

## Conservation units in north-eastern populations of the Alpine newt (*Triturus alpestris*)

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### Abstract

Genetic variation in 19 populations of the alpine newt, *Triturus alpestris*, was assessed at the north-eastern margin of its range (southern Poland). Allozyme variation at five polymorphic loci was used in order to evaluate the degree of intrapopulation variation and interpopulation differentiation and define conservation units. Three management units were established on the basis of significant differences in allele frequencies. These correspond to three separate mountain ranges, i.e. the Carpathian, Sudetes and Holy Cross Mts. Contemporary gene flow between them is limited. In the extensively sampled Carpathian Mts, genetic structure is less pronounced, although still significant. Among population differentiation may be attributed to genetic drift in accordance with the isolation by distance model. The consequences of recent changes in land use in Central and Eastern Europe for low vagility and genetically structured organisms are discussed.

### Introduction

Conservation efforts pinpointing single species commonly focus on information obtained from population genetic studies. Molecular markers are used to estimate standard measures of genetic variability which are in turn combined with geographical information in order to spatially define groups of populations that are genetically similar to each other (Moritz 1994; Manel et al. 2003). The rationale behind preserving conservation units stems from a compromise between limited resources and the need to preserve as much genetic diversity as possible in a given species. Indeed, maintenance of genetic diversity lays at the basis of biodiversity conservation (Avice 1994; Frankham 2003) and is one of the factors influencing the probability of a population's persistence over ecological and evolutionary time. A loss of genetic diversity, often coupled with

increased genetic load, diminishes the long-term prospects of populations (Frankham 2003, Rowe and Beebe 2003) and may lead to extinction (Saccheri et al. 1998).

Widespread amphibian population declines (Houlahan et al. 2000; Alford et al. 2001), have fuelled investigations into many aspects of amphibian biology (see Collins and Storfer 2003 for perspective on this issue), including population genetic approaches (Storfer 2003). Pertinent studies in Europe are confirmed mostly to the western part of the continent (e.g. Hitchings and Beebe 1997; Garner et al. 2003; Rowe and Beebe 2003; Brede and Beebe 2004). Information concerning the genetic variation among amphibian populations in Central and Eastern Europe is scarce and limited to a few species (Črnobrnja et al. 1992; Gonzalez-Candelas et al. 1992; Litvinchuk et al. 1994; Arntzen and Wallis 1999; Babik et al. 2004). Considering the recent land use changes in Eastern

and Central Europe (Bouma et al. 1998) and the regional diversity of these changes (Mander and Jongman 1998), the evaluation of regional genetic variation in amphibians is of immediate importance if appropriate conservation measures are to be undertaken.

Here, we assess the population genetic structure of a pond-breeding amphibian, the alpine newt *Triturus alpestris* (Laurenti, 1768) at the north-eastern margin of its range. This species can be found throughout Central Europe. An eastern, disjunct part of its distribution is centered in the Carpathian Mts (see inset in Figure 1). Although the alpine newt is relatively common in most of its broad geographical distribution (Gasc et al. 1997), anecdotal evidence suggests Polish populations have steadily declined in recent years (Głowaciński and Rafiński 2003). We use allozyme variation at

five polymorphic loci in order to evaluate the degree of intrapopulation variation and interpopulation differentiation and define conservation units.

#### Materials and methods

Tail-tips were biopsied from a total of 791 individuals from 19 breeding populations of the alpine newt in the Carpathian, Holy Cross and Sudetes Mts (southern Poland, Figure 1, Table 1; geographical coordinates available upon request) in 1981 and 1982. Variation at allozyme loci was analysed by horizontal starch gel electrophoresis optimized previously (Rafiński and Arntzen 1987). Variation at five loci was scored – *Gpi*, *Ldh-1*, *Ldh-2*, *Mdh-2*, and *Pgm-2*. The loci were chosen because they are known to be polymorphic in this

