MORPHOLOGICAL DIFFERENTIATION OF THE ALPINE NEWT (TRITURUS ALPESTRIS) IN THE BALKANS: TAXONOMIC IMPLICATIONS

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A comprehensive survey of the Balkan alpine newt was undertaken to describe morphological differentiation between populations, and to test the validity of previously described subspecies. Thirty population samples derived from the major part of the Balkans, excluding Bulgaria and Albania, were studied for patterns of both morphometric and qualitative trait variability. On the basis of morphology, separate taxonomic status cannot be allocated to any of the currently recognized Balkan subspecies, with the exception of the southernmost (T. a. veluchiensis). Paedogenesis affected morphological variability significantly. Variability among paedotypic populations was found to be lower than intrapopulation variability of metamorphosed individuals.

Key words: Triturus alpestris, morphological differentiation, taxonomy, paedogenesis, Balkan Peninsula

INTRODUCTION

Triturus alpestris (Laurenti, 1768), is a fairly widely distributed European newt species. Its range extends from north-east France to western Ukraine, and from southern Denmark to northern Italy and the Balkans, as far as the northern Peloponnese. Isolated parts of its range exist in northern and central Spain, as well as in southern and central Italy (Griffiths, 1996).

The alpine newt is a highly polytypic species. The status and nomenclature of the alpine newt subspecies have a confused taxonomic history lasting more than a century. Thirteen subspecies have been described, mainly according to external morphological features (head size and shape in particular) and coloration pattern. Currently, various subspecies are considered to be valid (see Griffiths, 1996 and Zuiderman, 1997 for the most recent accounts). The nomenotypical subspecies T. a. alpestris is by far the most widely distributed. The subspecies T. a. cyreni Wolterstorff, 1932 is confined to northern and central Spain; T. a. inexpectatus Dubois & Breuil, 1983, to central Italy and Calabria; while the range of T. a. apuamus (Bonaparte, 1839) includes south-eastern France and central-northern Italy.

The Balkan Peninsula is supposed to be the centre of alpine newt radiation (Arano & Arntzen, 1989; but see Herrero et al., 1989). Moreover, the Balkans have been considered to be a hot spot of alpine newt evolutionary diversification due to the description of numerous subspecies. In the western and central Balkans, subspecific status has been erected for a number of isolated populations exhibiting paedogenesis (sensu Reilly et al., 1997) which inhabit high-mountain glacial lakes.

These are: T. a. reiseri (Werner, 1902) – Prokosek lake (Bosnia); T. a. montenegrinus Radovanovic, 1951 - Bukumirsko lake (Montenegro); T. a. piperianus Radovanovic, 1961 - Kapetanovo and Manito lake (Montenegro); and T. a. serdarus Radovanovic, 1961 - Zminicko lake (Montenegro). Among Balkan alpine newt subspecies, T. a. veluchiensis Wolterstorff, 1935 exhibits the widest distribution. It has been proposed to be the only alpine newt in Greece (Breuil & Parent, 1988; Sotiropoulos et al., 1995) while its distribution extends to the south-eastern parts of Albania (Bruno, 1989).

The taxonomic status of Balkan subspecies is still uncertain. There are discrepancies in results obtained by limited morphological study (Ernst, 1952; Rocek, 1974a,b), electrophoretic investigations (Breuil & Guillaume, 1985; Arano & Arntzen, 1987) and cytogenetic analysis (Herrero et al., 1989). Concerning morphology, inter- and intragroup variability of morphometric trait relations over the broad species range have not yet been explored. Thus, only the morphometric variability of some populations has been examined, particularly populations consisting of both paedogenetic and metamorphic individuals (Dzukic & Kalezic, 1984; Kalezic et al., 1989; Kalezic et al., 1990), mainly to emphasize certain character differences between the two morphs.

In this paper we describe a study of the morphological differentiation of Balkan alpine newt populations, including taxonomical implications of this differentiation. Populations of all previously recognized Balkan subspecies along with populations from the margins and from the main body of the species range were examined by standard multivariate procedures. Paedogenetic population samples were included in order to examine the influence of paedogenesis on alpine newt morphological differentiation.

