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Relationship between morphometric and genetic variation in pure and hybrid populations of the smooth and Montandon's newt (*Triturus vulgaris* and *T. montandoni*)

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Abstract

The variation in 10 morphometric characters and the degree of sexual dimorphism in size and shape was studied in pure and hybrid populations of smooth *Triturus vulgaris* and Montandon's *T. montandoni* newts. Multivariate analyses showed pronounced interspecific differences in both sexes. Females differed mainly in traits related to general size, whereas males differed mainly in body proportions. *Triturus vulgaris* males possessed a relatively longer trunk and tail. Sexual size dimorphism was much stronger in *T. montandoni*, the females being the larger sex. In hybrid zone populations a general correlation between morphometric traits and nuclear genotypes was found. This does not, however, fully explain the variation in size and shape. Whereas the majority of genetically identified hybrids fell into the range of morphologically pure species, many genetically pure individuals were classified as morphometric intermediates. The linear relationship between the morphometric and genetic variation suggested no strong differential selection on the molecular markers and loci responsible for morphometric differences between species. The differences in the level of sexual size and shape dimorphism, however, suggest that females of both species show dissimilar mate preferences, and thus hybrid males may experience reduced fitness resulting from lower mating success.

Key words: *Triturus*, hybrid zone, sexual dimorphism, sexual selection, morphometry

INTRODUCTION

The smooth newt *Triturus vulgaris* and Montandon's newt *T. montandoni* are closely related species that hybridize in nature (Rafiński & Arntzen, 1987; Kotlík & Zavadil, 1999; Zajc & Arntzen, 1999). Recently we studied the extent of hybridization in an area of sympatry in the Carpathians (southern Poland) using molecular markers (Babik, Szymura & Rafiński, 2003). Hybrids were detected in almost all populations, with an average frequency of 23%. As most of the genetically identified hybrids were recombinants, F_1 and further generations must be both viable and fertile.

The study of natural hybridization provides information relevant to the processes of speciation and it may be in itself an important component of speciation through reinforcement or introgression (Hewitt, 1988; Harrison, 1990; Arnold, 1997). The strength and direction of natural selection acting in hybrid populations are key factors determining the evolutionary consequences of hybridization. Molecular traits are extremely useful tools in studies on natural hybridization owing to their simple mode of inheritance, allowing the analysis of hybrid populations for gene and genotype frequencies (Avise, 1994). On the other hand, variation of external phenotype in hybrid zones may be easier to interpret for natural/sexual selection as their association with fitness is usually more straightforward than that of molecular markers. Therefore a more comprehensive approach would consist of a genetic markers-based analysis paralleled by a study of variation in phenotypic traits (Nürnberger *et al*., 1995). For example, a close link between variation in body size and shape and the fitness of hybrids was shown for three-spined sticklebacks *Gasterosteus aculeatus* (Hatfield & Schluter, 1999; Vamosi & Schluter, 1999).

Most morphological differences between *T. vulgaris* and *T. montandoni* pertain to male secondary sexual characters. The males of the two species differ strikingly in qualitative epigamic traits, and also in body proportions. The females of the two species are generally much more similar, differing mainly in coloration, spotting pattern and size. Both species display different levels of sexual dimorphism in body size (Malmgren, 2001).

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†This paper is dedicated to the memory of Professor Jan Rafiński, who died suddenly, while doing fieldwork, in May 2003.

