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Modularity of the dorsal and lateral view of the skull in the European ground squirrel

Modularnost dorzalne in lateralne strani lobanje evropske tekunice

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Abstract: Modular organization is a general characteristic of biological systems from cellular to organismal level. The mammalian skull is a complex structure that can in general be divided into two functional components, the neurocranium and the viscerocranium. The two-module organisation of the skull of the European ground squirrel *Spermophilus citellus* (Linnaeus, 1766) has already been confirmed on the ventral cranium, while different studies of integration and modularity of squirrel skulls in general gave mixed results. Studies using 2D geometric morphometrics capture and analyse different views of the skull separately, and often the ventral cranial view is considered as the most suitable. In this study, the hypothesis of the two-module organisation of the *S. citellus* skull was re-evaluated and confirmed also on the dorsal and lateral cranial view. Nevertheless, the lateral cranium was more integrated than the dorsal cranium. Allometry had almost no effect on the pattern of modularity. This and the previous study of the *S. citellus* skull modularity show that different cranial views can give different results. Advisably, all three views should be considered also because the lateral view of the skull shows morphological variation in the sagittal plane that is not visible along the frontal plane, when only the ventral and/or dorsal views are considered.

Keywords: Sciuridae, modular organization, cranium, RV coefficient, *Spermophilus citellus*

Izvleček: Modularna organizacija je splošna značilnost biotskih sistemov od nivoja celic do organizmov. Lobanja sesalcev je kompleksna struktura, ki jo lahko razdelimo v dve funkcionalni komponenti, nevrokranij in viscerokranij. Organizacija lobanje evropske tekunice *Spermophilus citellus* (Linnaeus, 1766) na dva modula je bila potrjena na ventralni strani lobanje, medtem ko različne raziskave integracije in modularnosti lobanje veveric na splošno dajejo različne rezultate. Raziskave, ki temeljijo na dvo-dimenzionalni geometrijski morfometriji, ločeno zajemajo in analizirajo različne strani lobanje in pogosto obravnavajo ventralno stran lobanje kot najbolj primerno. V tej raziskavi je bila modularna organizacija lobanje *S. citellus* ponovno ovrednotena in potrjena tudi na dorzalni in lateralni strani lobanje. Vendar je bila stopnja integracije lateralne strani bolj izrazita kot dorzalne. Alometrija ni imela skoraj nobenega vpliva na vzorec modularnosti. Ta in predhodna raziskava modularnosti lobanje *S. citellus* kažeta, da lahko različne strani lobanje dajo različne rezultate. Iz tega sledi, da je priporočljiva obravnava vseh treh strani lobanje, tudi zato, ker je z lateralne strani lobanje vidna morfološka variabilnost v sagitalni ravnini, medtem ko lahko pri analizi samo ventralne in/ali dorzalne strani zajamemo variabilnost samo v frontalni ravnini.

Ključne besede: Sciuridae, modularna organizacija, kranij, RV koeficient, *Spermophilus citellus*

Introduction

Modular organization is a general characteristic of biological systems and is present at all biological levels, from cells to whole organisms (Klingenberg et al. 2003). Modules are units with a high degree of internal integration because of functional, developmental, genetic or other interactions, which are relatively independent of other such units (Klingenberg 2008). Integration and modularity have been most frequently studied in mammal skulls (for review see Klingenberg 2013). The mammalian skull is a complex structure that can be divided into two functional components, the neurocranium, composed of the braincase, eyes and ears, and the viscerocranium, consisting of the jaw apparatus (Emerson and Bramble 1993). Nevertheless, some studies have supported a more complex six-module organisation of the mammalian cranium (for review see Felice et al. 2018). Most studies of mammal skull modularity and integration are performed using linear measurements (traditional morphometrics) or 3D geometric morphometrics of the whole cranium (e.g. Goswami 2006, Drake and Klingenberg 2010). In 2D geometric morphometrics, the ventral view of the cranium is very suitable for analyses (Klenovšek and Jojić 2016). Thus, the hypothesis of modularity of the European ground squirrel *Spermophilus citellus* (Linnaeus, 1766) was tested and confirmed only on the ventral cranium (Klenovšek 2014a). Modular organisation of the *S. citellus* skull was in contrast to previous findings claiming that squirrels have a highly integrated skull without clear subdivisions into subunits (Olson and Miller 1958, Roth 1996). The aim of this study was to re-evaluate the hypothesis of the two-module organisation of the *S. citellus* skull on the dorsal and lateral cranium. Also the effect of allometry, as a possible strong integrating factor that can counteract modularity (Klingenberg 2009), has been estimated.

