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Geographic variation and taxonomy of crested newts (*Triturus cristatus* superspecies): morphological and mitochondrial DNA data

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Abstract

Within the newt genus *Triturus,* the large-bodied species in the *T. cristatus* (crested newt) superspecies show an unusual degree of variation in relative trunk length as a result of among-taxon variation in interlimb vertebral count. Here we examine the systematic value of this feature as assessed by both exterior measurement (Wolterstorff Index) and direct radiographic count of rib-bearing vertebrae, with particular reference to a number of confounding factors (sex differences, hybridisation, geographic variation, allometry, preservation effects). Using our mtDNA haplotype data, which are largely concordant with geographic distribution of species, we find that direct count of the rib-bearing vertebrae performs more reliably (14% misclassification) than external measurement (31% misclassification) as a species identifier. We therefore recommend this feature as a taxonomic tool, although (like external measurement) it breaks down near hybrid zones. To account for the observed biogeographical pattern and phenotype - genotype discrepancies, a scenario is presented that combines the movement of the contact zone between taxa with asymmetric hybridisation. This scenario applies to species interactions in eastern Yugoslavia and western France.

Introduction

Studies of geographic variation within and among closely related taxa have provided crucial information on the nature of species and the process of speciation (Mayr, 1963). Studies of spatial morphological heterogeneity are of interest from the point of view of adaptation since differences in morphology are likely to be functional or at least have consequences on fitness and ecology. A proper analysis of morphological variation requires independent assessment of the phylogenetic relationships of morphotypes (e.g. Losos, 1990). A central question to the systematist is: does geographically bounded, morphological differentiation reflect local adaptation (in which case convergence would be expected to be common), or is it a function of phylogeny (in which case morphotypes should be monophyletic) (Harvey and Pagel, 1991; Bernatchez, 1995)?

Adaptation and phylogeny are not, of course, mutually exclusive explanations of character distributions. Adaptive morphological change can occur and be proliferated by cladogenesis in the absence of reversal. To resolve this, the first step is to determine whether different character sets define the same taxa. Concordance of taxon boundaries resolved by different means, e.g., morphology and genetics, is strong evidence for their reality (Hillis, 1987; Avise and Ball, 1990). Indeed, morphological divergence often only becomes apparent after the taxa have been resolved genetically (e.g., Arntzen and García-París, 1995; McDowall and Wallis, 1996). Here we look at geographic variation in vertebral count in the *Triturus cristatus* superspecies (or Artenkreis *sensu* Rensch) and re-evaluate this character as a tool for species identification, with particular reference to the several hybrid zones present in the group and our genetic analyses of these zones (Wallis and Arntzen, 1989; Arntzen and Wallis, 1991; Arntzen, in prep.).

In his classic paper, Wolterstorff (1923) revised the taxonomy of the crested newt, *Triturus cristatus* using a morphological index. He distinguished four taxa at the subspecies level [*carnifex* (Laurenti, 1768), *cristatus* (Laurenti, 1768)*, dobrogicus* (Kiritzescu, 1903) and *karelinii* (Strauch, 1870)] and provided compelling phenotypic descriptions for each of these. He provided a crucial discriminator for taxon identification, which has become known as the Wolterstorff Index (WI). WI is the ratio between forelimb length (PaL) and interlimb distance (LiE) and is defined as WI = PaL * 100 / LiE. We have reviewed the available data on WI and have discussed some of the practical and theoretical pitfalls associated with its use (Arntzen and Wallis, 1994). WI succinctly summarises the morphological differentiation among taxa to much the same extent as more sophisticated methods of multivariate morphometric analysis used in crested newts and other urodeles (Kalezi_c et al., 1990; Arntzen and Wallis, 1994; Sket and Arntzen, 1994; Arntzen and Sket, 1997; Cvetkovi'c, Kalezi'c and Dzuki'c, 1997). From a statistical standpoint, indices may have certain undesirable properties (Atchley et al., 1976), but the WI has become a popular tool for species identification of crested newts in the field (for example Grillitsch et al., 1983). However, to avoid circular reasoning, the calibration of WI requires independent criteria, i.e., an identification without reference to body shape.

In the crested newt superspecies and its sister taxon *T. marmoratus* (Latreille, 1800), WI increases in the order: *dobrogicus - cristatus - carnifex - karelinii - marmoratus,* describing a morphological series from slender and short-legged to stout and long-legged. The taxa also possess characteristic patterns of colouration that are described on Plates I-II (for approximateive species distributions see Fig. 1). We previously gathered mtDNA RFLP data from female newts and concluded that each of the five phenotypes was associated with one or more characteristic mitochondrial genotypes (Wallis and Arntzen, 1989). Significant differences in WI were observed between groups carrying these genotypes, and therefore WI can be used as a tool for species diagnosis. Limb length and interlimb distance are sexually dimorphic characters, making it necessary to calibrate WI for males and females separately. For technical reasons, mtDNA data were available for (almost exclusively) female newts only (Wallis, 1987). However, we measured WI for males from the same populations and because (with a few exceptions) they belong to the same species, WI can be calibrated for males as well. The number of rib-bearing vertebrae (RBV) varies in parallel with the inverse of WI (Arntzen and Wallis, 1994; Crnobrnja-Isailovi'c et al., 1997). Similar patterns have been documented for lizards (Greer, 1987; Griffith, 1990; Caputo, Lanza and Palmieri, 1995), fish (Lindsey, 1975) and other salamanders (Jockush, 1997).

