

ORIGINAL ARTICLE

Patterns of morphometric variation in the alpine newt (*Mesotriton alpestris*) at the southern limit of its distribution: environmental correlates

Konstantinos SOTIROPOULOS,¹ Anastasios LEGAKIS² and Rosa Maria POLYMENTI¹

¹Section of Zoology-Marine Biology and ²Zoological Museum, Department of Biology, University of Athens, Athens, Greece

Abstract

We applied multivariate analyses to an array of body measures of alpine newt specimens derived from 11 local populations in Greece to describe, analyse and detect patterns and putative causes of within-population and among-population morphometric variation. The observed morphometric variation was partitioned into several independently varying aspects of the external phenotype, frequently following variation patterns in different environmental factors. The size and features of the aquatic habitat were found to affect body size, while altitude was found to affect head-shape variation in both sexes. At the intra-population level, variation in generalized body size and shape was found to be significantly lower when competitive newt species were present in the habitat, indicating stabilizing selection towards a decrease in inter-specific competition. No clear discrimination on body size and shape proportions was detected between the two genetic lineages examined, implying ecogenetic or environmentally induced variation rather than phylogeny.

Key words: body size/shape, environment, Greece, *Mesotriton alpestris*, morphometrics.

INTRODUCTION

Multivariate analyses of morphometric data have been widely used in the study of body size and shape variation in organisms as well as in the study of the spatial distribution and organization of this variation (Reyment *et al.* 1984; Bookstein *et al.* 1985; James & McCulloch 1990). Multivariate morphometrics can measure the variation of multi-gene characters, which are subject to the influence of micro-environmental and macro-environmental factors, cre-

ating a system that allows for the plasticity of the phenotype (Manly 1985; Endler 1986). However, because of a lack of indications of inherited variation, we can only hypothesize that the various body and shape proportions indicate considerable non-genetic variation produced by short-term adaptations of morphology in varying environmental conditions. In addition, as has been proposed by Lande and Arnold (1983), natural selection acts on phenotypes irrespective of their genetic background, producing immediate phenotypic change, even within a single generation, which can be measured without taking inheritance into account.

The alpine newt, *Mesotriton alpestris* (Laurenti 1768), is a polytypic amphibian species with a wide range across Europe. It inhabits a wide variety of habitats from sea level up to 2500 m a.s.l. at the southern limits of its distri-

Correspondence: Konstantinos Sotiropoulos, Section of Zoology-Marine Biology, Department of Biology, University of Athens, Panepistimioupolis, 157 84 Athens, Greece.
Email: ksotirop@biol.uoa.gr

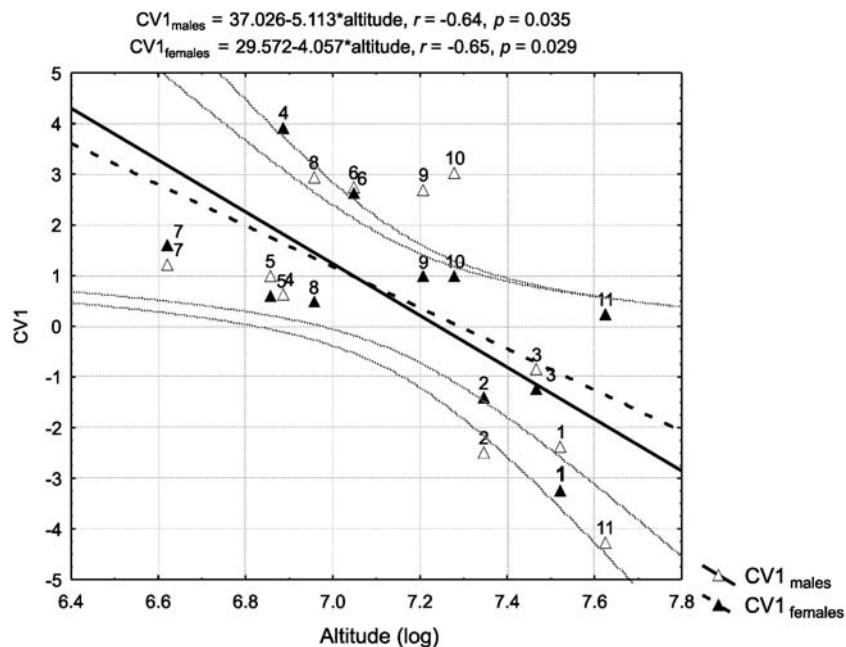


Figure 1 Correlation of canonical variate 1 (CV1) with altitude in both sexes of *Mesotriton alpestris* from Greece. Dashed lines represent the 95% confidence intervals for the regression lines. For population numbers and localities see Table 1.

bution (Griffiths 1996). It mostly displays a bi-phasic life cycle (aquatic larvae, terrestrial juveniles, bi-phasic adults), and facultative paedomorphosis is common (e.g. Denoël *et al.* 2001).

The southern limit of the alpine newt's distribution is located in Greece, where the species is represented by subspecies *Mesotriton alpestris velouchiensis* in the central mainland and in Peloponnisos, and another recently recognized lineage inhabiting northern Pindus extending to southern Serbia (Sotiropoulos *et al.* 2007). *Mesotriton alpestris velouchiensis* is further subdivided in two subclades in accordance with the geographic origin of populations. Populations from the mainland are genetically distant to populations from Peloponnisos bearing different mitochondrial haplotypes (Sotiropoulos *et al.* 2007), as well as alternative allozymic profiles (Sotiropoulos *et al.* 2008). It is found exclusively in mid to high altitudes over 750 m a.s.l., often inhabiting small ponds in dry limestone areas (Sotiropoulos *et al.* 1995). Geographic isolation in different mountain massifs, along with the low dispersal ability and breeding-site fidelity that alpine newts display (Joly & Miaud 1989; Joly & Grolet 1996), is proposed to explain the high levels of genetic divergence and low gene flow rates among local populations in Greece (Sotiropoulos *et al.* 2008).

In this study we sampled *M. alpestris velouchiensis* from 11 localities in Greece. We used multivariate analy-

ses on body proportions to describe spatial patterns in body size and shape variation, as well as to detect the possible influence of various environmental factors on the observed patterns. An additional aim was to explore the taxonomic value of the observed morphometric variation.

