Morphological integration of cranium and postcranial skeleton during ontogeny of facultative paedomorphic European newts

(Triturus vulgaris and T. alpestris)

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Abstract. The patterns of ontogenetic and interspecific variation of morphological integration (the relationships and correlation patterns of functionally and developmentally related morphological traits) in facultative paedomorphic species of two European newts (Triturus vulgaris and T. alpestris) were examined. The degree of morphological integration differed among ontogenetic stages. Variation in the level of morphological integration of cranium and postcranial skeleton during ontogeny of paedomorphic newts had the same pattern in both analysed species. The lowest level of cranium morphological integration characterises the larvae and the juveniles. Individuals that follow alternative life-history path (paedomorphs) remain at a relatively low level of morphological integration, while integration highly increased in metamorphosed adult individuals. Limb skeleton also showed a lower level of integration in paedomorphs in comparison with metamorphosed individuals. The hypothesis of morphological integration was confirmed for all stages of T. vulgaris, while the same hypothesis was confirmed only for larval and paedomorphic stages of T. alpestris. In T. vulgaris, metamorphosis changes the pattern of correlation among the cranial traits, while in T. alpestris, the patterns of phenotypic correlation among the cranial traits remain substantially similar for all analysed ontogenetic stages.

Introduction

Morphological integration (Olson and Miller, 1958) is a term usually used to describe the relation between morphometric correlation of phenotypic characters and their functional and developmental relationships. Empirical studies have shown that there is a significant association of developmentally and functionally related morphometric traits in the phenotype (see Cheverud, 1995 and references therein), and that the adjacent traits tend to be more integrated than spatially unrelated traits (Wagner, 1990). Furthermore, for morphometric traits, the pattern of phenotypic and genetic covariation should be similar and correlated (Cheverud, 1996; Roff, 1995; Marroig et al., 2004). The pattern and level of morphological integration, the causality and factors that produce such variation, attracted the interest of evolutionary biologist (see Wagner, 1990; Cheverud, 1995; Marroig et al., 2004, for a review). This wide interest has primarily resulted from the possible constrains that morphological integration could pose for ontogenetic and evolutionary changes. As morphological changes arise from changes in ontogeny, developmental integration may be responsible for coordinated changes in diverse parts of morphology on both the ontogenetic and the phylogenetic time scale (Zelditch and Fink, 1995). Also, morphological integration is significantly modifiable within a single pattern of development, which can thus be the root of more than one adaptive design (Zelditch, 1988; Zelditch and Carmichael, 1989; Cane, 1993).

To date, studies on ontogenetic aspects of morphological integration have overlooked groups with abrupt structural changes during postnatal development, such as metamorphosis. Tailed amphibians, with biphasic development of skeleton structures highly specialised in the larval and adult stage, offer special opportunities for such analyses. Moreover, it is of general interest to study morphological integration on individuals that follow different ontogenetic pathways. In some species facultative paedo-
morphosis occurs when larvae either metamorphose into terrestrial metamorphic adults (i.e., follow the common life history path), or attain sexual maturity retaining larval morphology (e.g., external gills) to become paedomorphic adults (e.g., Semlitsch & Wilbur, 1989).

In this study we analysed the morphological integration of the cranial and postcranial skeleton during ontogeny in two facultatively paedomorphic species of European newts to find out whether (1) paedomorphosis influences the pattern and level of morphological integration, and (2) do these possible influences extend across the species boundaries. Previous studies on the very same populations revealed that conspecific metamorphic and paedomorphic adults differ in body size and shape changes during ontogeny (Djorović and Kalezić, 1996), and that in both species paedomorphs have less prominent sexual dimorphism (Kalezić et al., 1992). Paedomorphosis also significantly affects the cranial skeleton – paedomorphic individuals of both species are characterised by cranial organisation that mostly corresponds to organisation of the cranial skeleton at late larval stage, before metamorphosis (Djorović and Kalezić, 2000).