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FIG. 1. Locations of the sampling sites. For locality numbers see the Appendix.

MATERIALS AND METHODS

STUDIED POPULATIONS

Locations of thirty alpine newt population samples from the Balkans are presented in Fig. 1. Locality names, their altitudes, UTM codes (10 x 10 km squares), and the numbers of males and females collected, are listed in the Appendix. Altogether, 1737 sexually mature individuals were subjected to analysis. The average numbers of males and females per sample (±SD) were 26.2±2.5 and 31.7±3.4, respectively.

Paedogenetic individuals were recognized by the presence of elongated external gills along with apparent reproductive maturity (enlarged testes in males and vitellogenic ova in females). Specimens were preserved in 70% ethanol for varying periods of time (1 to 7 years) before morphometric and qualitative characters were scored. Amphibians shrink when preserved in alcohol or formalin, especially during the first two years of preservation (Dolmen, 1983). Verrell (1985), reported a maximum body shrinkage of 3.2% in formalin-preserved smooth newts (T. vulgaris) during the first year of preservation. We believe that the bias introduced due to specimen preservation is evenly distributed and not systematic. The specimens analysed are deposited in the collections of G. Dzukic (Institute for Biological Research, Belgrade) and K. Sotiropoulos (Zoological Museum, University of Athens, Athens).

For each individual newt, eight external morphometric traits were measured to the nearest 0.1 mm, and fourteen qualitative characters of both sexes were scored (see Appendix for description).

DATA ANALYSIS

To determine the degree of variation in morphometric characters between (1) sexes and (2) a priori designated populations and intraspecific groups (i.e. presumed subspecies), a Multivariate Analysis of Variance (MANOVA) was used.

Paedogenetic populations were examined for patterns of morphometric variation, using a Principal Component Analysis (PCA) on pooled measurement data. The first component (PC1) of this analysis – which is highly positively correlated with the original data – was used as a latent size variable, while the second principal component (PC2) measures organism shape independent of size (Bookstein et al., 1985 and references therein). The analysis was performed on the variance-covariance matrix of log-transformed variables (in order to meet the assumption of homoscedasticity; Zar, 1984).

Discriminant Canonical Analysis (DCA), which maximizes variation between a priori groups, was used to characterize the degree of divergence among populations (James & McCulloch, 1990). Discriminant canonical variates were calculated and centroids of each population were plotted on the first three canonical axes. As a measure of morphometric distance, Mahalanobis’ generalized distance ($D^2$) was calculated between all pairs of the examined populations. This measure, which is the most popular for continuous variables, considers differences in means, variance, and covariance of characters among groups.

Variation of qualitative characters was analysed using a Correspondence Analysis following the algorithm of Greenacre (1984). Individuals received a score on each qualitative character consisting of a discrete value for the colour and/or shape trait. The output of such an analysis was the coordinates of the row (populations) and column (character states) on correspondence axes superimposed on the scatter plot.

All analyses were performed with STATISTICA 5.0 (Statsoft Inc., 1997) computer software.

RESULTS

SEX AND LOCATION EFFECTS ON MORPHOMETRIC VARIABILITY

The alpine newt displays a high degree of sexual dimorphism. Females are much larger than males (Kalezic et al., 1992). The effect of sexes on the morphometric variability of the Balkan populations appeared to be highly significant, as was the effect of different localities (geographic variation). Test criteria of the MANOVA statistic (Hotelling’s trace) showed a highly significant variation ($P<0.001$) between both sexes and locations, and included an interaction between these two factors. Intrapopulation paedotypic/metamorphic partitioning in terms of morphological variation was pronounced (Dzukic & Kalezic, 1984; Kalezic et al., 1989; Kalezic et al., 1990). Conse-
The first three discriminant axes (DA) explained almost equal amount of the total variation in metamorphic males (74.7%) and females (75.1%), as well as in paedogetic males (98.1%) and females (92.1%) (Table 1). The pattern of character correlation between population variability and canonical axes was considerably different especially between morphs. None of the previously described subspecies stood out as distinct, according to the discriminant analysis (Figs. 3-4). An exception, to some extent, was *T. a. veluchiensis* (Nos. 28-30). Plotting the second versus the third discriminant axis, provided clear separation of this subspecies on the ground of female affinities in comparison to other presumed intraspecific groups (Fig. 4), but with less clear separation for males (Fig. 3). The discrimination of female *veluchiensis* was

