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Biological Conservation 105 (2002) 127–130

BIOLOGICAL CONSERVATION

www.elsevier.com/locate/biocon

Short note

Low genetic diversity threatens imminent extinction for the Hungarian meadow viper (*Vipera ursinii rakosiensis*)

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Received 28 March 2001; received in revised form 10 July 2001; accepted 19 July 2001

Abstract

Meadow vipers (*Vipera ursinii*) are small venomous snakes whose range in Hungary has been greatly fragmented by anthropogenic habitat disturbance (especially, agriculture). We obtained DNA from a total of eight Hungarian snakes. Genetic variability at the major histocompatibility (Mhc) class I loci was much lower for these snakes than for specimens from two large Ukrainian populations. Within two Hungarian populations for which we had multiple individuals, band-sharing indices were 100 and 84.6% (versus 63.3 and 57% for the Ukraine populations). The Ukrainian snakes also displayed more RFLP fragments than the Hungarian vipers (mean 13.7 versus 9.0, respectively). In combination with reports of birth deformities, chromosomal abnormalities and low juvenile survival, these data strongly suggest that the Hungarian vipers are experiencing inbreeding depression. Genetic diversity is still present in the Hungarian vipers but among rather than within populations. Given the very low numbers of animals, the only feasible strategy to increase the genetic diversity and to save the Hungarian vipers from extinction is to implement a captive breeding program based on genetically screened animals.

Keywords: Population size; Genetic variability; Mhc; Snake

1. Introduction

Many taxa are declining worldwide, and the cause of this decline has attracted massive concern (Caughley, 1994). One major contributor to endangerment has been habitat fragmentation, where a formerly wide-ranging taxon persists only as a series of small isolated populations (Knick and Rotenberry, 1995). Small isolated populations are not only more vulnerable to extinction through factors such as demographic and environmental stochasticity, but the loss of genetic variation may further increase the risk of extinction (Lande and Barrowclough, 1987; Caughley, 1994).

From a pragmatic point of view, the first step in attempting to reverse a population decline is to identify the reasons for it. Only then can one implement the appropriate protective measures, which will differ according to the perceived threat. If the decline in population numbers is atributed to low genetic variation, then the amount of variation must somehow be increased (Caughley, 1994). Depressed genetic diversity may result in high frequency of stillbirths or neonatal deformities, and a continuing population decline even in areas not subjected to antropogenic modification (Madsen et al., 1999).

In the present study, we measured genetic variation at the Mhc Class I loci in a taxon, the Hungarian meadow viper (*Vipera ursinii rakosiensis*) that appears to be on the brink of extinction (Újvári et al., 2000). We compared levels of genetic variation in the few surviving Hungarian populations of *V. u. rakosiensis* with that of vipers collected in Ukraine which are considered as a different subspecies, *Vipera ursinii renardi*. The latter

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0006-3207/02/$ - see front matter
PII: S0006-3207(01)00176-8

taxon still occurs in large populations in southern Ukraine (Kotenko, 1989). Given the highly endangered status of the Hungarian vipers our sample sizes are very small and despite intensive field work from 1997 to 1999 we were only able to collect DNA samples from eight adult specimens. Nonetheless, the information available from these animals may offer the only opportunity to frame and implement measures to prevent the extinction of the Hungarian meadow viper.

2. Materials and methods

2.1. Study species and study populations

The meadow viper (*Vipera ursinii*) is a small (<60 cm) venomous snake, which is widely but discontinuously distributed through Europe and central Asia. Several subspecies have been described. The Hungarian meadow viper (*V. u. rakosiensis*) was formerly common through suitable habitats in Hungary, the easternmost part of Austria, Transylvania (Romania), and northern Bulgaria (Újvári et al., 2000). At present, the only extant populations occur in the Great Hungarian Plain between the rivers Danube and Tisza and in the Hanság Nature Reserve in the north-western part of the country (Újvári et al., 2000).

Five specimens were collected in an agricultural district 40 km south of Budapest, three individuals from a population close to Dög-hegy, one specimen from a population close to Dabas and one snake from the vicinity of Peszér. Each of these populations is separated from the others by 2–5 km of farmland. Three additional snakes were collected from the Bugac area, 45 km south of the other three populations and this locality is also surrounded by farmland.