Specifically, our aim was to examine whether (1) the morphological integration increases ontogenetically in European newts (see Zelditch et al., 1992), (2) rapid changes during newt ontogeny (metamorphosis) interrupt the order of integration from larvae to adults, and if so, are paedomorphs more like the larvae with respect to the level of morphometric integration than their intrapopulation counterparts (metamorphs), (3) the existence of truncated development (metamorphosis vs. metamorphic failure) influences the cranial and postcranial integration patterns during the postembriobrmal development. Furthermore, with regard to the level of developmental and even more so, the anatomical and functional relationships between the skeletal elements, we presume that the postcranial skeleton (e.g., limbs) should be differently integrated than the cranial skeleton (see Zelditch, 1988).

Materials and methods

Specimens and measurements

Morphometric analyses of the skull and limb characteristics were performed on different ontogenetic stages (larvae, juveniles, paedomorphs and metamorphic individuals) of the smooth newt (Triturus vulgaris) and the alpine newt (T. alpestris). The smooth newt specimens of all stages were collected from the Velika Osoječenica pond (42°41′N 18°37′E, 990 m a.s.l.). The alpine newt adults (paedomorphic and metamorphic) came from the Bukumirsko Lake (42°36′N 19°33′E, 1440 m a.s.l.), juveniles from the nearby temporary Black Lake, and larvae from the Širokor population (42°34′N 19°33′E, 1730 m a.s.l.). The very same individuals were used for studies on allometry (Djorović and Kalezić, 1996), fitness characteristics (Kalezić et al., 1996) and cranial morphology (Djorović and Kalezić, 2000). The cross-sectional data were obtained on a sample of larvae prior to metamorphosis (20 specimens per species), juveniles at the ontogenetic stage just after metamorphosis (20 individuals per species) and on samples of paedomorphic and metamorphic females and males (10 or 11 individuals per sex of each species). All 162 examined individuals were from Georg Džukić Batrachological Collection (Institute for Biological Research “Pimša Stanković”). Larvae and juveniles were staged according to their size, presence or absence of external gills, tail shape and skin structure and coloration. Paedomorphic and metamorphosed adult individuals were staged on the basis of external and internal morphological criteria (Denoël et al., 2001). All paedomorphs had well developed external gills with open gill slits. Maturity of paedomorphs and metamorphs was determined by the presence of yolk-filled ovarian follicles in females and by the development of secondary sexual characteristics (enlarged and pigmented cloaca, dorsal crest) and enlarged testes in males.

All specimens were cleared with trypsin (adults) and KOH (larvae and juveniles), and double stained with Alcian blue and Alizarin red for cartilage and bone, respectively (Wassersug, 1976; Digenkurs and Uhler, 1977). Whole-mount skulls were examined on same magnification, with the skull positioned at the centre of the optical field. Skull landmarks were drawn on paper using camera-lucida and then digitized and transferred to a computer file as two dimensional coordinates (see Djorović and Kalezić, 2000). Skull measurements are the distances between the chosen landmark points, calculated as Euclidean distances, except for the measurements of nasal elements, which were estimated from their areas. The paired measurements were taken on both sides of the skull and then averaged, to reduce potential measurement error. The 24 cranial traits (not all scored in every ontogenetic stage) were used for the study of morphological integration (fig. 1). Sexual size differences of the skull traits were found to be mostly negligible in both paedomorphs and metamorphs adults (Djorović and Kalezić, 2000). Therefore, the data from females and males were pooled in subsequent analyses.

