The Use of Geometric Morphometrics in a Study of Shape Diversity of Ground Beetles (Coleoptera: Carabidae)

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Abstract

Ground beetles are one of the largest groups in Coleoptera with variety of life styles. The aim of this study is to examine the diversity of head and pronotum shape of ground beetles, and the relationship with body size and phylogenetic relationships using geometric morphometrics. In addition, shape correlation between head and pronotum was examined by partial least square analysis. 300 species of family Carabidae were used in this study (280 for head, 290 for pronotum), covering 15 subspecies. Procrustes superimposition and principal component analysis was applied to head and pronotum independently to extract comparable shape information. Independent contrasts were used for comparison of shape and phylogeny to take into account the phylogenetic relationships. The main shape change associated with PC1 and PC2 (PC1=37.1%, 43.1% and PC2=21.6%, 33.9% for head and pronotum respectively) was in the slender-stout direction for both head and pronotum, and there was a significant correlation between head and pronotum shape change ($P<0.0001$). Confidence ellipses covering each subfamily showed large overlaps in the shape space constructed by the first two PCs, for both head and pronotum. However, Cicindelinae and Scaritinae were isolated from others due to their discriminative body form and other characteristic shape changes were observed in many clades. Shape variation due to evolutionary allometry, which is the relationship of shape and body size, was significant ($P<0.0001$ for head, $P=0.0137$ for pronotum) but small (2.8% for head, 1.3% for pronotum). Significant shape correlation was found associated with head and pronotum either getting slender or getting stout. Furthermore, significant phylogenetic signal was found from mapping the phylogeny onto the shape space and performing permutation test. These results indicate that phylogenetic relationships are considerably influencing the shape variation of ground beetles. Moreover, contribution of diet, flying ability and other behavioral factors should be considered to explain the underlying evolutionary processes of this shape variation.

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Introduction

Ground beetles

Ground beetles, family Carabidae, are one of the largest groups in Coleoptera, Adephaga, containing more than 40.000 species described from around the world. They are highly diverse and differ in behavior, ecology, function, diet and so on (Lövei and Sunderland 1996). Many of them are adapted to their terrestrial lifestyle and lose their ability to fly by decreased flight muscle power, jointed elytron, and devolution of wings (Roff 1990). Because of this aspect, ground beetles are good sample for understanding geographical isolation and evolution (Alibert et al 2001, Darlington 1943). However, some of them are able to fly around rapidly, for instance Cicindelids (Zerm and Adis 2002). Often their mandibles are well developed and many species are known as carnivores, although some of them are specialized in eating plants, especially genus Zabrus and Amara (Acorn and Ball 1991, Arndt and Kirmse 2002). Most of them are thought to be omnivores eating both plant and animal materials but the understandings of details of their diet are still poor.

Morphological adaptation and variation

Because of the rich diversity of ground beetle family Carabidae, it might be expected that their body form is affected by many kinds of factors. For instance, head shape and mandible structure show relationship with diet. Locomotion might affect the entire body form and lack of flight muscles can associate with pronotum shape. Phylogenetic relationships might also influence the body shape of beetles. However, all other ecological and behavioral factor has relationship with phylogeny so it is difficult to take them into account separately.

Morphological adaptation to different types of ecological factor is found in many studies of ground beetles (Barton et al 2011). The body form of snail eating species genus Carabus adapt to their diet style (Konuma and Chiba 2007). Diet and habitat association is found in diverse beetle assemblage including Carabidae (Barton 2011, Forsythe 1987, Forsythe 1991). Some studies explain geographical variation considering shape differences (Alibert et al 2001). Finding out the relationship of shape with variety of different factors and investigating the evolutionary process of it is an interesting context.

About Geometric Morphometrics

Geometric morphometrics is a great tool to study the diversification of shape of organisms and its relationship with ecology and phylogeny (Rohlf and Marcus 1993, Lawing and Polly 2010, Zelditch et al 2004). The general geometric morphometrics uses landmark coordinates to extract the shape information and these data can be used in variety of subsequent analysis mainly using multidimensional analysis (Lawing and Polly 2010, Zelditch et al 2004). One main purpose of geometric morphometrics is to quantify shape information and analyze it in subsequent mathematical procedure.