MATERIALS AND METHODS

A total of 258 (126 males and 132 females) *M. alpestris* individuals were measured at 11 localities in Greece (Table 1). The following 11 external morphometric characters were measured to the nearest 0.01 mm in each specimen: snout-vent length (SVL), tail length (TL), head width (LTC), head length (LCL), mouth opening (LC), eye diameter (LO), distance between front eye-corners (O1), distance between rear eye-corners (O2), front limb length (PA), hind limb length (PP), distance between limbs (D) (Appendix I).

Characters were first screened singly for significant differences between localities using a two-way analysis of variance (ANOVA) or covariance (ANCOVA), with the data grouped by locality and sex. As body proportions co-vary with size, it is necessary to take size into account. Where a univariate estimate of size is appropriate, SVL is used. An ANCOVA does this by performing an ANOVA on the residuals of the regression of the character in question against SVL. The relationships between size-dependent

characters and SVL were linearized by log-transforming SVL and the dependent variables prior to performing an ANCOVA with SVL as the covariate. The group means,

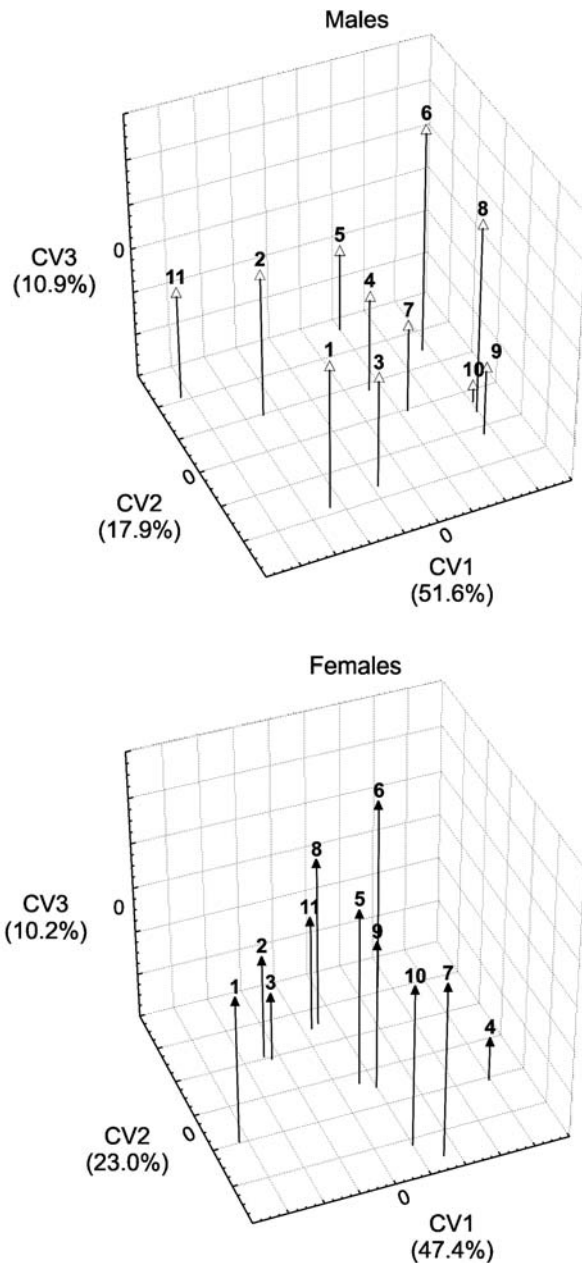


Figure 2 Plot of canonical variate 1–3 (CV1–CV3) scores showing separation of populations in multivariate space for male and female alpine newts from Greece. The proportion of the total variation summarized in the plots is 80.4% and 80.6% for male and female newts, respectively. For population numbers and localities see Table 1.

adjusted by the pooled within-group slope, resulting from this procedure were used for all other analyses.

Our aim was to detect any general trends within different aspects of the morphology rather than to relate (non-independent) characters to potentially causal environmental gradients. Therefore, emphasis was placed on obtaining and analysing independent linear combinations of characters. A canonical variate analysis (CVA) was used to study multivariate patterns of size and shape variations separately in males and females. This method ordonates a priori groups so that it maximizes the between-group variation in relation to the within-group variation, thus producing maximal separation between groups. This is a powerful technique in that it overcomes the problem of information redundancy in the character set by taking into account the within-group correlation/covariation between characters and is particularly suitable for the analysis of size-related characters. Site mean centroids of the first three canonical variates (CV) of both sexes were plotted in a three-dimensional space to examine grouping patterns of populations.

Overall (multivariate) variability in body size and shape at each site was estimated using Mahalanobis D^2 distances between individuals and group centroids. The mean D^2 distance at each site, therefore, estimates the degree of generalised within-population polymorphism (Báez & Brown 1997).

Abiotic data were collected on a range of environmental and ecological variables at all 11 localities. The variables were chosen because accurate and comparative data could be obtained for all sites: altitude in meters a.s.l., latitude and longitude, mean annual temperature, mean annual rainfall, mean annual humidity (%), pond seasonality, pond nature, pond area, vegetation cover, water turbidity and competition (Table 1).

To explore possible geographic and/or micro-geographic patterns of morphological variation, correlation coefficients between body size and shape components and the whole series of abiotic variables were calculated. When continuous variables were studied, Pearson's product-moment correlation coefficient was calculated; whereas Spearman's correlation coefficient was calculated when categorical variables were used.

In addition, t -tests were used to evaluate the differences in mean values of body size and shape components (expressed by SVL and CVs, respectively) between the two genetic groups of *M. alpestris velouchiensis* (i.e. between Peloponnesian and mainland individuals).