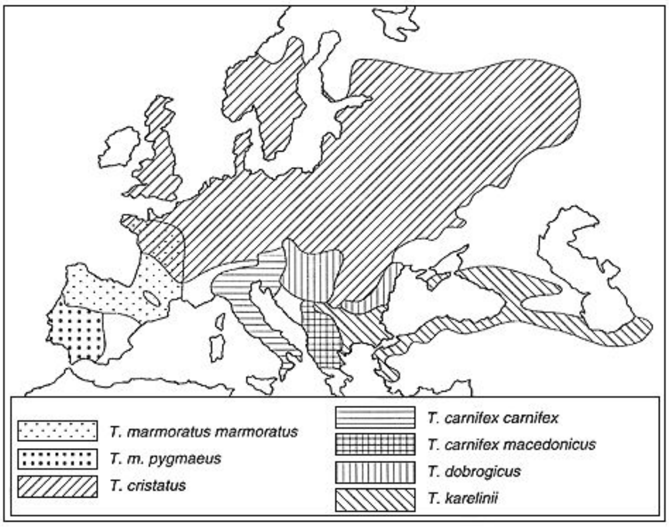


Fig.1. Distribution of *Triturus marmoratus* and five taxa in the *T. cristatus* superspecies (after Arntzen, 1995). The crossed hatching covering Yugoslavia and adjacent regions refers to *T. carnifex macedonicus,* a taxon recognition resurrected from Karaman (1922).

Here we present additional data to calibrate WI and RBV and document the existence of geographic variation across and within species. The results confirm the limited value of WI for diagnostic purposes. The character RBV on the other hand is shown to be diagnostic and without much intraspecific geographic variation. Moreover, it appears to be largely immune to observer bias, developmental or sexual variation, or preservation artefacts. Applying the character at the population level produces a coherent pattern of geographic variation that coincides with that of the species, although (like external measurements) it breaks down near hybrid zones. To account for the observed phenotype - genotype discrepancies and biogeographical patterns in eastern Yugoslavia and western France, a scenario is presented that combines the movement of the contact zone between taxa with asymmetric hybridisation.

Material and methods

Forelimb length and interlimb distance were measured with plastic callipers (0.1 mm accuracy) on preserved or live material, representing 142 populations across the total range. X-ray photographs were taken on preserved or sedated specimens (representing 116 populations) with an 'Elinax 90/20' and an exposure of 0.7 seconds at 38kV, 4mA on Agfa-Gevaert D10DW X-ray film. Additional data on WI (31 populations) and RBV (38 populations) were obtained from the literature (Vallée, 1959; Kalezi_c et al., 1990; Crnobrnja-Isailovi'c et al., 1997). Analysis of variance (ANOVA) and other tests followed Sokal and Rohlf (1981) and Siegel and Castellan (1988). Logistic equations were determined with SPSS (SPSS, 1990). Non-parametric tests were performed with StatView (StatView, 1988). Association of intraspecific morphological variation and geographical distance was tested for with the Mantel-test (NTSYS 1.80, Rohlf, 1993).

Results

We present estimates of Wolterstorff Index (Fig. 2) and the number of rib-bearing vertebrae in newts from populations characterised by mtDNA genotype (Table 1). For reference, we include published data from studies dealing with three or more taxa that were identified phenotypically (Table 1). Statistically significant differences were found between all species combinations for WI (four one-sided *t*- tests between consecutive groups for each sex:.$P < 0.001$ in all cases, except for males *T. cristatus* - *T. carnifex* and males *T. carnifex* - *T. karelinii* with $P < 0.05$ and females *T. karelinii* - *T. marmoratus* with $P < 0.01$) and for RBV (four Median*G*-tests between five consecutive groups with $P < 0.001$ in all cases). Males consistently have an average WI of 8-10 points greater than females of the same species. A weighted regression was used to determine the best fitting logistic curve separating species that are adjacent in morphometric space. The WI typical for each crested newt species is as follows: males: *T. dobrogicus* < 54.0, *T. cristatus* 54.0 - 63.69, *T. carnifex* 63.7 - 67.09, *T. karelinii* = 67.1; females: *T. dobrogicus* < 46.2, *T. cristatus* 46.2 - 53.89, *T. carnifex* 53.9 - 59.19, *T. karelinii* = 59.2. For RBV the modal values observed are: 13 in *T. marmoratus,* 14 in *T. karelinii,* 15 in *T. carnifex,* 16 in *T. cristatus* and 17 or 18 in *T. dobrogicus.* Limited intraspecific variation is observed with 86% of the investigated newts showing modal values. Ranges for the identification of populations are set as follows: *T. dobrogicus* = 16.5, *T. cristatus* 15.5 - 16.49, *T. carnifex* 14.5 - 15.49, *T. karelinii* 13.5 - 14.49 and *T. marmoratus* < 13.5. Median Fisher's exact tests demonstrated the absence of significant sexual dimorphism for this character, except in *T. dobrogicus* where females tend to have a higher count than males (modal RBV of 18 versus 17, $P < 0.05$). A trend was observed for increasing overlap of character states between species with increasing WI and decreasing RBV.

WI alone is usually sufficient to distinguish *T. dobrogicus* from all other species. On the basis of WI, three males (4.2%) and three females (2.9%) would have been classified as belonging to a species with non-corresponding (*T. cristatus*) phenotype. Much higher levels of misclassification were obtained over all species (31%), with no significant difference in misclassification between the sexes ($P > 0.05$; G-test of independence). Using RBV, good separation is obtained between: *T. dobrogicus* and *T.*

cristatus (6.6% misclassified), *T. cristatus* and *T. carnifex* (6.8%), *T. carnifex* and *T. karelinii* (16.4%) and *T. karelinii* and *T. marmoratus* (6.7%). Overall misclassification is 13.7%.