Material and Methods

Dorsal and lateral cranial views of *S. citellus* skulls were studied for modular organisation. The skulls originated from Burgenland (Austria) and Banat (Serbia) 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). Juvenile (< 5 months old) and very old (after the fourth hibernation) individuals were not used in the study. The sample included 65 skulls of *S. citellus*, from which 64 (Banat: N=44; Burgenland: N=20) were studied from the dorsal and 62 (Banat: N=43; Burgenland: N=19) from the lateral cranial view. Both sides of the crania were photographed under constant conditions following the protocol described in Klenovšek (2014b). Thirteen landmarks were digitized on the dorsal (Fig. 1A) and 12 on the lateral (Fig. 1B) cranium using the tpsDig2 program (Rohlf 2015).

For each side of the skull, landmark coordinates of all individuals were aligned using the Generalized Procrustes Analysis (GPA) (Rohlf and Slice 1990). GPA standardizes size and removes the differences in landmark configurations due to position and orientation. GPA also separates size and shape information. Shape information is retained in the form of Procrustes coordinates, which are shape variables containing the complete information on shape variation in the sample after superimposition. Because sexual shape dimorphism is not present in the skull of *S. citellus* (Klenovšek and Kryštufek 2013, Ramos-Lara et al. 2014) the sexes were pooled. Also the two populations were pooled to increase the number of studied objects compared to the number of shape variables (26 and 24 variables for dorsal and lateral crania, respectively) despite known significant shape differences between the populations (Klenovšek and Kryštufek 2013).

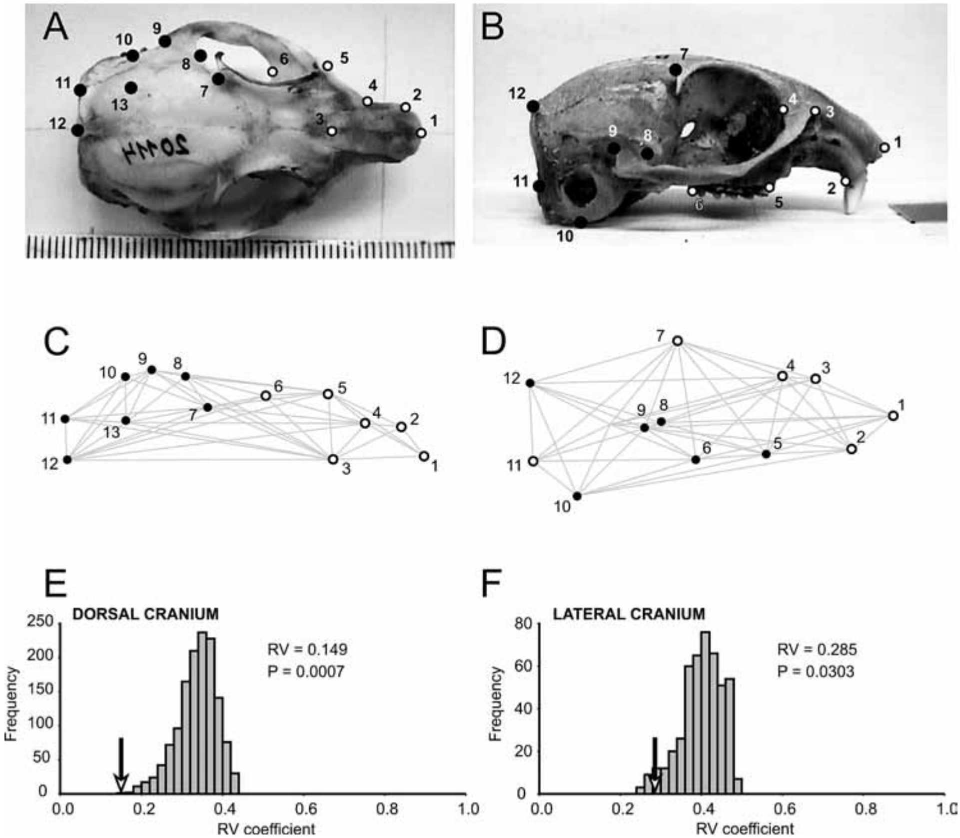

Figure 1: Dorsal (A) and lateral (B) cranial views of the *S. citellus* skull with 13 and 12 landmarks, respectively, divided into two hypothesized modules (black dots – neurocranium, white dots – viscerocranium). Adjacency graphs for the dorsal (C) and lateral (D) crania showing landmark partitions to the two subsets with the smallest RV coefficient. Histograms of the RV coefficinets for all spatially contiguous partitions of landmarks into two subsets for the dorsal (E) and lateral (F) cranium. The values of RV coefficients observed for the partition into viscerocranial and neurocranial regions are indicated by arrows. P- values present proportion of partitions with RV lower than or equal to the a priori hypothesis.