Table 1 Population localities, sample size (N), coordinates, altitude and environmental variables used in the study

No.	Locality	N ♂ / ♀	Coordinates	Region	Altitude (m a.s.l.)	Temperature (°C)	Rainfall (mm)	Humidity (%)	Pond seasonality [†]	Pond type [‡]	Pond area [§]	Vegetation cover [¶]	Water turbidity ^{**}	Competition ^{**}
1	Mt Velouchi	16/17	38° 57' N 21° 49' E	M	1850	17.0	1200	67.5	P	N	0.5	0	0	-
2	Mt Oeta 1	18/16	38° 46' N 22° 19' E	M	1550	17.5	1200	67.5	T	N	0.5	1	0.5	-
3	Mt Oeta 2	10/11	38° 50' N 22° 17' E	M	1805	17.5	1200	67.5	P	N	0.5	0.5	0.5	-
4	Mt Kallidromo	12/11	38° 45' N 22° 33' E	M	950	17.5	900	66.3	T	N	0.5	0	0	-
5	Belokomitis	10/10	39° 16' N 21° 44' E	M	900	17.0	1400	67.5	T	A	0	0.5	1	-
6	Mt Panachaiko	10/11	38° 10' N 21° 54' E	P	1150	18.0	1400	67.5	P	A	0	0	1	-
7	Kalavryta	9/8	38° 03' N 22° 02' E	P	750	18.0	1200	67.5	T	N	0.5	1	0.5	+
8	Mt Kerketio	14/15	39° 31' N 21° 31' E	M	1050	16.5	1400	70	P	N	0.5	0.5	1	-
9	Aoo's Springs Lake	11/11	39° 50' N 21° 12' E	M	1350	16.0	1400	71.3	P	N	1	0.5	0.5	-
10	Mt Zygos	8/14	39° 53' N 21° 17' E	M	1450	16.0	1400	71.3	P	N	0.5	1	0.5	+
11	Mt Tymphi	8/8	39° 59' N 20° 47' E	M	2040	16.0	1400	71.3	P	N	1	0.5	0.5/1	-

Temperature, rainfall and humidity refer to the mean annual values of the respective localities. M, mainland; P, Peloponnisos. [†]P, permanent; T, temporary. [‡]A, artificial; N, natural. [§]0, <100m²; 0.5, 100-1000m²; 1, >1000m². [¶]0, <35%; 0.5, 35-70%; 1, >70%. ^{**}0, turbid; 0.5, semi-transparent; 1, clear. ^{**}Absence (-) or presence (+) of other newt species in the pond.

♂ : Males, ♀ : Females

Table 2 Results of a two-way analysis of variance and covariance

Character	1 (Sex)	2 (Locality)	1 × 2
SVL	108.42***	8.75***	1.27
TL	7.30**	1.76	1.09
LTC	2.72	7.47***	3.24**
LC	0.58	3.25**	0.82
LCL	0.01	2.24*	1.01
O1	0.00	12.85***	0.94
O2	0.05	10.06***	1.69
LO	2.87	14.09***	0.84
PA	1.02	6.19***	0.36
PP	10.74**	2.98**	2.23*
D	0.38	3.97**	0.93

F-ratios are given for a two-way ANOVA for snout-vent length (SVL) and a two-way ANCOVA for the remaining characters (with SVL as the covariate). The grouping factors are sex (1) and locality (2), with 1 and 10 degrees of freedom, respectively. **p* < 0.05; ***p* < 0.01; ****p* < 0.001. TL, tail length; LTC, head width; LC, mouth opening; LCL, head length; O1, distance between front eye-corners; O2, distance between rear eye-corners; LO, eye diameter; PA, front limb length; PP, hind limb length; D, distance between limbs.

RESULTS

A two-way ANOVA on SVL, and two-way ANCOVAs on the 11 external morphometric characters showed that significant between-population differences exist for all but one (TL) of the characters (Table 2). Between sexes, significant differences were observed in three out of the 11 characters (SVL, TL, PP), while geographic variation in the degree of sexual dimorphism (given by the interaction between sex and locality) is significantly different for LTC and PP (Table 2). Because of the existence of sexual dimorphism all consequent analyses were carried out for each sex separately.

Body size variation

Body size, expressed by the character SVL, varied considerably between the populations examined (Table 2), increasing significantly in ponds with clear water in both males (Spearman *r* = 0.78, *P* = 0.004) and females (Spearman *r* = 0.61, *P* = 0.047) (Table 3). Moreover, male body size correlates significantly with the nature of the pond (Spearman *r* = -0.67, *P* = 0.024) and pond area (Spearman *r* = -0.67, *P* = 0.025) (Table 3).

Table 3 Correlation coefficients between various aspects of morphological variation of *Mesotriton alpestris* populations, with geographic and ecological factors

Factor	IPV		Body size		Body shape					
	♂	♀	SVL ♂	SVL ♀	CV1 ♂	CV2 ♂	CV3 ♂	CV1 ♀	CV2 ♀	CV3 ♀
Altitude	0.11	0.29	-0.48	-0.40	-0.64	-0.25	-0.01	-0.65	0.35	-0.33
Latitude	-0.40	-0.04	-0.23	-0.32	-0.03	-0.03	-0.45	-0.17	0.05	0.00
Longitude	0.42	0.24	-0.06	-0.18	0.00	-0.10	0.23	0.08	-0.09	-0.44
Temperature	0.44	0.10	0.24	0.27	-0.02	0.10	0.45	0.15	0.03	-0.10
Rainfall	-0.44	-0.27	0.39	0.55	0.24	0.13	0.06	-0.20	0.31	0.75
Humidity	-0.60	-0.27	-0.11	-0.07	0.15	-0.10	-0.34	-0.04	0.04	0.28
Seasonality	-0.18	0.12	-0.12	0.00	0.24	-0.48	0.24	-0.24	0.36	0.06
Pond nature [†]	-0.15	0.15	-0.67	-0.52	-0.22	-0.60	-0.15	-0.30	-0.37	-0.67
Pond area	-0.14	-0.19	-0.67	-0.48	-0.29	-0.33	-0.29	-0.24	-0.14	-0.52
Vegetation cover	-0.58	-0.43	0.12	0.08	0.12	0.08	-0.47	-0.12	-0.27	0.08
Turbidity	-0.28	-0.23	0.78	0.61	0.48	0.43	0.19	0.14	0.61	0.74
Competition [‡]	-0.67	-0.30	0.22	0.30	0.45	-0.07	-0.52	0.37	-0.67	0.30

Significant correlations ($P < 0.05$) are given in bold, IPV, intra-population variation of the generalized body size and shape (mean Mahalanobis D^2 distance). The factors “Temperature”, “Rainfall” and “Humidity” refer to the mean annual values at each locality. [†]Natural/artificial pond. [‡]Presence of other newt species in the pond. CV, canonical variate; SVL, snout-vent length. ♂: Males, ♀: Females

Body shape variation

Among-population variation was allocated to many different structures of the external morphology of both sexes. The first three CVs produced for males and females, summarised 80.4% and 80.6% of the total variation, respectively (Table 4). Correlation coefficients calculated between CVs and the 12 environmental variables (Table 3) indicated the existence of several spatial patterns of morphometric variation.