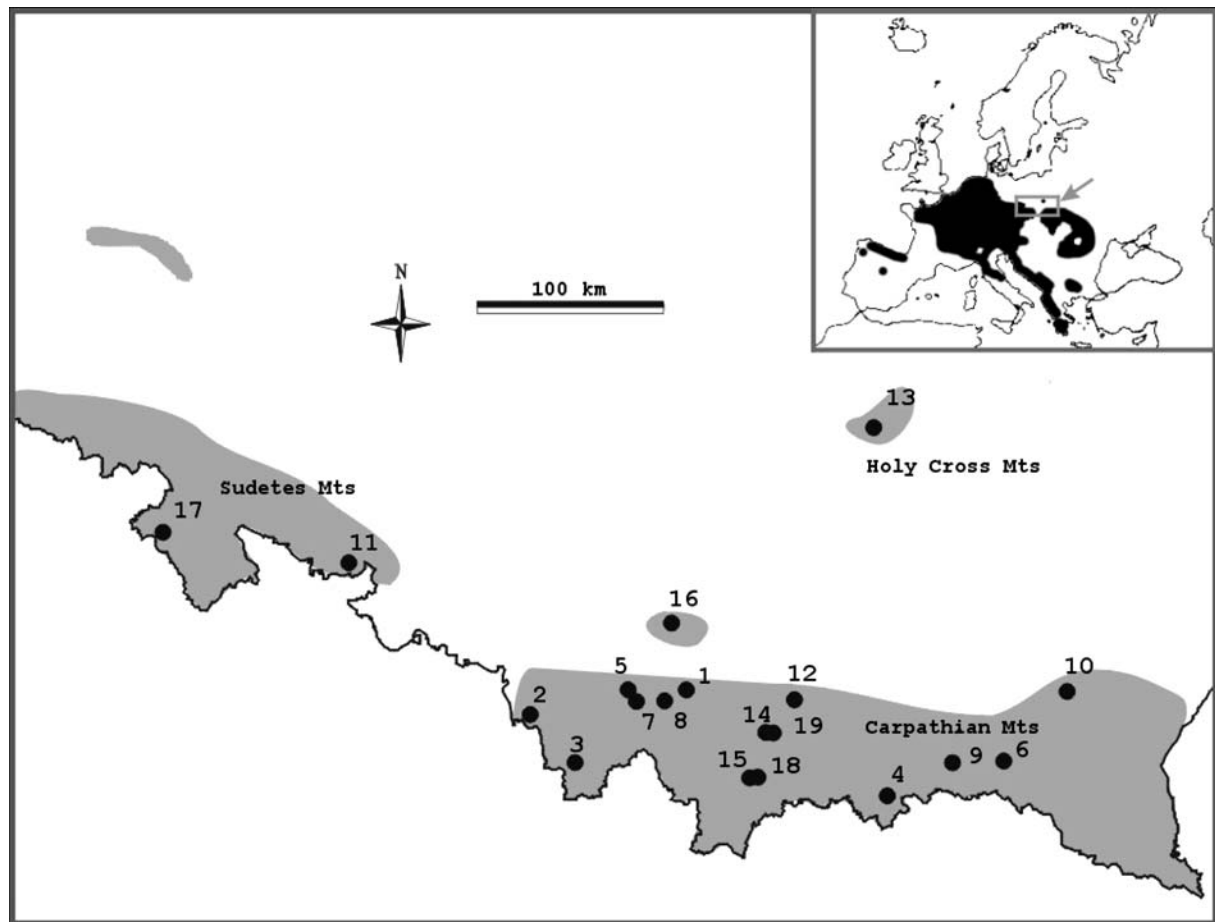


Figure 1. Distribution of the alpine newt *Triturus alpestris* in Poland and in Europe (inset). The locations of the studied populations are shown, numbered as in Table 1. The solid line represents the political border between Poland, the Czech Republic and Slovakia.

Table 1. Sampling localities of *Triturus alpestris*. Number of individuals ( $n$ ), mean number of alleles per locus ( $A$ ) and Nei's (1978) expected mean heterozygosity ( $H$ ). Mean  $F_{IT}$  and  $F_{ST}$  values (Wier and Cockerham 1984) with  $\pm 1$  SE and 99% CI in parentheses

Number	Locality	$n$	$A$	$H$
1	Lanckorona	60	2.2	0.416
2	Goleszów	42	2.2	0.440
3	Kasperki	30	2.2	0.413
4	Krynica	39	2.0	0.330
5	Andrychów	29	2.2	0.474
6	Dukla	39	1.8	0.322
7	Leskowiec	11	2.0	0.464
8	Skawce	35	2.4	0.427
9	Majdan	54	2.6	0.397
10	Wola Kąkolowska	45	2.4	0.401
11	Prudnik	50	2.2	0.396
12	Kamionna	46	2.2	0.417
13	Święta Katarzyna	46	1.8	0.239
14	Staw Zgniły	36	2.2	0.426
15	Staw Pucułowski	47	2.0	0.418
16	Tenczynek	39	2.0	0.405
17	Duszniki Zdrój	50	2.0	0.377
18	Staw Zawadowski	41	2.0	0.379
19	Mogielica	52	2.2	0.406
$F_{IT}$ all	0.111 $\pm$ 0.048 (0.035, 0.294)			
$F_{IT}$ carp	0.057 $\pm$ 0.026 (0.015, 0.136)			
$F_{ST}$ all	0.114 $\pm$ 0.036 (0.059, 0.247)			
$F_{ST}$ carp	0.043 $\pm$ 0.012 (0.023, 0.070)			

All, values for all populations; carp, values for populations from the Carpathian Mts.

species and because they could be unambiguously scored.

