

Biogeography of elytral ornaments in Palearctic genus *Carabus*: disentangling the effects of space, evolution and environment at a continental scale

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Abstract Members of large genus *Carabus* (Insecta, Coleoptera, Carabidae) show ornamental and conspicuously diverse morphological sculptures on the surfaces of their elytrae. Origin and significance of these sculptures is unknown. Theories usually explain emergence of such structures as a result of sexual selection or as an adaptation to environment, but almost never as an outcome of neutral evolution. We explore the latter two possibilities by analyzing the relationship between morphospace of elytral sculptures of 1,177 sub-species of genus *Carabus* and environmental variables across the whole Palearctic, while taking into account spatial and evolutionary autocorrelation. We used principal coordinates analysis (PCoA) to identify two main axes of variability in elytral morphospace (20 morphological traits) and we also classified the sub-species according to the homogeneity of morphological elements. We used the fourth-corner method, generalized linear models and variation partitioning to assess if the morphology is driven by environmental conditions, by spatial autocorrelation, and/or by evolutionary relatedness. We found two main axes of morphological variability which separated the sub-species according to elytral grain size and distinctness of the grains. These axes and also the homogeneity of morphological elements correlated weakly with climate. However, when

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we accounted for the spatial component and evolutionary relatedness there was nearly no independent effect of environment on elytral sculptures. Our results indicate that morphological variability of the sculptures is not influenced by environmental factors and we hypothesize that, at continental scales, the elytral ornaments of genus *Carabus* are an outcome of environmentally unconstrained neutral evolution—something that has been rarely demonstrated in empirical study. A surprising by-product of our study is the finding that sub-species within the genus *Carabus* have an extremely high degree of endemism which highlights the conservation value of the group.

Keywords Evolution · Geographic distribution · Elytra · Fourth-corner · Climate · Multidimensional scaling · SEVM

Introduction

The geographic variation of life has predominantly been studied using taxonomy, but this focus is changing. There is a growing interest in understanding patterns in the distribution not only of taxa but also of the traits those taxa possess (Green et al. 2008; McGill et al. 2006), with most of the research focusing on geographic variation of functional traits (i.e. traits that directly influence organismal performance; (McGill et al. 2006). The classical textbook examples are geographic patterns of body size (Bergmann 1847), length of extremities in ectotherms (Allen 1877), or clutch size in birds (Lack 1947). Recent studies reveal geographic trends in wood density in trees (Swenson and Enquist 2007) or leaf architecture in plants (Wright et al. 2004).

Apart from clearly functional traits, there are traits with less straightforward influence on organisms' performance such as structural ornamentation or colour variation. These can emerge as a result of sexual selection (e.g. Emlen 2001), but there are also cases for which we have no adaptive evolutionary hypothesis explaining their purpose (Gould and Lewontin 1979). These traits indeed influence our perception of natural diversity in a particular area. However, studies trying to geographically map the variation of these features are scarce. These comprise the studies of geographic patterns of fruit colours (Burns et al. 2009), plumage colours in hummingbirds (Schmitz-Ornes 2006) and bumblebee colour patterns (Williams 2007).

The ornamental sculptures on elytrae of carabid beetles (Fig. 1) may be taken as a classic case of a trait with unclear significance. The sculptures usually consist of three kinds of longitudinal parallel and symmetrically arranged arrays that may differ in their morphological appearance (Deuve 2004). The geometric combinations of these elements are responsible for the high ornamental variety of sculptural patterns. Approximately 97 percent of all *Carabus* species have a developed sculpture on their elytrae. The evolutionary origin of elytral sculptures in genus *Carabus* have been discussed but few times during last 150 years (Deuve 2004; Diez 1896; Kraatz 1878; Reitter 1908; Richter 1936). There are anecdotal proposals that these sculptures represent imitations of fluid secretions of abdominal defence glands (Portchinskij 1892) or mimicry of decomposed beechnuts in *Hygrocarabus variolosus* (Jeřátko 1938). It is highly improbable that the sculptures originated via sexual selection, as there is no behavioural evidence that the sculptures serve as a sexual display (Brandmayr et al. 2000). Moreover, the sculptures are identical in both males and females and most of the species are active after dusk, which means that they cannot serve as a visual cue (Hůrka 1996).

In this paper we explore the possibility that the elytral sculptures and patterns of their geographic distribution are influenced by abiotic environmental conditions (climate in particular). Specifically, we propose the following hypotheses:

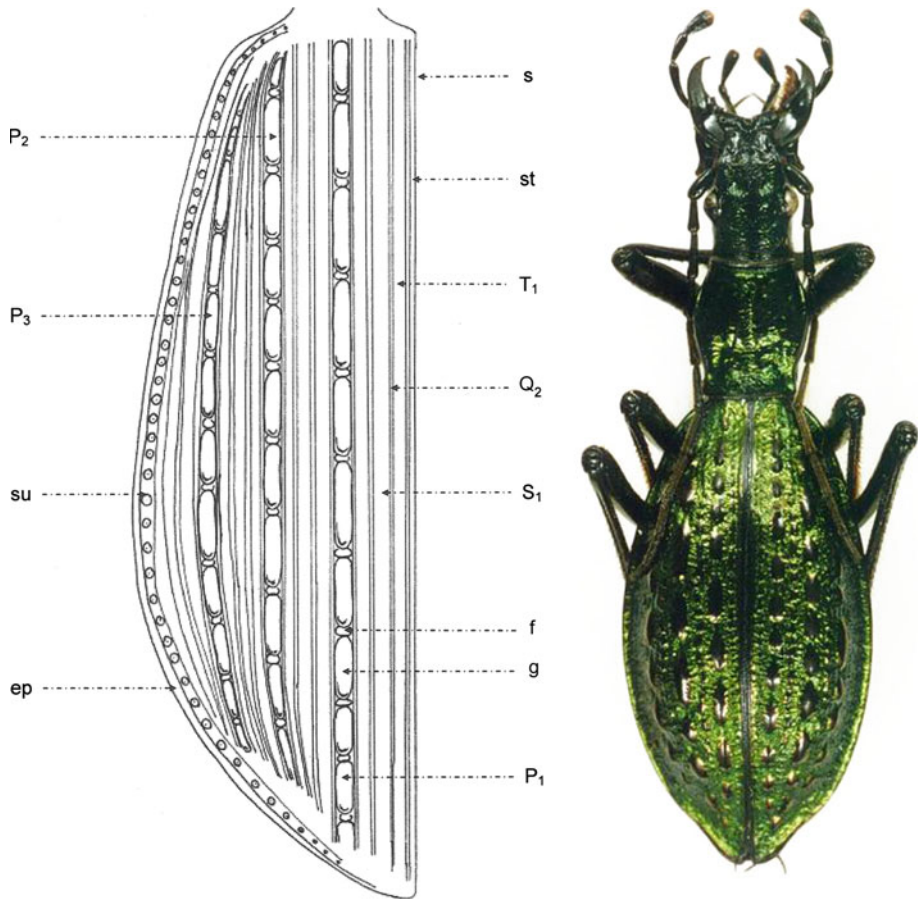


Fig. 1 *Left*: The schematic description of sculptural elements on elytrae of genus *Carabus*: primary intervals (P_1 , P_2 , P_3), first secondary interval (S_1), first tertiary interval (T_1), second quaternary interval (Q_2), epipleuron (ep), suture (s), series umbilicata (su), stria suturalis (st), grain (g), fovea (f). The state of primary, secondary, tertiary, and quaternary intervals were used to describe the morphology of sculpture for purposes of further analysis. *Right*: An example of the *Carabus* phenotype, *Carabus haeckeli* (photo by Boleslav Březina)

H1 The sculptures are independent of environmental conditions—they are a result of environmentally unconstrained evolution. In this case, we expect no independent effect of environmental conditions on the geographic distribution of sculptural morphology. Moreover, because members of the genus *Carabus* are poor dispersers (Deuve 2004), evolutionarily closely related species should occur geographically close to each other and the variation in elytral morphology should be attributable to how related the species are.

H2 The sculptures are linked to the environment, which can constrain them in several ways: (1) environmental conditions are selective forces and the sculptures are an adaptive trait, (2) the elytral sculptures are neutral traits that are, however, costly; and their emergence is constrained by environmental suitability or (3) the sculptures are formed by the effects of environmental variables on a beetle's ontogenetic development. However, we are aware that it is not possible to separate the three proposed mechanisms only by

exploring geographic distributions of traits. Hence, we simply test if there is a fraction of geographic variation in elytral morphology that can be explained by abiotic environmental conditions.

We present a unique and extensive dataset on large genus *Carabus* in Palaearctic (1,177 sub-species, 20 morphological traits, 121 regions). The members of large genus *Carabus* Linné (Carabidae, Coleoptera) represent the extant group of more than 700 species that inhabit the Holarctic region, with the centre of distribution and species diversity unequivocally in the Palearctic (Březina 1999; Deuve 2004; Hůrka 1996). The sculptural patterns in genus *Carabus* represent highly conspicuous structures that traditionally made the members of this group attractive for collectors and amateur entomologists, which means that we have reliable distributional information for all of the *Carabus* sub-species. Using this dataset, we (1) explore the trait morphospace of elytral sculptures and extract the principal coordinates that characterize the position of each taxon in this morphospace, (2) we map geographic patterns of the elytral morphology over the whole Palaearctic, and finally, (3) we attempt to partition the variation in morphological traits among variables representing evolutionary relatedness, purely spatial variables and variables describing environmental conditions.

Materials and methods

Distributional data

For purpose of this study, we followed the traditional interpretation of the *Carabus* as one large genus *Carabus sensu lato* (Březina 1994; Deuve 2004). We gathered information about the occurrence of 1,177 (of total 1,433) sub-species of Palearctic genus *Carabus* in 121 regions. Information concerning the presence/absence of each sub-species was taken from the checklist and world catalogue of the genus *Carabus* (Březina 1994; Březina 1999). These do not include 258 sub-species for which we have no morphological characteristics. The complete distributional dataset is provided in Appendix 1 of supplementary material. We acknowledge that the regions in our study have unequal areas, which is something that can be problematic in biodiversity studies (Keil and Hawkins 2009). However, we found no significant correlations between log-transformed area and any of the morphological traits, neither did variance in the trait values decrease with increasing area. For these reasons and for the sake of simplicity we do not consider the area of the regions in our analyses.