The first CV of male alpine newts explained 51.6% of the total variation and was dominated by a high negative loading of LTC, with parallel increases in SVL, while head width decreased (Table 4). In female alpine newts, the first CV explains 47.4% of the total variation because of the major contribution of O2 and PA with positive loadings (Table 4). In both sexes, the first CV correlates significantly with the altitude of the respective localities (males: $r = -0.64$, $P = 0.035$; females: $r = -0.65$, $P = 0.029$) (Table 3, Fig. 1), implying that male newts derived from lower altitudes express narrower heads relatively to males from higher altitudes with similar body size, while female newts derived from lower altitudes express longer front limbs and wider inter-ocular distance than females from higher altitudes.

The second CV (17.9%) of male alpine newt variation is

dominated by a high negative loading of SVL, with parallel increases in O2, while body size decreases (Table 4). Female CV2 explains 23.0% of the observed variation describing the relative LO compared to D (Table 4), which seems to be affected by habitat characteristics (water turbidity and competition) (Table 3). Female alpine newts load negatively, thus expressing larger eyeballs and trunk in turbid aquatic habitats ($r = 0.61$, $P = 0.047$) where competitive newt species exist ($r = -0.67$, $P = 0.024$) compared with females from habitats with clear water and in the absence of competitors.

Male CV3 (10.9%) is dominated by the relative contribution of PP and SVL with contrasting signs (Table 4), without any detectable spatial pattern (Table 3). In females, CV3 explains 10.2% of the observed variation and is dominated by a high negative loading of LTC, with parallel increases in SVL, while head width decreases (Table 4). This variation in relative head width seems to be affected by both climatic factors (rainfall) and habitat characteristics (pond nature, turbidity) (Table 3). Females from areas with low rainfall levels load negatively in CV3, while females from areas with higher rainfall levels load positively ($r = 0.75$, $P = 0.008$) (Table 3).

The overall shape variation of male alpine newts (expressed by the mean Mahalanobis D^2 distance of each individual to site centroid) decreases significantly in habi-

Table 4 Standardized coefficients of the first three canonical variates (CVs) for male and female *Mesotriton alpestris*

Character	CV1	Males			Females		
		CV2	CV3	CV1	CV2	CV3	
SVL	1.8362	-1.9660	1.8494	-0.7435	1.0539	1.7577	
TL	-0.2185	-0.7288	-0.4222	0.1493	0.4848	-0.1540	
LTC	-2.0665	0.6479	0.9322	-0.7602	0.2041	-1.6306	
LCL	-0.0256	-0.4148	-0.7022	0.1873	-0.5212	-0.7805	
LC	-0.1441	0.3132	-0.6877	0.0208	1.1196	-0.5780	
O1	-0.0914	-0.8152	0.6829	-1.1453	1.1781	0.2570	
O2	-0.0364	1.6318	-0.0500	1.9383	0.6186	0.0840	
LO	-0.8066	0.2218	-0.7429	-0.9170	-1.4202	-0.5833	
PA	1.2452	-0.4881	0.9039	1.5820	-0.5708	-0.4898	
PP	0.2777	0.6956	-1.6478	-0.5743	-0.4948	1.5000	
D	0.0301	0.9026	-0.1156	0.2627	-1.6521	0.6175	
Eigenvalue	6.83	2.36	1.44	5.03	2.44	1.09	
Variation (%)	51.63	17.85	10.88	47.39	23.00	10.24	
Cumulative variation (%)	51.63	69.47	80.36	47.39	70.39	80.63	

SVL, snout-vent length; TL, tail length; LTC, head width; LC, mouth opening; LCL, head length; O1, distance between front eye-corners; O2, distance between rear eye-corners; LO, eye diameter; PA, front limb length; PP, hind limb length; D, distance between limbs.

tats where competitive newt species exist (Table 3).

No significant morphometric differences were found between the two genetic groups in both males and females (Table 5). Projection of population centroids in the morphospace delimited by the first three CVs (for males and females separately) results in a grouping pattern irrespective of the geographic origin of populations in which Peloponnesian populations are well within mainland (Fig. 2).

DISCUSSION

Body size variation: influence of the habitat

Body size is often correlated with numerous life-history traits of organisms, particularly to those contributing to overall fitness (Hespenheide 1973; Atchley 1983; Barbault 1988; LaBarbera 1989; Calder 1996; Malhotra & Thorpe 1997; Morrison & Hero 2003).

Growth rate before maturity along with size at metamorphosis are among the major factors determining the adult body size of newts (Bell 1977; Bell & Lawton 1975; Halliday & Verrell 1986). Fluctuations in growth rate can result from fluctuations in food quality and availability, or can relate to habitat features, foraging behaviour, population density and predation pressures (Gamradt & Kats 1996; Gamradt *et al.* 1997; Arntzen 2000; Van Buskirk & Schmidt

2000; Schmidt & Van Buskirk 2005). The observed reduction in body size in large ponds probably indicates an influence of some or a combination of factors that co-vary with pond area. For example, predation on newts could be intensive in large ponds, while in smaller ponds predation could be limited or even absent because most of the time the newts are the major predators (e.g. Cooke & Frazer 1976; Giacoma *et al.* 1988; Bringsøe 1994; Denoël *et al.* 2001). It has been reported that intensive predation on

Table 5 Differences in body proportions between the two lineages of *Mesotriton alpestris veluchiensis* from Greece

	Mean mainland	Mean Peloponnisos	<i>t</i> -value	df	<i>p</i>
SVL _{males}	76.23556	81.67000	-1.43891	9	0.184030
CV1 _{males}	0.036667	1.981550	-0.97657	9	0.354299
CV2 _{males}	0.089689	0.700350	-0.49928	9	0.629558
CV3 _{males}	-0.524156	0.599200	-1.10693	9	0.297029
SVL _{females}	89.53111	96.00000	-1.90439	9	0.089260
CV1 _{females}	0.146722	2.112450	-1.32524	9	0.217747
CV2 _{females}	-0.007111	-0.253800	0.18861	9	0.854585
CV3 _{females}	-0.237200	1.034950	-1.64440	9	0.134506

CV, canonical variate; SVL, snout-vent length.