The vertebrate cranial skeleton is a complex structure consisting of skeletal elements of diverse embryonic origin.
Its development is induced by multiple cell and tissue interactions, and under hormonal influences (e.g. Hanken and Hall, 1993). Despite the vast complexity and variability of the vertebrate skull design and its functional differences, the conservative developmental skull formation processes, structural similarity and a common basic plan of skull formation remain substantially similar across vertebrate groups (Hanken and Hall, 1993), including the tailed amphibians (Ericsson, 2003). Three main components, originating from different embryonal primordia, form the cranial skeleton in all vertebrates: the neurocranium that protects the brain and the main sensory organs, the splanchnocranium that forms the visceral arches and their derivatives, and the dermal skeleton that contributes to the cranium in close association with neurocranial and visceral elements. The skull development, differentiation and growth largely depend on epigenetic factors such as tissue interactions, hormonal influences and biomechanical factors (see Hanken and Hall, 1993). The amphibian cranial skeleton is composed of: (1) endocranium, which includes the neurocranium, auditory and olfactory capsules; (2) exocranium, with dermal bones that form the skull roof, the snout (anterior part of the skull roof and palate), and the upper jaw; (3) composite endo- and exocranial unit, which includes the mandible and the suspensorium, and (4) hyobranchial apparatus (Trueb, 1993).

The elements of the neurocranium and the associated dermal bones that form the braincase and the skull roof are linked with brain development and growth. The posterior neurocranium (the otic end exooccipital bones that fuse and form the otic capsules) is associated with growth and development of the inner ear. All other parts of the newt cranial skeleton are directly or indirectly functionally related to foraging (snout, jaws, mandible, suspensorium and hyobranchial apparatus). The dermal bones that form the skull roof anterior to the orbits, which primarily protect the orbits and olfactory capsules, are in lateral conjunction with the tooth bearing upper jaw bones. The upper jaw bones along with the vomers are also involved in protection of olfactory capsules. Premaxillae, maxillae and vomers are the tooth bearing skull bones, while the squamosum, the pterygoideum and the palatine suspend the jaws from the neurocranium. During metamorphosis, the snout, the suspensorium and the jaws, along with the hyobranchial apparatus, go through significant changes, and the development and remodeling of these skeletal elements is under hormonal control (Smirnov and Vassilieva, 2003). Based on the hypothesis that parts of the skeleton affected by growth of the same or related soft tissues should be morphologically integrated (Cheverud, 1995), we divided the newt skull into two major cranial regions with different biological roles, that growth and develop corresponding to different associated soft-tissues. These two parts are:
The neurocranial component was subdivided into two sets: 1) the cranial vault, composed of two dermal bones that ossify during the mid-larval stage (d4, d5, d6, d12), and 2) the otic and occipital region (d7, d8, d9, d10, d11, d20, d21, d22, d24) formed of endochondral bones that appear simultaneously just prior to ossification of the cranial vault, and protect the hind brain and the otic capsules. The paired dermal bones, squamosums, that ossify earlier, are topographically closely related with exoccipitals and prooticums and therefore included in this region. The oronal traits were composed of two functional sets also: 1) the nasal (d1, d2, d3, d13, d23) and 2) traits that describe the jaws and the palate (d13, d14, d15, d16, d17, d18, d19). The vomers and palatines ossify early during postembryonic development, just after hatching. By the onset of exogenous feeding, the maxillae and pterygosideums ossify. The bones that develop last in the late larval stage are the maxilla, the prefrontal and the nasal bones, and their development is induced by the underlying nasal cartilage and under hormonal control (Smirnov and Vassilieva, 2003). The resorption and remodeling of cranial skeletal elements during metamorphosis is also under hormonal control (Rose, 1996; Smirnov and Vassilieva, 2003). During metamorphosis and transition from aquatic to land environment, the oronasal part goes through significant morphological and functional changes. Resorption of the palatines, vomer transformation, development of the maxillae, prefrontal and nasal bones, along with the transformation of the hyobranchial apparatus (Djorovic and Kalezić, 2000; Smirnov and Vassilieva, 2003), change the cranial kinematics and the feeding performance (Reilly, 1996; Denoel, 2004). However, beside intrapopulation variation in ossification sequences (Djorovic and Kalezić, 2000), geographical variations in ossification sequences and paedomorphosis influences on the cranial skeleton of *T. vulgaris* (Djorovic and Kalezić, 2000; Smirnov and Vassilieva, 2003) and *T. alpestris* were found (Roček, 1996; Djorovic and Kalezić, 2000).

