Skull modularity of the European ground squirrel *Spermophilus citellus* (Linnaeus, 1766)

Modularnost lobanje evropske tekunice *Spermophilus citellus* (Linnaeus, 1766)

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Abstract: The skull is a complex structure that has frequently been studied for the patterns of morphological integration and modularity. The ventral side of the skull can be divided into two functional modules, the neurocranium composed of the braincase, eyes and ears, and the viscerocranium composed of the jaw apparatus. The aim of this study was to test the ventral cranium of the European ground squirrel *Spermophilus citellus* (Linnaeus, 1766) for this partitioning as sciurid skull is believed to be highly integrated without clear divisions into subunits. Additionally, I compared the degree of modularity between juveniles and adults. Hypothesized modularity was tested on 159 (43 juveniles and 116 adults) skulls by applying geometric morphometric method based on Escoufier RV coefficient. In adults, the results yielded strong support to the hypothesis that the viscerocranium and neurocranium are separate modules. In juveniles, two-module organization of the skull was also confirmed, but the hypothesized modules were much more integrated with each other. Although allometry can be a strong integrating factor, it had very little influence on the hypothesized modularity of the *S. citellus* skull. A permutation test for the difference in the degree of modularity between juveniles and adults was marginally significant. The change in the strength of integration between the viscerocranium and neurocranium in the *S. citellus* skull during ontogeny, with the higher level of modularity in adults than in juveniles, is probably a consequence of the transition from suckling to gnawing of food and greater specialisation of the two functional modules.

Keywords: neurocranium, viscerocranium, ontogeny, allometry, Escoufier RV, morphological integration, geometric morphometrics

Izvleček: Lobanja je kompleksna struktura, pri kateri se pogosto preučujejo vzorci morfološke modularnosti in integracije. Ventralno stran lobanje lahko razdelimo v dve funkcionalni podenoti; nevrokranij, ki ga sestavljajo kosti možganskega dela lobanje, oči in ušes, in viscerokranij, ki je iz kosti čeljustnega aparata. Cilj raziskave je bil preveriti prisotnost te delitve na ventralni strani lobanje evropske tekunice *Spermophilus citellus* (Linnaeus, 1766), saj za lobanje veveric velja, da imajo visoko stopnjo integracije brez jasnih delitev na podenote. Primerjala sem tudi stopnjo modularnosti med mladimi in odraslimi osebki. Hipotetično modularnost sem testirala na 159 (43 mladih in 116 odraslih) lobanjah z metodami geometrijske morfometrije na osnovi

Escoufierjevega RV koeficienta. Pri odraslih osebkih so rezultati podprli hipotezo o delitvi lobanje na viscerokranij in nevrokranij. Hipotezo o modularnosti sem potrdila tudi pri mladih osebkih, le da sta bila modula med seboj veliko bolj povezana. Čeprav je alometrija lahko močan integracijski faktor, je imela na hipotetično modularnost lobanje *S. citellus* zelo majhen vpliv. Permutacijski test, s katerim sem testirala razliko v stopnji modularnosti med mladimi in odraslimi osebki, je bil na meji signifikantnosti. Sprememba v stopnji povezanosti med viscerokranijem in nevrokranijem pri lobanji *S. citellus* med ontogenijo, z višjim nivojem modularnosti pri odraslih kot pri mladih, je verjetno posledica prehoda s sesanja na glodanje hrane ter večje specializacije obeh funkcionalnih modulov.

Ključne besede: nevrokranij, viscerokranij, ontogenija, alometrija, Escoufier RV, morfološka integracija, geometrijska morfometrija

Introduction

The skull is a complex structure composed of many parts that have different embryonic origins and functions (Klingenberg et al. 2004). To function as a whole, the parts of the skull are integrated (Olson and Miller 1958). The integration is not evenly distributed, but rather structured into modules (Klingenberg et al. 2004) that are internally tightly correlated and relatively independent from other modules (Klingenberg 2008). Integration and modularity of a structure can be studied by analyzing the covariation among its traits (Drake and Klingenberg 2010). In morphometrics, traits are usually measured by the use of lenghts or landmarks. It has been shown that in studies of integration and modularity the two methodologies, i. e. traditional, using linear measurements, and geometric, using landmarks, give similar results (Goswami and Polly 2010, Jojić et al. 2012). Goswami and Polly (2010) also compared different exploratory and confirmatory approaches for studying integration and modularity and did not find statistically distinguishable differences among them.