Appendix I. Mean (mm) and standard error (\pm S.E.) of 12 external morphometric characters in 11 alpine newt populations from Greece. For population numbers and locations see Table 1. ♂: Males, ♀: Females

		L	SVL	TL	LTC	LC	LCL	O1	O2	LO	PA	PP	D
1 ♂	<i>Mean</i>	71.37	41.01	30.36	7.27	6.41	9.69	4.25	5.90	3.19	13.80	14.37	20.16
	<i>S.E.</i>	2.11	1.03	1.13	0.16	0.26	0.26	0.16	0.14	0.06	0.36	0.39	0.78
♀	<i>Mean</i>	89.34	50.07	39.27	7.96	7.22	11.62	4.52	6.38	3.47	15.83	17.28	25.96
	<i>S.E.</i>	2.73	1.42	1.40	0.22	0.22	0.39	0.10	0.13	0.11	0.45	0.42	0.78
2 ♂	<i>Mean</i>	74.28	42.47	32.06	7.73	6.03	10.27	4.40	6.65	3.14	14.54	15.33	21.22
	<i>S.E.</i>	1.44	0.75	0.65	0.14	0.19	0.22	0.11	0.15	0.06	0.23	0.22	0.43
♀	<i>Mean</i>	87.54	48.05	39.48	8.25	7.07	11.43	4.79	6.94	3.23	15.99	16.59	25.13
	<i>S.E.</i>	2.03	1.08	1.35	0.15	0.41	0.29	0.11	0.17	0.10	0.50	0.39	0.55
3 ♂	<i>Mean</i>	73.01	41.47	31.55	7.37	6.42	10.49	4.12	5.70	3.20	15.71	15.52	20.79
	<i>S.E.</i>	0.92	0.43	1.21	0.08	0.23	0.13	0.09	0.29	0.12	0.32	0.61	0.48
♀	<i>Mean</i>	87.01	49.22	37.79	8.13	7.03	11.98	4.44	6.58	3.30	16.46	16.07	23.80
	<i>S.E.</i>	1.89	0.72	1.30	0.16	0.29	0.19	0.14	0.26	0.05	0.52	0.76	0.44
4 ♂	<i>Mean</i>	74.27	42.14	32.13	7.30	6.47	10.11	3.82	6.42	2.90	15.48	16.03	22.40
	<i>S.E.</i>	1.88	1.06	0.90	0.16	0.35	0.33	0.09	0.25	0.12	0.43	0.36	0.61
♀	<i>Mean</i>	85.20	45.90	39.30	8.14	7.41	10.75	3.91	6.86	2.82	16.72	15.42	26.08
	<i>S.E.</i>	2.61	1.56	2.20	0.11	0.40	0.19	0.05	0.16	0.07	0.66	0.86	0.97
5 ♂	<i>Mean</i>	83.88	48.51	35.37	8.20	7.44	11.24	4.54	7.28	3.16	18.15	18.84	25.98
	<i>S.E.</i>	2.80	1.09	1.72	0.22	0.39	0.29	0.05	0.15	0.04	1.04	0.47	1.55
♀	<i>Mean</i>	98.36	53.91	44.45	8.59	7.76	12.24	4.84	7.32	3.23	18.96	18.32	30.44
	<i>S.E.</i>	2.40	1.15	2.01	0.14	0.13	0.45	0.11	0.17	0.05	0.35	0.33	0.77
6 ♂	<i>Mean</i>	82.61	48.89	33.72	8.23	6.77	10.82	4.32	7.08	2.56	17.16	17.39	25.84
	<i>S.E.</i>	2.57	1.04	2.11	0.23	0.09	0.34	0.19	0.22	0.11	0.74	0.61	0.97
♀	<i>Mean</i>	95.47	54.20	41.26	8.95	7.20	11.66	4.65	7.81	2.78	17.98	17.57	28.51
	<i>S.E.</i>	3.55	1.52	2.12	0.39	0.18	0.42	0.23	0.35	0.07	0.52	0.52	1.50
7 ♂	<i>Mean</i>	80.73	45.37	35.35	7.65	6.78	10.33	3.70	6.12	3.01	16.51	16.86	24.20
	<i>S.E.</i>	2.11	1.21	0.90	0.10	0.19	0.38	0.08	0.21	0.17	0.39	0.26	1.08
♀	<i>Mean</i>	96.53	54.35	42.18	8.94	7.21	11.07	4.26	7.57	3.49	19.20	18.55	29.66
	<i>S.E.</i>	0.81	0.10	0.91	0.16	0.21	0.29	0.15	0.34	0.14	0.18	0.66	0.20
8 ♂	<i>Mean</i>	79.82	45.64	34.18	7.45	5.92	10.21	4.04	6.23	2.63	15.94	16.67	24.07
	<i>S.E.</i>	1.71	0.72	1.04	0.13	0.14	0.18	0.06	0.09	0.05	0.26	0.23	0.40
♀	<i>Mean</i>	95.52	51.90	43.62	8.65	7.10	12.00	4.56	6.96	2.84	17.00	17.21	28.53
	<i>S.E.</i>	1.46	0.54	1.38	0.16	0.30	0.30	0.09	0.11	0.05	0.24	0.34	0.50
9 ♂	<i>Mean</i>	68.54	39.18	29.43	6.58	6.36	9.82	3.50	5.75	2.56	14.53	15.61	20.02
	<i>S.E.</i>	1.98	0.56	1.29	0.10	0.18	0.20	0.09	0.13	0.09	0.13	0.32	0.47
♀	<i>Mean</i>	85.74	47.75	36.75	7.52	7.20	11.37	4.04	6.38	2.90	16.04	16.57	24.93
	<i>S.E.</i>	1.25	1.35	0.60	0.15	0.15	0.31	0.07	0.09	0.02	0.38	0.23	0.84
10 ♂	<i>Mean</i>	80.68	45.55	35.14	7.32	7.57	10.83	4.04	6.22	2.91	17.09	18.39	23.98
	<i>S.E.</i>	3.78	2.04	1.75	0.53	0.30	0.69	0.22	0.43	0.10	1.32	0.88	0.49
♀	<i>Mean</i>	86.29	49.36	36.93	7.77	7.45	11.05	4.12	6.60	3.16	17.42	17.56	26.08
	<i>S.E.</i>	1.37	0.92	0.88	0.15	0.13	0.20	0.07	0.15	0.05	0.42	0.38	0.48
11 ♂	<i>Mean</i>	80.27	44.27	36.00	8.33	6.71	11.12	4.16	6.83	3.08	14.44	15.29	24.33
	<i>S.E.</i>	9.31	6.45	2.86	1.02	0.64	0.18	0.19	0.50	0.07	1.41	1.57	4.29
♀	<i>Mean</i>	90.78	50.74	40.04	8.39	6.99	11.90	4.45	7.05	3.07	16.36	16.25	26.36
	<i>S.E.</i>	5.86	3.58	2.28	0.48	0.26	0.10	0.14	0.29	0.20	0.60	0.05	4.65

