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Population size and genetic diversity in sand lizards (*Lacerta agilis*) and adders (*Vipera berus*)

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Abstract

Because low genetic diversity may threaten the viability of isolated populations, conservation biologists have devoted much effort to quantify genetic variation. Two techniques routinely used involve levels of mini- and microsatellite polymorphism, with the assumption that levels of variation at these parts of the genome will be reflected in levels of variation at other loci. Our data challenge this assumption. We studied six populations of sand lizards (*Lacerta agilis*) and five populations of adders (*Vipera berus*), differing considerably in size and degree of isolation. They, therefore, offer an opportunity to examine how population parameters affect genetic variation at different parts of the genome. Relative population size (based on degree of isolation and number of animals) was not correlated with either minisatellite variability or microsatellite heterozygosity. However, our measures of genetic diversity at the Mhc class I loci of both sand lizards and adders revealed a significant correlation between relative population size and Mhc polymorphism: non-isolated/larger populations exhibited higher genetic diversity than did isolated/small populations. Consequently, only the Mhc-based estimates of genetic diversity yielded results in agreement with population genetic theory.

Keywords: Population size; Population genetics; Minisatellites; Microsatellites; Mhc; Reptiles

1. Introduction

Advances in molecular biology during the last decade have stimulated many conservation biologists to quantify genetic variation both within and among populations. Two techniques routinely used to document genetic diversity in a multitude of vertebrate taxa involve levels of variability at mini- and microsatellite loci (e.g. Roelke et al., 1993; Haig and Ballou, 1995; Paetkau et al., 1995, 1998; Tegelström and Sjöberg, 1995; Houlden et al., 1996; Madsen et al., 1996; Sanjayan et al., 1996; Kretzman et al., 1997; Mundy et al., 1997; Prior et al., 1997; Scribner et al., 1997; Bouzat et al., 1998; Bushar et al., 1998; Gullberg et al., 1998a,b, 1999; Hitchings and Beebee, 1998; Komdeur et al., 1998; O'Ryan et al., 1998; Nader et al., 1999; Negro and Torres, 1999). Many of these studies have focused on endangered populations, and their results have often provided the basis for management recommendations.

Two central questions in conservation genetics are the degree to which "bottlenecks" and low effective population size will reduce genetic variability within a population, and the impact of this reduction on long-term viability of the population. In particular, will genetic variability be reduced to a similar degree throughout the genome, or will some loci exhibit more or less reduction?

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The strength of the relationship between genetic variation and population size is likely to vary for different categories of loci as they are subject to different intensities of selection (Frankham, 1996). If some loci are disproportionately important for the maintenance of viability, selection may retain a higher variance at these loci despite considerable reduction in variation in other parts of the genome. To test this prediction, we need to compare levels of genetic variability among populations with histories of high versus low effective population sizes, and compare variation at "critical" versus "non-critical" parts of the genome. Presumably, most mini- and microsatellite loci fall into the latter category, so the challenge is to identify a few "critical" loci. Recent studies suggest that the major histocompatibility complex (Mhc) loci may be ideally suited to this role (Hedrick, 1994; von Schantz et al., 1996; Vincek et al., 1997; Hedrick and Parker, 1998; Paterson et al., 1998).

The major histocompatibility complex (Mhc) codes for polymorphic membrane glycoproteins that play a key role in the T-cell mediated immune system (Klein, 1986). There are two distinct classes of Mhc molecules, class I and class II, which are encoded by separate but tightly linked loci. The antigen-binding properties of the Mhc class I and II molecules determine which foreign peptides can be identified for triggering an immune response and these Mhc determined differences are thought to influence disease susceptibility (Klein, 1986). In many vertebrate species the Mhc class I and class II loci exhibit an extraordinarily high degree of polymorphism (Klein, 1986). This variation possibly reflects balancing selection related to interactions of the immune system with pathogens (Parham and Ohta, 1996), although it is not resolved whether the selection is overdominant, frequency dependent or a combination of these factors (Hughes and Hughes, 1995).

In this study we document mini- and microsatellite polymorphism in six sand lizard (*Lacerta agilis*) populations, minisatellite polymorphism in five adder (*Vipera berus*) populations and compare these data with Mhc class I polymorphism recorded in all of the 11 populations. The study populations differ considerably in size and degree of isolation and, therefore, offer an opportunity to examine how these parameters affect genetic variation at different parts of the genome.

2. Materials and methods

2.1. Study populations

We sampled six sand lizard and five adder populations which were ranked according to their degree of isolation and size (below referred to as "relative population size"). The population estimates refer to adult lizards and snakes. A summary of the population demography data is presented in Table 1.