Allele frequencies, the mean number of alleles per population and mean heterozygosity using the algorithm of Levene (1949), equivalent to Nei's (1978) unbiased expected estimate, were calculated in POPGENE (Yeh et al. 1999). Deviations from Hardy–Wienberg equilibrium were assessed by Fisher exact tests or their Markov chain Monte Carlo approximations (Guo and Thompson 1992) using GENEPOP (Raymond and Rousset 1995). Wier and Cockerham's (1984)  $F$  statistics, genotypic disequilibria and pairwise tests of differentiation between populations were calculated in FSTAT (Goudet 2001). The sequential Bonferroni procedure was applied where appropriate to keep the Type I error level at  $\alpha = 0.05$  (Rice 1989). Nei's (1978) genetic distances ( $D_N$ ) were calculated in BIOSYS 1 (Swofford and Selander 1981). Mantel tests in GENEPOP (Raymond and Rousset 1995) were performed in order to test the effects of iso-

lation by distance for all populations and for only the Carpathian group.

Two tree building methods were used to determine among-population relationships: an unrooted neighbour-joining tree constructed from the matrix of Nei's (1978) genetic distances and a maximum likelihood tree from the matrix of allele frequencies; the robustness of both trees was tested with 1000 bootstrap replicates in PHYLIP 3.6 (Felsenstein 2004). Non-metric multidimensional scaling (NMDS) was also used to define genetically similar groups of samples. The calculations were performed in STATISTICA (StatSoft 2001).

## Results and discussion

Allele frequency data are available from the authors upon request. No deviations from Hardy–Wienberg equilibrium were detected and no significant linkage disequilibria existed between

any pair of loci. The populations from the Carpathian Mts were the most variable. The population from the Holy Cross Mts (13) was monomorphic in *Ldh-1* and had a high frequency of a single allele in *Gpi*. Populations from the Sudetes Mts (11 and 17) were monomorphic in *Gpi*. Nei's (1978) expected mean heterozygosity ( $H$ ) and the mean number of alleles per polymorphic locus ( $A$ ) are given in Table 1. Genetic differentiation among populations as measured by  $F$  statistics was significant (Table 1,  $F$  statistics for individual loci available upon request). As expected, geographically distant populations had significantly different allele frequencies (data not shown).  $F_{ST}/(1 - F_{ST})$  values were correlated with geographical distance when all populations were analysed ( $r = 0.116$ ,  $P < 0.001$ , 1000 permutations), correlation was much lower albeit still significant when only Carpathian populations were included ( $r = 0.013$ ,  $P = 0.021$ , 1000 permutations). Pairwise Nei's (1978) genetic distances are available from the authors. Genetic differentiation was not detected between several population pairs ( $D_N < 0.001$ ), while the highest  $D_N$  value (0.351) was between Święta Katarzyna (13) and Duszniki (17).

A similar overall pattern was revealed by the NJ tree, ML tree and NMDS analysis, therefore only the latter is shown (Figure 2). Clusters corresponding to the Sudetes and Carpathian ranges were evident and well supported. Despite low bootstrap support, the Święta Katarzyna (13) population from the Holy Cross Mts was consistently placed on a long branch from other populations using both dendrogram techniques. In the NMDS analysis, this population was also distant from others. The existence of three clusters is corroborated by the results of a previous study using a skin grafting method (Rafiński 1974). Contemporary gene flow between the clusters is probably very limited. In fact very few, if any, populations of the alpine newt exist at lower altitudes separating the Holy Cross Mts from the Sudetes or Carpathian ranges (Głowaciński and Rafiński 2003) and a gap in the distribution of this species is also apparent between the Carpathian and Sudetes Mts (Figure 1). The alpine newt populations inhabiting different mountain ranges in southern Poland meet the "discrete and significant" criteria of management units (Moritz 1994), meaning that they are physically separated and genetically