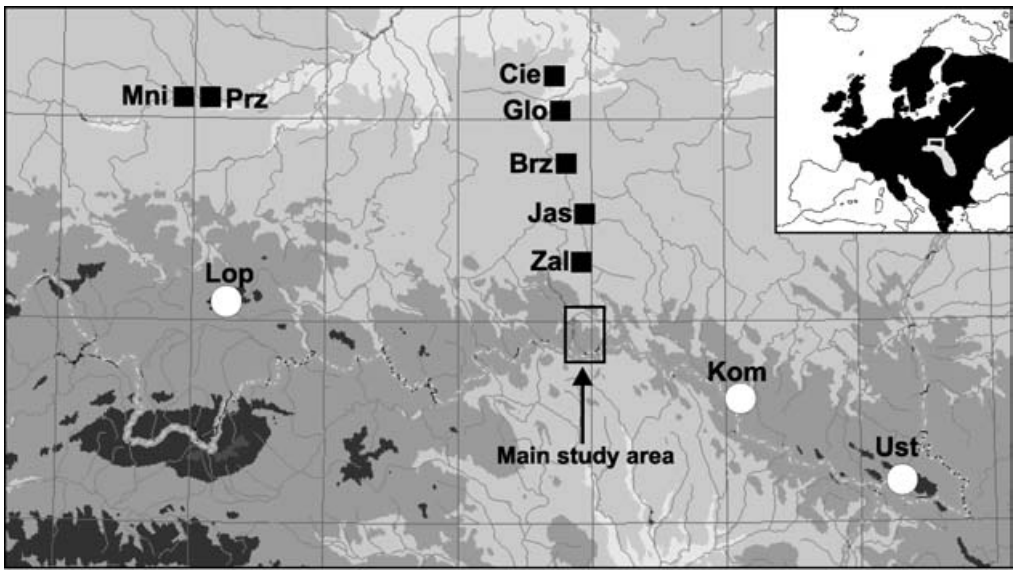

Fig. 1. Distribution of pure populations of *Triturus vulgaris* (black squares) and *T. montandoni* (white circles) reported in the present paper. Colours from light to dark represent altitudes from low to high (200, 500, 1000 m a.s.l.). Inset, distribution of *T. vulgaris* (black) and *T. montandoni* (grey) in Europe.

Our previous analysis was concentrated on the patterns seen at presumed neutral nuclear markers and qualitative sexually dimorphic traits in males (Babik *et al*., 2003). In the present paper data are provided on morphometric variation in hybrids and these are compared with the genetic data. To set a frame of reference for the analysis of morphological variation in the hybrid zone, the level of variation was first assessed in genetically pure populations of both species. Several studies on morphometric variation in *T. vulgaris* (Kalezić *et al*., 1992; Malmgren & Thollesson, 1999) and *T. montandoni* (Kminiak, 1971; Dandova, Weidinger & Zavadil, 1998) have been published. However, as these studies were carried out on populations distant from our area of hybridization and because of the possibility of interpopulation differences and variation in measurement methods, they could not be treated as reference data for our study.

The main goal of the present study was to investigate the relationship between molecular nuclear markers and morphometric traits in *T. vulgaris* × *T. montandoni* hybrid populations. Our focus was on morphometric traits reflecting species-specific sexual dimorphism and size/shape differences. As these traits may be under a greater influence of natural/sexual selection compared to molecular markers, discordant patterns of variation between morphometric and genetic traits were expected.

MATERIALS AND METHODS

Newts were sampled from 7 allopatric *T. vulgaris* populations. Two of these have been reported elsewhere (Babik *et al*., 2003), their location together with the geographic position of 5 additional populations (Zal, Jas, Brz, Glo, Cie) is indicated in Fig. 1. Allopatric *T. montandoni* samples were collected from 3 sites, 1 of which (Ust) was reported in Babik *et al*. (2003), with the 2 additional populations being Lop and Kom (Fig. 1).

The newts were dip-netted, anaesthetized with 0.2% MS 222, and photographed in side and bottom views. After collecting the tail tips for genetic analyses, the newts were released to their breeding sites within 3 days. A total of 10 morphometric measurements was taken from the digital photographs of the ventral side of animals with 0.1 mm accuracy by one of the authors (WB): HW, maximum head width; HL, head length from snout to narrowest point of neck; PHL, distance from snout to the point of maximum head width; HS, distance from snout to base of forelimb; LL, arm length; IL, distance from posterior base of forelimb to anterior base of hindlimb; SVL, snout–vent length from tip of snout to anterior vent margin; TL, tail length from anterior vent margin to tail tip (excluding the tail filament in *montandoni* males); FLW, arm width; HLW, thigh width. The tail length measurements were not fully comparable for the males of the 2 species. *Triturus montandoni* males develop a tail filament several mm long during the breeding season, its length being highly dependent on the condition of the male, so it was excluded from the tail length measurement; thus in both species, tail length corresponds to the part of the tail that bears the tail-fin.

Age-dependent changes in body proportions were not a serious problem because only adults were sampled, and growth rate in newts slows down considerably after attaining sexual maturity (e.g. Halliday & Verrell, 1988).

Before further analyses, all the measurements were log-transformed, which enhanced normality of the distributions (Shapiro–Wilk test). The differences in means between the species for each morphometric variable were checked using *t*-tests; type I error level in multiple tests was controlled using a sequential Bonferroni correction (Rice, 1989). The statistical analyses were always performed for each sex separately.

Transformed measurements were subjected to multivariate analyses. For all the PCAs, the correlation matrices were used. To assess the level of among-population variation in general size we performed principal component analyses (PCAs) for each species separately. Then the PC 1 scores were subjected to 1-way ANOVAs with the population as a grouping variable. In all the population-based analyses only samples with $n \geq 5$ for either sex were included. For further analyses of interspecific differences the population samples were pooled for each species.

