temperature in Sweden which was 2–2.5 °C warmer than today (Hultqvist & Mörner, 1991). In this favourable climate, the sand lizard spread in the southernmost and central parts of Sweden. When the climate deteriorated during the Holocene, the average temperature decreased to about 1 °C below the present one (Kullman & Hofgaard, 1987; Kullman, 1988). At this point, it is likely that the sand lizard became extinct except in the most favourable localities. This scenario is supported by ^{14}C-dated fossil findings of another heat-loving European reptile, the pond turtle (*Emys orbicularis*) (Kurck, 1917; Aaris-Sørensen, 1988; Persson, 1992).

Today, the distribution of the sand lizard is more or less continuous in the southernmost and south-eastern part of Sweden, and there are isolated relict populations scattered mainly on glaciofluvial sand dunes in the central part of Sweden (Fig. 1, Table 1). The primary threat to Swedish sand lizard populations is the deterioration of
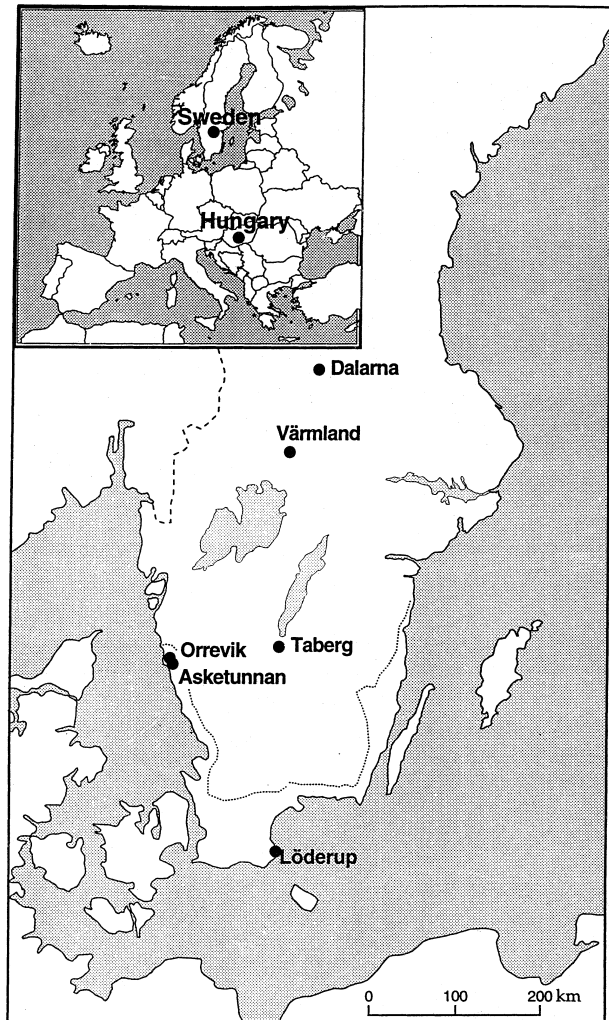

Fig. 1 The geographical locations of the sampling sites of the sand lizards in the present study. In the southernmost part of Sweden (south of the dotted line) the sand lizard occurs more or less continuously.

their habitats (Dent & Spellerberg, 1987; Corbett, 1989). This egg-laying species, on the northern border of its distribution, is restricted to areas with a high sunshine index (Dent & Spellerberg, 1987), and may have survived

Table 1 Distances (km) between pairs of populations. The distance between Asketunnan and Orrevik in Halland is about 3 km.

	Löderup	Halland	Taberg	Värmland
Löderup	–			
Halland	260	–		
Taberg	250	130	–	
Värmland	470	270	220	–
Dalarna	620	430	370	155

Table 2 Estimated population sizes (Gullberg, 1996), sample sizes (number of individuals) and the degree of isolation of the different populations.