Contrary to the cranial skeleton, the limb skeletal elements appear almost simultaneously (Vorobyeva and Mednikov, 2002). The limb bones form before metamorphosis, and the development and ossification of appendicular skeleton is independent of hormonal influences (Smirnov and Vassilieva, 2003). The limb measures (humerus length, humerus width, radius length, ulna length, length of third metacarpal, femur length, femur width, tibia length, tibia width and length of third metatarsal bone) of paedomorphs and metamorphosed adult individuals (i.e., metamorphs) were analysed separately. A notable sexual dimorphism in size of the analysed limb bones was found only between Alpine newt metamorphs. Alpine newt metamorphic females had significantly longer humerus, ulna, femur and fibular bones (t-test, all \( P < 0.05 \)). Among smooth newt individuals, statistically significant differences between the sexes (t-test, all \( P < 0.05 \)) in the analysed limb traits were found only for the length of the third metacarpal (among metamorphs), and for the length of the third metatarsal bone (among paedomorphs). Despite these differences between the sexes, because of small sample sizes, the data for females and males were pooled.

**Statistical analyses**

The degree of phenotypic integration of the cranial and limb skeleton was measured by the variance of the eigenvalues (Wagner, 1984; Cheverud et al., 1989), which represents the overall level of correlation of the analysed traits. (Eigenvalues were obtained from Principal Component Analysis, PCA.) The pattern of correlation and the hypothesis of morphological integration (the hypothesized degree of functional and developmental relationships among morphological characters) were analysed by using empirically derived correlation matrices of the analysed traits, and by comparing these matrices with theoretically derived matrices that reflect developmental or functional interrelation among the analysed traits. The 24 cranial traits were grouped into six functional and developmental sets (neurocranial region, cranial vault, occipital, oronasal, nasal region and jaws and palate) based on their functional, anatomical and developmental relationships. This classification was used to derive a series of connectivity matrices (24 × 24), one for each set separately. In connectivity matrices, the linked traits that belong to the same functional set were indicated by 1 (correlated), while the unlinked traits that belong to different functional sets were indicated by 0 (uncorrelated). To test the overall integration (total integration) the connectivity matrices of six specific cranial sets were combined as a sum of all separate connectivity matrices (Cheverud et al., 1989; Cheverud, 1995). The relationship between the empirically derived correlation matrices and theoretical connectivity matrices was used to test the hypotheses of morphological integration (Cheverud et al., 1989; Wagner, 1990; Cheverud, 1996). The statistical significance of the matrix correlation R (R was calculated as a Pearson product moment correlation between the elements of empirically derived and connectivity matrix) and the statistical significance of mean correlations between the elements of empirically derived and connectivity matrices was used to test the hypotheses of morphological integration (Cheverud et al., 1989; Wagner, 1990; Cheverud, 1996). The significance test is based on the null hypothesis of no similarity in correlation patterns between the compared matrices. The procedure consists of random permutation of one matrix (1000 iterations) and then correlation of each randomized matrix with the unaltered matrix to generate a distribution of matrix correlations. The observed matrix correlation was then compared to the derived distribution. The proportion of correlations greater than or equal to the observed correlation is an estimate of probability that the null hypothesis is true. The same test was used for comparative
analyses of phenotypic matrices among different ontogenetic stages.

The robust validity and repeatability of observed correlation matrices was estimated using the resampling or bootstrapping method (Cheverud et al., 1989; Marroig and Cheverud, 2001). For each ontogenetic stage of the sample size of \( n \) individuals were randomly resampled with replacements from the original dataset. This procedure was repeated 500 times using the software program Poptools 2.62 (Hood, 2004). New correlation matrices from resampled data sets were calculated and compared with the correlation matrix obtained from the original data using a matrix correlation. The frequency distributions of matrix self correlations were used to estimate robust validity of correlation matrices. As the individual matrix elements of the observed matrix correlations are always estimated with errors, the correlation between two estimated matrices could be quite downwardly biased. The repeatability of matrix self correlations were used to estimate the theoretical maximum matrix correlation \( (R_{\text{max}}) \) and to obtain adjusted matrix correlation \( (R_{\text{adj}}) \) between two observed matrices (Cheverud, 1996; Marroig and Cheverud, 2001). The value of \( R_{\text{max}} \) was calculated as \( (t_AtB)^{0.5} \), where \( t_A \) and \( t_B \) denote the mean of the matrix self correlations of matrices A and B, respectively. Adjusted matrix correlation \( (R_{\text{adj}}) \) was calculated as observed correlation between two matrices \( (R) \) divided by the maximum matrix correlation \( (R_{\text{max}}) \).