Slika 1: Dorzalna (A) in lateralna (B) stran lobanje *S. citellus* s 13 in 12 oslonilnimi točkami razdeljenimi na dva hipotetična modula (črne pike – nevrokranij, bele pike – viscerokranij). Žični graf za dorzalno (C) in lateralno (D) stran lobanje z delitvijo oslonilnih točk na dve podskupini z najmanjšim RV koeficientom. Histogram RV koeficientov vseh prostorsko sosednih delitev konfiguracij oslonilnih točk za dorzalno (E) in lateralno (F) stran lobanje. Vrednosti RV koeficientov med hipotetičnima moduloma (viscerokranijem in nevrokranijem) so označene s puščico. P vrednosti predstavljajo delež delitev na podskupine z nižjim RV koeficientom kot tistim za hipotetična modula.

To correct the data for the effect of population affiliation, the pooled within-populations covariance matrix could be used (Klingenberg 2009). Because this method assumes that the groups have the same covariance matrix, the Box's M test for the equality of covariance matrices was performed (SPSS Statistics 2008). The Box's M test was carried out on the first 15 Principal Components that explained 95.4 % and 95.8 % of total variance in the dorsal and ventral cranium, respectively. The differences between covariance matrices were not statistically significant either for the dorsal or lateral crania. Therefore, the pooled within-populations covariance matrices were computed for each cranial view by subtracting differences between the population means (Klingenberg 2009).

To re-evaluate the hypothesis of modularity of the *S. citellus* skull (Klenovšek 2014a), landmark configurations of the dorsal and lateral cranium were divided into subsets describing the anterior (viscerocranium) and posterior (neurocranium) parts of the skull (Fig. 1). The degree of covariation between the hypothesized modules was compared to all alternative spatially contiguous partitions with the same number of landmarks as in the hypothesized modules (Klingenberg 2009).

The strength of association between the subsets of landmarks was estimated with the Escoufier RV coefficient (Escoufier 1973), which represented the amount of covariation scaled by the amount of variation within the two subsets of variables. The RV coefficient takes the value of zero if the two sets of variables are completely uncorrelated 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). To estimate the effect of allometry on modularity, I repeated the analyses of modularity for each cranial view using residual data of a multivariate regression of Procrustes coordinates onto centroid size. All analyses of modularity were performed using the MorphoJ software (Klingenberg 2011).

Results

The hypothesis of 2-module organization was confirmed both in the dorsal and lateral cranium. The RV coefficient obtained for the hypothesized partition into the viscerocranium and neurocranium was 0.149 for the dorsal cranium, and 0.285 for the lateral cranium. In the dorsal cranium, none of the 1352 alternative partitions to two subsets had a lower RV coefficient than the partition into the hypothesized modules. The hypothesized partition (Fig. 1A) was the same as the partition with the smallest RV coefficient (Fig. 1C). In the lateral cranium, 14 (or 3.037 %) of the 461 alternative spatially contiguous partitions had a lower or equal RV coefficient. Partition to two subsets with the minimal covariation (Fig. 1D) was different from the hypothesized one (Fig. 1B).

To match the number of landmarks in all hypothesized modules, i.e. viscero- and neurocranium in dorsal and lateral view, landmark 13 (Fig. 1A) was omitted and the hypothesis of modularity in the dorsal cranium was repeated on 12 landmarks. The new RV coefficient was 0.219 and again none of the 453 alternative partitions to two subsets had a lower RV coefficient than the a-priori hypothesis.

To sum up, in both cranial views the covariation between the viscero- and neurocranium was significantly weaker than it would be expected for a random partition of the dorsal and lateral cranium into two parts with the pre-defined number of landmarks. This is illustrated in histograms of RV coefficients, where for both cranial views the hypothesized subdivisions to two modules were clearly in the lower extreme of the distribution of RV coefficients for the alternative partitions (Fig. 1E, F).