Population	Estimated population size	Sample size	Degree of isolation
Hungary	>5000	15	With gene flow
Löderup	300	10	With gene flow
Asketunnan	500	30	With gene flow
Orrevik	50	9	With gene flow
Taberg	100–150	15	Isolated relict
Värmland	30–40	15	Isolated relict
Dalarna	2–300	30	Isolated relict

at several localities as a result of forest fires that frequently created succession areas suitable for basking and reproduction (Zackrisson, 1977). Thus, there is reason to believe that the very small numbers of individuals in some present relict populations (Table 2) are a consequence of reductions in population size that have occurred during the last 100 years.

To estimate the remaining genetic variation in these populations we used DNA fingerprinting, which is based on information from a variable number of tandem repeat (VNTR) loci; the hypervariable minisatellite regions. These loci consist of repetitive units of a common core sequence that makes it possible to screen multiple loci simultaneously. The extraordinarily high mutation rates within these regions (up to 0.001 or 0.05 per locus per generation; Jeffreys *et al.*, 1988) create the potential for a high level of variability. The exact mechanism behind the mutations is still unclear (Jeffreys *et al.*, 1994). The extreme variability demonstrated in minisatellite loci makes DNA fingerprinting a suitable method to examine evolutionary and ecological issues in inbred populations, which may have become almost monomorphic at allozyme loci.

Materials and methods

Samples were collected from a total of 122 individuals representing six Swedish localities and one Hungarian population (Fig. 1). Characteristics for the populations, sample sizes and the geographical distance between the Swedish populations are given in Tables 1 and 2, respectively. The populations within parentheses represent five Swedish regions: south-east Sweden (Löderup); Halland (Asketunnan and Orrevik); Taberg (one population); Värmland (one population) and Dalarna (one population). The two populations in Halland are separated by a distance of 3 km and migration is possible between these two localities. The samples from the remaining three regions, Värmland, Taberg and Dalarna, represent isolated relict populations. The population in the Värmland region is one of several small isolated populations, and the distance to the closest population

outside the region is ≈160 km. Population size was estimated by counting numbers of lizards observed per man hour under ideal basking conditions within the distribution boundaries of local populations (Gullberg, 1996). These numbers were compared with corresponding estimates for the Asketunnan population, which has been monitored in detail. This admittedly only generates an approximate population size; however, this is all we need to rank the populations by size, and these ranks can then be submitted to the statistical analysis (Table 2).

Blood was sampled from the corner of the mouth (*v. angularis*) and transferred to Eppendorf tubes with $1 \times$ SSC buffer (0.15 M NaCl, 0.15 mM trisodium citrate, 0.5 mM EDTA pH 7.0) and stored at -70 °C. Genomic DNA was purified by incubation of ≈30 μL of blood with 2.5 mL of SET-buffer (0.15 M NaCl, 0.05 M Tris-HCl, 1 mM EDTA pH 8.0), 50 μL of 25% SDS (w/v) and 70 μL of proteinase K (10 mg mL^{-1}) for 3–4 h at 55 °C. Proteins were removed by precipitation with 1.0 mL of saturated (6 M) NaCl, chloroform extraction and centrifugation. DNA was precipitated with ethanol and dissolved in 0.4–1.5 mL 0.01 M Tris-HCl, pH 8.0, for at least 24 h. DNA was digested with 30 units of restriction enzyme Alu I for 4 h at 37 °C, extracted once with phenol/chloroform, once with chloroform and precipitated with ethanol. The digested DNA was dissolved in 25 μL 0.01 M Tris-HCl, pH 8.0. DNA fragments (6 μg) were separated in 0.8% agarose gels for 1680 volt-hours and transferred to Pall Biodyne A transfer membranes by Southern blotting.

For the DNA fingerprints, we used the insert of the human minisatellite clone 33.15 (Jeffreys *et al.*, 1985a) as a probe, which was isolated by preparative restriction enzyme digestion and electrophoresis in low-melting-temperature agarose. The probe DNA was purified from the agarose using Gene Clean (Bio 101). We labelled 50 ng of probe DNA with ^{32}P-dCTP using the random primer method (Feinberg & Vogelstein, 1983) or 250 ng of the synthetic $(TC)_n$ polydinucleotide (Pharmacia LKB Biotechnology, Sweden), labelled with ^{32}P-dCTP by standard nick translation (Promega).