**Results**

**The robust validity of correlation matrices**

Even though the phenotypic correlation matrices for each ontogenetic stage of *T. vulgaris* and *T. alpestris* were calculated on relatively small sample sizes (\( n = 20 \) or 21 individuals per stage), the estimated matrices show a considerably high degree of repeatability, and therefore reliability. Exception is the sample of *T. alpestris* larvae, where the obtained bimodal distribution of matrix correlations indicates a high estimation error and an inconsistent sample. Matrix correlations between the observed matrices and matrices obtained from resampled data (500 iterations) for *T. vulgaris* larvae have a geometric mean correlation of 0.79, while 95% of iterations have a matrix correlation coefficient between values 0.65 and 0.95. In juveniles, the geometric mean correlation is 0.79, with 95% of the correlations falling between 0.54 and 0.90. The correlation matrices of paedomorphs and adult metamorphs also show a relatively high reliability (geometric mean correlation 0.83, with 95% of correlations between 0.65 and 0.97 for paedomorphs, and geometric mean correlation 0.86, with 95% of correlations between 0.72 and 0.97 for metamorphs). Matrix correlations for the sample of *T. alpestris* larvae range from 0.16 to 0.97, and have a bimodal distribution, indicating instability and unreliability of the estimated correlation matrix. Matrix correlations of the juvenile stage have a geometric mean correlation of 0.77, with 95% matrix correlations between 0.63 and 0.87. The geometric mean correlation for *T. alpestris* paedomorphs is 0.75, with 95% correlations falling between 0.57 and 0.90, and for metamorphs 0.85, with 95% of correlations between 0.68 and 0.95.

**The intensity of integration**

There is an evident change in the intensity of integration during the postembryonic development of newt craniums, but it is differently expressed in paedomorphs vs. metamorphs, as well as across the species (table 1). It appears that in the smooth newt, metamorphosis impacts considerably on the level of morphological integration. The degree of morphological integration of each cranial component, as well as overall integration, was much lower in juveniles compared to the larval ontogenetic stage. Adult metamorphs have the highest overall integration, although the nasal region, the jaws and the palate (the oronasal region) show higher integration in paedomorphs. Unlike the smooth newt, larval and juvenile ontogenetic stages of the alpine newt have approximately the same level of total integration, just like the paedomorphs. However, the level of total integration in metamorphs increases almost three times over the level of other stages. In paedomorphic alpine newt individuals the nasal set of traits has a somewhat higher level of integration than in metamorphs.

The first principal component (size component) explains 45.1% and 34.3% variability of skull traits in the smooth newt metamorphs and paedomorphs, respectively. In the alpine newt these figures are for metamorphs 47.0%
and 39.0% for paedomorphs. The first principal component explains much less variation in smaller, larval and juvenile stages (29.3% in the smooth newt larvae and 20.5% in juveniles, and 26.3% in larvae and 28.3% in juveniles from the alpine newt population). Principal component analysis of the limb traits showed that the first principal component explains even more variability: 62.7% in the smooth newt metamorphs, 34.3% in paedomorphs, 63.9% in the alpine newt metamorphs and 57.7% of the whole variability in paedomorphs from same population.