Integration and modularity of the skull have been the most frequently studied in primates, whereas in rodents, analyses of the mandible modularity have been more popular (e. g. Klingenberg and Leamy 2001, Jojić et al. 2007, Klingenberg 2009, Swiderski and Zelditch 2010, Jojić et al. 2012). The ventral side of the skull can be divided into two functional components, the neurocranium composed of the braincase, eyes and ears, and the viscerocranium composed of the jaw apparatus (Emerson and Bramble 1993). However, different analyses of the rodent skull showed that patterns of modularity can be inconsistent and sometimes unclear (Klingenberg 2013). Among rodents, modularity and integration in sciurid skulls are, especially compared to murid skulls, poorly investigated. Olson and Miller (1958) studied the fox squirrel *Sciurus niger* and discovered that its skull is well integrated without clear subdivisions into subunits. Moreover, Roth (1996) suggested that high integration of the sciurid skull is a general feature of the family; probabaly because of its conservative evolution.

European ground squirrel, *Spermophilus citellus* (Linnaeus, 1766), inhabits dry grasslands and open woodland throughout central and southeastern Europe (Ramos-Lara et al. in press). It is a relatively well-studied species with clear phylogenetic stucturing and reasonably well-known ecology and life history (ibid.). Because of its longevity and well defined age stages, *S. citellus* is also a good organism for studying changes in the strength of integration over postnatal ontogeny, a process that has been previously investigated in some rodent skulls (Willmore et al. 2006, Zelditch et al. 2006, Gonzalez et al. 2011, Klingenberg 2013).

In this study, I applied geometric morphometric methods to test the *S. citellus* ventral cranium for hypothetical partitioning to two functional modules, the viscerocranium and neurocranium, despite the previous findings that the sciurid skull is highly integrated (Olson and Miller 1958, Roth 1996). Additionally, based on prediction that the shift in diet during ontogeny could influence the strength of integration between modules, I compared the level of modularity between juveniles and adults.

Material and Methods

I studied 159 skulls of *S. citellus* from Burgenland (Austria) and Banat (Serbia). Specimens are deposited in the Slovenian Museum of Natural History (Ljubljana, Slovenia), the Museum of Natural History (Vienna, Austria), and the Zoological Research Museum Alexander Koenig (Bonn, Germany). Individuals were categorized either as juveniles (1 - 5 months old, caught after natal emergence to the end of September) or adults (> 5 months old, caught just before or after the first hibernation). Age was estimated on the basis of molar tooth wear (Ružić 1966) and the date of collection. Very old individuals (after the fourth hibernation) were excluded. The sample comprised 24 juveniles and 70 adults from Burgenland, and 19 juveniles and 46 adults from Banat. Adults from different seasons were pooled because a previous study of the *S. citellus* skull ontogeny (Klenovšek and Kryštufek 2013) showed that the skull shape does not change after the age of five months,

which means that the majority of shape changes during growth are correlated with the shift from a liquid to a solid diet that takes place before the first hibernation.

The ventral side of the skulls was photographed under constant conditions, following Cardini and Tongiorgi (2003). Twenty-two two-dimensional landmarks were digitized on the left side of the skull (Fig. 1), using the tpsDig program (Rohlf 2010). Landmark precision was tested for digitizing error as described in Klenovšek and Kryštufek (2013). The digitizing error was low.