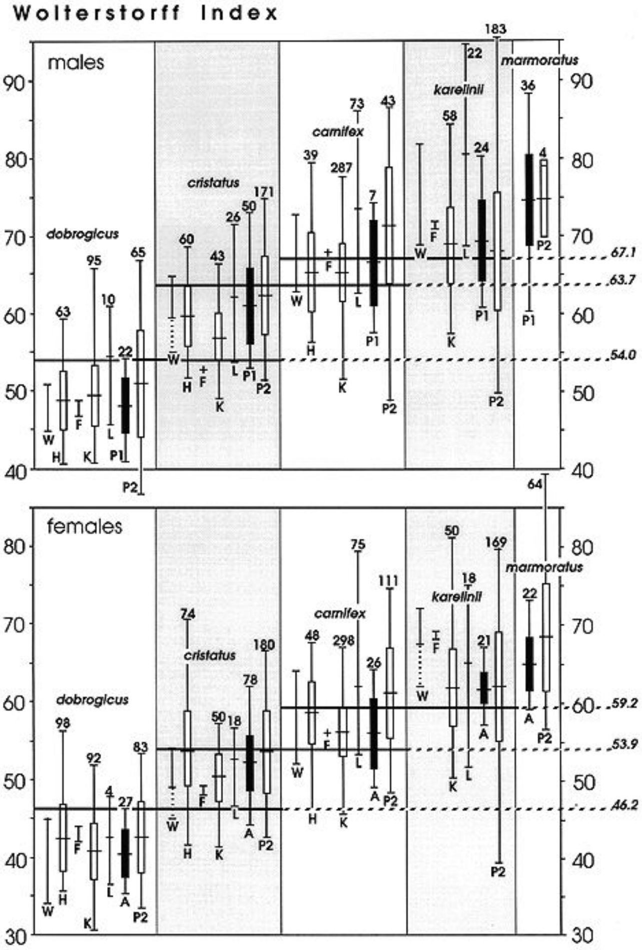


Fig. 2. Reported values of the Wolterstorff Index for seven studies that involved three or more taxa in the crested newt superspecies. W = Wolterstorff (1923): range, interrupted lines refer to extreme values; H = Herre (1932): mean ± SD and range; F = Fachbach (1974): range; K = Kalezi'c and Stevanovi'c (1980) and Kalezi'c et al. (1990: tables 1 to 3 on page 34-35, with populations allocated to species following Kalezi'c et al. (1997) but excluding the population from Tre'snja which Wallis and Arntzen (1989) identified as a genetically mixed *T. dobrogicus - T. karelinii* population: average weighted mean ± SD and range; L = Lanza, Gentile and Torricelli (1991): mean and range; A = Arntzen and Wallis (1994): mean ± SD and range; P1 and P2 = present paper: mean ± SD and range. Populations figuring in 'P2' were identified a *posteriori* , following the species distributions as in Fig. 3. Solid bars refer to populations classified on the basis mitochondrial DNA genotype. Sample sizes (if known) are presented at the top of the bars. Horizontal bars indicate optimal interspecies boundaries with respect to WI (details see text). Data on *T. marmoratus* are given for comparison.

Table I: Number of rib bearing vertebrae observed in crested and marbled newts, genus Triturus. Sample size in brackets. The populations used in the present study were idententified to the species on phenotype and mitochondrial DNA (Wallis & Arntzen, 1989).

	Herre, 1932	Lanza et al., 1991	present study
T. dobrogicus	17 (3)	17.6 ± 0.51 (14)	17.5 ± 0.58 (47)
T. cristatus	16 (3)	16.0 ± 0.15 (44)	16.0 ± 0.41 (122)
T. carnifex	15 (3)	15.1 ± 0.30 (148)	14.9 ± 0.33 (26)
T. karelinii	14 (1)	14.1 ± 0.30 (40)	14.2 ± 0.44 (41)
T. marmoratus			13.1 ± 0.34 (49)

Application of the RBV cut-off point criteria to population means (Table 2) provides inferred species distributions that are largely internally consistent, in line with prior knowledge, and hence reflect documented distributions with some exceptions as follows. Populations 1 and 2 are inferred *'T. karelinii'* and *'T. marmoratus',* but are within the known geographic and phenotypic ranges of *T. marmoratus* and *T. karelinii,* respectively (Fig. 3). Similarly, populations 3 - 5 are obviously wrongly classified (*'T. dobrogicus'* within the *T. cristatus* range and *'T. karelinii'* within the range of *T. carnifex carnifex*). Sites 6 - 8 are known syntopic *T. cristatus - T. marmoratus* and *T. cristatus - T. dobrogicus* populations with bimodal character state distributions (Vallée, 1959; Wallis and Arntzen, 1989;