2.1.1. Sand lizards

Three of the populations that we studied are isolated relicts situated in south-central Sweden. These populations have been isolated for at least several hundred years (Gislén and Kauri, 1959). The number of lizards in these populations range from 85 to 115 (Värmland), 100 to 150 (Taberg) and 200 to 300 (Dalarna) (Gullberg et al., 1998b, 1999). A fourth population sampled is located on the west coast of Sweden (Asketunnan). This population has been studied since 1984 and consists of approximately 500 lizards (Gullberg et al., 1998b, 1999). However, several other populations occur within a few kilometers from this population and considering the high mobility exhibited by male sand lizards during the spring mating season (Olsson, 1986) migration/gene flow between these populations is, therefore, highly probable.

The fifth population sampled is located on the south coast of Sweden (Löderup). In this region sand lizards are very abundant over large areas and exhibit a con-

Table 1
Demographic and molecular genetic data of six sand lizard and five adder populations

Population	Population structure	Estimated population size	Relative population size	Minisatellite band sharing (%)	*N*	Microsatellite heterozygosity (%)	*N*	Mhc band sharing (%)	*N*
Sand lizards:									
Värmland	Isolated relic	25–40	1	49.7	15	60.0	21	84.0	14
Taberg	Isolated relic	100–150	2	53.9	15	61.0	14	90.6	12
Dalarna	Isolated relic	200–300	3	65.2	30	47.0	27	83.0	15
Asketunnan	Meta population	500	4	63.3	30	44.0	66	71.5	16
Löderup	Within a continium	>300	5	60.4	10	21.0	12	64.4	12
Hungary	Within a continium	>5000	6	19.4	15	70.0	25	51.6	15
Adders:									
Smygehuk	Isolated	15–40	1	79.3	9			95.6	9
Jukkasjärvi	Isolated relic	30–50	2	51.0	9			90.2	9
Hallands Väderö	Isolated island pop.	30–200	3	49.4	9			87.6	9
Genarp	Meta population	50–250	4	65.7	9			84.8	9
Lovön	Within a continium	>100	5	60.1	9			81.2	9

tinuous distribution (Gislën and Kauri, 1959). The population in Löderup consists of more than 300 lizards (Gullberg et al., 1998b, 1999). The last population consists of a sample collected in central Hungary where sand lizards are extremely abundant over vast continuous areas (Olsson, pers. obs).

2.1.2. Adders

The first adder population studied is located on the south coast of Sweden (Smygehuk). This population is totally isolated from other populations and has been studied since 1981. Over this period the number of snakes has ranged from less than 15 to 40 snakes (e.g. Madsen et al., 1996). Our samples were taken prior to the introduction of new males (Madsen et al., 1999). The second population sampled is the northernmost recorded adder population in Sweden (Jukkasjärvi). This population is an isolated relict. Since 1994 the number of adders has ranged from 30 to 50 snakes (Andersson and Madsen, in prep).

The third population is found on an island situated approximately 3 km from the southwest coast of Sweden (Hallands Väderö). Since 1983 population numbers have ranged from 60 to 200 adders (Madsen and Stille, 1988). The island has probably been separated from the mainland for several thousand years (Devoy, 1987). Adders have been recorded migrating between islands in the Swedish east coast archipelago, where sea salinity is about 0.6% (Forsman, 1995). However, along the Swedish west coast sea salinity ranges between 1.4 and 2.5%. Adders appear to avoid highly saline water and during a 10-year mark-recapture study of adder populations on two west coast islands, separated by less than 100 m, no adders were recorded migrating between the islands (Andrén and Nilson, pers. com.). We, therefore, assume that migration to and from Hallands Väderö will be extremely limited.

The fourth adder population studied is situated in an area of southern Sweden which harbours several subpopulations separated by 1–5 km (Genarp). These populations have been studied since 1984 and recaptures have documented inter-population movements of adult male adders (Madsen, unpublished). The number of adders in this area has ranged from 50 to more than 250 snakes. The last population sampled is located in a region of central Sweden (Lovön) where adders have a more or less continuous distribution (Gislén and Kauri, 1959). We have not undertaken any population studies in this area but our observations indicate that adders are extremely abundant (Madsen and Stille, unpublished).

2.2. DNA and statistical analyses

Genomic DNA was isolated from whole blood by salt-chloroform extraction (Mullenbach et al., 1989) and/or phenol-chloroform extraction (Sambrook et al., 1989). The methods used to document mini- and microsatellite polymorphism in sand lizards are thoroughly described in Gullberg et al. (1998a,b) and, therefore, only a brief account is given below.

Microsatellite polymorphism was only documented in the six sand lizard populations. Sixteen microsatellite clones were sequenced and primers were synthesised for amplification of six variable loci (Gullberg et al., 1998a,b). The number of alleles was determined by direct counting and expected heterozygosity was calculated assuming Hardy–Weinberg equilibrium.