larval stages, as well as on the adults of many amphibians, has a negative effect on growth rate, leading to reduced size at metamorphosis and to an increment in mortality (Gamradt & Kats 1996; Gamradt *et al.* 1997; Kiesecker & Blaustein 1998; Lawler *et al.* 1999; Tyler *et al.* 1998; Nyström *et al.* 2001; Schmidt & Van Buskirk 2005). In addition, a negative effect of predation on size at metamorphosis has been suggested on many occasions to result from an alteration of behaviour. Cryptic behaviour is followed by reduced activity and feeding, which dramatically affects the adult body size of many amphibians (Gamradt & Kats 1996; Gamradt *et al.* 1997; Tyler *et al.* 1998; Van Buskirk & Schmidt 2000; Kats & Ferrer 2003). Consequently, small aquatic habitats could possibly offer conditions of reduced intra-specific competition and predation pressures that have a direct effect on newt body size.

Body shape variation: influence of the altitude

Our results show that approximately 70% of the observed morphometric variation in males (summarized by CV1-2) and 81% of the observed morphometric variation in females (summarized by CV1-3) is attributed to variation in characters closely related to the animals' head shape. The heads of newts are involved in feeding, foraging and courtship behaviour (Himstedt 1979; Malacarne & Giacoma 1986; Cogălniceanu 1992, 1994). Experiments with newts from the genus *Hynobius* suggest that a relatively wide head offers an adaptive advantage that maximizes fitness, especially in temporary aquatic habitats with hostile, unpredictable environments, as well as in cases of increased population density where cannibalism takes place (Kohmatsu *et al.* 2001). Cannibalism in overcrowded situations and increased population densities has also been reported for *Triturus* newts (Griffiths 1996; K. Sotiropoulos, personal observations). Consequently, a relatively wider head in such conditions possibly facilitates efficient foraging and feeding.

Our results show variation in head shape in both sexes in relation to altitude and consequently to factors that covary with altitude. Male alpine newts had wider heads at higher altitudes, which possibly facilitate efficient foraging of the limited food resources in high altitude areas. In contrast, in females, relative head width shows the opposite pattern and is narrower in high altitude areas. In addition, a small portion of the observed variation in female head width is related to the mean annual rainfall, as well as to the nature and turbidity of the aquatic habitat. This discrepancy in variation patterns between the sexes implies differential selection pressures in the sexes. However, and because altitude constitutes probably the

most important factor of ecological and climatic differentiation in Greece (Mavrommatis 1978), the observed altitudinal clines might indicate the influence of a number of other factors that co-vary with altitude, which in turn could influence the external morphology as a result of modifications at developmental, physiological and population levels (Mani 1990; Miaud *et al.* 2000; Miaud & Merilä 2001; Morrison & Hero 2003).

Water turbidity and the incidence of competitive newt species in the habitat were found to influence the eye size of female alpine newts. In amphibians, vision along with olfaction are the chief senses used to detect prey movement (Duellman & Trueb 1994; Stebbins & Cohen 1995). The eyes are also involved in other physiological processes, such as breathing movements, gulping down air into the lungs and sound production (De Jongh & Gans 1969; Duellman and Trueb 1994). In newts, vision plays a significant role during courtship behavior and spermatophore transfer (Halliday 1974). Thus, an increased eye size might offer increased foraging performance as well as effective courtship display in the low-visibility conditions of turbid aquatic habitats, or in cases of intense competition, for limited prey resources.

However, the available data cannot support a causal hypothesis of the observed variation without diminishing the possible influence of other factors that have not been measured in the present study. It has been shown that turbidity displays seasonal and/or diel fluctuations, and is also correlated to a series of abiotic (e.g. physicochemical and climatic features) and biotic (e.g. planktonic and benthic organisms and decomposing vegetation) factors (Toetz 1967; Mitchell & Stapp 1994). It is, therefore, possible that some or a combination of factors actually affects the morphological variability of local populations in a way that is still unknown to us.

Intra-population variation of body proportions

Local alpine newt populations express different levels of variation in the generalized size and shape of males that correlate significantly with the incidence of competitive newt species in the aquatic habitat. In cases where a competitive species lives in sympatry, the deviation of specimens from the mean phenotype is significantly lower. Competition between syntopic newt species has been reported in many cases and is often attributed to the utilization of common food resources from the competitive species (Griffiths & Mylotte 1987; Zuiderwijk & Bouton 1987; Dolmen 1988; Bringsøe 1994; Babik 1998). It is, therefore, tempting to hypothesize that this increased homogeneity of phenotypes might occur because of the action of stabi-

lizing selection towards a decrease in inter-specific competition (e.g. Williamson 1987; Merilä *et al.* 2001).

Taxonomical implications of body size and shape variation

No clear taxonomical implications or any type of phylogenetic origin were detected in the morphometric variation of the alpine newts from Greece. In both sexes, geographically distant and genetically differentiated populations (e.g. populations 4 and 7) (Sotiropoulos *et al.* 2007) appear more uniform with respect to body proportions than geographically neighboring and genetically similar populations (e.g. populations 6, 7) (Sotiropoulos *et al.* 2007). These results are in accordance with other studies that report a lack of a clear morphometric discrimination between alpine newt taxa, despite their significant genetic differentiation (Rocek 1974; Breuil 1986; Giacoma *et al.* 1988; Sotiropoulos *et al.* 2001), indicating a rapid response of the external phenotype to the altering conditions and environments that lead to the plasticity of the phenotype, however, within the variation of the species. It appears that the alpine newt is an exception among other polytypic newt species (i.e. *Lissotriton vulgaris*) in that its intraspecific taxonomic subdivision is not accompanied by sufficient morphological differentiation.

In conclusion, it appears that the various components of shape variation in both sexes vary independently to each other, following the spatial patterns of different environmental factors. Although the absence of any type of parallelism between the variation patterns of the external phenotype has been shown to reflect ecogenetic causation rather than phylogeny (Thorpe 1987; Brown & Thorpe 1991; Thorpe & Brown 1991; Thorpe *et al.* 1991, 1995; Malhotra & Thorpe 1994, 1997, 2000), further research is needed to unveil the possible adaptive value of the observed shape variation of alpine newts in Greece.