Landmark coordinates were superimposed using the generalized Procrustes analysis (GPA) (Rohlf and Slice 1990) to standardize size and remove the differences in landmark configurations due to position and orientation. With GPA, I obtained centroid sizes (CS) and Procrustes coordinates for all skulls. The CS is a geometric measure of size calculated as the square root of the sum of squared distances between each landmark and the centroid of the landmark configuration
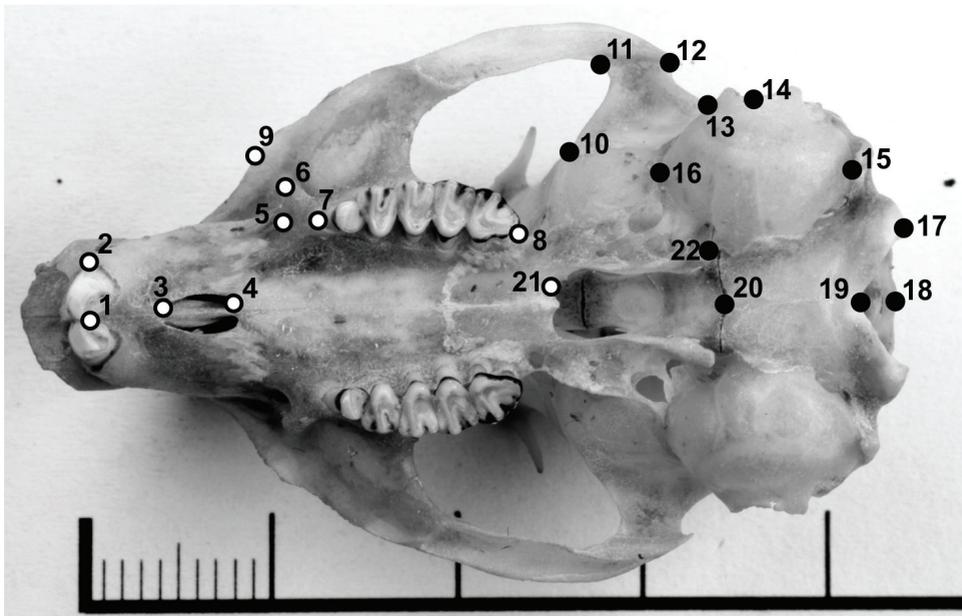

Figure 1: Ventral side of the cranium of *S. citellus* with 22 landmarks divided into two hypothesized modules (white dots – viscerocranium, black dots – neurocranium). For definitions of landmarks see Klenovšek and Kryštufek 2013

Slika 1: Ventralna stran lobanje *S. citellus* z 22 oslonilnimi točkami razdeljenimi na dva hipotetična modula (bele pike – viscerokranij, črne pike – nevrokranij). Za opis točk glej Klenovšek in Kryštufek 2013

(Bookstein 1991). Procrustes coordinates are shape variables containing the complete information on shape variation after superimposition.

A previous study of the ventral side of the skull of *S. citellus* (Klenovšek and Kryštufek 2013), performed on the same material, detected sexual dimorphism (SD) in the size of the skull in adults. Because there was no SD in the skull shape, the sexes were pooled in the current study. Population differences between Burgenland and Banat were also significant in skull size as well as shape (for results see Klenovšek and Kryštufek 2013). Nevertheless, morphometric distances between populations at different ages showed that differences between juveniles and adults exceeded the differences between populations (ibid.). I therefore pooled juveniles and adults from Burgenland and Banat.

To evaluate the hypothesis that in *S. citellus* the anterior part of the ventral cranium (the upper jaw bones with the palate or the viscerocranium) and the posterior part (the skull base or the neurocranium) are modules, the configuration of 22 landmarks was divided into subsets of 10 (viscerocranium) and 12 (neurocranium) landmarks (Fig. 1).

I compared the degree of covariation between the hypothesized modules to alternative spatially contiguous partitions with the same number of landmarks as in the hypothesized modules (Klingenberg 2009).

The strength of association between the sets of landmarks was estimated with the RV coefficient, a multivariate generalization of the Pearson correlation coefficient (Escoufier 1973), which represented the amount of covariation scaled by the amount of variation within the two sets of variables. If the two sets of variables are completely uncorrelated, the RV coefficient takes the value of zero, and the value of one, if the two sets of variables are completely interdependent (Klingenberg 2009). If the hypothesis of modularity holds, the RV coefficient for the selected partition should be the lowest value, or at least near the lower extreme of the distribution of RV coefficients of all partitions (Klingenberg 2009). I separately computed RV coefficients for the hypothesized modules for juveniles and adults. Because allometry can have a major effect on detection of modularity (e. g. Hallgrímsson et al. 2006, Klingenberg 2009), I afterwards corrected the data for allometry and repeated the analyses of modularity with the residuals from the multivariate regression of shape on the centroid size for each age group. Because the value of RV coefficient can depend on the sample size as well as on the difference in sample size between groups (Fruciano et al. 2013), I computed the Escoufier RV coefficients (Escoufier, 1973) for both age classes, and performed a permutation test of the null hypothesis of no difference in the RV coefficient between juveniles and adults (Fruciano et al. 2013).

Statistical analyses were performed using the IBM SPSS Statistics (2008), and analyses of morphological modularity with the MorphoJ software (Klingenberg 2011) and RVComparison 1.0 (Fruciano et al. 2013).