There are many ways to point landmarks on an image; using homologous points which correspond to each other in every specimen, using equally spaced points to extract the boundary, using outline contour, and so on (Bookstein 1997). Once the landmarks are taken, Procrustes superimposition is applied. Procrustes superimposition takes away three redundant information, scale, position, and rotation (Kendall

1977, Rohlf and Slice 1990, Goodall 1991). Scale is often eliminated by setting the centroid size, square root of sum of squared distances between the centroid and each landmark, the same in all specimens. Position is excluded by putting the centroid of every specimen at the same point. Orientation is taken away by rotating specimens so that square root of sum of squared distances between corresponding landmarks would be the minimum. By eliminating this information, only the shape is extracted and the landmark coordinates can be used as quantified shape information in the subsequent mathematical analysis.

The next step is to generate covariance matrix. Because shape data are multidimensional, many shape variables has to be analyzed simultaneously. Thus, the covariance of every pairs of variables is summarized in the covariance matrix.

To explore the patterns and tendency of the shape variation principal component analysis (PCA) is often used. PCA generates new sets of variables which account for the maximum amount of variation and are uncorrelated with each other. Every specimen is represented as a single point in a multidimensional shape space. In this shape space, the first PC axis is taken in the direction which accounts for the largest amount of variation. The second PC axis is taken perpendicular to the first PC axis and in the direction which has the most amount of variation. New sets of variables are taken successively by this procedure and create new multidimensional space. The amount of variance of the sum of all new variables is equal to the total variance. Therefore, the contribution of each PC can be expressed as eigenvalues. The coordinate made from PC1 and PC2 is the best-fitting plane and the main variation can be observed in this coordinate.

In this study, phylogenetic tree obtained from molecular data is mapped onto the scatter plots on the coordinates made by PC axes to examine the shape difference between each clade and the variation within each clade. Phylogeny was also used to compute phylogenetic signal to tell whether there is a significant association between phylogeny and shape (Klingenberg and Gidaszewski 2010). Furthermore, phylogeny can be used to fix for the emphasized phylogenetic relationship by using independent contrasts (Felsenstein 1985).

Allometry, correlation of size with shape, can be detected by multivariate regression (Drake and Klingenberg 2008). The amount of variation due to size can be computed. Effect of size can be fixed by using the residuals of size-shape multivariate regression. There are three types of allometry; static allometry, ontogenetic allometry, and evolutionary allometry (Gould 1966, Klingenberg 1996). Static allometry is the shape difference due to size in same species same ontogenetic stage. Ontogenetic allometry is the shape difference due to growth of same species. Evolutionary allometry is the shape and size variation among different species that indicates evolutionary processes that formed its' shape (Drake and Klingenberg 2008, Klingenberg 1992). In this study we focus on evolutionary allometry because we have widespread sample covering the family and the phylogenetic tree.

Shape correlation between different structures of an organism can be explained by using partial least squares (PLS) analysis (Rohlf and Corti 2000). Moreover, correlation between ecological, physiological, behavioral, phylogenetical factors and shape can be observed by many kinds of multidimensional analysis and can emphasize biological perspective of the study (Angielczyk et al 2010, Claude et al 2004).

Here I picked up analysis that is used in this study. However, there are large numbers of different type of analysis that can be applied to investigate biological shape (Zelditch et al 2004). Moreover, by interpreting the results of these analyses from a biological point of view, we can improve our understandings and a fascinating question may arise.

Material and Methods

Data collection

Total of 300 species of ground beetle family Carabidae (Coleoptera) are used in this study, all collected from Manchester Museum (The University of Manchester) Entomology store.

A single image was taken by a camera attached to Leica microscope for each specimen, head and pronotum separately. 8 landmarks on the pronotum and 15 landmarks on the head were digitized once for each image using ImageJ (Figure1). The landmark coordinates of 280 species were used for the head shape analyses and 290 species for the pronotum. The sample size difference is due to difficulty of locating landmarks in some species. The sample in this study covers 15 subfamilies described by Beutel et al (2008). Further analyses of landmark configurations were done by using MophoJ v1.03a (Klingenberg 2011).