Prehybridization and hybridization with 33.15 were performed according to Georges *et al.* (1988) and hybridization with $(TC)_n$ was performed according to Ellegren (1991). Membranes hybridized with 33.15 were washed for 2×15 min in $2 \times$ SSC, 0.1% SDS at room temperature, 2×15 min in $2 \times$ SSC, 0.1% SDS at 58 °C and finally 10 min in $1 \times$ SSC at room temperature and autoradiographed at -70 °C for 1–6 days using Kodak X-omat AR and intensifying screens. For the $(TC)_n$ probe, the stringency wash was performed for $2 \times$ 15 min in $0.1 \times$ SSC, 0.1% SDS at 56 °C. The DNA probes were removed from the membranes by washing in 0.4 M NaOH and 0.2 M Tris-HCl, pH 7.5, and checked for remaining radioactivity before being rehybridized with the next probe.

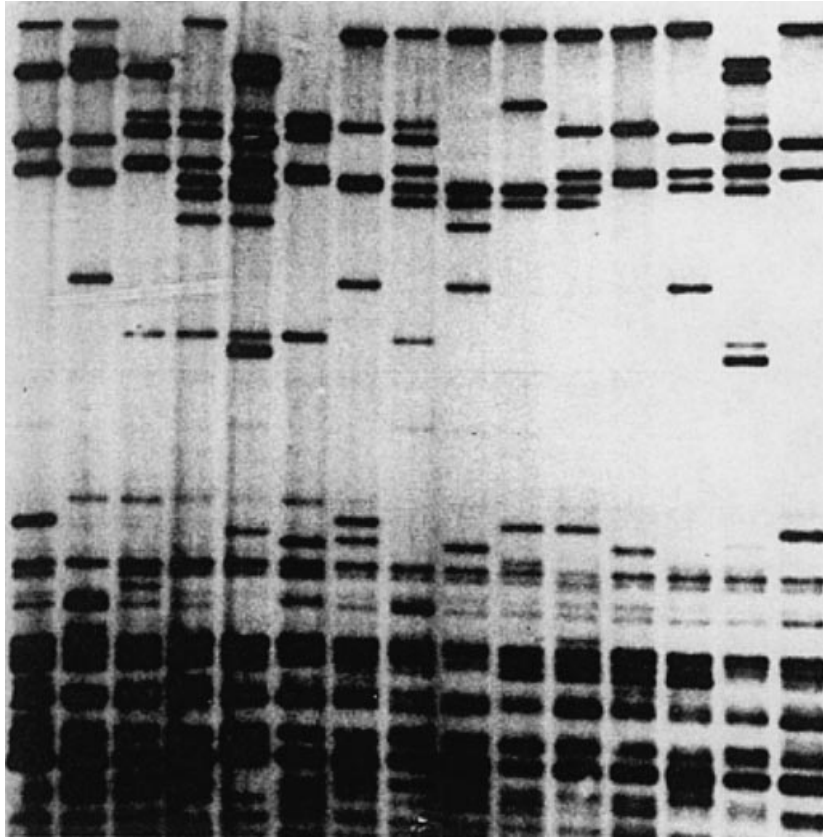Fragments >3–4 kb were scored for each probe. To be able to score and compare all lanes on a gel, we included

Fig. 2 DNA fingerprints with probe 33.15 for comparison of individuals within the population in Dalarna.

5 μg of Hind III-digested λ-DNA as an internal standard in each lane. After hybridizations with the DNA finger-printing probe (Fig. 2), we rehybridized the membranes with ^{32}P λ-DNA, using the same method as described above, to visualize the internal standard. The DNA fingerprint bands were scored as the same if their centres were within 0.5 mm of each other. No comparisons between individuals on different gels were performed. Due to limited amounts of DNA and number of lanes per gel, not all populations could be compared.