Results

In juveniles, 294 (or 3.9 %) of the 7460 partitions had a lower RV coefficient than the partition into the hypothesized modules, and in adults, none of the 7460 alternative partitions had a lower RV coefficient (Fig. 2). Both age groups had similar minimal RV values (juveniles: RV = 0.282, adults: RV = 0.212). In adults, the RV coefficient for the hypothesized subdivision was clearly in the lower extreme of the distribution of RV coefficients, which was consistent with the hypothesis that viscerocranium and neurocranium of the *S. citellus* ventral side of the skull are distinct modules. A higher RV coefficient in juveniles (RV = 0.330) for the hypothesized partition, and the percent of partitions with lower RV coefficients, indicated that juveniles, compared to adults, are characterized by a lower level of modularity.

Regressions of shape variables onto CS showed statistically significant effect of size on shape in both age groups. In juveniles, the allometry accounted for 10.77 % of shape variation, and in adults, for 4.82 %. After the correction for allometry, the values of RV coefficients between the viscero- and neurocranium were in both age groups higher than before the correction (Fig. 3). In both age groups, the range of the distribution of RV coefficients for all alternative partitions broadened mainly to the right side, towards the higher values of RV coefficients. In juveniles, the P-value slightly lowered.
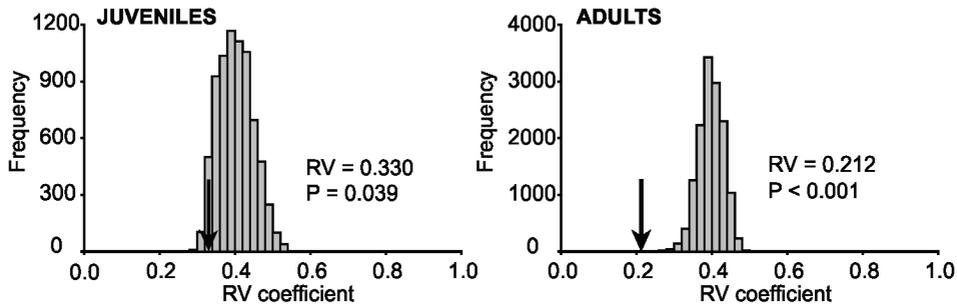
Figure 2: Histograms of the RV coefficients of all spatially contiguous partitions of the ventral skull landmark configurations for juveniles and adults. The arrows indicate the values of RV coefficients beween the hypothesized modules (viscerocranium vs. neurocranium). The P value is the proportion of partitions with lower RV than observed for the hypothesized modules

Slika 2: Histograma RV koeficientov vseh prostorsko sosednjih delitev konfiguracije oslonilnih točk ventralne strani lobanje za mlade in odrasle osebke. Puščice označujejo vrednosti RV koeficientov med hipotetičnima moduloma (viscerokranijem in nevrokranijem). P-vrednost je delež delitev z nižjim RV koeficientom kot tistim za hipotetična modula
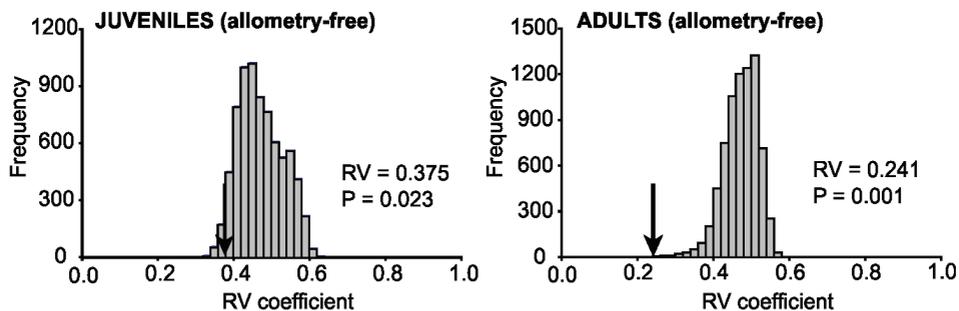

Figure 3: Histograms of the RV coefficients of all spatially contiguous partitions of the ventral skull landmark configurations for juveniles and adults after the correction for allometry. The arrows indicate the values of RV coefficients beween the hypothesized modules (viscerocranium vs. neurocranium). The P value is the proportion of partitions with lower RV than observed for the hypothesized modules