Interspecific size and shape differences as well as interspecific levels of sexual dimorphism were evaluated by PCA. Discriminant analysis (DA) and canonical variate analysis (CVA) were used to find the combinations of variables that best separated the 2 species. The pooled within-group correlations of variables with CV 1 were taken as measures of the variable contributions to discriminate between groups (Dillon & Goldstein, 1984). The CV 1 coefficients were derived from allopatric populations and subsequently CV 1 scores were computed for each individual. An individual was regarded as a morphometric intermediate when its CV 1 score fell outside the 95 percentiles computed for the allopatric populations. All the statistical analyses were performed using Statistica 6 package (StatSoft, 2001).

Descriptions and the scoring methods for the nuclear genetic markers are given in Babik *et al*. (2003). Briefly, variation was scored at 6 allozyme loci and 1 microsatellite, and then combined to form the maximum likelihood genetic hybrid index (GenHI) (Rieseberg, Baird & Desrochers, 1998), which can roughly be regarded as a proportion of *vulgaris* alleles for each individual. The GenHI value for *T. vulgaris* reference populations Prz and Mni ranged from 0.808 to 1.000, whereas for the *T. montandoni* reference population Ust GenHI equalled 0.000 for all individuals (Babik *et al*., 2003). The additional allopatric populations (Brz, Cie, Glo, Jas, Kom, Lop, Zal) were scored for the same set of markers (W. Babik, pers. obs.), revealing a frequency of foreign alleles of < 0.05. These populations were treated as non-hybrid in morphometric analyses.

The concordance between morphometric and genetic data in sympatric populations was assessed using the transect-independent method of Kruuk (1997) for each sex. The standardized population-mean CV 1 score value was regressed on the standardized population-mean GenHI. The relative excess and differential introgression of either species character set would be indicated by the significance of the quadratic and cubic components of the regression, respectively (Kruuk, 1997; Rohwer, Bermingham & Wood, 2001).

Table 1. Means of the morphometric measurements (in mm), their standard errors (SE) and the Bonferroni-corrected *P*-values from *t*-tests of differences between means for *Triturus vulgaris* and *T. montandoni* from allopatric populations. Results are given for each sex separately. For an explanation of acronyms see Materials and methods

	T. vulgaris		*T. montandoni*		
Variable	Mean	± SE	Mean	± SE	*P*
Females					
	$n = 103$		$n = 26$		
HW	6.32	0.05	8.18	0.08	< 0.0001
HL	10.10	0.08	11.78	0.13	< 0.0001
PHL	5.57	0.05	6.76	0.08	< 0.0001
HS	13.83	0.12	16.76	0.11	< 0.0001
LL	4.01	0.05	5.14	0.12	< 0.0001
IL	20.44	0.22	23.70	0.36	< 0.0001
SVL	37.84	0.34	44.71	0.48	< 0.0001
TL	37.63	0.42	46.26	0.47	< 0.0001
HLW	1.84	0.02	2.38	0.06	< 0.0001
FLW	1.15	0.02	1.62	0.03	< 0.0001
Males					
	$n = 65$		$n = 56$		
HW	6.17	0.05	7.05	0.09	< 0.0001
HL	10.01	0.09	10.41	0.12	< 0.05
PHL	5.49	0.07	6.28	0.08	< 0.0001
HS	13.74	0.13	14.76	0.14	< 0.0001
LL	4.12	0.05	4.89	0.06	< 0.0001
IL	19.60	0.17	18.92	0.18	< 0.05
SVL	37.11	0.30	37.26	0.38	NS
TL	40.76	0.51	37.32	0.40	< 0.0001
HLW	1.68	0.02	1.86	0.03	< 0.0001
FLW	1.15	0.02	1.36	0.03	< 0.0001

RESULTS

Morphometric differences between species

The total number of *vulgaris* individuals studied for variation in morphometric traits was $n = 103$ for females and $n = 65$ for males. In *T. montandoni* $n = 26$ females and $n = 56$ males were measured. The means, their standard errors and the results of *t*-tests for all measurements from the reference populations are given in Table 1. In *T. vulgaris*, significant differences in body size as measured by PC 1 scores were found for females only ($F_{5,93} = 7.844$, $P < 0.0001$), whereas in *T. montandoni* the among-population variation was found for males only ($F_{4,53} = 30.816$, $P < 0.0001$). However, species-specific differences for each sex assessed by PCA and DA were clear-cut even when the populations were pooled (see below, Fig. 3). This indicated that interspecific differences were large in respect to within-species interpopulation variation and justified the subsequent pooling of the data.