REFERENCES

- Accordi F, Massarek A, Nobili G (1990). Ecological responses in a population of Smooth newts (*Triturus vulgaris meridionalis*) in an unpredictable environment. *Herpetological Journal* **1**, 509-13.
- Arantzen JW (2000). A growth curve for the newt *Triturus cristatus*. *Journal of Herpetology* **34**, 227-32.
- Atchley WR (1983). Some genetic aspects of morphometric variation. In: Felsenstein J, ed. *Numerical Taxonomy: Proceedings of a NATO Advanced Studies Institute*. NATO ASI Series, Vol. G1. Springer-Verlag, Berlin-Heidelberg, pp. 346-363.
- Babik W (1998). Intrageneric predation in larval newts (*Triturus*, Salamandridae, Urodela). *Amphibia-Reptilia* **19**, 446-51.
- Báez M, Brown RP (1997). Testing multivariate patterns of within-island differentiation in *Podarcis dugesii* from Madeira. *Journal of Evolutionary Biology* **10**, 575-87.
- Barbault R (1988). Body size, ecological constraints, and the evolution of life-history strategies. *Evolutionary Biology* **22**, 261-86.
- Bell G (1977). The life of the Smooth newt (*Triturus vulgaris*) after metamorphosis. *Ecological Monographs* **47**, 279-99.
- Bell G, Lawton JH (1975). The ecology of the eggs and larvae of the Smooth newt (*Triturus vulgaris* L.). *Journal of Animal Ecology* **44**, 393-423.
- Bookstein FL, Chernoff BC, Elder RL, Humphries JM, Smith GR, Strauss RE (1985). Morphometrics in evolutionary biology. *Academy of Natural Sciences, Philadelphia* **15**, 1-277.
- Breuil M (1986). Biologie et différenciation génétique des populations du Triton Alpestre (*Triturus alpestris*) (Amphibia, Caudata) dans le Sud-est de la France et en Italie. Thèse du 3eme cycle, Université de Paris-Sud.
- Bringsøe H (1994). New records of *Triturus alpestris* (Amphibia, Caudata) in south Greece, with information on feeding habits, ecology and distribution. *Annales Musei Goulandris* **9**, 349-74.
- Brown RP, Thorpe RS (1991). Within-island microgeographic variation in body dimensions and scalation of the skink *Chalcides sexlineatus*, with testing of causal hypotheses. *Biological Journal of the Linnean Society* **44**, 47-64.
- Calder WA III (1996). *Size, Function, and Life History*. Dover Publications, Mineola, New York.
- Cogălniceanu D (1992). A comparative ethological study of female chemical attractants in newts (genus *Triturus*). *Amphibia-Reptilia* **13**, 69-74.
- Cogălniceanu D (1994). The relative importance of vision and olfaction in mate recognition in male newts (genus *Triturus*). *Herpetologica* **50**, 344-9.
- Cooke AS, Frazer JFD (1976). Characteristics of newt breeding sites. *Journal of Zoology* **178**, 223-36.
- De Jongh HJ, Gans C (1969). On the mechanism of respiration in the bullfrog, *Rana catesbeiana*: a reassessment. *Journal of Morphology* **127**, 259-90.

- Denoël M, Duguet R, Dzukic G, Kalezic M, Mazzotti S (2001). Biogeography and ecology of paedomorphosis in *Triturus alpestris* (Amphibia, Caudata). *Journal of Biogeography* **28**, 1271-80.
- Dolmen D (1988). Coexistence and niche segregation in the newts *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti). *Amphibia-Reptilia* **9**, 365-74.
- Duellman WE, Trueb L (1994). *Biology of Amphibians*, 2nd edn. Johns Hopkins University Press, Baltimore, MD.
- Endler JA (1986). *Natural Selection in the Wild*. Princeton University Press, Princeton, New Jersey.
- Gamradt SC, Kats LB (1996). Effect of introduced crayfish and mosquitofish on California newts. *Conservation Biology* **10**, 1155-62.
- Gamradt SC, Kats LB, Anzalone CB (1997). Aggression by non-native crayfish deters breeding in California newts. *Conservation Biology* **11**, 793-6.
- Giacoma C, Picariello O, Puntillo D, Rossi F, Triperi S (1988). The distribution and habitat of the newt (*Triturus*, Amphibia) in Calabria (Southern Italy). *Monitore Zoologico Italiano* **22**, 449-64.
- Griffiths RA (1996). *Newts and Salamanders of Europe*. T & AD Poyser, Natural History, London.
- Griffiths RA, Mylotte VJ (1987). Microhabitat selection and feeding relations of smooth and warty newts, *Triturus vulgaris* and *T. cristatus*, at an upland pond in mid-Wales. *Holarctic Ecology* **10**, 1-7.
- Halliday TR (1974). Sexual behaviour in the smooth newt, *Triturus vulgaris* (Urodela, Salamandridae). *Journal of Herpetology* **8**, 277-92.
- Halliday TR, Verrell PA (1986). Sexual selection and body size in amphibians. *Herpetological Journal* **1**, 86-92.
- Hespenheide HA (1973). Ecological inferences from morphological data. *Annual Review of Ecology and Systematics* **4**, 213-29.
- Himstedt W (1979). The significance of colour signals in partner recognition of the newt *Triturus alpestris*. *Copeia* **1979**, 40-43.
- James FC, McCulloch CE (1990). Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annual Review of Ecology and Systematics* **21**, 129-66.
- Joly P, Miaud C (1989). Fidelity to the breeding site in the alpine newt, *Triturus alpestris*. *Behavioural Processes* **19**, 47-56.
- Joly P, Grolet O (1996). Colonization dynamics of new ponds, and the age structure of colonizing alpine newt, *Triturus alpestris*. *Acta Oecologica* **17**, 599-608.
- Kats LB, Ferrer RP (2003). Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9**, 99-110.
- Kiesecker JM, Blaustein AR (1998). Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs (*Rana aurora*). *Conservation Biology* **12**, 776-87.
- Kohmatsu Y, Nakano S, Yamamura N (2001). Effects of head shape variation on growth, metamorphosis and survivorship in larval salamanders (*Hynobius retardatus*). *Ecological Research* **16**, 73-83.
- La Barbera M (1989). Analyzing body size as a factor in ecology and evolution. *Annual Review in Ecology and Systematics* **20**, 97-117.
- Lande R, Arnold SJ (1983). The measurement of selection on correlated characters. *Evolution* **37**, 1210-26.
- Lawler SP, Dritz D, Strange T, Holyoak M (1999). Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. *Conservation Biology* **13**, 613-22.
- Malacarne G, Giacoma C (1986). Chemical signals in European newt courtship. *Bollettino di Zoologia* **53**, 79-83.
- Malhotra A, Thorpe RS (1994). Parallels between island lizards suggest selection on mitochondrial DNA and morphology. *Proceedings of the Royal Society of London B* **257**, 37-42.
- Malhotra A, Thorpe RS (1997). Size and shape variation in a Lesser Antillean anole, *Anolis oculatus* (Sauria: Iguanidae) in relation to habitat. *Biological Journal of the Linnean Society* **60**, 53-72.
- Malhotra A, Thorpe RS (2000). The dynamics of natural selection and vicariance in the Dominican Anole: patterns of within-island molecular and morphological divergence. *Evolution* **54**, 245-58.
- Mani MS (1990). *Fundamentals of High Altitude Biology*, 2nd edn. Oxford & IBH Publishing, New Delhi, Bombay, Calcutta.
- Manly BJB (1985). *The Statistics of Natural Selection*. Chapman & Hall, London.
- Mavrommatis GN (1978). *Bioclimate of Greece. Relations between climate and vegetation, bioclimatic maps*. Institute of Forestry Research, Athens, Greece