Arntzen and Wallis, 1991; Arntzen et al., 1997). Individuals with mixed *T. dobrogicus - T. carnifex* phenotype at site 9 and 10 had RBV scores typical for *T. cristatus* (i.e., in between the values typical for *T. dobrogicus - T. carnifex*) and hence remain unclassified in Fig. 3. Populations 11 and 12 were in the field identified as *T. carnifex,* showed RBV counts typical for *T. cristatus*, an mtDNA haplotype typical for *T. dobrogicus* and also remain unclassified. Individuals with mixed *T. carnifex - T. karelinii* phenotype (site 13) were classified as *T. carnifex* on the basis of RBV. Similarly, a newt with mixed *T. cristatus - T. karelinii* (site 14) phenotype was classified as *T. karelinii.* Newts from sites 15 - 20 are phenotypically *T. karelinii,* but have RBV scores higher than average for that species. Sites 6 - 19 are all located at the fringe of species distributions and close to another species, where one might expect interbreeding if the various taxa of crested newts were not genetically isolated. No *T. dobrogicus* or *T. cristatus* samples (except for those mentioned above) had RBV scores that did not correspond with phenotype. However, among the larger ($N = 5$) samples, four *T. cristatus* populations with relatively low RBV scores (populations 30 - 33, Fig. 3c; mean RBV = 15.9) are all situated at the fringe of the *T. cristatus* range, close to *T. karelinii* which typically has low RBV counts. Among 16 *T. dobrogicus* populations with $N = 5$, those with relatively low counts (mean RBV < 17. 5, see table 2) are, with one exception (population Alap) situated along the southern edge of the *T. dobrogicus* range, whereas those with higher counts (mean RBV = 17.5) are, with one exception (population Jamena) situated away from the edge of the species range.

Application of the WI criterion to the identification of individuals and populations produces mixed results. For *T. dobrogicus,* the distribution pattern obtained with WI identifications neatly fits the documented distribution, whereas for the other species the results are erratic (compare Table 2 with Fig. 1). On the basis of the reconstructed species distributions (Fig. 3) the frequency of incorrect identification of individual crested newts applying the WI can be estimated in *post hoc* manner as 11% in *T. dobrogicus,* 42% in *T. cristatus,* > 50% in *T. carnifex* and 39% in *T. karelinii.* No significant differences in classification scores are observed between the sexes, except for *T. karelinii* for which males are more often misclassified than females (G -test of independence, $P < 0.001$). Classification success is not significantly affected by sample size, except in *T. carnifex* ($P < 0.05$, Mann-Whitney U- test, one-tailed).

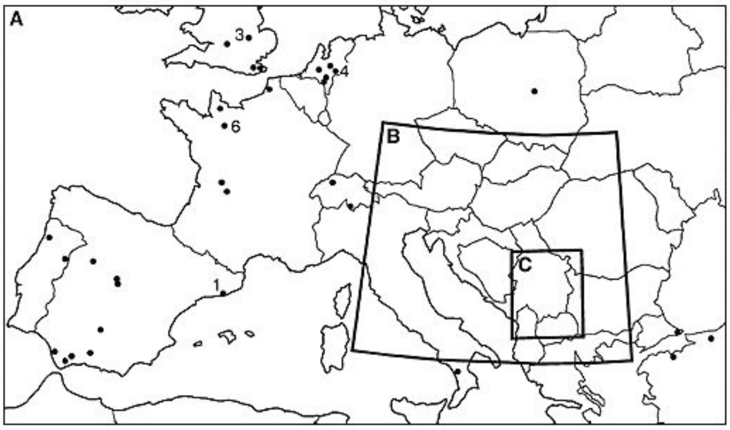
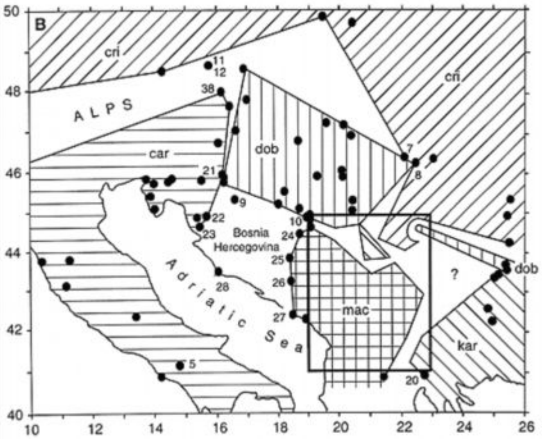


Fig. 3ABC. Newt populations for which mean NVR (number of rib bearing vertebrae) and mtDNA haplotype was determined. Solid lines connect records describing the edge of species ranges: car = *Triturus carnifex carnifex,* cri = *T. cristatus,* dob = *T. dobrogicus,* kar = *T. karelinii,* mac = *T. carnifex macedonicus.* Numbered populations are discussed in the text. No big-bodied newts are known for the area approximately coinciding with Bosnia-Hercegovina. The area marked with a '?' in northwestern Bulgaria - adjacent parts of Yugoslavia is not devoid of crested newts (see Kalezi'c et al., 1997), but the taxon to which they belong is undetermined. The interrupted heavy line on map C describes an area with newt populations possessing mtDNA characteristic for *T. karelinii* (Wallis and Arntzen, 1989); studied populations shown by triangular symbols. Arrows refer to hypothesized dispersal events discussed in the text. Note that the River Danube connects the Pannonian and Dobrogean Plains through the Iron Gate. For the more detailed distribution of *T. dobrogicus* see Arntzen et al., 1997.