Two probes were used to analyse sand lizard minisatellite variability, 33.15 (Jeffreys et al., 1985) and a synthetic (TC)n polydinucleotide. The probes did not exhibit any overlapping band pattern and therefore the number of bands were pooled in the subsequent analysis. When scoring adder minisatellite polymorphism only one probe (33.15) was employed. In both species genomic DNA was digested with Alu I restriction enzyme.

Restriction Fragment Length Polymorphism (RFLP) of sand lizard and adder Mhc class I genes was analysed using Mhc class I species specific probes. The probes are a cloned and sequenced PCR fragment (21.207 and 21.141 respectively) spanning 261 base pairs of the hypervariable exon 3 of a class I gene. Initially three adders were tested in a Southern blot analysis using five different restriction enzymes; Hind III, Pst I, Sac I, Taq I and Pvu II. All enzymes revealed polymorphism in combination with the Mhc class I probe. However, Pvu II revealed the highest degree of polymorphism and were subsequently used in all of the analysis. Southern blots were performed as described by Wittzell et al. (1994).

Mhc and minisatellite band sharing was calculated as the number of RFLP fragments shared between each pair of individuals divided by the total number of bands scored for both individuals. The different molecular data were obtained from the same individual sand lizards and adders.

Spearman rank correlations were employed in all statistical analyses.

3. Results

The three molecular methods revealed a substantial among-population variation in genetic diversity in both sand lizards and adders (Table 1). However, relative population size was not significantly correlated with minisatellite variability either in sand lizards ($r_s = 0.09$, $p = 0.85$, $n = 6$) or adders ($r_s = 0.20$, $p = 0.69$, $n = 5$; Fig. 1). Likewise, we did not detect any correlation between relative population size and microsatellite heterozygosity among the sand lizard populations ($r_s = 0.14$, $p = 0.85$, $n = 6$; Fig. 2). On the contrary, the five Swedish sand lizard populations exhibited a trend for a *negative* correlation between relative population

size and microsatellite heterozygosity ($r_s = 0.90$, $p = 0.07$, $n = 5$; Fig. 2).

The pattern was very different when we used Mhc class I polymorphism as our measure of genetic variability. Genetic diversity was significantly correlated with relative population size. That is, the less isolated and larger the population, the higher its genetic diversity (for sand lizards: $r_s = 0.94$, $p = 0.035$, $n = 6$; adders: $r_s = 1.0$, $p = 0.045$, $n = 5$; Fig. 3). To increase the power of our statistical analyses we pooled the data for the two species by standardising the estimates of genetic diversity (i.e. setting the mean to zero and the standard deviation to one). There was, however, still no correlation between population size and minisatellite variability ($r_s = 0.24$, $p = 0.48$, $N = 11$), whereas Mhc polymorphism was almost perfectly correlated with population size ($r_s = 0.96$, $p = 0.0001$, $N = 11$). Consequently we did not record any correlation between genetic diversity of mini-, microsatellites and Mhc class I polymorphism (adders: minisatellite band sharing versus Mhc band sharing; $r_s = 0.20$, $p = 0.68$, $N = 5$. Sand lizards: minisatellites band sharing versus Mhc band sharing; $r_s = 0.14$, $p = 0.75$, $N = 6$; microsatellite heterozygosity versus Mhc band sharing; $r_s = 0.14$, $p = 0.75$,$N = 6$).

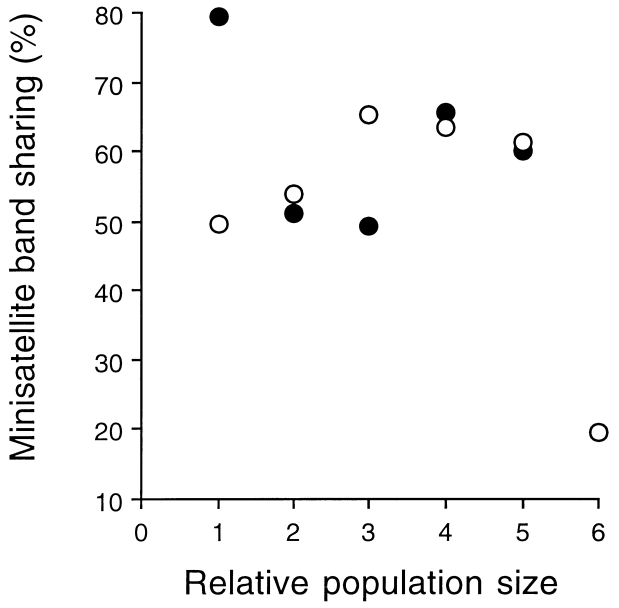

Fig. 1. Mean minisatellite band sharing and relative population size of six sand lizard populations (open circles) and five adder populations (filled circles). Populations denoted as in Table 1.