- (in Greek).
- Merilä J, Sheldon BC, Kruuk LEB (2001). Explaining stasis: microevolutionary studies in natural populations. *Genetica* **112-113**, 199-222.
- Miaud C, Merilä J (2001). Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. *Biota* **2/1**, 31-50.
- Miaud C, Guyétant R, Faber H (2000). Age, size, and growth of the alpine newt, *Triturus alpestris* (Urodela: Salamandridae) at high altitude and a review of life-history trait variation throughout its range. *Herpetologica* **56**, 135-144.
- Mitchell MK, Stapp WB (1994). *Field Manual for Water Quality Monitoring*, 8th edn. Thomson-Shore, Dexter, MI.
- Morrison C, Hero J-M (2003). Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* **72**, 270-79.
- Nyström P, Svensson O, Lardner B, Brönmark C, Granéli W (2001). The influence of multiple introduced predators on a littoral pond community. *Ecology* **82**, 1023-39.
- Reyment RA, Blackith RE, Campell NA (1984). *Multivariate morphometrics*, 2nd edn. Academic Press, London.
- Roček Z (1974). Biometrical investigations of Central European populations of the Alpine newt - *Triturus alpestris alpestris* (Laurenti, 1768) (Amphibia: Urodela). *Acta Universitatis Carolinae - Biologica* **1972**, 295-373.
- Schmidt BR, Van Buskirk J (2005). A comparative analysis of predator-induced plasticity in larval *Triturus* newts. *Journal of Evolutionary Biology* **18**, 415-25.
- Sotiropoulos K, Legakis A, Polymeni RM (1995). A review of the knowledge on the distribution of the genus *Triturus* (Rafinesque, 1815) in Greece (Caudata, Salamandridae). *Herpetozoa* **8**, 25-34.
- Sotiropoulos K, Tomovic L, Dzukic G, Kalezic ML (2001). Morphological differentiation of the alpine newt (*Triturus alpestris*) in the Balkans: taxonomic implications. *Herpetological Journal* **11**, 1-8.
- Sotiropoulos K, Eleftherakos K, Dzukic G, Kalezic ML, Legakis A, Polymeni RM (2007). Phylogeny and biogeography of the alpine newt *Mesotriton alpestris* (Salamandridae, Caudata), inferred from mtDNA sequences. *Molecular Phylogenetics and Evolution* **45**, 211-26.
- Sotiropoulos K, Eleftherakos K, Kalezic ML, Legakis A, Polymeni RM (2008). Genetic structure of the alpine newt, *Mesotriton alpestris* (Salamandridae, Caudata), in the southern limit of its distribution: implications for conservation. *Biochemical Systematics and Ecology* **36**, 297-311.
- Stebbins RC, Cohen NW (1995). *Natural History of Amphibians*. Princeton University Press, Princeton.
- Thorpe RS (1987). Geographic variation: a synthesis of cause, data, pattern and congruence in relation to subspecies, multivariate analysis and phylogenesis. *Bollettino di Zoologia* **54**, 3-11.
- Thorpe RS, Brown RP (1991). Microgeographic clines in the size of mature male *Gallotia galloti* (Squamata: Lacertidae) on Tenerife: causal hypotheses. *Herpetologica* **47**, 28-37.
- Thorpe RS, Brown RP, Malhotra A, Wüster W (1991). Geographic variation and population systematics: distinguishing between ecogenetics and phylogenetics. *Boletino di Zoologia* **58**, 329-35.
- Thorpe RS, Malhotra A, Black H, Daltry JC, Wüster W (1995). Relating geographic pattern to phylogenetic process. *Philosophical Transactions of the Royal Society of London B* **349**, 61-8.
- Toetz DW (1967). *Ecological factors affecting turbidity and productivity in prairie ponds in the Southern Great Plains*. Oklahoma State University, Environmental Institute Technical Report, Publ. Nr: A-001.
- Tyler T, Liss WJ, Ganio LM, Larson GL, Hoffman R, Deimling E, Lomnický G (1998). Interaction between introduced trout and larval salamanders (*Ambystoma macrodactylum*) in high-elevation lakes. *Conservation Biology* **12**, 94-105.
- Van Buskirk J, Schmidt BR (2000). Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. *Ecology* **81**, 3009-28.
- Williamson PG (1987). Selection or constraint: a proposal on the mechanism for stasis. In: Campbell KSW, Day MF, eds. *Rates of Evolution*. Allen & Unwin, London, pp. 129-42.
- Zuiderwijk A, Bouton N (1987). On competition in the genus *Triturus* (Caudata, Salamandridae). In: Van Gelder JJ, Strijbosch H, Bergers PJM, eds. *Proceedings of the 4th Ordinary General Meeting*. Societas Europaea Herpetologica, Nijmegen 1987, pp. 453-8.