The allometry accounted for 6.65% ($P < 0.0001$) and 4.20% ($P = 0.0042$) of shape variation in the dorsal and lateral cranium, respectively. After the correction for allometry, the values of RV coefficients between the viscero- and neurocranium were 0.169 for the dorsal cranium, and 0.292 for the lateral cranium. In the dorsal cranium, the hypothesized partition was the same as the partition with the smallest RV coefficient. In the lateral cranium, 18 (or 3.905 %) of the 461 alternative spatially contiguous partitions had a lower or equal RV coefficient.

Discussion

The hypothesis of two-module organisation of the dorsal and lateral view of the *S. citellus* skull was confirmed, which is in agreement with a previous study performed on the ventral cranium (Klenovšek 2014a). This is important because studies of integration and modularity in the Sciuridae family yielded mixed results. Olson and Miller (1958) and Roth (1996) claimed that squirrels have a highly integrated skull without clear subdivisions into subunits. Porto et al. (2013), on the other hand, discovered that Sciuridae have compared to some other rodent families less integrated skulls, but modular organisation becomes more pronounced after removing size variability. Moreover, a skull is a complex three-dimensional structure, therefore most studies of skull modularity and integration use morphometric methods that consider the skull as a whole; e.g. traditional morphometrics uses linear measurements of the skull in all three dimensions, while the more sophisticated 3D geometric morphometrics uses landmarks on 3D scans of the skull. In 2D geometric morphometrics, skulls are photographed from different sides and analyses are performed separately for different views of the skull, from which the ventral view is mainly used (examples in Sciuridae: Roth 1996, Cardini and O'Higgins 2004, Klenovšek and Kryštufek 2013, Klenovšek 2014a). This is because the ventral cranium is genetically and functionally diverse (Caumul and Polly 2005), and due to its complexity, contains a large number of anatomical landmarks (Kryštufek et al. 2012). In this study, skull modularity of the lateral view was less obvious that of the ventral and dorsal. In the lateral cranium, the two-subset partition with the minimal covariation (Fig. 1D) was different from the hypothesized partition to viscero- and neurocranium (Fig. 1B). Contrary to the hypothesis, the molar tooth row (LM 5 and 6) was in stronger covariation with the posterior part of the skull, while the base of the caudal supraorbital process (LM 7) and the occipital bone (LM 11) were in stronger covariation with the anterior part. In both ventral (Klenovšek 2014a) and dorsal cranial view (Fig. 1C), the hypothesized partition to viscero- and neurocranium had the lowest covariation compared to all alternative partitions to two subsets. Also, the difference in RV coefficients between the studied cranial views suggests that in *S. citellus* the lateral cranium is more integrated than the dorsal cranium. This was supported by higher RV values for all alternative partitions in the lateral (RV range up to approx. 0.5) compared to the dorsal cranium (RV range up to approx. 0.4) (Fig. 1F and 1E). Although allometry can be a strong integrating factor (Klingenberg 2009), it had very little influence on the pattern of modularity in *S. citellus*. After the correction for allometry, the values of RV coefficients between the viscero- and neurocranium were in all cranial views a little higher than before the correction (this study and Klenovšek 2014a). Moreover, in the lateral cranium, the P-value slightly incerased. This is contrary to findings of Porto et al. (2013), who discovered that modular organization becomes more pronounced after removing size variability.

Because the ventral and dorsal surfaces of the skull are opposite sides along the frontal plane, the 2D geometric morphometric analyses of these cranial views do not capture morphological variation in the sagittal plane. Addition of the lateral cranial view to studies of modularity and integration using 2D geometric morphometrics is valuable and meaningful; because it allows the analysis of morphological variation of the skull in the ventral-dorsal axis, which is in an additional dimension that is not observable in the frontal plane.

Conclusions

1. The analysis of the covariation among landmarks in the dorsal and lateral cranium supported the hypothesis that in adult specimens of the European ground squirrels *S. citellus* the viscerocranium and neurocranium are separate modules.

2. Modular organisation of the dorsal and lateral view of the *S. citellus* skull was in agreement with a previous study performed on the *S. citellus* ventral cranium (Klenovšek 2014a).