Band sharing between individuals (S_{xy}) was calculated as the number of bands shared between each pair of individuals (n_{xy}) divided by the total number of bands scored for both individuals ($n_x + n_y$),

$$S_{xy} = 2n_{xy}/(n_x + n_y).$$

The bandsharing value is identical to the similarity index (Lynch, 1990), the *D* value for unrelated individuals (Wetton *et al.*, 1987) and equivalent to Jeffreys *et al.*'s (1985a,b) value of *x* for unrelated individuals. For the bandsharing values within populations, a total of 774 pairwise comparisons were performed with the same individual involved in 8–14 comparisons. To estimate the bandsharing value between the Swedish populations, 202 comparisons were performed with the same individual involved in 3–5 comparisons.

The bandsharing values between pairs of lizards from four of the Swedish populations and the Hungarian population were estimated from 86 comparisons with the same lizard involved in 3–5 comparisons. Because the use of the same individuals in more than one comparison leads to a nonindependence of pairwise similarity values, we used a jack-knifing procedure to adjust for potential bias (Sokal & Rohlf, 1995). The calculation of the pseudovalues in the jack-knifing procedure was based on sequential removal of groups of pairwise similarity values involving each individual.

To investigate whether mutations in the minisatellites could interfere with the interpretation of our results, we looked for mismatched bands (i.e. bands that could not be traced to either of the parents) in a laboratory experiment with controlled parentage (Olsson *et al.*, 1994). We found no mismatch band in 92 offspring with a total of 879 bands in their DNA fingerprints. Therefore, the mutation rate in the minisatellites used for our DNA fingerprinting of the sand lizard is probably not higher than 10^{-3}.

The proportion of alleles (*A*) remaining after a reduction in the number of alleles compared with an original number of alleles can be estimated by the index $A = (n' - 1)/(n - 1)$, where *n* is the original number of alleles and *n'* is the number of remaining alleles (Jeffreys *et al.*, 1985b; Allendorf, 1986). We used the number of

alleles in the Hungarian population as an estimate of the original number of alleles.

We calculated the bias-corrected expected heterozygosity values and the estimated number of loci according to Jin & Chakraborty (1993). The mean group heterozygosity (H_S) for the populations being compared were calculated as the average of bias-corrected estimates of heterozygosity. To estimate the distribution of variability within and among populations, Wright's fixation index (F_{ST}) was calculated as $F_{ST} = (H_T - H_S)/H_T$ according to Nei (1973). The total heterozygosity (H_T) was estimated as the heterozygosity for the pooled data for all the compared populations. The heterozygosity values that were used to calculate the F_{ST} value were corrected for sampling bias (Webb *et al.*, 1995).

We used the PHYLIP v3.5 computer package (Felsenstein, 1993) to construct an UPGMA phenogram (Sneath & Sokal, 1973) from the dissimilarity indicies ($D = 1 - S$) between individuals.

Results

Number of alleles, and degree of bandsharing

Within populations

The total number of scorable bands per individual detected by the two probes ranged from 12.3 to 19.4 with an average (SD) of 15.6 ± 1.8 over all populations. For the probe 33.15, the average number of detected loci ranged between 5.5 and 9.7 with a mean of 8.1 ± 1.7. With probe $(TC)_n$, we detected on average 3.6 ± 2.4 loci per individual, ranging from 2.1 to 8.8. When both probes were used in combination, the average number of detected loci was 11.7 ± 3.5 per individual. We found the lowest number of loci for both probes combined in the Hungarian population (7.7), and the highest number in the Swedish populations Dalarna (9.7) and Asketunnan (8.8).

The Hungarian population showed the highest mean number of alleles, 9.8, per individual. In the Swedish populations we found a considerably lower number of alleles (mean 2.7, range 2.1–3.4), about one-fifth (19%) of the number of alleles found in the Hungarian population.

The bandsharing similarity between pairs of individuals within Swedish populations was on average 0.61 ± 0.08, and ranged between 0.26 and 0.94 (Fig. 3A). The corresponding value in the Hungarian population was 0.19 and ranged between 0.00 and 0.67 (Fig. 3B).