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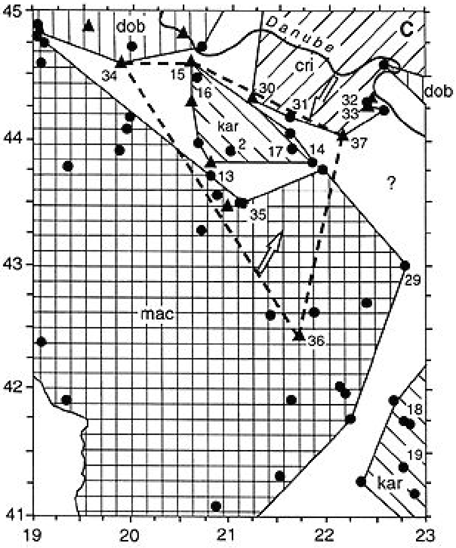

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Mantel tests indicate a marginally significant association between geographic distance and WI for males and females *T. cristatus* and male *T. dobrogicus* (P _ 0.05), while the association of geographical distance with RBV is significant ($P < 0.05$ for *T. cristatus* and $P < 0.01$ for *T. dobrogicus*). A series of ANOVA's with 'sex' nested under 'group' confirmed the difference in WI between the sexes ($P < 0.001$) and between the following *a priori* identified groups (cf. Fig. 1): *T. m. marmoratus* versus *T. m. pygmaeus* (Wolterstorff, 1905) ($P < 0.01$), Pannonian versus Dobrogean *T. dobrogicus* ($P < 0.01$) and *T. carnifex carnifex* versus *T. carnifex macedonicus* (Karaman, 1922) ($P < 0.05$), whereas no significant difference was observed for Asian versus European *T. karelinii.* A significant difference was also found between *T. carnifex carnifex* and *T. carnifex macedonicus* in RBV (Median G -test,$P < 0.05$) but not between *T. m. marmoratus* and *T. m. pygmaeus* ($P > 0.05$). The limited sampling of Dobrogean (versus Pannonian) *T. dobrogicus* and Asian *T. karelinii* (versus European *T. karelinii*) precluded their testing.

Discussion

Performance of WI and RBV for species diagnosis

In our previous work concerning the capacity of the Wolterstoff Index to discriminate females of the taxa, we identified several potential problems (Arntzen and Wallis, 1994): 1) statistical representation (the need for a mean value), 2) non-biological variation (preservation and measurement differences), 3) sexual variation (WI is higher for males than for females), allometric variation (WI decreases with size), 4) geographic variation (nearby animals may tend to be more similar within species), 5) hybridisation (hybrids between two species can have intermediate values typical of a third species), and 6) circular reasoning (the need for an independent character set to determine the significance of WI). All of these factors to some extent reduce the efficacy of WI and compromise classification made solely on this basis. The WI purports to capture information useful in taxonomy but in fact confounds the variables limb length, vertebral number, and possibly vertebral length.

Using mtDNA haplotype (Wallis and Arntzen, 1989) we showed that WI makes a good approximation to species classification, but can only be used with confidence for discriminating adult *T. dobrogicus* from the other species. In contrast, the number of rib-bearing vertebrae (RBV) as assessed by radiography eliminates all but one of these problems. Because RBV is a direct discrete meristic count, stable through the lifetime of the individual, and with limited intraspecific geographic differentiation its use in conjunction with a diagnostic genetic character leaves only the issue of hybridisation to be addressed. Variation caused by hybridisation near regions of parapatry is difficult to disentangle from intraspecific geographic variation on the basis of morphological data alone. Under both scenarios, variation will be most clearly expressed when samples from remote parts of the geographic range are compared. However, the observation that character state changes are consistently in the direction to that of the neighbouring species supports the hypothesis of hybridisation, rather than that of intrinsic geographic variation. For example, the significantly different values in *T. carnifex macedonicus* (high WI, low RBV compared to *T. carnifex carnifex*) may well be a result of introgression from *T. karelinii,* whose mtDNA prevails in some *T. carnifex macedonicus* populations (Wallis and Arntzen, 1989). Note, however, that the meristic count in hybrids is not necessarily intermediate to that of the parental species, as documented for salmonid fishes (Leary, Allendorf and Knudsen, 1985). If hybridisation between taxa of crested newts is a common phenomenon, it should be possible to find genetic markers for the species covarying with interspecific morphological variation. The observed breakdown of the diagnostic power of RBV in areas where taxa meet may reflect the true nature of characters in a contact zone.

It is possible that RBV is influenced early on in development. Indeed, vertebral count is often highly labile in fish and salamanders (e.g. McDowall, 1970; Jockush, 1997), with cooler conditions generally slowing development and increasing several meristic counts (Barlow, 1961 and references in Jockush, 1997). In *Triturus vulgaris* the average RBV count increases with the temperature at which the embryos are raised (Orska and Imiolek, 1962), while for other salamander species more complicated environmental effects were found (Lindsey, 1966; Peabody and Brodie, 1975). To address the question to what extent the variation in RBV is genetically determined and to gain insight into the relationship between embryonic development and adult morphology requires experimental work. *Triturus dobrogicus* might be the best species to work with because it is naturally polymorphic for RBV.

Adaptation

Elongation of the body and a reduction in the length of limbs in vertebrates generally indicates a more piscine locomotion by sinusoidal body undulation. Although this can be associated with some terrestrial habitats (as in sand-swimming skinks), it is more usually an adaptation to a more aquatic mode of life. Reduction of trunk size and the development of robust legs is more unequivocally associated with a terrestrial mode of life where the body requires more support (Young, 1950; Lande, 1978). The wide variation in body shape in the leg-less Gymnophiona, ranging from stout to thread-like, may also be associated with locomotory behaviour and ecological adaptation (Renous and Gasc, 1989). In the salamander genus *Batrachoseps,* extensive geographic variation in RBV, possibly related to fossoriality, has been observed, most of which was shown to be genetically determined (Jockush, 1979). Selection on female fecundity (correlated with interlimb length) and male sexual performance (stature at display correlated with leg length) may also play a role (Arntzen and Wallis, 1994).