Data on morphology

The dorsal surface of elytra of genus *Carabus* is usually structured in parallel striae and deep points (Fig. 1). The geometrized systems of longitudinal striae (intervals) typically differ in the degree of their development, and can be classified as primary (P), secondary (S), tertiary (T), and quaternary intervals (Q) (see Fig. 1). Dorsal sculpture is composed of three primary intervals that often bear setae or foveae, and of a more external fourth interval, marked as *series umbilicata*, which is homologous to but morphologically differs from the three former primary intervals. The space between the two primary intervals is most frequently filled with one secondary and two tertiary intervals; in some exceptions, a further two or four quaternary intervals are interposed between the primary and secondary, and/or secondary and tertiary intervals. For more detailed description of the morphology of sculpture on elytron see Deuve (2004).

For character coding, we used the presence and morphology of all intervals between the first primary (P_1) and first secondary interval (S_1). In practice, this describes the morphology of the entire sculptural motif because intervals are arranged in the form of laterally repeated central symmetry systems. Every interval may acquire four different states: (1) interval not present, (2) interval dominated by developed foveae, (3) interval dissipated to row of grains, (4) interval consist of grains and foveae of intermediate development. Based on whether the intervals of different development and size are present, or whether all intervals reach the same development, two basic groups of sculpture were defined: *heterodynamic* sculptures consist of morphologically diversified intervals and *homodynamic* sculptures with structurally uniform elements. The complete dataset on these morphological characteristics is given in Appendix 2 of supplementary material.

We encoded the traits into a sub-species \times trait matrix where each of the 20 traits was either present (1) or absent (0). Because many of the traits co-occur with each other, we reduced the number of traits and identified the most important axes of trait variability by Principal Coordinates Analysis (PCoA; sometimes called Metric Multidimensional Scaling; Legendre and Legendre 1998) based on Manhattan distances between objects (sub-species). Additionally to the PCoA-derived traits we also classified the sub-species according to the homogeneity of morphological elements as homodynamic or heterodynamic as this is visually the most straightforward classification. We calculated mean scores on the PCoA axes for each country and ratios of homodynamic and heterodynamic sub-species and we plotted these on maps.

Environmental data

We selected four climatic variables for our analysis. These were mean annual temperature, mean annual precipitation, seasonality of temperature, and seasonality of precipitation (expressed as coefficient of variation of mean monthly values). The climatic variables were selected because (1) they are well known to influence many aspects of biodiversity; (2) they are readily obtainable and interpretable at large geographic scales; (3) they represent crucial abiotic factors that influence the life of ectothermic organisms such as insects (Dixon et al. 2009; Hawkins and Porter 2003). We also examined the effect of mean altitude and variability of altitude in a region. The altitudinal variables were included because they describe topographic variability of a region, which can be regarded as a proxy for habitat heterogeneity and also dispersal permeability (the more mountains and valleys there are, the more isolated local populations are).

We extracted the data on mean annual temperature and precipitation from the UEA Climatic Research Unit (<http://www.cru.uea.ac.uk>) 10 min grid (New et al. 2002). The data on mean temperature seasonality and precipitation seasonality were taken from the WorldClim (<http://www.worldclim.org>) 10 min grid (Hijmans et al. 2005). For each of these variables we took an average over all of the 10 min grid cells within the region of interest. We also extracted latitudinal and longitudinal coordinates of the centroids of each region in order to look for any geographical trends. All mapping procedures and manipulations with environmental and geographic variables were done in ESRI ArcGIS 9.2 (<http://www.esri.com>). We provide the complete environmental dataset in Appendix 3 of supplementary material.

To avoid co-linearity between variables and to facilitate the interpretation of the analyses, we performed Principal Components Analysis (PCA, centred, standardized; package *ade4* in R) on all climatic and topographic variables (Dray and Dufour 2007; R Development Core Team 2009). This resulted in two PCA axes (Fig. 2). We call the first