Between populations

Among the Swedish populations, the isolated northern relict Värmland population showed, somewhat surprisingly, the lowest bandsharing value between individuals (0.50), and the small population Orrevik on the southwest coast the highest degree of bandsharing (0.73). The
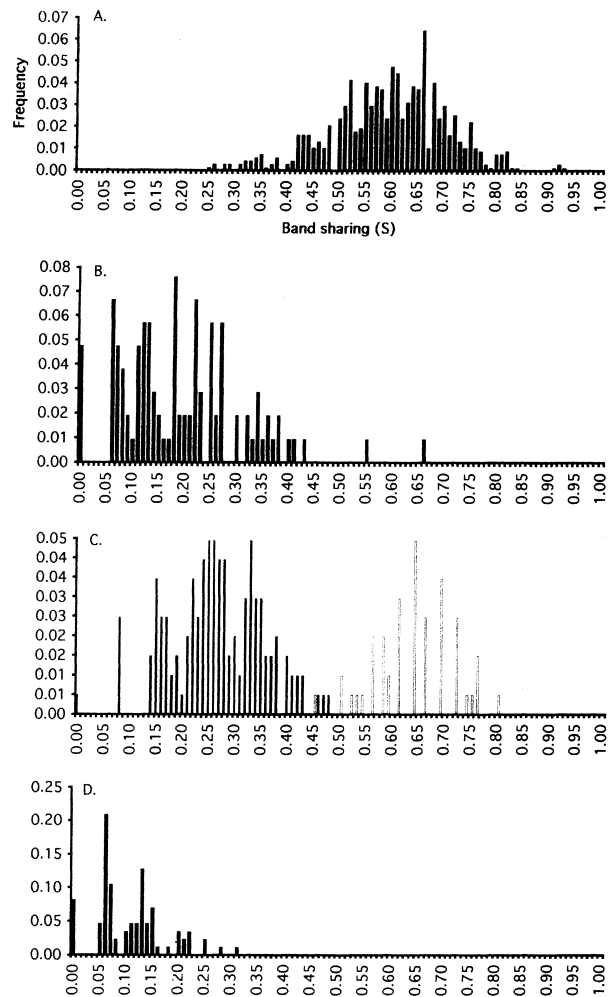

Fig. 3 The frequency of the bandsharing similarity between pairs of individuals (A) within the Swedish populations, (B) within the Hungarian population, (C) between the Swedish populations (□ comparisons between Asketunnan and Orrevik) and (D) between Swedish and Hungarian populations.

reference population in Hungary was extremely variable with a bandsharing value between individuals of 0.19, which is significantly lower ($P < 0.05$) than for all Swedish populations (Table 3).

The similarity between individuals from different populations ranged between 0.00 and 0.80 (Fig. 3C). Among the Swedish populations, the isolated relict Dalarna, the northernmost of all the populations, and Taberg, a relict in the centre of the Swedish distribution, showed the largest differentiation (similarity 0.24). The most similar populations were the two geographically closest populations Asketunnan and Orrevik on the Swedish west coast between which there is migration (similarity 0.65, Fig. 3C). The mean bandsharing between the Swedish populations was 0.33 ± 0.19, where-

Table 3 The average similarity (SE) for within and between population comparisons of individuals from Dalarna, Värmland, Asketunnan, Orrevik, Taberg, Löderup and Hungary based on DNA fingerprints with probes 33.15 and TC combined. Asterisks (*) indicate a significant ($P < 0.05$) difference in bandsharing similarity between populations.

Population	Dalarna	Värmland	Asketunnan	Orrevik	Taberg	Löderup	Hungary
Dalarna	0.652						
	(0.013)						
Värmland	0.260*	0.497					
	(0.033)	(0.021)					
Asketunnan	–	–	0.633				
	–	–	(0.011)				
Orrevik	–	–	0.650	0.729			
	–	–	(0.021)	(0.040)			
Taberg	0.245*	0.249	–	–	0.539		
	(0.031)	(0.051)	–	–	(0.036)		
Löderup	0.319	0.284	0.294	–	–	0.604	
	(0.048)	(0.065)	(0.038)	–	–	(0.036)	
Hungary	0.078*	0.127*	0.111*	–	–	0.120*	0.194
	(0.025)	(0.025)	(0.044)	–	–	(0.027)	(0.027)

as the corresponding figure for the four Swedish populations compared with the Hungarian population was on average 0.11 ± 0.02 and ranged between 0.00 and 0.31 (Fig. 3D).