Morphology predicts the aquatic period of *T. marmoratus* to be short, that of *T. carnifex* to be intermediate and that of *T. cristatus* - and *T. dobrogicus* in particular - to be long. While of course the phenology of breeding may be different from year to year, this prediction appears to be corroborated by field data. *Triturus marmoratus* spends annually approximately three months in the water, *T. carnifex* four months, and *T. cristatus* five months (Bouton, 1986; Griffiths and Mylotte, 1987; Andreone and Giacoma, 1989), while the aquatic phase of *T. dobrogicus* usually lasts six months (Karaman, 1948; Jehle et al., 1996). No data are available on the phenology of *T. karelinii.* This species is predicted to have a short aquatic phase, of intermediate length to that of *T. carnifex* and *T. marmoratus.* In terms of performance, the sinusoidal swimming ability should be best in the lowest WI / highest RBV count taxon, i.e., *T. dobrogicus* and relatively poor in *T. marmoratus*. Indeed, the ecological niche of *T. dobrogicus* is different from that of the other big-bodied species. It may co-exist with fish in oxbows, river margins and other non-temporary water bodies (Arntzen et al., 1997). The observed ventral aposematic colouration pattern in the *T. cristatus* superspecies versus the dorsal aposematic colouration of *T. marmoratus* (particularly evident in the entirely terrestrial juveniles) provides further support to our interpretations. Predictions about performance, such as in the gathering of food or predator avoidance behaviour in the aquatic versus terrestrial habitat could be tested experimentally.

Taxonomy and phylogeny

The depth of the differences among taxa, and the relative sharpness of the contact zones led us to follow earlier suggestions to raise the taxa to full species status (Bucci-Innocenti, Ragghianti and Mancino, 1983) as have others (Frost, 1985). The available data, unfortunately, do not support a single phylogenetic hypothesis for the four taxa comprising the *T. cristatus* superspecies. RBV is primitively 14 in the genus *Triturus* (B. Lanza et al., in prep.), rendering RBV of 13 an autapomorphic character state for *T. marmoratus* and RBV of 15 - 18 a synapomorphic character state series for *T. carnifex* - *T. cristatus* - *T. dobrogicus*. This character alone would suggest that *T. karelinii* represents the oldest extant crested newt lineage, followed by *T. carnifex*, *T. cristatus* and *T. dobrogicus*. This evolutionary classification is supported somewhat ambiguously by the phenetic analysis of protein electrophoretic data (Crnobrnja, Kalezi'c and D'zuki'c, 1989) but contradicted by another such study (Litvinchuk et al., 1994). The phylogenetic analysis of molecular data (mtDNA RFLP's) suggests a different phylogeny. Looking at the most-parsimonious mtDNA tree (Wallis and Arntzen, 1989: Fig. 4), a tree that optimizes RBV character-state change [tree : character structure

(MAR,PYG:13)/ancestor:14/(KAR?,KAR:14(CAR?,CAR:15(CRI:16,DOB:17,18)))]

involves moving only the 'DOB' branch (with terminal taxon number 11). [CAR? and KAR? refer to deeply differentiated haplotype lineages within *T. carnifex* and *T. karelinii*. We now recognize the first of these as belonging to *T. carnifex macedonicus* (see below) while the other will be subject to taxonomic description at the subspecific level (S. Litvinchuk et al., in. prep.)]. If DOB were placed with the *T. cristatus* (CRI) haplotypes, increased RBV becomes a derived character interior to the tree, with the more massive built newts basal. Although this haplotype tree has 70 steps as opposed to 67 in the published maximum parsimony tree (Wallis and Arntzen, 1989: Fig. 4), there is no bootstrap support above 50% for any of the crested newt species-level structure. That is to say, the relationship DOB(KAR(CAR,CRI)) is only defined by three synapomorphies in total and the tree could more conservatively be depicted as a four-way polychotomy at this level. This incomplete resolution is appreciated by Wallis and Arntzen (1989: 99) and emphasised by further analysis (Faith and Cranston, 1991; Faith, 1992). However, strong support is obtained from the 'CAR' and 'CAR?' mtDNA haplotypes for the sister taxon status of Italian and central Balkan crested newts. These groups of populations are also united by the synapomorphic character state RBV = 15. We therefore consider the crested newts from the central Balkan to belong to *T. carnifex*. The range of this species is disjunct (see below). Newts from both parts of the range are phenotypically distinct: *T. carnifex* from the western (Italian and Slovenian) part of the range typically have few, large, ill-defined black dots on the bellies, whereas *T. carnifex* from the eastern part of the range (F. R. Yugoslavia and Greece) have ventral coloration patterns with many sharp-edged spots, as Freytag (1988) observed, not unlike that of *T. cristatus* (Plates I-II). Crested newts of the eastern group were described as *Molge karelinii* var. *macedonica* Karaman, 1922. Considering the morphological and genetic differentiation between the forms, we propose raising this taxon to the subspecies level and, supported by phylogenetic arguments, classifying it as belonging to *T. carnifex* (not *T. karelinii* as suggested by Karaman, 1922). Therewith, *Triturus carnifex* var. *albanicus* Dely, 1959 is a junior synonym of *T. carnifex macedonicus* (Karaman, 1922). Following our correspondence with co-author J. Crnobrnja-Isailovi'c (J. W. Arntzen, in letter, 1996) this taxonomic solution is accepted by Kalezi'c et al. (1997), although the taxon is incorrectly referred to in feminine gender.

The two more massive newt species, *T. karelinii* and *T. carnifex*, show much greater restriction site variation than the other two species (Wallis and Arntzen, 1989). They also have slightly larger mitochondrial genomes and a greater tendency for insertions in the control region (Wallis, 1987). These factors suggest that the two northern species may have been subjected to long-term small population size during glaciations (Wallis and Arntzen, 1989), and it is conceivable that the evolutionary change in vertebral count is related to this population genetic feature.

