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*

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).
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. viscerocranium and neuro-
cranium 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 covaria-
tion between the viscerocranium and neurocranium
was significantly weaker than it would be expected
for a random partition of the dorsal and lateral
cranial 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, re-
spectively. After the correction for allometry, the
values of RV coefficients between the viscerocranium and neurocranium were 0.169 for the dorsal cranium,
and 0.292 for the lateral cranium. In the dorsal
cranial, 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
cranial (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 viscerocranium 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 viscerocranium 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 viscerocranium 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 increased. 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,

Moduli so notranje tesno povezani deli strukture, ki so med seboj relativno neodvisni. Hipoteza modularnosti (delitev lobanje na nevro- in viscerokranij) je bila testirana iz izraženem stopnje kovariabilnosti med hipotetičnima moduloma. Stopnjo kovariabilnosti se primerja z vsemi alternativnimi delitvami oslonilnih točk na dve podskupini z takimi 14 od 461 alternativnih delitev na dve podskupini (3.037%) je imela nižjo vrednostRV 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 obravnavana vseh treh strani lobanje, med drugim tudi zato, ker je z lateralne strani lobanje vidna morfološka variability v sagitalni ravnini oziroma dimenziji, ki je z analizo samo ventralne in/dorzalne strani ne moremo zajeti.

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**References**