The two species differed markedly in the amount of sexual dimorphism as assessed by PCA (Fig. 2, Table 2). In *T. vulgaris* there was no clear separation of the sexes on the PC 1–PC 2 plane. However, there was some separation along the PC 3 axis (not shown), which correlated with the variables FLW, HLW, TL and PHL. In contrast, PC 1

Table 2. Factor loadings for the first three principal components and the percentages of variance explained by the individual components. Analyses performed for the individuals from allopatric populations of *Triturus vulgaris* (Brz, Cie, Glo, Jas, Mni, Prz, Zal – see Fig.1) and *T. montandoni* (Kom, Lop, Ust), for each species separately. For an explanation of acronyms see Materials and methods

	T. vulgaris			*T. montandoni*		
Variable	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
HW	0.777	−0.079	−0.120	0.902	−0.015	0.054
HL	0.880	0.160	0.221	0.908	−0.130	−0.086
PHL	0.711	0.402	0.323	0.709	−0.204	−0.648
HS	0.916	0.185	0.120	0.951	−0.049	−0.040
LL	0.526	−0.767	0.039	0.346	0.917	−0.170
IL	0.780	−0.160	−0.180	0.919	−0.028	0.163
SVL	0.945	−0.013	0.002	0.974	−0.070	0.087
TL	0.733	−0.190	0.394	0.893	−0.041	0.203
HLW	0.692	0.304	−0.512	0.865	0.013	0.014
FLW	0.698	−0.095	−0.367	0.811	0.153	0.194
% of variance explained	60.1	9.8	7.7	71.6	9.3	5.7

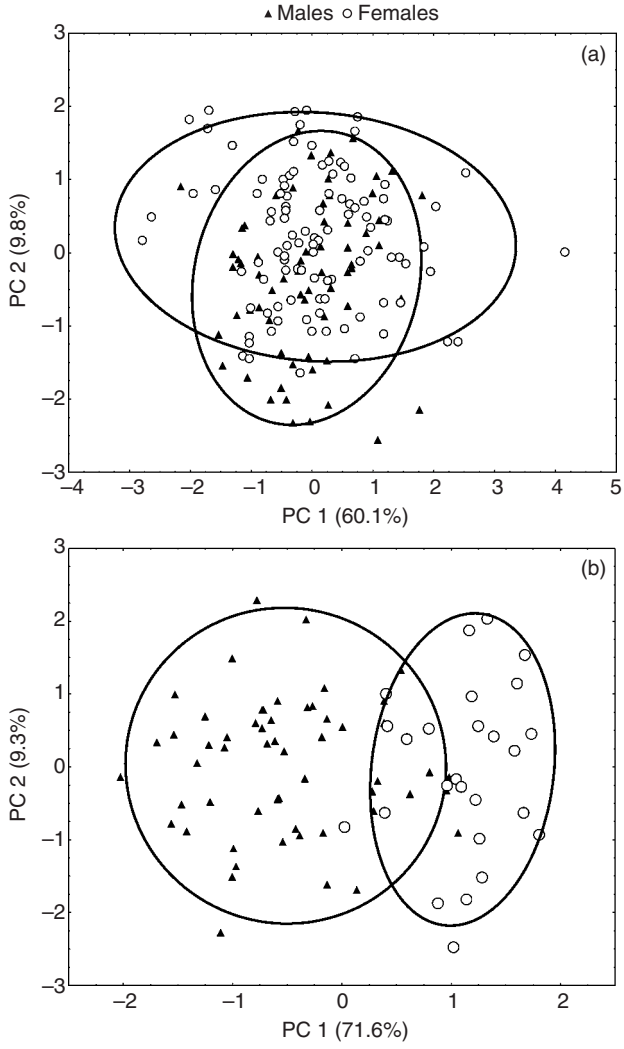

Fig. 2. Scatterplots of individual PC 1 *vs* PC 2 scores from analyses performed for: (a) *Triturus vulgaris*; (b) *T. montandoni*. Percentages of variance explained by PC 1 and PC 2 together with 95% concentration ellipses are given.

clearly differentiated the sexes in *T. montandoni*. High positive loadings for all but one variable (LL) allow PC 1 to be interpreted as a general size component.

The females of the two species were clearly separated along the PC 1 axis, which explained 78.4% of the total variance. Since all loadings were of similar magnitude and had a positive sign, PC 1 can be regarded as a general size component (Fig. 3a, Table 3). PCs 2–4 explained roughly similar amounts of variance. They revealed no clear grouping, which indicates that shape variation between females was slight.

In discriminant analysis 96.9% of females were correctly classified to the genetically determined species. Head width (HW) showed the highest correlation with the CV 1 axis, the correlations of the other variables were broadly similar (Table 4). *Triturus montandoni* females showed higher values for all variables (Table 1).