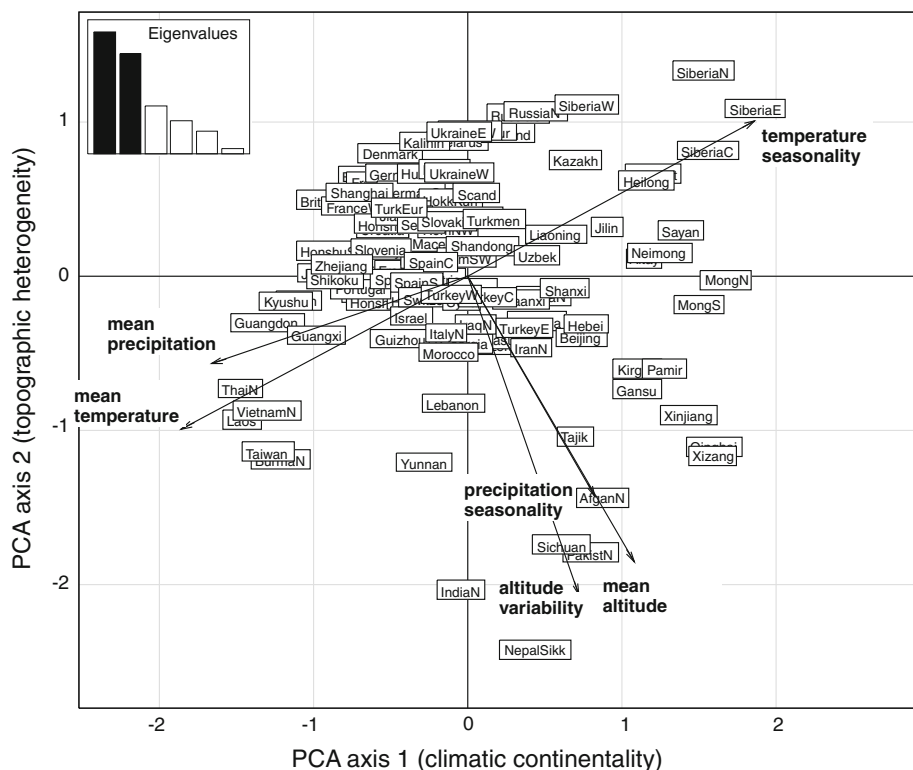


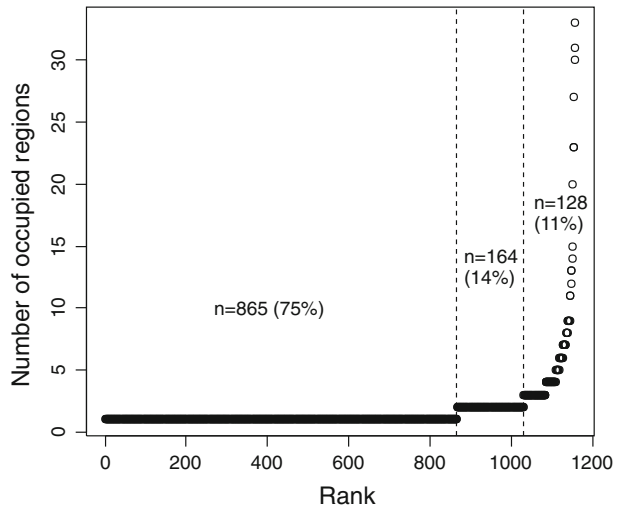
Fig. 2 PCA of environmental variables (*standardized, centred*). We term the first (*horizontal*) axis *continentality of climate*. The second axis (*vertical*) describes the seasonality (or annual variability) of precipitation conditions and also topographic variability—we will use term *topographic heterogeneity* for this axis. The first and second axes explain 37 and 30.3% of variability in environmental data respectively. Note that we use both of the axes (environmental variables) together in some analyses as *environment*

axis *climatic continentality* and the second axis *topographic heterogeneity*. In the GLM analyses (see the following section) we used both of these variables together and we call them simply *environment*.

Autocorrelation issues

Although spatial autocorrelation has usually been regarded as a problem in ecology (Dormann et al. 2007; Lennon 2000), it can also provide useful insights into the origins of the geographic patterns of species' traits. There are several kinds of autocorrelation that can be present in our data. First, sets of taxa in two adjacent regions may have similar morphology simply because these taxa are phylogenetically related. This phylogenetic relatedness can generate spatially structured and autocorrelated continental gradients of morphological traits. We expect that this kind of autocorrelation should disappear after controlling for the phylogenetic relationships between sub-species. Phylogenetic relationships are usually controlled for by including phylogenetic information in statistical models (Desclaves et al. 2003; Harvey and Pagel 2000). Currently, there is no completely resolved phylogeny for the large genus *Carabus*. Therefore, we used simple taxonomy

Fig. 3 Rank-occupancy diagram for the 1,177 sub-species of genus *Carabus* used in our analyses. The diagram shows that the majority of sub-species occupy only one (75%) or two (14%) geographic regions. Therefore, only a very limited fraction of sub-species' geographic ranges can cause spatial autocorrelation in the patterns of morphological traits



(sub-genus identity of each sub-species) as a proxy for phylogeny. The taxonomic classification follows Březina (1999) and is provided in Appendix 1 of supplementary material.

Apart from the phylogenetically driven spatial autocorrelation, there can be spatial autocorrelation caused by the sizes of sub-species' geographic ranges (Dormann et al. 2007). Taxa in one region may have similar morphology to taxa in another region simply because the lists of taxa in both regions are similar (geographic ranges of many taxa overlap both regions). This would generate smooth and autocorrelated continental gradients of morphological traits, regardless of phylogeny. However, this is rarely the case in our data as most of the sub-species are present only in one region (Fig. 3) and hence we expect only a weak effect of this kind of spatial autocorrelation.

Finally, the third source of spatial autocorrelation of morphological traits can be caused by spatially autocorrelated environment (but only in cases where there is a link between traits and environment). In this case we expect the autocorrelation to disappear after the effect of environmental variables is taken into account.