Distribution and biogeography

Crested newts appear to be absent from the largest part of Bosnia-Hercegovina (see for example Schmidtler and Schmidtler, 1983; Kalezi'c, D'zuki'c and Tvrtkovi'c, 1990; Kalezi'c et al., 1997). The southeasternmost localities of *T. carnifex* to the northeast of the perceived gap in the species distribution are sites 21 - 23 [Belovar Moravce (Table 2); Plitvice (Fejervary-Langh, 1943) and Licki Osik (Kalezi'c et al, 1990). Further to the southeast *T. carnifex* is found at sites 24 - 27 [Donja Dubrava (Kalezi'c et al., 1990), Sarajevo (Bolkay, 1929; communicated by G. D'zuki'c), the Zelengora Mountain (Bolkay, 1928) and Dobrsko Selo (Kalezi'c and D'zuki'c, 1990). The easternmost recorded locality is site 29 at Dimitrovgrad (Radovanovi'c, 1964). Crested newts of unknown taxonomic affinity were recorded at the Dalmatian coast [site 28, situated in between Sebenico (= S'ibenik) and Spalato (= Split) (Werner, 1897, also mentioned by Buresh and Zonkov, 1941), but with no clearly independent confirmation for over a century we doubt the validity of this record. Dzuki'c (1993) considers the distribution of *T. carnifex* not to be interrupted but continuous, following a strip of land to the south of the Sava river, without, however, presenting data supporting this view. The area where crested newts are absent coincides with the core area of the karst (Sket, 1994), where most natural water bodies are ephemeral and do often not support the larval development of species with a prolonged larval phase, such as crested newts. The small-bodied newts such as *T. alpestris* and *T. vulgaris* in contrast are widespread and locally abundant. They may reproduce successfully in shallow and temporary ponds such a wheel ruts (Winkler and Brauns, 1990) and the dispersal rate for the small newt *T. vulgaris* is estimated to be higher than that for the big newt *T. cristatus* (Stensjö, 1998). While most contemporary newt ponds are man-made and rarely desiccate (i.e., watering holes for cattle), the puddles formed by fallen trees and springs may originally have been the typical breeding habitat for the small bodied species.

The distribution of the four crested newt species in F. R. Yugoslavia is complex (Fig. 3c). *Triturus dobrogicus* is found all over the Pannonian and Dobrogean Plains. Both parts of the range are probably connected by the Danube where flowing through the Iron Gate (Arntzen et al., 1997). *Triturus cristatus* has a wide European range, is widespread over Romania and reaches southwards over the Iron Gate into Yugoslavia. *Triturus carnifex macedonicus* is widespread over most of Yugoslavia, the Former Yugoslavian Republic of Macedonia, Albania, and northern Greece. *Triturus karelinii* is found immediately south and southeast of Belgrade. The available evidence suggests that the local distribution is in a small pocket - an enclave, geographically isolated from the main *T. karelinii* distribution in Bulgaria, Thrace, and Turkey (Fig. 3c). However, a link between the parts, along a narrow strip in northeastern Yugoslavia (as in Arntzen, 1995 and in Kalezi'c et al, 1997: Fig. 6), cannot be excluded. The further surveying of eastern Yugoslavia and northwestern Bulgaria is required to settle this issue.

On a gross geographic scale, phenotype distributions, and mtDNA haplotype distributions are concordant. However, in northern Yugoslavia the 'KAR?' mtDNA haplotype is more widespread than the *T. karelinii* phenotype distribution would suggest (Fig. 3) (Wallis and Arntzen, 1989). The 'KAR?' mtDNA haplotype is locally found in *T. dobrogicus*, *T. cristatus,* and *T. carnifex macedonicus* populations. The reverse situation, with a foreign haplotype in *T. karelinii,* has been observed once (the 'DOB' haplotype in population 15). Populations with foreign haplotypes possess either two haplotypes - the original plus an alien, such as at site 15 and 34 in *T. karelinii* and *T. dobrogicus*), or just the alien haplotype ['KAR?' in *T. cristatus* (site 30 and 37, *N* = 5) and in *T. carnifex macedonicus* (site 35 and 36, *N* = 16; Wallis and Arntzen, 1989). To account for these observations we suggest the following scenario. In former times *T. karelinii* was more widespread than at present, with a range approximately coinciding to the present day distribution of the 'KAR?' haplotype. By dispersing southwards and northwards, respectively *T. cristatus* and *T. carnifex macedonicus* superseded *T. karelinii*, in which process the range of *T. karelinii* south of Belgrade became isolated from the main stock (see the arrows in Fig. 3c). The genetic interactions between *T. karelinii* at one side and *T. cristatus* and *T. carnifex macedonicus* at the other where such that the formation of F1 hybrids was asymmetric, with hybrid offspring and the subsequent backcrosses possessing the (maternally inherited) *T. karelinii* mtDNA. This scenario is surprisingly similar to the one we described for *T. cristatus* - *T. marmoratus* interactions in western France (Arntzen and Wallis, 1991). In France, *T. cristatus* supersedes *T. marmoratus*, forming *T. marmoratus* enclaves in the process. Hybridisation between the species is strongly asymmetric, with F1 adults derived from matings of *T. cristatus* mothers and *T. marmoratus* fathers significantly outnumbering the reverse combination. The facts responsible for this phenomenon are largely unknown but may involve the genetic incompatibility of the nuclear and mtDNA genomes (J. W. Arntzen et al., in prep.). By comparing past and present distributions, the rate at which *T. cristatus* takes over from *T. marmoratus* has been estimated as averaging one km a year. The process may be triggered, or accelerated, by the removal of hedgerows, modifying a landscape with terrestrial features favourable to *T. marmoratus*, the most terrestrial among the big-bodied newt species, into one favourable to the more aquatic *T. cristatus.* The habitat preferences of the various crested newt species in eastern Europe, with the exception of *T. dobrogicus*, are poorly understood and it is unclear which ecological parameters affect their distribution or change in distribution. Another area of complexity is that around Vienna, where *T. carnifex, T. cristatus* and *T. dobrogicus* meet (Fig. 3b). At sites 12 and 36 newts were found with *T. carnifex* phenotype and the mtDNA haplotype typical for *T. dobrogicus*, matching a similar observation at Tasovice in the Czech Republic (48°49' N, 16°09' E; J. Pialek, V. Zavadil and J. W. Arntzen, unpubl.).