The relative trunk length (logIL–logSVL) did not differ between females of the two species ($t_{127} = 1.249$, $P = 0.214$). At the same time, *T. vulgaris* females had relatively shorter tails (logTL–logSVL) ($t_{127} = 2.795$, $P = 0.006$).

The males formed non-overlapping clusters on the plane defined by PC 1 and PC 2 (Fig. 3b). PC 1 accounted for 55.7% of variance and can be regarded as representing the general size (Table 3). PC 2 explained 17.5% of variance with high loadings for TL, IL and LL, emphasizing the shape differences between males of the two species. *Triturus vulgaris* males have relatively longer tails and trunks and relatively shorter arms. PC 3 and PC 4 explained a much smaller amount of variance and did not separate the species (not shown).

The classification of males in DA was almost perfect (99.2% of correct classifications, only one *T. vulgaris* male misclassified as *T. montandoni*). The variables LL, HW, PHL and FLW exhibited highest correlations with CV 1.

The *T. vulgaris* males had relatively longer trunks (logIL–logSVL) and tails (logTL–log SVL) than did

Table 3. Factor loadings for the first four principal components and the percentages of variance explained by the individual components. Analyses performed separately for males and females from allopatric *Triturus vulgaris* and *T. montandoni* populations. For an explanation of acronyms see Materials and methods

	Females				Males			
Variable	PC 1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3	PC 4
HW	0.921	0.164	0.052	−0.078	0.787	−0.370	−0.142	0.122
HL	0.920	−0.156	0.082	−0.180	0.880	0.134	0.251	0.045
PHL	0.879	0.063	0.249	−0.208	0.721	−0.307	0.443	0.278
HS	0.951	−0.104	0.104	−0.128	0.936	−0.063	0.163	0.056
LL	0.779	0.326	−0.507	−0.125	0.506	−0.570	−0.496	0.250
IL	0.817	−0.383	−0.244	0.288	0.636	0.608	−0.312	0.004
SVL	0.954	−0.194	−0.019	0.014	0.879	0.383	−0.084	0.044
TL	0.887	−0.146	−0.058	−0.087	0.443	0.739	−0.066	0.121
HLW	0.849	0.219	0.220	0.356	0.772	−0.103	0.164	−0.422
FLW	0.882	0.255	0.041	0.188	0.746	−0.309	−0.209	−0.382
% of variance explained	78.4	4.9	4.5	3.7	55.7	17.5	7.3	5.0

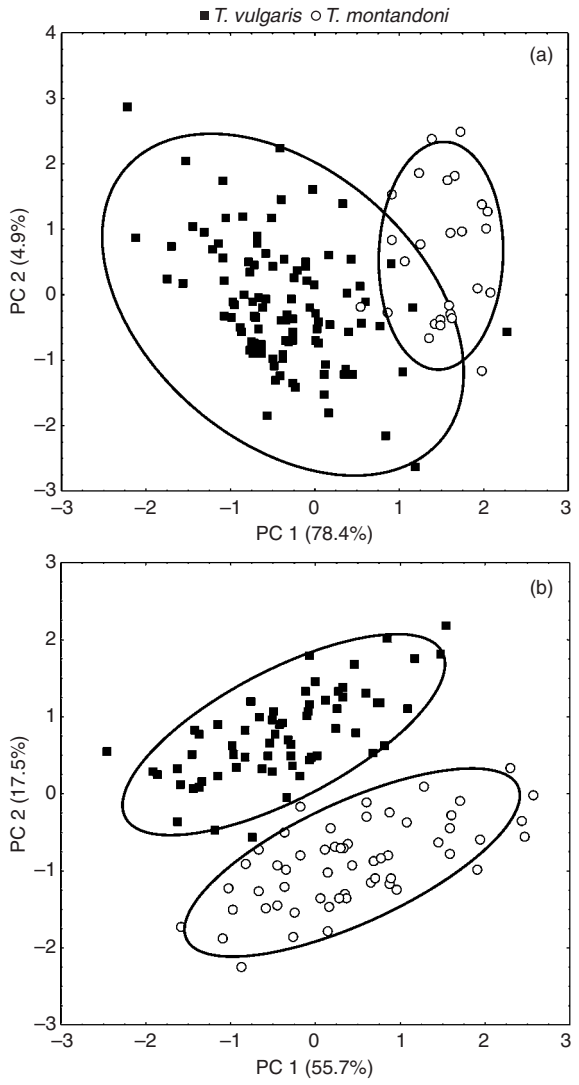

Fig. 3. Scatterplots of individual PC 1 *vs* PC 2 scores from analyses performed for: (a) females of *Triturus vulgaris* and *T. montandoni*; (b) males of both species. Percentages of variance explained by PC 1 and PC 2 together with 95% concentration ellipses are given.