**INTERGROUP DIFFERENCES**

The Mahalanobis’ distances ($D^2$), calculated between all analysed population samples, appeared to be statistically significant (at least at $P=0.05$ level) in the majority of pairwise comparisons. Metamorphic newts of two geographically remote populations (Nos. 2 and 15, both sexes), as well as paedogetic males of two population pairs (Nos. 6 and 17; 17 and 18), expressed non-significant $D^2$ values. The UPGMA phenograms (available from authors upon request), constructed on the basis of Mahalanobis’ distances, clustered populations irrespectively of their presumed subspecific designation. As an exception to some degree, *T. a. veluchiensis* populations clustered together when metamorphosed females were compared. Generally, population relations did not follow their geographical proximity in the cases of either sexes or morphs.

FIG. 2. Plot of the average values of PC1 and PC2 scores of paedoletic (p) and metamorphic (m) males and females of Bukumirsko, Manito and Zminicko lakes.

FIG. 3. Combined plot of population centroids for paedogetic (solid triangle) and metamorphic (open circle) males on the second and third discriminant axes (DA). For population numbers see Appendix.

FIG. 4. Combined plot of population centroids for paedogetic (solid triangle) and metamorphic (open circle) females on the second and third discriminant axes (DA). For population numbers see Appendix.
TABLE 1. Standardized coefficients for the first three discriminant axes (DA) of variation in morphometric characters, for metamorphic and paedogenetic females and males respectively. For character abbreviations and description, see Appendix.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Females</th>
<th>Males</th>
<th>Females</th>
<th>Males</th>
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<td></td>
<td>DA1</td>
<td>DA2</td>
<td>DA3</td>
<td>DA1</td>
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<tr>
<td>L</td>
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</tr>
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<td>0.2411</td>
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</tr>
<tr>
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<td>-0.1262</td>
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</tr>
<tr>
<td>Pa</td>
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<tr>
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<tr>
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<tr>
<td>% explained variation</td>
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FIG. 5. Plot of populations for metamorphic females on the first and second correspondence axes (DIM). For population numbers see Appendix.

FIG. 6. Plot of populations for metamorphic females on the second and third correspondence axes (DIM). For population numbers see Appendix.
based primarily on the influence of Ltc (negative) and Pp (positive) on the third discriminant function (Table 1). Female veluchiensis are characterized by small Ltc value (= narrow head) and large value of Pp (= long hind limbs) in relation to values of other characters. Less clear discrimination of male veluchiensis was influenced by L (negative), Pa and Pp (positive) on the second discriminant function (Fig. 3, Table 1). Male veluchiensis exhibit a large relative body size (L) and short fore- and hind-limbs (Pa, Pp). Another obvious population grouping included the previously described ‘one-lake’ subspecies from Zminicko, Manito and Bukumirsko (Nos. 18, 19 and 21) (Figs. 3-4). These populations bore a considerable morphometric resemblance to each other for both sexes and compared axes pairs, especially in the case of metamorphic individuals. Metamorphic males of these populations exhibit the same pattern as veluchiensis males, while females are less clearly discriminated in DA2, exhibiting relatively large inter-limb distance (D) (Table 1). Apparently, the same morphs of these paedogenetic populations appeared more uniform than the alternative of males in which some of the traits (IX -1, XI -2 and XIV -1) appeared to be monomorphic. Correspondence analysis revealed that the males were grouped in an unrecognizable manner, without obvious taxonomic meaning. However, the females of the three T. a. veluchiensis populations appeared to be distinct from the other populations, and were grouped in the morphospaces delimited by DIM1/DIM2 and DIM2/DIM3 axes (Figs. 5 and 6). Females of this subspecies were mainly discernable from the females of other populations on the basis of the dominant phenotype characterized by the following set of qualitative traits: olive-greenish colour of the upper body surface and flanks; presence of black spots on the belly zone; the skin flaps of the upper jaw not reaching the lower jaw; snout from the top view narrow and convex; the flank colour pattern; and sparse dark spots on the lower edge of the tail. Discrimination of other populations did not reveal any spatially consistent pattern.