We focused primarily on the spatial autocorrelation in morphological traits measured between pairs of adjacent regions (first distance class). We measured this autocorrelation using Moran's I (Legendre and Legendre 1998), which we calculated for all morphological variables and for residuals of all environmental and spatial models (see below).

Linking morphology with space, taxonomy and environment

A variety of approaches have been proposed to relate trait and environment variables via the taxa occurrence (or abundance) matrix for overview, see (Webb et al. 2010). We first used the fourth-corner method (Dray and Legendre 2008) to assess the correlation between *Carabus* morphological traits and regional environmental characteristics. The fourth-corner analysis directly relates a matrix of environmental variables to a matrix of taxa traits (in our case, the PCoA axes), by way of a matrix of taxa occurrences. The analysis allows for statistical tests of the significance of the links between all combinations of traits and environmental variables. A Pearson correlation coefficient is computed for each pair of environmental variables and traits. The significance of this correlation can be tested by five permutation models that follow five different ecological hypotheses (Dray and Legendre

2008). Here, we used the model 1 (environmental control model; Dray and Legendre 2008), which tests the hypothesis that taxa are found in areas where they encounter optimal living conditions versus the null hypothesis that all parts of the environment are equally suitable.

Although the fourth-corner method is frequently used, it cannot deal with problems related to space and phylogeny, and it does not allow us to partition independent effects of various explanatory variables. Therefore, we also used a complementary approach based on generalized linear models (GLM in R) and spatial and phylogenetic eigenvector filtering (Dormann et al. 2007; Diniz-Filho et al. 2007; Kühn et al. 2009) which allows us to explicitly incorporate spatial, phylogenetic and environmental variables into statistical models. Moreover, it allowed us to partition their independent effects on elytral morphology. Response variables were (1) proportions of homodynamic and heterodynamic sub-species in each region (binomial error structure), (2) mean values of PCoA axes in each region (normal error structure). Explanatory variables were: *Environmental*: climatic continentality and topographic heterogeneity. *Spatial*: latitude, longitude and 10 broad-scale spatial eigenvector filters (SEVs) that we generated using latitude and longitude of centroids of each region. The SEVs were created in SAM 4.0 (Rangel et al. 2010). We then fitted only pure spatial models and eliminated all redundant SEVs based on stepwise deletion and AIC as the selection criterion. The resulting SEVs represent pure spatial autocorrelation that emerges without any underlying biological mechanism. *Taxonomic*: we multiplied the sub-species \times genus matrix by the region \times sub-species matrix and obtained a matrix that characterized taxonomic distinctness of each region. This matrix was then subject to PCoA on a Chi-square distance matrix (function `cmdscale` in R). We then chose the 10 resulting eigenvectors with highest eigenvalues to represent the taxonomic information (see Kühn et al. 2009 for more details).

We fitted GLMs using only spatial, taxonomic and environmental variables, as well as GLMs with all combinations of these. We assessed independent effects of spatial, taxonomic and environmental variables using variation partitioning (Legendre and Legendre 1998).

Results

The results of the PCoA performed on elytral morphological traits are illustrated in Fig. 4. We were able to assign a clear interpretation to the first two PCoA axes. The first PCoA axis explained 39.5% of variability in morphological traits and represents morphological transformation between fully developed grained sculptures and sculptures with undifferentiated motifs at primary intervals (Fig. 4). The second axis explained 19.4% of trait variability and represents a transition from smooth grains to elytrae with prominent compact ribs positioned in primary intervals (Fig. 4).

We mapped geographic patterns of elytral morphology (mean values of the traits) in Fig. 5. After a simple visual inspection, it was apparent that the patterns were non-random. Also, the values of Moran's I at first distance class showed moderate (but significant) autocorrelation in each of the three morphological traits (Table 1).

Based on the fourth-corner analysis, the correlations between morphology and environment were significantly different from the values expected in a randomly organized environment for most morphological and environmental variables (Table 2). The significant morphology-environment correlations were relatively weak, roughly between -0.2 and 0.2 (Table 1). Morphological traits correlated more tightly with climatic continentality

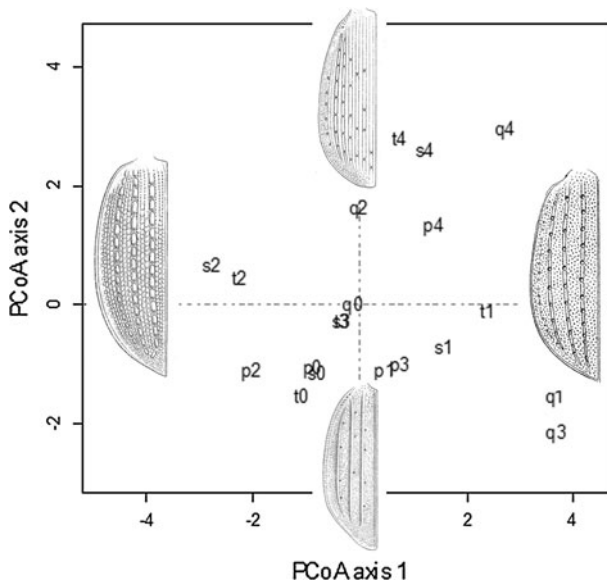


Fig. 4 Results of the PCoA analysis performed on Manhattan distance matrix based on 20 morphological traits and 1,177 sub-species of Palaeartic genus *Carabus*. Variability explained by the first and second PCoA axes (percentage eigenvalues) was 39.5 and 19.4% respectively. Units of the axes are the Manhattan distances between given individual sub-species, which are equal to the number of categorical morphological traits in which the sub-species differ along the given axis. Numbered letters indicate mean PCoA scores of all sub-species which possess the given trait