**The patterns of integration**

The average correlation within the functionally and developmentally related cranial traits is higher than the average correlations within unrelated cranial traits. In ontogenetic series of the smooth newt, the average correlation within the developmentally and functionally linked traits in comparison with unrelated traits increases 37.4% in the larval stage, more than three times in the juvenile stage, 58.9% in paedomorphs and 29.0% in metamorphs. The same figures for the alpine newt are as follows: 57.0% in larvae, 22.9% in juveniles, 43.3% in paedomorphs and only 15.2% in metamorphosed individuals. The average correlations between the functionally and developmentally related cranial traits (r), and the test of morphological integration with matrix correlation (R) for the analysed ontogenetic stages of smooth and alpine newts are given in table 2. With the complete trait set (total), the obtained correlations in all smooth newts and in the alpine newt larval and paedomorphic ontogenetic stages are significantly correlated with the pattern of hypothesised morphological integration. In the smooth newt, the patterns of correlation in the larval and paedomorphic stages are quite different from the patterns of correlation in metamorphosed individuals (juvenile and adult stage, table 3). However, in the alpine newt the pattern of correlation is significantly similar between all compared ontogenetic stages.

We also compared the matrix correlation patterns among the species (only for metamorphosed individuals). The average matrix correlation between the smooth and the alpine newt is \( R = 0.61 \), the estimated maximal matrix correlation is \( R_{\text{max}} = 0.86 \), and adjusted value of matrix correlation is \( R_{\text{adj}} = 0.71 \) \( (P < 0.01) \), indicate similarity in the correlation pattern of cranial traits between these two newt species.

**Discussion**

Our results indicate obvious differences in the level of morphological integration between the two morphs and that paedomorphosis considerably affected the whole of the individual skeleton. Paedomorphic individuals are characterised by a lower level of morphological integration than adult metamorphs, regardless of the part
Morphological integration of cranium

Table 2. The average correlations between functionally and developmentally related traits (r) and test of morphological integration with the matrix correlation (R) in analysed ontogenetic stages of the smooth (T. vulgaris) and the alpine newt (T. alpestris). The probability was derived from Mantel’s test with 1000 iterations. *P < 0.05.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Larvae</th>
<th>Juveniles</th>
<th>Paedomorphs</th>
<th>Metamorphs</th>
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<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cranial vault</td>
<td>0.273</td>
<td>0.038</td>
<td>0.055</td>
<td>−0.001</td>
</tr>
<tr>
<td>otic and occipital</td>
<td>0.237</td>
<td>0.043</td>
<td>0.127</td>
<td>0.100</td>
</tr>
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<td>nasal</td>
<td>0.442</td>
<td>0.175*</td>
<td>0.016</td>
<td>−0.030</td>
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<tr>
<td>jaws and palate</td>
<td>0.211</td>
<td>0.003</td>
<td>0.157</td>
<td>0.106</td>
</tr>
<tr>
<td>total</td>
<td>0.261</td>
<td>0.121*</td>
<td>0.114</td>
<td>0.127*</td>
</tr>
<tr>
<td>T. alpestris</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cranial vault</td>
<td>0.215</td>
<td>0.040</td>
<td>0.227</td>
<td>0.017</td>
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<td>jaws and palate</td>
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<td>0.214</td>
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<td>total</td>
<td>0.190</td>
<td>0.108*</td>
<td>0.231</td>
<td>0.078</td>
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</table>

Table 3. Comparative analysis of correlation matrix patterns of cranial traits between successive ontogenetic stages. The observed coefficient of correlation between matrix (R), the maximum possible correlation between matrix compared (Rmax), and the values of adjusted matrix correlations (Radj) are presented. The probability was derived from the Mantel’s test with 1000 iterations. Correlation matrices were compared under null hypothesis of no association between them. **P < 0.01; ***P < 0.001.