Procrustes ANOVA

First, measurement error and the comparison of

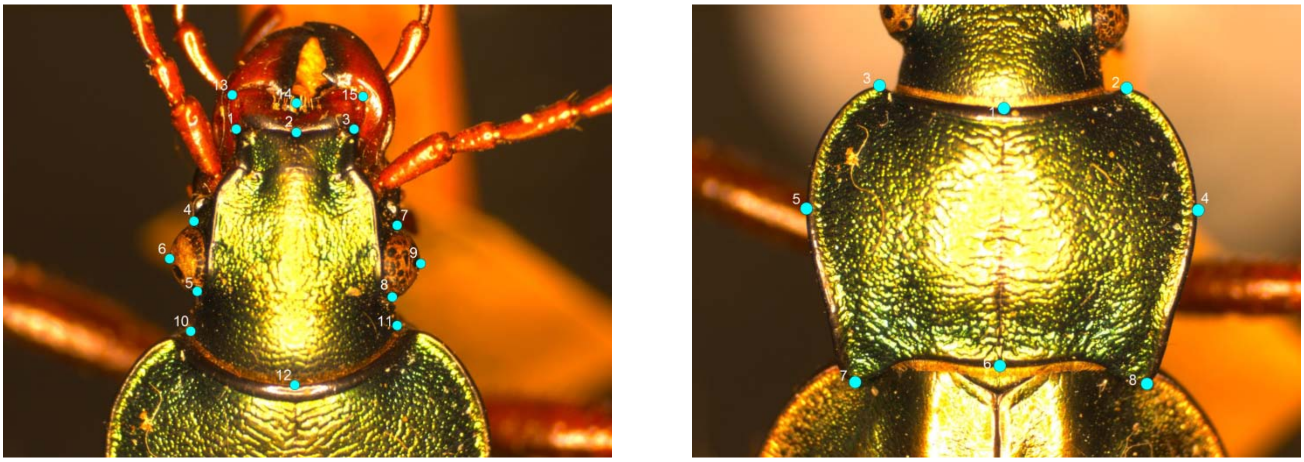

Figure1. Landmark position of head and pronotum

15 landmarks for the head and 8 landmarks for the pronotum placed on *Carabus auratus*. Definition of each landmark is 1; the most curvature of the anterior left side of the clypeus, 2; center point of the anterior part of the clypeus, 3; the most curvature of the anterior right side of the clypeus, 4; anterior intersection of the left eye and the frons, 5; posterior intersection of the left eye and the frons, 6; the most curvature of the left eye, 7; anterior intersection of the left eye and the frons, 8; posterior intersection of the left eye and the frons, 9; the most curvature of the right eye, 10; intersection of the left posterior part of the head and the pronotum, 11; intersection of the right posterior part of the head and the pronotum, 12; center point of the posterior part of the head, 13; the most curvature of the anterior left side of the labrum, 14; center of the anterior part of the labrum, 15; the most curvature of the anterior right side of the labrum

Table1. Procrustes ANOVA results

Mean squares (MS) are the amount of variation from the one higher level in the hierarchy. The F value represents the comparison of each MS to the one lower level of MS which could be the source of error.

Effect	SS	MS	df	F	P
Species	0.1428	0.002746	52	5.09	<.0001
Individual	0.1403	0.000539	260	17.71	<.0001
Side	0.0025	0.000195	13	6.4	<.0001
Ind * Side	0.0095	0.000030	312	4.52	<.0001
Image	0.0044	0.000007	650	1.54	<.0001
Digitizing	0.0057	0.000004	1300		<.0001