Level of heterozygosity and differentiation between populations

The heterozygosity within populations ranged from 0.32 to 0.89 (Table 4). In Sweden, Orrevik showed the lowest heterozygosity (0.32) and Taberg the highest (0.59). For the Hungarian population the heterozygosity estimate was (0.89) and, hence, considerably higher than in the Swedish populations (average 0.45 ± 0.11).

The F_{ST} values for pairwise comparisons between the Swedish populations ranged from 0.141 (Asketunnan–Orrevik) to 0.412 (Asketunnan–Löderup), with a mean

of 0.322 ± 0.095, and revealed a strong subdivision of the genetic variability between the populations (Table 4). A genetic separation between the Swedish and the Hungarian populations is supported by the UPGMA tree (Fig. 4), demonstrating that individuals from two of the Swedish populations were more similar to each other than to the individuals from the Hungarian population.

To evaluate the robustness of the results of our heterozygosity estimates obtained with DNA fingerprinting (mean 0.514 ± 0.192, range 0.32–0.89), we compared these scores with heterozygosity estimates arrived at by using six microsatellite loci (mean 0.48 ± 0.169, range 0.21–0.70, Gullberg, 1996) for all seven populations. This correlation analysis was statistically significant (Spearman correlation coefficient, $r_s = 0.7857$, $P = 0.036$), which confirms that the two methods yield results in close agreement.

Table 4 Heterozygosity within populations, shown in bold type, and the F_{ST} value, shown in normal figures, for both probes combined. The number of comparisons are given within parentheses.

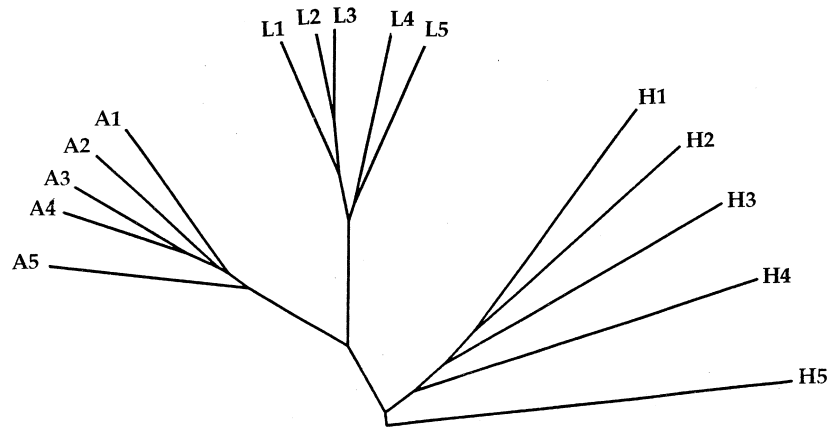| Population	Dalarna	Värmland	Asketunnan	Orrevik	Taberg	Löderup	Hungary
Dalarna	**0.407**						
	(210)						
Värmland	0.223	**0.570**					
	(39)	(105)					
Asketunnan	–	–	**0.380**				
	–	–	(210)				
Orrevik	–	–	0.141	**0.319**			
	–	–	(54)	(36)			
Taberg	0.167	0.187	–	–	**0.586**		
	(25)	(25)	–	–	(105)		
Löderup	0.276	0.268	0.412	–	–	**0.440**	
	(16)	(16)	(25)	–	–	(45)	
Hungary	0.239	0.108	0.267	–	–	0.237	**0.891**
	(12)	(12)	(25)	–	–	(37)	(105)

Fig. 4 UPGMA tree used on distance data obtained from DNA fingerprints of five individuals from Asketunnan (A), Löderup (L) and Hungary (H) hybridized with the probes 33.15 and (TC)$_n$.