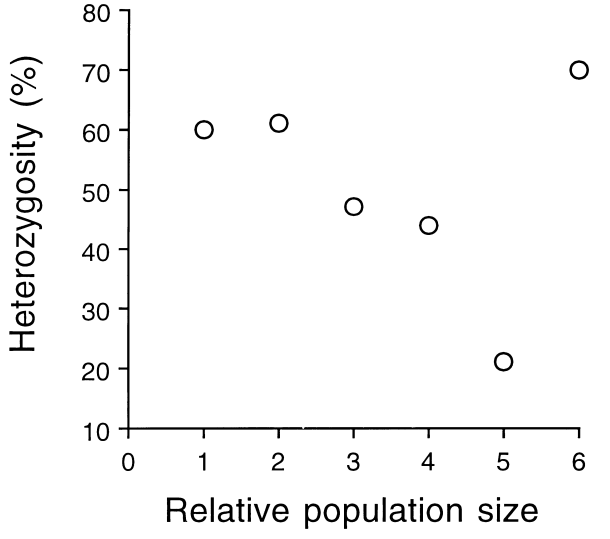

Fig. 2. Mean microsatellite heterozygosity and relative population size in six sand lizard populations.

4. Discussion

The different ways of measuring genetic diversity provided congruent results only for two of our eleven study populations; polymorphism was high in all three measures in the largest population of sand lizards (the Hungarian population), and was low for both minisatellites and Mhc variability in the smallest most isolated adder population (Smygehuk; Table 1, Figs. 1, 2 and 3). We have previously documented strong inbreeding depression in this latter population (e.g. reduced litter size and a high proportion of stillborn offspring; Madsen et al., 1996). The low level of Mhc polymorphism exhibited by the Smygehuk adders supports our previous interpretation.

In contrast to our expectations, we did not observe any correlations between relative population size and minisatellite variability/microsatellite heterozygosity

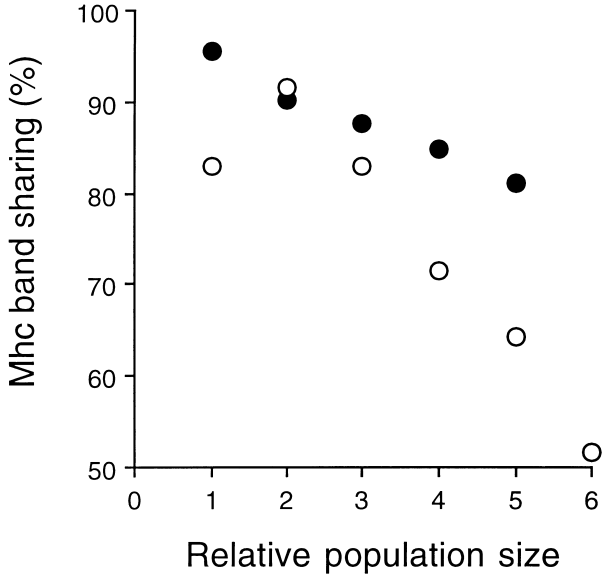

Fig. 3. Mean Mhc Class I band sharing and relative population size in six sand lizard populations (open circles) and five adder populations (filled circles).

among the six sand lizard populations or the five adder populations (Table 1, Figs. 1 and 2). For example, the three isolated relic populations of Swedish sand lizards (Taberg, Värmland and Dalarna) exhibited higher genetic diversity in both minisatellite polymorphism and microsatellite heterozygosity than the larger non-isolated populations (Asketunnan and Löderup; Table 1, Figs. 1 and 2). Among the five Swedish sand lizard populations there was in fact a trend for a negative correlation between relative population size and levels of microsatellite heterozygosity i.e. the larger the populations the lower the levels of heterozygosity (Fig. 2). Similarly, two of the isolated adder populations (Hallands Väderö and Jukkasjärvi) exhibited higher minisatellite variability than did the non-isolated populations at Genarp and Lovön (Table 1, Fig. 1).

However, our measures of genetic diversity at the Mhc class I loci revealed a significant correlation between relative population size and Mhc polymorphism among both the sand lizard and the adder populations: larger/less isolated populations exhibited higher genetic diversity than the smaller/isolated populations (Fig. 3). These results are in agreement with population genetic theory (Soulé, 1976; Frankham, 1996). Our results suggest that mini- and microsatellite techniques may provide ambiguous information concerning the relationship between population size and genetic variability. Thus, population genetic diversity estimates (and management decisions that spring from those estimates) may depend as much upon the method being used, as upon the underlying pattern of genetic variation within the study population. We, therefore, urgently need more work to compare the results from different techniques. Until such research has been conducted, data presented by Hedrick (1999) and our present results suggest that we should be cautious about framing conservation policies solely on data generated from studies of mini- and microsatellites.

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