Slika 3: Histograma RV koeficientov vseh prostorsko sosednjih delitev konfiguracije oslonilnih točk ventralne strani lobanje za mlade in odrasle osebke po izločitvi vpliva alometrije. Puščice označujejo vrednosti RV koeficientov med hipotetičnima moduloma (viscerokranijem in nevrokranijem). P-vrednost je delež delitev z nižjim RV koeficientom kot tistim za hipotetična modula

Finally, the Escoufier RV coefficients of juveniles and adults were 0.331 and 0.158, respectively. Permutation test for the difference in modularity between *a priori* defined groups disclosed that the difference in RV coefficient between juveniles and adults (0.173) was marginally significant (P = 0.050).

Discussion

The ventral skull is a complex structure that can be divided into two functional components, the neurocranium and the viscerocranium (Emerson and Bramble 1993). Olson and Miller (1958) and Roth (1996), on the other hand, discovered that squirrels have a highly integrated skull without clear subdivisions into subunits. Nevertheless, in

the current study, the analysis of the covariation among landmarks in the ventral cranium yielded strong support to the hypothesis that in *S. citellus* the viscerocranium and neurocranium are separate modules. I also analyzed the strength of integration between the hypothesized modules of the *S. citellus* ventral cranium during ontogeny and observed that the level of modularity was higher in adults than in juveniles, i.e. the hypothesized modules in the juvenile skull were more integrated with each other. In adults, the partition to viscerocranium and neurocranium had the lowest RV value from all alterative partitions and therefore the lowest degree of covariation. In juveniles, the RV of the hypothesized modules was near the lower extreme of the distribution of RV coefficients. Nevertheless, both age groups had similar minimal RV values. Minimal RV value applies minimal covariation between sets of landmarks and was in adults congruent with the partition to viscerocranium and neurocranium. In juveniles, a pattern of modularity emerged that did not match the subdivision into functional modules. A further study might discover a model of association between cranial traits different from conventional modules. Allometry can be a strong integrating factor (Klingenberg 2009), but it had little influence on the hypothesized modularity of the *S. citellus* skull in juveniles as well as in adults, probably because the amount of shape variation explained by allometry was low. Although marginally significant, observed difference in RV coefficients between the two age classes also suggests that in *S. citellus* the strength of association between viscerocranium and neurocranium was higher in juvenile than in adult skull.

The relationship between the developmental determinants of integration and phenotypic covariance is very complicated, therefore the integration cannot be studied through phenotypic covariance patterns alone (Hallgrímsson et al. 2009). Nevertheless, it is commonly known that functionally and developmentally related traits are more integrated than traits that do not share functional and developmental influences (Leamy et al. 1999, Willmore et al. 2006). Most studies of morphological integration showed high covariation patterns between functionally related traits (e. g. Cheverud 1995, Marroig and Cheverud 2001, Klingenberg et al. 2004, Ivanović and Kalezić 2010, Jojić et al. 2012). In skulls, modularity is

well studied and defined in primates (e. g. Marriog and Cheverud 2001, González-José et al. 2008), whereas in rodents the correlations between functionally and developmentally related structures are less consistent and obvious (Willmore et al. 2006, Klingenberg 2013). For instance, Monteiro et al. (1999) discovered that the orofacial region of the *Thrichomys apereoides* (Lund, 1839) skull is less integrated than the basicranium. Willmore et al. (2006), on the other hand, found out that the facial regions of mice are weakly but significantly integrated, while no integration was found for the cranial vault. Jojić et al. (2011), who used the same methods as the present study, confirmed the hypothesis of a face-basicranium organization of the *Apodemus flavicollis* (Melchior, 1834) skull. Tests of integration and modularity can yield mixed results also because of the differences in methodology (Jojić et al. 2012, Klingenberg 2013). Olson and Miller (1958) studied morphological integration of the cranium and mandible of the fox squirrel using linear measurements and methods based on statistical correlation. Roth (1996) studied the lateral view of the cranium of several squirrel species using landmark based geometric morphometric methods and preseneted only descriptive preliminary results with no statistical analyses of morphological integration. Therefore, it is possible that support for the two-module organization of the ventral cranium observed herein or for a highly integrated sciurid skull reported in previous studies (Olson and Miller 1958, Roth 1996) could depend upon the methodology.