Table 4. The correlation coefficients of the morphometric variables with the canonical variate 1. Analyses performed separately for males and females of *Triturus vulgaris* and *T. montandoni* from allopatric populations. For an explanation of acronyms see Materials and methods

Variable	Females	Males
HW	−0.887	−0.318
HL	−0.477	−0.094
PHL	−0.563	−0.278
HS	−0.585	−0.189
LL	−0.509	−0.345
IL	−0.330	0.097
SVL	−0.470	−0.009
TL	−0.473	0.183
HLW	−0.508	−0.165
FLW	−0.612	−0.231

T. montandoni males ($t_{119} = 6.765$, $P < 0.0001$, and $t_{119} = 7.187$, $P < 0.0001$, respectively).

Hybrid populations

All 33 populations from the area of sympatry were those studied by Babik *et al.* (2003), totalling $n = 292$ females and $n = 393$ males. Hybrids were detected in 31 of these populations, with a mean frequency of 23%.

There was a significant linear relationship between individual GenHI and CV 1 scores for both sexes, though it was much stronger for hybrid males ($R^2 = 0.756$, $F_{1,90} = 279.591$, $P < 0.0001$) than for hybrid females ($R^2 = 0.496$, $F_{1,77} = 75.685$, $P < 0.0001$) (Fig. 4).

CV 1 in females was strongly correlated with general size, while in males it expressed mainly differences in body proportions. Since the relative tail length seems to be a target of sexual selection (see below), we specifically looked for a correlation between individual GenHI and relative tail length in males (lnTL–lnSVL). This relationship was significant ($R^2 = 0.240$, $F_{1,90} = 28.492$, $P < 0.0001$), but weaker than the correlation between GenHI and CV 1.

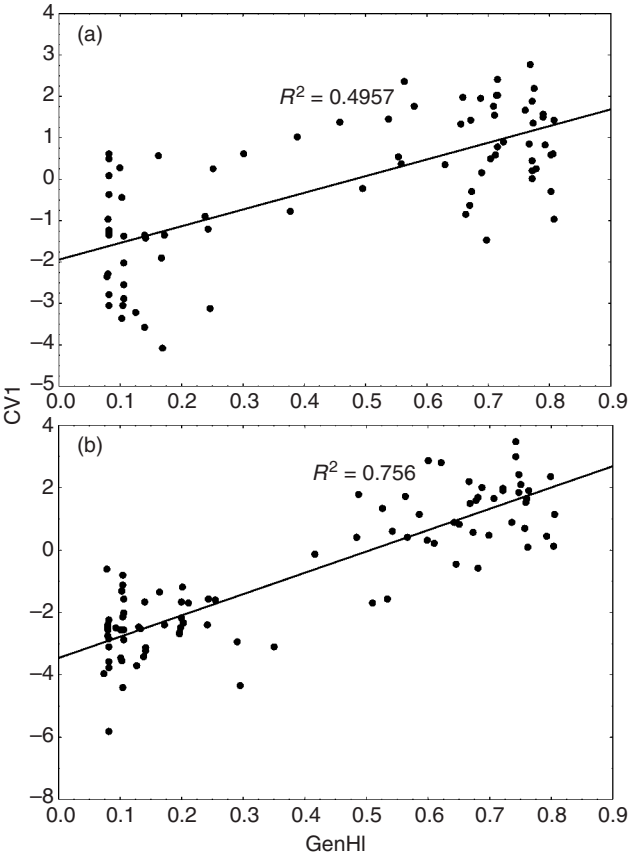

Fig. 4. Relationships between the individual CV 1 scores and GenHI values for hybrid individuals (0.000 < GenHI < 0.808): (a) females; (b) males. Both variables were standardized to mean 0 and variance 1. The linear regression lines and R^2 values are shown.