Determinants of genetic diversity and similarity

Different population genetics models make different predictions about how genetic variation is eroded and re-established in space and time (e.g. Hanski & Gilpin, 1991; Slatkin, 1993; Avise *et al.*, 1995; Hanski *et al.* 1996; Lande & Shannon, 1996). To make a discussion of such scenarios possible, we looked for geographical patterns in how the remaining genetic diversity in the Swedish populations is distributed and how it relates to the source population in central Europe.

There were no strong trends between geographical closeness and genetic similarity between Swedish populations (Tables 1 and 3). However, a pattern with respect to between-population genetic similarity emerged when we categorized the populations depending on whether they were isolated relict populations or were more likely to have genetic interchange with other populations via migration (Table 2). Using this categorization of populations, we could consequently assign between-population bandsharing scores to either of two classes depending on whether the two compared populations were both isolated relict populations, or if they were scores between an isolated and a nonisolated population. The rationale for this was to contrast the similarity between relict populations alone, and between relict populations and populations from which relicts were likely to have received immigrants in the past. A Kruskal–Wallis test revealed that isolated relict populations had significantly lower bandsharing on average (mean 0.387, SD = 0.176, $n = 4$) than did the relict-interchange populations (mean = 0.251, $P = 0.008$, $n = 3$; $\chi^2 = 4.5$, DF = 1, $P = 0.03$). For F_{ST} values, none of the corresponding analyses was statistically signficant at the 0.05 level.

In summary, no clear relationship could be established between populations based on geographical separation. However, more importantly, relict populations are less similar to each other in genetic structure than they are to

other populations from where founding members of the population are likely to have come.

Because our data suggest that there is gene flow between the closely situated populations Asketunnan and Orrevik, separated by only a few kilometres, we calculated average F_{ST} and bandsharing scores for these populations and cumulated their population sizes.

Surprisingly, there was a strong positive correlation between population size and degree of bandsharing ($r_s = 0.90$, $P = 0.037$, $n = 5$), with a corresponding negative trend between F_{ST} values and population size ($r_s = -0.80$, $P = 0.10$, $n = 5$). One factor that could explain this paradoxical result is a confounding negative correlation between population size and distance to the source population in central Europe, and a strong trend for such a correlation was present ($r_s = -0.77$, $P = 0.07$, $n = 6$, correlation between latitude and population size, Table 2). To control for this confounder, we held latitude constant in a series of Spearman's partial rank-order correlation analyses (SAS, 1987). The result of these analyses further strengthened our conclusion that there was a strong positive relationship between population size and level of genetic similarity ($r_s = 0.99$, $P = 0.005$, $n = 5$). In the corresponding partial correlation analysis between F_{ST} value and population size, there was a trend towards a negative correlation ($r_s = -0.90$, $P = 0.097$, $n = 5$).

Discussion

The sand lizard populations in central Sweden might have been fragmented for more than 5000 years and the present population sizes of the relict populations are of the order of less than 100 animals. From this perspective, we would have expected a much lower level of genetic variability than we found in the present investigation; the close agreement between our minisatellite and microsatellite analyses (which will be reported elsewhere) confirms the robustness of our results. Indeed,

low genetic variation in bottlenecked populations has previously been demonstrated using multilocus DNA fingerprinting in birds (Tegelström & Sjöberg, 1995) and mammals (Gilbert *et al.*, 1990; Ellegren *et al.*, 1993).

The main fraction of the variability in Swedish lizards was among populations (Table 4). For example, the F_{ST} values between the Swedish and Hungarian populations indicate that the variability *within* the Hungarian population was higher than the variability *among* all Swedish populations. The distribution of the genetic variability between the Swedish populations probably reflects the present small population sizes and the absence of gene flow between the isolated populations, where stochastic drift of allele frequencies, combined with high mutation rates, has contributed to the genetic divergence of the populations. This is supported by the higher genetic similarity between individuals within Swedish populations (mean 0.61) than between individuals from different populations (mean similarity 0.33).