than with topographic heterogeneity, which indicates that perhaps climate has a stronger influence on elytral morphology than habitat heterogeneity and dispersal barriers. According to the directions of the correlations (Table 2, Figs. 4, 5) we can see that regions with continental climate are more frequently inhabited by homodynamic sub-species and by sub-species with fully developed grain structure and smooth grains. On the other hand, regions with oceanic climate have higher proportions of heterodynamic sub-species and also of sub-species with an undifferentiated motif.

The generalized linear models incorporating space, taxonomy and environment revealed further details. According to the values of AIC, all explanatory variables (space, taxonomy, environment and their combinations) performed better than null models (Table 3). All morphological traits were best predicted by models that incorporated pure spatial components (Table 3). Models operating with pure environmental components performed relatively poorly (in case of PCoA axis 2 and Homodynamic/heterodynamic), or were indistinguishable from other models (PCoA axis 1; Table 3).

The clearest information is provided by the results of variation partitioning (Fig. 6), which comprehensively shows both independent and combined effects of all explanatory variables on the geographic variation in morphological traits. There were hardly any independent effects of pure environment; it always had a joint effect with spatial eigenvectors. Additionally, there was nearly always an additional distinct independent effect of space per se (with the exception of PCoA axis 1), there was always an independent effect

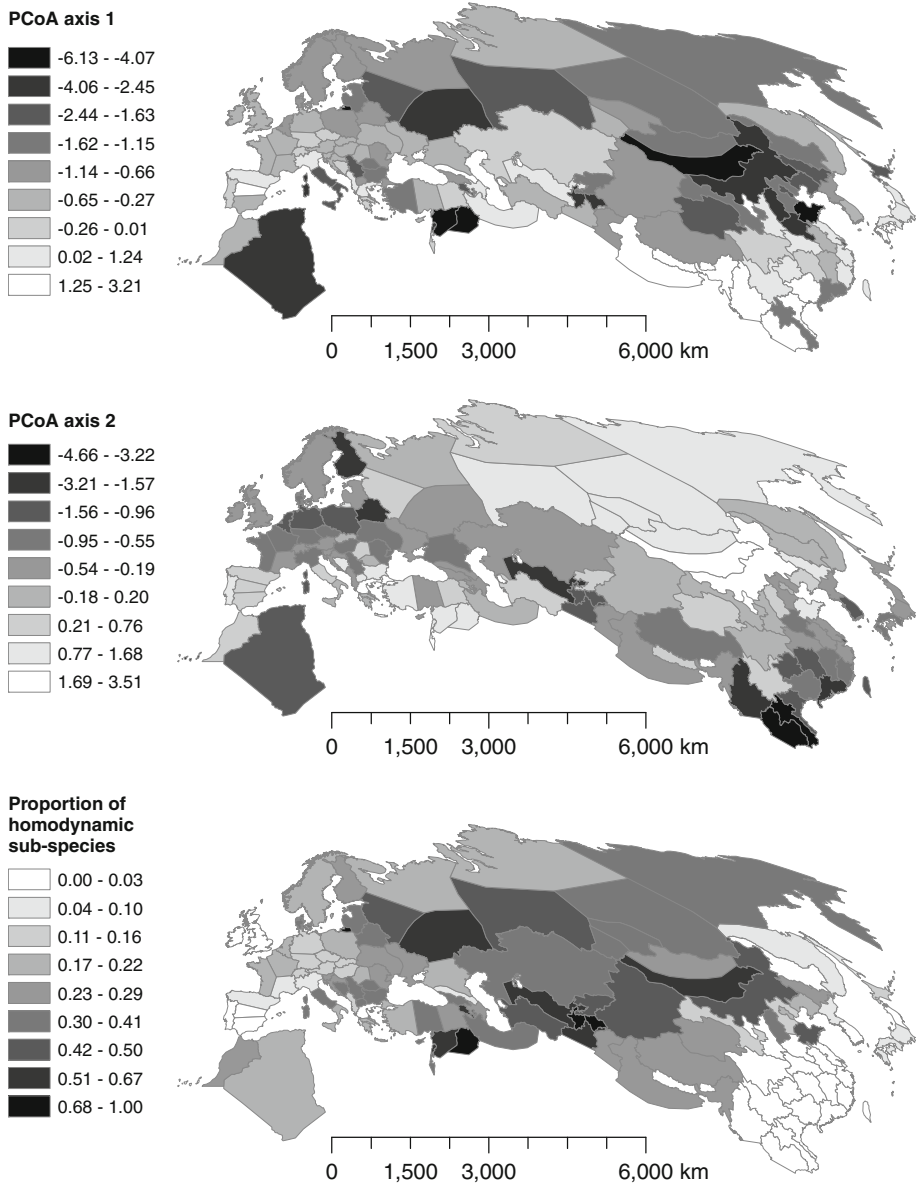


Fig. 5 Maps showing the distribution of mean values of morphological traits (PCoA axes 1–2) and proportions of homodynamic sub-species in Palearctic. All maps use Mollweide Equal Area projection

of taxonomy per se and finally, there was always a distinct joint effect of all explanatory variables together (space, taxonomy and environment) (Fig. 6).