<table>
<thead>
<tr>
<th>Ontogenetic stages compared</th>
<th>T. vulgaris</th>
<th></th>
<th>T. alpestris</th>
<th></th>
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<tr>
<td></td>
<td>R</td>
<td>Rmax</td>
<td>Radj</td>
<td>R</td>
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<td>Larvae – juveniles</td>
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<td>0.791</td>
<td>−0.047</td>
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<td>Juveniles – metamorphs</td>
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<td>0.824</td>
<td>0.231</td>
<td>0.353***</td>
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<td>Larvae – paedomorphs</td>
<td>0.319**</td>
<td>0.811</td>
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<td>Paedomorphs – metamorphs</td>
<td>0.163</td>
<td>0.846</td>
<td>0.163</td>
<td>0.356***</td>
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</table>

of the skeleton and the species studied. Paedomorphic individuals have a loosely integrated skull, at almost the same level of integration as larvae. It seems that a relatively higher level of integration of the nasal region at larval and paedomorphic stages corresponds to late and simultaneous ossification of the maxillae, prefrontal and nasal bones. The intensity of cranial morphological integration at the juvenile stage, which goes through significant morphological and functional changes during metamorphosis, remained at the same level or decreased in comparison with the larval stage. The level of morphological integration highly increased with metamorphosed adults in both species. It seems that in the course of ontogeny of European newts, phenotypic integration of the cranium increases during post-metamorphic growth. Studies of morphological integration in mammalian (Zelditch, 1988) and avian skeletons (Cane, 1993) showed that the general body size is the dominant, but not the only, source of phenotypic integration. According to our study, size does influence morphological integration, but it is not the dominant source of integration. The first principal component (the size component) explained less than 50% of skull traits variability in each of the analysed ontogenetic stages. Also, our results indicated that the contribution of size to cranial morphological integration increased in the course of ontogeny of European newts. The proportion of variance explained by the first component increased in adults (paedomorphs and metamorphs) compared with the larval and juvenile stages in both species. The highest proportion of variation explained by size was found in metamorphosed adult individuals.
As regards the limb skeleton, the size had more influence on its morphological integration than with the cranial skeleton. Here, the proportions of variation explained by size were up to 64%, with higher values for metamorphs than paedomorphs in both species.

The hypothesis of morphological integration seems to be confirmed in all ontogenetic stages of the smooth newt and for the branchiate stages of the alpine newt. The relatively high correlations among the functionally and developmentally related traits in the smooth newt larval stage are largely due to a closer correlation among the nasal characteristics or between the jaws and the palate in the paedomorphic stage. However, the nasal traits have a very low, even negative, average correlation with the hypothesised connectivity matrix in metamorphosed smooth newt individuals. In the alpine newt, the nasal traits in the larval stage and the characteristics of the skull vault in paedomorphs are more correlated with each other than with other characteristics. In the alpine newt metamorphs, the jaws and the palates display a high correlation, but in total, the functionally and developmentally related traits are not significantly more correlated with one another than the random pairs of characteristics.

Overall, the functionally and developmentally linked traits had a higher average correlation than unrelated traits. The relations of average correlations between the linked and the unlinked traits vary among ontogenetic stages. The average correlation of the functionally and developmentally related traits is higher in ontogenetic stages with a relatively low level of overall morphological integration. For example, the average correlations among the functionally related cranial traits are three times higher than among unrelated traits in the *T. vulgaris* juvenile stage, which is characterised with the lowest level of morphological integration (table 1). In metamorphosed adults with the highest level of cranial morphological integration, the difference between the average correlations among the functionally and developmentally linked and unlinked traits is only 29% for *T. vulgaris*, or even 15.2% for *T. alpestris*. The small differences in average correlations between the related and unrelated traits at the terminal adult metamorphosed stage could be the result of a decrease in regionalisation as a consequence of the increasing overall integration.