Small populations are sensitive to stochastic biotic and abiotic factors. In this context, the positive correlation between population size and genetic similarity between lizards within the Swedish populations contradicts the theoretically predicted inverse relationship; genetic diversity is supposed to increase, not decrease, with increasing population size. However, this prediction relies on the assumption that a large population becomes fragmented into smaller units of varying size and genetic diversity, which are then sampled at random for empirical analysis. This is unlikely to be true because at the fragmentation stage, when the population is going from outbreeding to inbreeding, relatively small populations with high genetic similarity are likely to go extinct first. Larger populations would have a higher buffering capacity against stochastic threats to survival and, hence, after a long exposure to such threats to population survival we may be left with relatively smaller populations with low bandsharing and larger populations with a varying degree of bandsharing from high to low. A correlation analysis between bandsharing and population size based on such data would result in a positive correlation coefficient, a result which offers a cautionary tale. Empirical studies of extinction phenomena may not always conform to classic population genetics theory simply because there may be no small populations with high bandsharing left at the sampling event.

Some populations may have been separated for such a long period that they are genetically different due to selective local adaptation or intrinsic coadaptation of the genome (Wilmsen-Thornhill, 1993). Our results are consistent with such a scenario in several ways. First, Värmland, being situated in the periphery of the Swedish distribution, is likely to have been among the oldest relict populations (i.e. first separated). Thus, selection may have had relatively longer time, compared with more southern populations, to purge the genome of recessive lethals. This is supported by a second observation. The Värmland populations and Asketunnan have been monitored in detail for conservation purposes and for a study of evolutionary ecology (e.g. Olsson *et al.*, 1996). The Värmland population, with low population size, does not show signs of inbreeding depression, such as high frequencies of young with deformities and low viability, whereas in Asketunnan, a relatively large population, the frequency of inviable young is over 10% (Olsson *et al.*, 1996).

The estimated fraction of remaining alleles in the Swedish populations was on average 19% compared with the Hungarian population. The low allelic diversity in Sweden, not only within but also among populations, suggests a historical population bottleneck common to all the Swedish populations. Allendorf (1986) showed that a population bottleneck of short duration had a limited effect on heterozygosity, but could drastically reduce the number of alleles, especially in highly polymorphic loci such as VNTR loci. Our observations in the sand lizard are congruent with Allendorf's results. Because of the rapid population growth which is likely to have occurred after the population bottleneck, the reduction in heterozygosity was probably not as severe as that for the number of alleles.

The similarity between individuals in the Hungarian population (0.19) was within the range found for other outbred species (0.1–0.3, Burke, 1989), and was low compared with the Swedish populations (mean = 0.61). Large populations in the centre of a geographical distribution are predicted to have higher genetic diversity compared with marginal populations (Sage & Wolf, 1986), and, hence, our results agree with this prediction. Furthermore, the heterozygosity in the Swedish populations (average 0.45) was almost half of that in the Hungarian population (0.89). We expected to find most of this variability in the populations from the more continuous distribution in south-east Sweden. However, the populations in that area (Löderup, Asketunnan and Orrevik) showed a lower heterozygosity (0.44, 0.38 and 0.32, respectively) than the average for Sweden (0.45), indicating that migration between subpopulations, even within a continuous distribution, is limited.

In summary, this investigation shows that the genetic variability in minisatellite loci in the Swedish sand lizard populations is considerably reduced, compared with a more central population in the geographical distribution range. A possible explanation to the reduced genetic variability is a population bottleneck during the immigration to Sweden subsequent to the last glacial period. Still, the populations showed a considerable level of variability in relation to the long period of isolation, which suggests that until relatively recently these populations were much larger than at present. The minisatellite variation in the Swedish sand lizard is strongly subdivided between populations also in regions where sand lizards are believed to have a more continuous distribution. This suggests that

different populations may be genetically unique and may harbour different deleterious alleles and/or may be adapted to different ecological conditions.

Acknowledgments

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