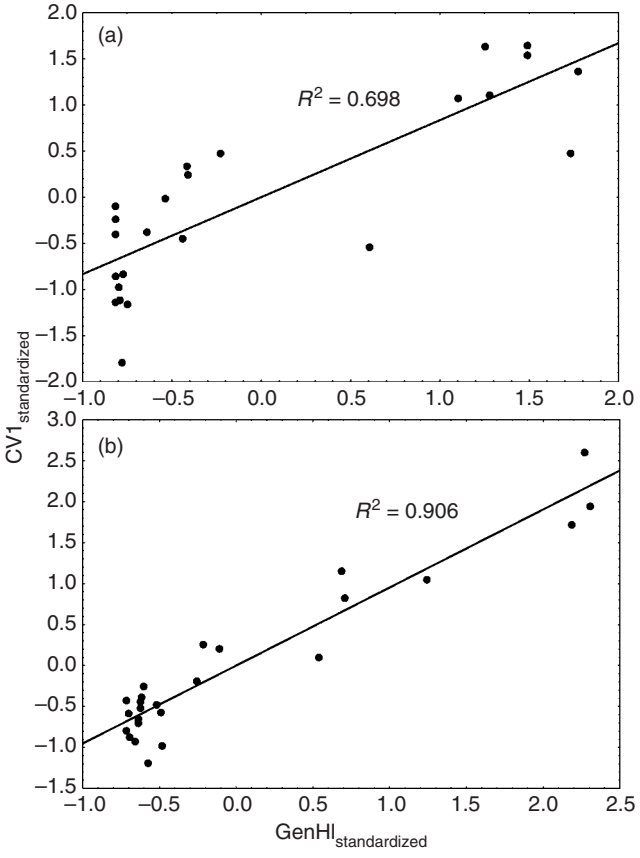

Fig. 5. Relationships between the population-mean CV 1 and GenHI for the populations from the area of sympatry: (a) females; (b) males. Both variables were standardized to mean 0 and variance 1. The linear regression lines and R^2s are shown.

An association between the frequency of hybrid individuals (as assessed by their GenHI values) and frequency of morphological intermediates was checked for. The 2×2 contingency table test showed a significant association for females ($\chi^2_1 = 11.83$, $P = 0.0006$) and indicated that morphologically intermediate females were under-represented among genetic hybrids. Only 10 of 79 genetic hybrid females were in the range of morphologically intermediate individuals. In other words, 87.4% of genetic hybrids would be classified as morphologically pure individuals. Unexpectedly, as many as 70 of 213 (32.4%) genetically non-hybrid females fell into the morphometrically intermediate class.

The results of the analogous test for males was also significant ($\chi^2_1 = 5.47$, $P = 0.0193$). As in females, similarly low proportion of genetic hybrids (15 out of 92) were in the range of morphologically intermediate individuals (test of difference between proportions, $P = 0.503$). In contrast to females, only 24 of 301 (7.97%) of non-hybrid males fell into the morphometrically intermediate class. This difference between sexes was highly significant ($P < 0.0001$).

The linear relationship between population-mean GenHI and CV 1 values was stronger for males ($R^2 = 0.906$, $F_{1,23} = 222.12$, $P < 0.0001$), than for females ($R^2 = 0.698, F_{1,22} = 50.821, P < 0.0001$) (Fig. 5). Neither adding quadratic nor cubic terms significantly improved the regression fit.

DISCUSSION

In general, our results confirm earlier reports on the size differences between the females of *T. vulgaris* and *T. montandoni*. However, the existing studies (Kminiak, 1971; Kalezić *et al*., 1992; Dandova *et al*., 1998; Malmgren & Thollesson, 1999) did not aim specifically at examining morphometric differences between these two species and used methods not comparable to ours. Univariate analyses and PCA revealed that all of the metric variables had higher values for *T. montandoni* females (Table 1). DA disclosed that the head width (HW) contributed most to the discrimination between females of the two species (Table 4). The remaining characters exhibited relatively high and similar correlations with CV 1, which resulted from the general size difference between females of the two species.

In contrast to females, the males did not differ significantly in SVL. *Triturus montandoni* males showed significantly higher values in all but two measurements. These were tail length (TL) and the trunk length (IL)

which had significantly higher values for the *T. vulgaris* males (Table 1). Unlike the females, males differ mainly in shape as confirmed by PCA and DA (Fig. 3b, Tables 3, 4).

The degree of sexual dimorphism in body size and shape differed markedly between species. This is evident both from the inspection of Table 1 and from the results of PCA (Fig. 2a, b, Table 2). For *T. vulgaris*, we obtained much weaker separation of the sexes than Malmgren & Thollesson (1999). This discrepancy might have resulted from a number of factors: different origins of the specimens, differences in the set of traits included, differences in the measurement methods or the use of fixed *vs*. live individuals. *Triturus montandoni* males are much smaller than females, whereas the sizes of both sexes in *T. vulgaris* are similar (sexual dimorphism index *sensu* Lovich & Gibbons (1992) equals 0.200 and 0.020, respectively). This confirms the results of Malmgren (2001). In *T. vulgaris* females the trunk length is positively correlated with fecundity (Verrell & Francillion, 1986; Baker, 1992; Nobili & Accordi, 1997). The same relationship seems to pertain to interspecific differences; *T. montandoni* females are more fecund than *T. vulgaris* females (Pecio, 1992; Osikowski & Rafiński, 2001). Since fecundity is strongly correlated with fitness, body length may be an important target of selection in hybrid populations. Body size in newt females is also under sexual selection, larger, more fecund females being preferred by males (Verrell, 1986). The species also differ in the size and shape of the head, which is smaller and relatively more narrow in *T. vulgaris* than in *T. montandoni*. This feature may be correlated with the differences in gape size and thus with prey size. An adaptation to different trophic niches may constitute another factor influencing the fitness of hybrids (Schluter, 2000).