DISCUSSION

Our study of geographic patterns of morphometric and qualitative trait variability in the Balkan alpine newt does not support currently recognized intraspecific taxonomic differentiation. Two ‘one-lake’ subspecies, T. a. montenegrinus and T. a. piperianus, have been found to be genetically virtually indistinguishable from each other and from the nominotypical subspecies from the Balkans (Breuil & Guillaume, 1985). Electrophoretic analysis (Arano & Arntzen, 1987) and cytogenetic study (Herrero et al., 1989) revealed that T. a. reiseri, another ‘one-lake’ subspecies, is genetically most similar to Serbian T. a. alpestris. We found no relevant morphological characters allowing for the distinction of these populations as separate taxonomic units. Our results are in agreement with Rocek’s (1974a, b) observations that the degree of variability in nominotypical populations may be higher than the observed interpopulation variability of separate subspecies, making the existence of numerous Balkan subspecies questionable.

The only exception is the southernmost subspecies, T. a. veluchiensis, which appeared to be distinct from the other populations, especially on the grounds of qualitative morphological traits. This taxon belongs to a group of five subspecies (T. a. cyreni, T. a. apuanus, T. a. inexpectatus, T. a. serdarus and T. a. veluchiensis) which, according to the analyses of intraspecific genetic differentiation, may warrant full subspecific status (Breuil, 1986; Arano & Arntzen, 1987; Herrero et al., 1989; Arano et al., 1991). Another subspecies from this group, T. a. serdarus, failed to show morphological distinctiveness as a separate taxonomic unit. The population from Zminicko displays discordant patterns of morphological and genetic differentiation. Neither have clear morphological differences been identified for three Italian subspecies (T. a. alpestris, T. a. apuanus and T. a. inexpectatus; Giacoma et al., 1988) in spite of their considerable genetic differentiation (Breuil, 1986). It seems that T. alpestris is an exception among highly polytypic European newts in that intraspecific taxonomic subdivision is not accompanied by substantial morphological differentiation. In contrast, the smooth newt (T. vulgaris) is very different, as it expresses well-defined male epigenetic characteristics for each subspecies along with a morphologically recognizable zone of intergradation (Raxworthy, 1990; Krizmanic, Mesaros, Dzukic & Kalezic, 1997). Studies of biochemical polymorphisms definitely provide the first widely applicable means of evaluating the genetic structure and continuity of populations in a manner independent of morphological variation (Larson, 1989). However, it is well established that the morphological differentiation among populations is not always closely correlated with their genetic compatibility (Cracraft, 1989).

Among European newts, the alpine newt frequently displays paedogenesis. Apart from a number of Dinaric populations (Dzukic et al., 1990), paedogenesis has been reported also in T. a. apuanus (Andreone & Dore, 1991; Denoël, 1997; Bovero, Giacoma & Andreone, 1997), T. a. veluchiensis (Breuil & Parent, 1987, 1988) and T. a. inexpectatus (Dubois & Breuil, 1983; Andreone & Dore, 1991). As in other newt species, the incidence of T. alpestris paedogenesis is a pond-dependent, temporary and variable trait, as paedogenetic individuals can metamorphose, bypassing the eft stage.
in their individual life cycle. The two morphs interbreed freely and there is no genetic distinctiveness, as has been revealed by electrophoresis (data for Manito; Breuil & Guillaume, 1985). As mentioned above, many Balkan subspecies have been described exclusively on the basis of morphological differences between paedogenetic and nominotypical specimens, without taking into account the within-pond morphological dimorphism. Apparently, the diagnostic features used by previous authors to describe new paedogenetic taxa were mere within-morph – or even individual – variations. Paedogenesis is generally regarded as a notorious source of morphological convergence between what are otherwise dissimilar forms, i.e. paedogenetic alpine newts of different populations are generally more similar to each other than metamorphosed adults of the same populations. This statement is valid, with a few exceptions, for Balkan paedogenetic populations. In conclusion, current evidence indicates that previously described ‘one-lake’ alpine newt subspecies from the Dinaric region – with the possible exception of T. a. serdarius – are populations that do not merit subspecific distinction.