Because the shape of bones is influenced by the mechanical forces during ontogeny (Sun et al. 2004, Young and Badyaev 2007) and the bones of the viscerocranium are directly involved in the mechanics of feeding, they undergo prominent shape transformations after weaning when juveniles change their diet form suckling to gnawing. Based on the results, I suppose that modularity in the *S. citellus* ventral cranium is largely driven by masticatory forces that apply to the bones of the viscerocranium and form a functional module that is in weak covariation with the neurocranium, which grows relatively slow during postnatal ontogeny (Herring 1993, Monteiro et al. 1999). For a better understanding of modularity, variability and development of the skull of *S. citellus* more research is needed, ideally on bigger samples and

postnatal longitudinal data including the study of fluctuating asymmetry, which can be a useful tool for determination of the boundaries of developmental modules (Klingenberg and Zaklan 2000, Klingenberg et al. 2001).

Conclusions

1. Unlike previous studies of sciurid skulls, the analysis of the covariation among landmarks in the ventral cranium yielded strong support to the hypothesis that in *S. citellus* the viscerocranium and neurocranium are separate modules.

2. The level of modularity was higher in adults than in juveniles, i.e. the hypothesized modules in the juvenile skull were more integrated with each other.

3. Although allometry can be a strong integrating factor, it had very little influence on the hypothesized modularity of the *S. citellus* skull.

4. The change in the strength of integration between the viscerocranium and neurocranium in the *S. citellus* skull during ontogeny is probably a consequence of the change in diet, from liquid to solid food.

Povzetek

Članek obravnava morfološko integracijo in modularnost lobanje evropske tekunice *Spermophilus citellus* in primerja stopnjo modularnosti med mladimi in odraslimi osebki vrste. Moduli so notranje tesno povezani deli strukture, ki so med seboj relativno neodvisni. Integracijo in modularnost morfoloških struktur analiziramo z ugotavljanjem stopnje povezanosti med posameznimi deli strukture. Ventralno stran lobanje lahko razdelimo v dve funkcionalni podenoti; nevrokranij, ki ga sestavljajo kosti možganskega dela lobanje, oči in ušes, in viscerokranij, ki je iz kosti čeljustnega aparata. Cilj raziskave je bil preveriti prisotnost te delitve na ventralni strani lobanje evropske tekunice, saj je za lobanje veveric

znano, da imajo visoko stopnjo integracije brez jasnih delitev na podenote (Olson and Miller 1958, Roth 1996). Testirala sem 159 lobanj, od tega 43 mladih in 116 odraslih osebkov. Obliko ventralne strani lobanje sem opisala z 22 oslonilnimi točkami. Konfiguracije koordinat oslonilnih točk vseh lobanj sem poravnala s posplošeno Procrustovo analizo (GPA), ki konfiguracije koordinat premakne, zavrti in skalira, tako da je vsota kvadratov razlik na koncu v vzorcu minimalna. Nato sem z delitvijo oslonilnih točk na viscerokranij in nevrokranij postavila hipotezo o modularni zgradbi lobanje. Hipotezo testiramo s izračunom stopnje kovariabilnosti med hipotetičnimi moduli, ki jo nato primerjamo z vsemi alternativnimi delitvami točk na podskupine z enakim številom točk, kot jih imajo hipotetični moduli. Hipoteza o modularnosti drži, če je kovariabilnost med hipotetičnimi moduli izrazito nižja kot med vsemi ostalimi alternativnimi delitvami točk na module. Stopnjo integracije med moduli sem ovrednotila z metodami, ki temeljijo na RV koeficientu. Primerjala sem tudi stopnjo modularnosti in integracije med mladimi in odraslimi osebki. Tako pri mladih kot odraslih osebkih so rezultati podprli hipotezo o delitvi lobanje *S. citellus* na viscerokranij in nevrokranij, le da je bila lobanja mladih osebkov izraziteje integrirana. Razliko v stopnji integracije in modularnosti lobanje med mladimi in odraslimi osebki je potrdil tudi permutacijski test. Čeprav je alometrija lahko močan integracijski faktor, je imela majhen vpliv na vzorec modularnosti in integracije. Sprememba v stopnji modularnosti pri lobanji evropske tekunice med ontogenetskim razvojem je najverjetneje posledica spremembe v prehrani, t. j. prehoda s tekoče na trdo hrano, in večje specializacije obeh funkcionalnih modulov.

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