Vipera ursinii renardi were collected from two sites in the Ukraine. One was at Orlov Island, in the Black Sea, 4 km west of the Yagorlitshky Kut Penisula in southern Ukraine. Meadow vipers are abundant on this small island (19 ha), attaining densities of 10–25 adult individuals per ha (Kotenko, 1989). We also sampled individuals from the coastal region of north-eastern Crimea where meadow vipers are extremely common (pers. obs.), though we have no data concerning their actual population numbers. We extracted DNA from a random subsample of eight individuals from each of the two populations.

2.2. DNA and statistical analyses

All captured vipers were marked (by micro chips), measured (snout-vent length), weighed, and ca. 50 μl of blood was collected from the caudal vein in each snake after which all specimens were released at their site of capture.

Genomic DNA was isolated from whole blood phenol-chloroform extraction (Sambrook et al., 1989). Restriction fragment length polymorphism (RFLP) of Mhc class I genes was analysed using Mhc class I probes developed for adders (*Vipera berus*). The probe is a cloned and sequenced PCR fragment (21.141) spanning 261 base pairs of the hypervariable exon 3 of a class I gene. Madsen et al. (2000) found that the restriction enzyme Pvu II revealed the highest degree of genetic polymorphism in adders and was therefore used in the present study. Southern blots were performed as described by Wittzell et al. (1994).

Mhc band-sharing was calculated between a pair of individuals as twice the number of bands shared between each pair of individuals divided by the total number of bands scored in both individuals. However, band-sharing data created using this method are not independent, which thus violates an underlying assumption of parametric hypothesis testing. To overcome this problem, Danforth and Freeman-Gallant (1996) suggested sub-sampling of independent comparisons of the overall data. We therefore restricted our analyses to independent observations and compared the Ukrainian and the Hungarian populations from which more than one individual was recorded. Pitman's permutation tests (available in StatXact 4) were employed in all statistical calculations.

3. Results

RFLP revealed that the three individuals from the Bugac population in Hungary were identical at their Mhc Class I loci, band-sharing index 100% (Table 1). Two of the three individuals sampled from the Dög-hegy population shared identical Mhc Class 1 loci (100% band-sharing) whereas the third individual differed from the other two. Thus, the mean band-sharing index among the Dög-hegy population was 84.6% (Table 1). The Bugac-Dög-hegy between-population band-sharing index was also high (71.1%) although the populations are separated by 45 km (Table 1).

However, the single individuals from Dabas and Peszér exhibited dramatically different band-sharing indices from each other and compared to the Dög-hegy and the Bugac populations (Table 1). These genetic difference are quite remarkable as the former three populations are separated by only a few kilometres.

In contrast to the Hungarian populations, the Ukrainian populations exhibited much lower within-population band-sharing indices (Orlov Island 63.3%; coastal region of Crimea 57.0%, Table 1). Band-sharing indices did not differ between the two Ukrainian populations (Pitman's permutation test, $P = 0.74$) and we therefore pooled these observations. Likewise there was no significant difference in band-sharing indices between the

Table 1
Mean band-sharing among the four Hungarian and two Ukrainian populations. Data are presented as mean ±S.E. (number of pair-wise comparisons)[a]

	Hungary				Ukraine	
	Bugac ($n=3$)	Dög-hegy ($n=3$)	Dabas ($n=1$)	Peszér ($n=1$)	Orlov Island ($n=8$)	Crimea ($n=8$)
Bugac	100±0 (3)	71.1±0.13 (9)	36.4±0 (3)	43.4±0 (3)	51.0±1.30 (24)	52.0±2.10 (24)
Dög-hegy	–	84.6±7.70 (3)	38.1±4.77 (3)	36.2±4.67 (3)	41.7±1.98 (24)	46.2±1.62 (24)
Dabas	–	–	–	44.4 (1)	44.3±1.65 (8)	45.5±2.34 (8)
Peszér	–	–	–	–	59.9±2.17 (8)	41.0±2.21 (8)
Orlov Island	–	–	–	–	63.3±2.81 (28)	48.8±1.30 (72)
Crimea	–	–	–	–	–	57.0±1.70 (28)

[a] S.E. is uncorrected for interdependence among band-sharing indices.

two Hungarian populations from which we have multiple samples (Bugac and Dög-hegy, Pitman's permutation test, $P=0.62$) and thus these observations were also pooled. A comparison of the pooled data showed that the Hungarian vipers exhibited significantly higher band-sharing indices compared to the Ukranian snakes (exact inference, $P=0.02$).