We mentioned that all three morphological traits were significantly spatially auto-correlated (Table 1). This autocorrelation was no longer present in residuals from all of the GLMs (regardless to the kind of explanatory variables), with the exception of PCoA axis 2 where environment and taxonomy were not able to eliminate the autocorrelation from model residuals (Table 1).

Table 1 Values of Moran's I (strength of spatial autocorrelation) calculated for mean values of morphological traits and for residuals from spatial, taxonomic and environmental generalized linear models (GLM) performed on the morphological trait data

	PCoA axis 1	PCoA axis 2	Homodynamic/ heterodynamic
Mean value of a trait	0.124***	0.227***	0.33**
Space residuals	−0.038 NS	0.05 NS	0.016 NS
Taxonomy residuals	0.054 NS	0.094**	0.052 NS
Environment residuals	0.035 NS	0.202***	0.004 NS
Space and environment residuals	0.036 NS	0.053 NS	0.018 NS
Space and taxonomy residuals	−0.011 NS	−0.026 NS	0.001 NS
Environment and taxonomy residuals	0.035 NS	0.062*	0.031 NS
Space, taxonomy and environment residuals	−0.005 NS	−0.026 NS	0.001 NS

Bold letters indicate values with $P < 0.05$

Significance codes are as follows: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant

Table 2 Results of the fourth-corner analysis performed using sub-species occurrences, morphological traits (which are the PCoA axes or homodynamic/heterodynamic status here) and environmental variables

Environmental variable	Morphological trait	Pearson's r	Prob.
PCA axis 1 (climatic continentality)	PCoA axis 1	−0.12	***
	PCoA axis 2	0.13	***
	Homodynamic/heterodynamic	0.17	***
PCA axis 2 (topographic heterogeneity)	PCoA axis 1	−0.097	***
	PCoA axis 2	−0.039	NS
	Homodynamic/heterodynamic	0.055	**

Pearson's r is the correlation between the environmental variable and the trait. The probabilities are obtained using the environmental control permutation Model 1 (999 permutations, Dray and Legendre 2008)

Significance codes are as follows: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS not significant

Discussion

Elytral sculptures and environment

We showed that the effect of environmental conditions on elytral structures is either weak or none at all. Chapman (2010) showed that if climatic gradients and an ecological variable (elytral morphology in our case) are completely unrelated but have similar scale of autocorrelation, then they can show significant but false correlation with each other. This seems to be the case of our results as well—the relationships between morphology and climate revealed by the fourth-corner method appeared significant but they nearly disappeared when the pure spatial component was introduced to the GLM models. Although we cannot totally discard a very small effect of environment on sculpture formation, we suggest that environmental conditions certainly do not represent an important driver of their evolution. Moreover, the greatest proportion of explained variation was shared between space and taxonomy. Therefore, the evolution of sculptures at continental scales is probably independent of environmental selection pressures (H1) and we suggest that the sculptures variation is the result of neutral evolution. From a selectionist point of view,

Table 3 Akaike Information Criterion (AIC) values of generalized linear models (GLM) in which we explained values of given trait in each region by environmental variables (climatic continentality and topographic heterogeneity), spatial variables (spatial eigenvector filters) and variables representing taxonomy (taxonomic eigenvector filters)

	PCoA axis 1	PCoA axis 2	Homodynamic/ heterodynamic
Null model	451.28	380.55	665.4
Space	429.8	330.1	450
Taxonomy	446.3	376.9	458.32
Environment	430.8	363.6	600.683
Space and environment	429.4	333.58	448.5
Space and taxonomy	435.5	335.8	415.53
Environment and taxonomy	428.9	374.14	437.84
Space, taxonomy and environment	431.3	338.8	409.66

In case of PCoA Axis 1 and 2 (Fig. 4) we used means of the given trait in each region as response variable (gaussian error structure). In case of homodynamic/heterodynamic we used proportions of numbers of homodynamic and heterodynamic sub-species in each region as response variable (binomial error structure). Null model is a model in which we only fitted the mean of the response variable. Bold numbers indicate best models according to AIC values (there can be more than one of them in case that the AIC differences between the models were <3)