3. Lateral cranium was more integrated than the dorsal cranium.

4. Allometry had almost no effect on the modularity of the dorsal and ventral cranium of the *S. citellus* skull.

5. In studies of modularity and integration using 2D geometric morphometrics, the lateral cranial view should be analysed together with the ventral or dorsal view, because it shows morphological variation in the sagittal plane or ventral-dorsal axis that is not visible along the frontal plane.

Povzetek

Modularna organizacije je splošna značilnost biotskih sistemov od nivoja celic do organizmov. Lobanja sesalcev je kompleksna struktura,

ki jo lahko razdelimo v vsaj dve funkcionalni komponenti, nevrokranij in viscerokranij. Za lobanjo veveric naj bi bila značilna visoka stopnja integracije brez jasnih delitev na module (Olson in Miller 1958, Roth 199). Medtem ko je novejša raziskava primerjave stopnje integracije in modularnosti lobanje med različnimi družinami sesalcev pokazala, da imajo veverice v primerjavi z ostalimi glodavci manj integrirane lobanje (Porto et al. 2013). Organizacija lobanje evropske tekunice *Spermophilus citellus* (Linnaeus, 1766) na nevrokranij in viscerokranij je že bila potrjena na ventralni strani lobanje (Klenovšek 2014a). Namen pričujoče raziskave je bil ovrednotiti morfološko integracijo in modularnost lobanje tudi na dorzalni in lateralni strani. Ovrednotenih je bilo 65 lobanj odraslih osebkov *S. citellus*. Oblika dorzalne strani lobanje je bila opisana s 13 oslonilnimi točkami in lateralne strani lobanje z 12 (Slika 1A in 1B). Z delitvijo točk na nevro- in viscerokranij je bila postavljena hipoteza o modularni organizaciji lobanje. Analiza morfološke variabilnosti lobanje in s tem modularnosti je temeljila na metodah geometrijske morfometrije, ki konfiguracije oslonilnih točk poravnana s posplošeno Procrustovo analizo (GPA) in ki omogoča ločeno obravnavo velikosti in oblike objektov.

Moduli so notranje tesno povezani deli strukture, ki so med seboj relativno neodvisni. Hipoteza modularnosti (delitev lobanje na nevro- in viscerokranij) je bila testirana z izračunom stopnje kovariabilnosti med hipotetičnima moduloma. Stopnjo kovariabilnosti se primerja z vsemi alternativnimi delitvami oslonilnih točk na dve podskupini z enakim številom točk, kot jih imata hipotetična modula. Hipoteza o modularnosti drži, če je kovariabilnost med hipotetičnimi moduli izrazito nižja kot med vsemi ostalimi alternativnimi delitvami točk na podskupine. Analiza je temeljila na Escoufier RV koeficientu, ki obsega vrednosti od 0 do 1. Nižja kot je vrednost RV koeficienta, nižja je stopnja kovariabilnosti med hipotetičnima moduloma in večja je njuna neodvisnost z vidika morfološke variabilnosti. Če hipoteza o delitvi lobanje na dva modula drži, ima delitev na hipotetična modula v primerjavi v vsemi alternativnimi delitvami najnižjo vrednost RV koeficienta.

Hipotetična delitev na neuro- in viscerokranij lobanje *S. citellus* je bila potrjena tako na dorzalni kot na lateralni strani (dorzalno: RV = 0,149; $p < 0,001$ in ventralno: RV = 0,285; $p = 0,030$) . Vendar je bila modularnost lateralne strani lobanje slabše izražena, saj je bila vrednost RV koeficienta višja v primerjavi z dorzalno stranjo in tudi 14 od 461 alternativnih delitev na dve podskupini (3.037%) je imela nižjo vrednost RV koeficienta kot hipotetična modula. Čeprav je alometrija lahko močan integracijski faktor, je imela zelo majhen vpliv na vzorec modularnosti.

Raziskave, ki temeljijo na dvo-dimenzionalni geometrijski morfometriji, ločeno zajemajo in analizirajo različne strani lobanje in pogosto obravnavajo samo ventralno stran lobanje zaradi njene kompleksnosti in relativne ploščatosti. Rezultati raziskave modularnosti lobanje *S. citellus* kažejo, da lahko različne strani lobanje dajo različne rezultate. Iz tega sledi, da je priporočljiva obravnava vseh treh strani lobanje, med drugim tudi zato, ker je z lateralne strani lobanje vidna morfološka variabilnost v sagitalni ravnini oziroma dimenziji, ki je z analizo samo ventralne in/ali dorzalne strani ne moremo zajeti.

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