As noted by Crnobrnja-Isailovi'c et al. (1997), the remarkable variability in Balkan crested newts should provide valuable insights into the evolution of the group. Palaeontological and various molecular methods have provided some clues towards the timing of the radiation of the *T. cristatus* superspecies (reviewed in Oosterbroek and Arntzen, 1992). This period, which can be placed at 2-5 Ma, was one of great geographical and geological complexity in southeastern Europe (Crnobrnja-Isailovi'c et al., 1997 and references therein). However, our ability to associate the historical patterns of fragmentation, speciation and dispersal with palaeogeography is, as yet, hampered by the absence of a well-supported phylogeny.

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Appendix

Plates I, II. Ventral surface of five taxa of crested newts in the *Triturus cristatus* superspecies.

Plate I: b, c, e, *T. dobrogicus*; a, d, f, *T. cristatus*; j, *T. carnifex carnifex*; k, *T. carnifex macedonicus*; g - i, *T. karelinii*. Geographical origin of the individuals is: *T. dobrogicus* from Tadten, Austria; *T. cristatus* from Mayenne, France; j, Farma, Italy; k, Vis'egrad, Bosnia-Hercegovina; g, Djurinci, Federal Republic of Yugoslavia; h, Istanbul, Turkey, and i, Karacabey, Turkey.

Plate II: l, o, p, *Triturus carnifex carnifex*; n, q-w, *T. carnifex macedonicus*, and m, *T. karelinii*. Geographical origin of the individuals is: l and p, Benevento, Italy; o, Farma, Italy; n, t, v, w, Divcibare, Federal Republic of Yugoslavia; q, r, Tavna Monastire, Bosnia-Hercegovina; s, Karan, Federal Republic of Yugoslavia; u, Vis'egrad, Bosnia-Hercegovina, and m, Istanbul, Turkey. For geographical coordinates see Table 2. Note that males (a, e, g, h, j, n - r, u, v) and females (the others) can be distinguished by shape, colour and size of the cloaca, by the colour of the underside of the tail and by digit and limb length and body shape. In males the cloaca is large, black and two-lobed and in the female it is small, yellow and flattened. In males the ventral side of the tail is yellow only directly behind the cloaca or it is entirely black; in females the tail underside is yellow along most or all of its lenght. Digits and limbs are longer and inter-limb distance is shorter in males than in females. Note that some toes are not original size (such as one of the inner toes in newt 'a') but regenerating from marking by toe-clipping. Photography by L. van der Laan.

Description of phenotypes

Triturus dobrogicus. Lean build, short legs, narrow tail base, heavily white-stippled sides, black throat with large angular white spots, ventral surface deep orange with many sharp, roundish black spots. Notes : Ventral spots tend to line-up and fuse as in (b). Throat spots are larger in males than in females. A possible function of the conspicuous throat coloration is shown by Plate III.

Triturus cristatus. Lean-medium build, medium sized legs, narrow tail base, heavily white-stippled sides, throat a muddied mix of black and yellow with fine white stippling, ventral surface yellow-orange with irregular black spots. Notes : The black spots grow and the spot pattern becomes denser with age (Arntzen & Teunis, 1993) and individual (a) is likely to be younger than the others.

Triturus carnifex carnifex. Medium build, large legs, tail base medium wide, little or no white stippling on sides, throat colour variable with white stipples. Ventral surface yellow with few large, roundish, ill-defined and muddy-gray to black spots. Notes : Males tend to have darker throats with more and larger white stipples than females. The newts 'l' and 'o' represent opposite sides of the range.

Triturus carnifex macedonicus. Medium to heavy build, large legs, tail base medium wide, sides densely white-stippled, throat a muddied mix of black and yellow with many, medium sized white stipples. Ventral surface yellow to orange-yellow with a dense pattern of small, irregular spots. Notes : The coloration characteristics are particularly variable and individuals may resemble *T. dobrogicus* (n), *T. cristatus* (s), *T. carnifex carnifex* (t) or *T. karelinii* (v).

Triturus karelinii. Heavy build, large legs, wide tail base, heavily white-stippled sides, ventral surface yellow-orange with many small to medium-sized black spots, extending on to the tail (especially in females) and continuous with throat where spots tend to be angular. Notes: no obvious coloration characters distinguish between *T. karelinii* possessing different mtDNA haplotypes (KAR and KAR?, see Wallis and Arntzen, 1989).

Plate III. Sexual display by male *Triturus dobrogicus*. Symptomatic of the species are the bulging black and white throat and high head crest, giving the appearance of increased size. Photo by M. Sparreboom.

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