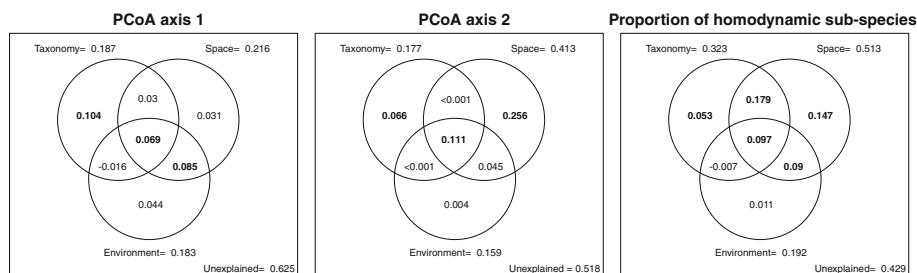


Fig. 6 Venn diagrams showing partitioning of the variation in morphological traits among variables representing taxonomy (taxonomic eigenvector filters), spatial variables (spatial eigenvector filters) and environmental variables (climatic continentality and topographic heterogeneity together). Values may not add up exactly because of rounding errors. Bold numbers represent values >0.05 (an arbitrary criterion selected to highlight variables with a more distinct effect)

neutral evolution has traditionally been regarded as a source of controversy or at least as phenomenon of negligible importance for phenotypic evolution (Simpson 1964; Mayr 2001). By contrast, Nei (2005, 2007) recently argued that neutral or nearly neutral mutations have played a significant role in morphological evolution. In beetle evolution, the transformation of the first pair of wings into elytrae was accompanied by the reduction of wing venation, which is lacking in all modern beetles except of the members of the basal group Archostemata (Grimaldi and Engel 2005). As the function of the forewings changed from locomotory to protective, it very likely led to a relaxation of the evolutionary constraints set upon the morphogenetic processes responsible for the development of wing venation. Any particular phenotypic character arises by complex processes of temporal and spatial expression of many interacting genes in development. The genes formerly partaking in the development of venation could have found a new role in sculpture patterning. In the case that there is no selection on a specific phenotype of sculpture, the new mutations and

random fluctuations in allele frequencies would lead to the fixation of phenotypic traits that are not conflicting with the protective function of the elytra.

Methodological remarks

We used two approaches to relate traits to environment. The first was the fourth-corner method (Dray and Legendre 2008), which has been used quite extensively in recent years (Aubin et al. 2009; Besemer et al. 2009; Gallardo et al. 2009), together with a similar method called RLQ analysis (Ribera et al. 2001; Thuiller et al. 2006; Cleary et al. 2007). A huge disadvantage of these methods is that they do not take into account spatial and phylogenetic structures of the data. We show that ignoring such structures would lead to misinterpretations of the results because taxonomy (or phylogeny) and space do matter. We detected the magnitude of the trait-environment correlations (r) by using the fourth-corner analysis ($-0.2 < r < 0.2$); it is low but comparable to trait-environment correlations found in other studies (Hooper et al. 2004; Lacourse 2009; Roy and de Blois 2006; Urban 2004). All these studies claim that the trait-environment correlations are low due to the noisy character of ecological data, and they usually treat these low values as important and significant results. By incorporating the spatial and simple taxonomic structures into our models (Kühn et al. 2009), we increase the amount of explained trait variability (to approx. 50%; Fig. 6) and reduce the amount of variability independently attributable to the environment. Therefore, (1) we warn that the results of any fourth-corner and RLQ analysis that ignores space and phylogeny should be treated with caution, (2) we predict that the incorporation of space and phylogeny should lead to more informative models, and (3) we call for a modification of fourth-corner and RLQ methods that would allow us to link traits, phylogeny, space and environment without the averaging of the values of species traits causing a loss of information.

Conservation remarks

All major lineages of genus *Carabus* originated in a relatively short period during the late Eocene; dated approximately 40 million years ago (Osawa et al. 1999). The members of the large genus *Carabus* have metathoracic wings typically reduced to rudimentary stumps. Very rare exceptions occur only within some populations of a few species such as *C. granulatus*, and *C. clathratus* wherein individuals capable of flight have been found. This indicates a low dispersal ability of the *Carabus* species compared to members of highly mobile flying insects (Deuve 2004).

A striking by-product of our study is the finding that most of the sub-species occupy only a single geographic unit. This suggests that, in history, sub-species evolve quite easily into new sub-species as they colonize new areas. As a result, each of the geographic units shows a unique composition of *Carabus* sub-species. Moreover, when we look at the global distribution of genus *Carabus*, it is characterized by a high level of species (and sub-species) diversity mainly in Eurasia, with contrasting absence of *Carabus* in continents in the southern hemisphere (Sota and Ishikawa 2004). We claim that this taxon should be given a priority in biodiversity conservation in Eurasia because of its omnipresent and high degree of endemism, its globally restricted occurrence, its low dispersal abilities and subsequent vulnerability to abrupt environmental disturbances, and finally because of the fact that *Carabus* members are among the most charismatic insects (comparable to butterflies).

Conclusion

We conclude that elytral sculptures probably do not represent an adaption to environmental conditions. The sculptural ornaments are rather the result of random sampling drift and mutational processes of neutral evolution resulting in a genus-specific pattern of variation which is probably canalized by internal developmental constraints. The exact evolutionary causes and processes of the diversification of sculptures and their specific relation to site environmental conditions still remain to be discovered.

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