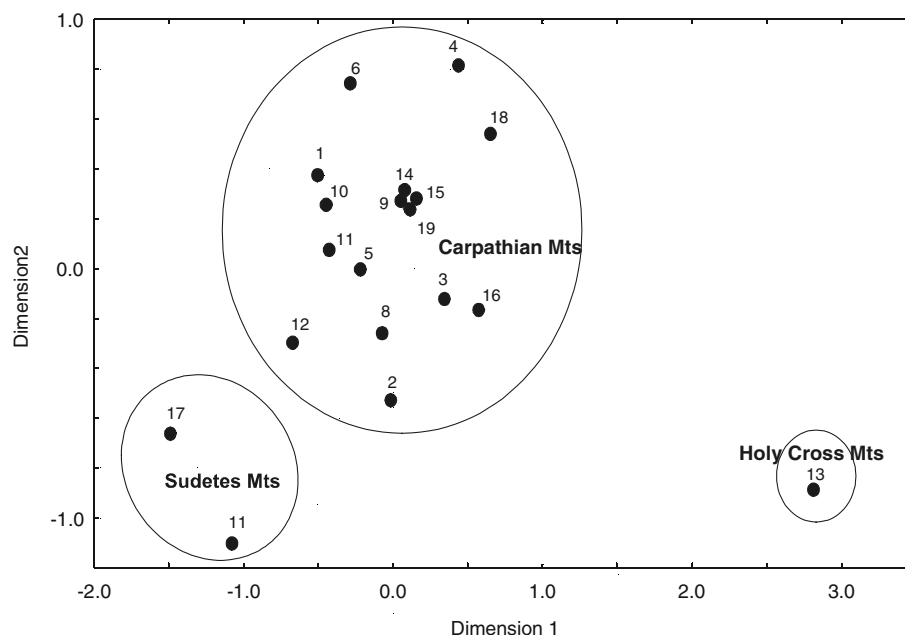


Figure 2. Non-metric multidimensional scaling analysis using Nei's (1978) genetic distances among 19 populations of the alpine newt *Triturus alpestris* in southern Poland. Stress value = 0.0597. Populations numbered as in Table 1.

distinct and therefore warrant separate conservation status. This sort of genetic substructuring may promote local adaptation in marginal populations (Garcia-Ramos and Kirkpatrick 1997) which is important in maintaining the adaptive potential of a species.

Colonization of the northern limits of the distribution of the alpine newt most probably occurred after the last glaciation. Unpublished mtDNA sequence data confirm that alpine newt populations in southern Poland are derived from a single colonization event (M. Pabijan and W. Babik, unpublished data). If so, the previous range of the species must have incorporated lowland areas adjoining the mountainous regions that harbour contemporary populations. Today, this part of Europe has a more continental climate compared to western Europe (where the alpine newt exists at lower altitudes), particularly lowlands that do not experience the buffering effect of the Carpathian Mts. An increase in climatic continentality in north-central Europe took place during the mid-Holocene (8000–4500 cal yr BP, Seppa and Poska 2004). This may have reduced the number of available habitats in lowland areas of the species range and resulted in the isolation of the three population clusters found in our study. Alternatively, isolation may have been induced by human pressure in more recent times, as many historical populations have ceased to exist (Głowaciński and Rapiński 2003, unpublished data).

No clear structure within the Carpathians was revealed by any of the implemented methods and relationships among populations were for the most part inconsistent with their geographical locations. The pattern of variation is shaped mainly by isolation by distance with moderate levels of gene flow between neighbouring subpopulations. Amphibians, in general, exhibit a low dispersal ability and consequent high levels of among population genetic variation (Beebe 1996) and urodeles, in particular, are known for high levels of genetic differentiation at a small scale (Larson et al. 1984; Highton 1999). Although we could not readily compare the variation in our samples with those of other studies because we had only considered polymorphic loci, mean levels of allozyme variation reported previously in this species, even in formerly glaciated areas, are similar to those of other amphibian species (Arano and Arntzen 1987; Rapiński and Arntzen 1987; Arano et al. 1991).

Our results show the existence of discrete genetic units at a relatively small geographical scale and point to the need for increased resolution in investigations into the genetic variation of organisms with limited dispersal capacity such as amphibians. This type of detailed genetic information can be particularly valuable in countries undergoing socio-economic change, reflected in different patterns of land use. For example, traditional small-scale farming has predominated in the mountainous regions of southern Poland. The diversified landscape was presumably favourable for low vagility species, when compared with lowland regions subjected to monocultural, collective agricultural practice. However, the pressures of infrastructural development and increased urbanization that enveloped Poland after 1989 have negatively affected the environment. The effects include a drop in subterranean water level and further fragmentation of the landscape. Additionally, global climate change can be expected to influence the distribution and/or the sustainability of populations, especially ones at the borders of species ranges (Opdam and Wascher 2004). In fact, the amplitude of mean annual temperatures is increasing in Central and Eastern Europe (Schär et al. 2004), and the resulting hot and dry summers will most probably decrease even further the available habitat for newts and other species associated with small, temporary water bodies.

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