The cause of alpine newt differentiation processes might be the high breeding site fidelity of adult newts, as has been confirmed by homing experiments (Joly & Miaud, 1989). Dispersal behaviour of the alpine newt is still unknown, but – according to studies on other newt species – a distance of 500 to 1000 m from a potential source is a reasonable dispersal estimate (see Joly & Grolet, 1996). As a result of adult newts homing to a particular aquatic site, gene flow between populations must have been impeded for a long time. Bottleneck effects and random drift in allele frequencies have been proposed as plausible factors which have facilitated genetic isolation even between geographically adjacent populations of T. a. cyreni in Spain (Arano et al., 1991). This fact, along with species range fragmentation during Pleistocene glaciations, might have caused a substantial differentiation between subspecies. The apparent morphological similarity of the majority of Balkan alpine newt populations can be attributed to the action of stabilizing selection which remains the most commonly invoked explanation of morphological evolutionary stasis (Williamson, 1987). Stabilizing selection on an optimal phenotype leads to the adaptation of this phenotype in constant environments. However, adaptation due to uniform selection does not always prevent the genetic divergence of conspecific populations, but sometimes accelerates it (Cohan, 1984).

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Prof. Dr. Milutin Radovanovic, the distinguished herpetologist who worked extensively on alpine newt paedogenesis, on the 30th anniversary of his death (August 1968). Our colleague, Ruza Radonjic, contributed considerably in measuring most of the former-Yugoslav populations. The technical assistance of Dragana Novakovic is highly appreciated. Dr Clive Cummins and two anonymous referees considerably improved the manuscript. Tanya Hall kindly improved the English language.

REFERENCES


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APPENDIX


Morphometric characters. L - total length, Lsv - snout-vent length (from the snout to the posterior edge of the cloacal base), Lcd - tail length (from the anterior edge of the cloacal base to the tail tip), Ltc - head width, Lc - head length (from the snout to the corner of the mouth), Pa - fore-limb length, Pp - hind-limb length, D - distance between fore- and hind-limb. The length of the tail in damaged individuals was estimated as the expected value from the regression of Lcd on Lsv.

Qualitative characters. 1. Colour of the upper body surface and flanks - (1) dark, (2) olive-greenish, (3) brownish, (4) dove-blue, (5) whitish-grey, (6) other colour; II. Belly colour - (1) deep yellow to bright orange, (2) dark-red or reddish, (3) other colour; III. Throat colour pattern - (1) unspotted, (2) sparse black spots and/or blotches dispersed all over the throat, (3) numerous spots and/or blotches dispersed all over the throat, (4) spots and/or blotches concentrated close to gular fold; IV. Black spots and/or blotches on the belly zone - (1) present, (2) absent; V. Skin flaps of the upper jaw - (1) extend over the lower jaw; (2) do not reach lower jaw; VI. Snout from the dorsal view - (1) wide and flat, (2) narrow and convex, (3) wide and concave; VII. Flank colour pattern (scored in females only) - (1) reticulate, (2) numerous denticulate blotches present, (3) other ornamentation; VIII. Dorsal crest (scored in males only) - (1) high (>2 mm), (2) medium (1-2 mm), (3) low (<1 mm); IX. Colour of the dorsal crest (scored in males only) - (1) dark zones completely separated by narrow lightly coloured stripes, (2) above the dark zones a wavy white band underlines a row of well separated dark spots, (3) other colour pattern; X. Number of dark spots in the lower edge of tail - (1) numerous (>10), (2) moderate (3-10), (3) sparse (<3); XI. Size of dark blotches on the flanks - (1) regularly decrease toward the forelimbs, (2) no regularity; XII. The flank zone with dark blotches is - (1) equally wide along the trunk, (2) wide close to hindlimbs, and narrow close to forelimbs; XIII. Cloaca colour pattern (scored in males only) - (1) with spots, (2) with blotches, (3) completely black; XIV. Dorsal crest origin (scored in males only) - (1) on the rear part of the head, (2) between forelimbs.