What mechanism accounts for the observed pattern of functionally related and unrelated traits during ontogenies? Conceptual models developed by Wagner (1996), Wagner and Altenberg (1996) and Winther (2001) hypothesized that a functionally integrated phenotypic unit (a phenotypic module) should be based on a genetic module, where pleiotropic effects of genes or linkage disequilibrium act as genetic mechanisms of modularity. It was suggested that, among the traits within a phenotypic module, pleiotropy plays an important role in morphological integration (Cheverud et al., 1997). However, this does not mean that pleiotropic effects of genes are constant during ontogeny. The variability of gene pleiotropic effects and nonconstant genetic variance-covariance matrices are biochemically justified and empirically well supported phenomena (e.g. Gromko, 1995; Tucić et al., 1998). It appears, therefore, that the observed decrease in differences among correlations between the functionally related and unrelated traits during ontogeny of the *Triturus* species could be the result of an increased number of pleiotropic effects among the genes influencing different phenotypic modules. Thus, our data support the notion that modularity and integration act, as suggested by Wagner and Altenberg (1996), as opposites, i.e. they are inversely proportional to each other. Such relationship between modularity and integration could underlie the observed pattern of relations between the cranial and limb skeletons. In view of the complexity of developmental and even more anatomical and functional relationships among the cranial skeletal elements compared to limb skeletons, we presumed that limbs, as a compact unit, might be much more integrated than the cranial skeleton. However, the limb
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skeleton shows a lower level of integration than the cranial part of the skeleton, except with T. alpestris paedomorphs. Also, we consider that the most unexpected and puzzling result is that these two species showed differences in changes of ontogenetic patterns of morphological integration.

The observed integration patterns in these newts may reflect diversification in functional requirements. A different functional ability related to the foraging of paedomorphs and metamorphs was found for some other salamanders (Lauder and Shaffer, 1986) and for paedomorphs and metamorphs of the alpine newt (Denoël, 2004), as well.

The data on differences in fitness components of alternative morphs are of crucial importance for testing of different hypotheses about the mechanisms that produce and maintain this polymorphism in populations (Semlitsch, 1985; Kalezić et al., 1996; Whiteman, 1994; Whitman et al., 1996; Whiteman, 1997; Ryan and Semlitsch, 1998; Rot-Nikčević et al., 2000; Denoël et al., 2002; Denoël and Schabetsberger, 2003; Denoël, 2004). However, for the moment it is safe to presume that the maintenance of ontogenetic polymorphism in tailed amphibian populations depends on environmental characteristics (e.g. heterogeneity of aquatic and terrestrial habitats, drying probability, etc.). The ontogenetic polymorphism can be approached from different tactics, such as: metamorphosis vs. paedomorphosis, progenesis vs. neoteny, resource partitioning or not, and could be variable within and between the taxa.

Theoretically, morphological integration may facilitate adaptive evolution as a developmental system which integrates the functionally and developmentally related characters that could be selectively favored over those that exhibit largely independent control of such characters (Cheverud, 1995; Marroig et al., 2004). If this generalisation holds, possible differences in the level and pattern of morphological integration of individuals following different life-history paths might appear with possible consequences on both survival and reproduction. However, the currently available data for the relationship between heterochronic changes and skull integration patterns in tailed amphibians are insufficient and yield only tentative conclusions.

Finally, it is interesting to point out similarities between the basic pattern of the skull phenotypic integration observed in this study and the described patterns of phenotypic integration of the mammalian skull. The hypothesis of mammalian skull integration, based on well documented eutherian mammal ontogeny, assumed that disjunction of the early (neural) and late (somatic) growth is a general developmentally integrating factor that produces similar patterns of cranial morphological integration across mammals (see Cheverud, 1995). The studies of phenotypic integration of cranial characteristics in various species of rats (Zelditch, 1988; Zelditch and Carmichael, 1989) and primates (Cheverud, 1995, 1996; Marroig and Cheverud, 2001) found significantly higher correlations among neurocranial traits and among traits that describe the oral and facial regions. The higher correlations among functionally and developmentally related neurocranial traits, and cranial traits related with foraging, were also obtained in this study. These results could indicate that the basic modularity of the skull, the dividing neural and somatic component, remains the same during vertebrate evolution. Such two different vertebrate groups, mammals (endotherms with determined growth) and amphibians (ectotherms with undetermined growth), share the same, obviously, conservative processes in skull formation, homology of the skull skeletal parts, and similarity in biological roles of skull portions (Hanken and Hall, 1993). The same basic modularity of vertebrate skull could be the source of similarity in the patterns of cranial morphological integration across vertebrates. However, the ontogenetical variation in the patterns of morphological integration and different selective pressure on particular correlated character sets during ontogeny could produce diversification and

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change the patterns of morphological integration within and among species.

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