The Ukrainian snakes exhibited a significantly higher number of bands than did the Hungarian vipers (13.7 versus 9.0 respectively, exact inference, $P=0.0002$).

4. Discussion

We are well aware that our study is based on very small samples of Hungarian snakes but, as mentioned above, these were the only specimens from which we could obtain DNA samples. In contrast, meadow vipers were readily collected in the Ukraine. We thus conclude that the Hungarian populations are genuinely at low densities, and—given that they have decreased so rapidly over the last decade (Újvári et al., 2000)—may well be in imminent danger of extinction. The major factor for this decline is certainly caused by agricultural activities which have severely reduced and fragmented the few meadow viper habitats that now exist in Hungary. The dramatic difference in Mhc genotypes of the four specimens collected in the Dög-hegy-Dabas-Peszér region (where all the populations are separated by only a few kilometres) suggest that small barriers of farmland can reduce migration among populations. Another factor that has probably contributed to the decline of the Hungarian meadow viper is the collecting for scientific purposes and for the pet trade. In the Hanság region of north-western Hungary, collecting may have been a major factor in reducing the number of vipers in this area during the 1970s (Újvári et al., 2000).

Today, however, we consider that low genetic diversity, possibly through inbreeding depression, is the prime contributor to the present endagered status of these populations. In keeping with this interpretation, recent studies have documented severe neonatal deformities in four out of seven offspring in a brood from Bugac (an example depicted in Fig. 1), chromosomal abnormalities in the malformed offspring in the Bugac brood and from vipers captured in Dabas (Liptói et al., 1999), and low juvenile survival (Újvári et al., 2000).

The case for genetic factors influencing the viability Hungarian meadow viper populations is strengthened by our extensive studies on an isolated Swedish population of a closely related species, the adder (*Vipera berus*; Madsen et al., 1999). This population displayed much lower genetic variability than did adder populations from areas of continuous habitat, and stillbirths and neonatal deformities were more frequent (Madsen et al., 1996). These results thus echo those obtained from the Hungarian viper populations. Compelling evidence that population declines may be caused by genetic factors was demonstrated by the introduction of novel genes into the adder population which reversed the decline and eliminated the still-births (Madsen et al., 1999).

The Swedish study also offers a clear blueprint for remedial action to save the Hungarian meadow viper.

Fig. 1. Example of the severe deformities, e.g aberrant scalation, huge "misplaced" eye and deformed snout, exhibited by offspring from a female captured in Bugac, Hungary.

Given the extremely low numbers of snakes in wild Hungarian populations, simply moving snakes between the populations is unlikely to be successful. Instead, we advocate that a captive breeding program should be implemented. To do nothing is a recipe for the extinction of this subspecies. However, as our data demonstrate extremely low within-population variability but also large genetic difference between some of the populations, the vipers used in the captive breeding program should be genetically screened to avoid deleterious effects of inbreeding and outbreeding depression (Marshall and Spalton, 2000). The captive breeding program should thus be based on carefully selected individuals in consultation with relevant experts to increase the genetic variability and numbers of viable offspring, before rearing, releasing and monitoring the captive-bred vipers in suitable restored areas.

Acknowledgements

We thank Z. Korsós and T. Péchy for their assitance in obtaining DNA samples. Permits for the work were provided by the Directorate of the Kiskunság National Park and the Budapest Nature Protection Authority. The project was supported by the Hungarian National Research Fund (F23454) and the Hungarian Natural History Museum (to B.Ú.), the Hungarian Science Academy and the National Academy of Sciences of Ukraine (to B.Ú. and T.K.), the Australian Research Council (to T.M. and R.S.), the Swedish Natural Research council (to M.O.) and the Swedish Research Council for Forestry and Agriculture (to H.W.).

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