Sexual selection is probably responsible for the different levels of sexual dimorphism in these species. *Triturus vulgaris* is the only species among the small-bodied newts (subgenus Palaeotriton; Zajc & Arntzen, 1999) in which males are not smaller than females (Malmgren, 2001). At the same time, *T. vulgaris* is the only species within the group whose males possess a conspicuous skin crest along the tail and trunk. This is especially evident in the nominal subspecies (Raxworthy, 1990), which is the form that hybridizes with *T. montandoni* in the area that was studied. The lack of sexual size dimorphism and a relatively long tail in *T. vulgaris* males have probably evolved by sexual selection. It was shown that *T. vulgaris* females prefer males with higher crests (Green, 1991; Gabor & Halliday, 1997). A relatively large body and long tail in males are apparently correlated with the total area of the crest and thus may provide a stronger visual signal.

A wide range of morphological intermediates in both sexes from sympatric populations points to a multilocus genetic background of morphometric traits, and suggests little departures from additivity. Furthermore observations on laboratory-produced F_1 newt hybrids (Geyer, 1953; Cogălniceanu, 1994) and our own data on genetically F_1-like individuals do not indicate dominance to be important, although this was shown for qualitative species-specific traits only.

We expected that the relative tail length in males might be one of the key features associated with the fitness of hybrids, as it shows a significant difference between the species and thus may constitute a possible target for differential sexual selection. However, the data do not support this hypothesis as there is no evidence for departures from a linear relationship between the TL and GenHI in hybrid males.

In spite of a general correlation between morphometric traits and the nuclear genotypes, the latter does not fully explain the variation in size and shape (Fig. 4). This is especially evident for females. An over-representation of genetically pure females in the morphometrically intermediate class as shown by contingency table tests most probably results from the relatively small number of *T. montandoni* females sampled from reference populations, which might have underestimated the true range of morphometric variation present in this species. An alternative explanation is that the pure females classified as morphometric intermediates were in fact introgressed individuals undetected by our limited set of the genetic markers. This interpretation is unlikely as genetically only slightly introgressed individuals were generally classified as pure parentals on the basis of their morphology. Hybrid males as often as females fell into the morphometrically intermediate class. In contrast to the females, only a relatively small proportion of pure males was classified as morphometric intermediates. This reflects a clearer morphometric separation between the males of both species. Rather poor performance of morphometric data in predicting nuclear genotypes in the zone of sympatry emphasizes the need for a more complete understanding of the processes influencing genotype–phenotype interactions (e.g. Badyayev, 2002). This is especially important in studies of natural hybridization, as various parts of the genome exhibit differing potential for introgression between hybridizing species (Brumfield *et al*., 2001; Martinsen *et al*., 2001).

There was a significant linear relationship between the population-mean CV 1 and GenHI (Fig. 5). Neither quadratic nor cubic terms of the regression equations were significant, indicating no strong differential action of selection on morphometric traits and molecular markers. The observed covariance may have several causes. Selection acting on many loci interspersed throughout the genome may produce the shared selective background resulting in the concordant pattern of the allele frequency at various loci even if they are differentially associated with fitness (Barton, 1986). The non-random association between genes responsible for morphometric traits and other markers in the hybrid populations can also arise from the influx of the parental genotypes into the area of hybridization or from assortative mating. In the case of the *T. vulgaris* × *T. montandoni* hybrid zone, the results obtained previously (Babik *et al*., 2003) support the latter explanation.

In summary, no evidence was found for strong selection acting differentially on morphometric traits and nuclear genetic markers in the *T. vulgaris* × *T. montandoni* hybrid populations. However, this does not mean that there are no

fitness differences between the various hybrid genotypes. In analyses based on the distribution of phenotypic and genotypic variation even relatively strong differential selection may be difficult to reveal because of linkage disequilibria, which produce concordance among different sets of loci (Nürnberger *et al*., 1995). The differences in the level of sexual size and shape dimorphism suggest that the females of *T*. *vulgaris* and *T*. *montandoni* show dissimilar mate preferences. Since hybrid males are intermediate in the expression of epigamic characters, inferior fitness resulting from lower mating success may be expected. Indeed, a comparison of the distributions of GenHI and qualitative male secondary sexual traits seemed to indicate that hybrids may experience lower reproductive success (Babik *et al*., 2003).

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