Effect	SS	MS	df	F	P
Species	0.3230	0.013460	24	19.08	<.0001
Individual	0.0847	0.000706	120	10.72	<.0001
Side	0.0045	0.000753	6	11.45	<.0001
Ind * Side	0.0095	0.000066	144	5.46	<.0001
Image	0.0036	0.000012	300	3.09	<.0001
Digitizing	0.0023	0.000004	600		<.0001

interspecific and intraspecific variation were estimated in a pilot study using Procrustes ANOVA. Procrustes ANOVA estimates the variation at each level (individual, image, digitizing) from the deviation from the mean shape which corresponds to the one higher level in the hierarchy (Klingenberg and McIntyre 1998). Procrustes ANOVA was performed for total of 25 specimens, 5 individuals for 5 species of genus Carabus. Each specimen was imaged twice and each image was digitized twice producing 100 raw coordinate data for both head and pronotum. Measurement error was estimated from this Procrustes ANOVA by considering individual as the main source of variation, continuously nested by individual*side variation, variation in different image of a same specimen served as the first source of error, and residuals representing variation in digitized replicates as the second source of error. The individual*side variation stands for fluctuating asymmetry which is defined as the variation of asymmetric pattern from the mean asymmetry (Klingenberg and McIntyre 1998). Comparisons of intraspecific and interspecific variation were derived by the same Procrustes ANOVA by setting species as a source of variation above individual (Table1).

Procrustes superimposition

A generalized Procrustes superimposition was performed on the configuration of head and pronotum separately. In order to analyze the shape change exclusively, we need to exclude redundant information that is included in the size, position, and orientation of the configurations (Kendall 1977, Rohlf and Slice

1990, Goodall 1991). To extract the shape information, generalized Procrustes superimposition will be applied. First, the size of all configurations will be scaled to unit centroid size defined as the sum of the squared distances between each landmark and the centroid (center of gravity). Second, the configurations are moved so that the centroid overlaps, and this will take away the information of the position. Finally, the configurations are rotated so that the sum of the squared distances among each landmark minimizes. This procedure will eliminate the orientation information, and only the shape change remains for the subsequent analysis. A covariance matrix of the coordinates of landmarks was generated and principal component analysis (PCA) was performed on this covariance matrix. The first two PCs were chosen to construct a coordinate and PC scores were plotted against the PC axis to visualize the shape space and the position of each species in the shape space. Also, amount of contribution of each PCs were calculated.

Independent contrasts

A phylogenetic tree including all the species in this study (except for a few genera) were made based on the tree of Beutel et al (2008). Construction of the tree was done by Mesquite v2.74. The tree was made at genus level by species from the same genus clustered together making a polytomy. The length of the branches was not considered. The data of the landmark configurations were mapped onto this phylogeny to generate an independent contrast of shape variation in the sample. The residuals of the independent contrast adjusted for phylogeny takes into account the relationship of the species, whereas the original data treat each species completely independently with no relations to each other (Felsenstein 1985). Independent contrasts can be obtained by comparing the systematic node of each branch originating from a common internal node, avoiding the redundant information caused by the duplicate counting of branches (Felsenstein 1985). To test the existence of phylogenetic signal, permutation test was performed by permuting the terminal taxa 1000 times and mapping onto the phylogeny each time. Phylogenetic signal is defined as the relationship between species, and when phylogenetic signal exist related species in the phylogenetic tree shows similar phenotypic traits (shape change) than between distant species (Klingenberg and Gidaszewski 2010). None of the 1000 permuted mapping had equal or less amount of changes compared to the original mapping ($P<0.0001$) indicating an apparent existence of phylogenetic signal. Therefore, the use of the residuals of the independent on this is expected to show slightly different result from the analyses of the original dataset if phylogeny plays a large role in phenotypic changes.

Evolutionary allometry

To examine evolutionary allometry, regression of shape variable against log transformed centroid size was done for head and pronotum independent contrasts respectively. Shape variable that has the most correspondence with size is extracted for this analysis. Centroid size is defined as the square root of the sum of the squared distances between each landmark and the centroid. Evolutionary allometry concerns the covariation of shape change and size among phylogenetic branches that derive from a common ancestor (Klingenberg 1996). From evolutionary allometry the association of shape and size in the evolutionary process can be examined and interpretation of how they are formed can be provided.

Correlation of shape change

In addition, to examine the correlation of the shape change between head and pronotum, partial least-square was performed comparing the symmetric shape changes of head and pronotum independent contrasts. PLS analysis creates a new variable axis that accounts for the largest amount of correlation between two datasets by singular value decomposition (Rohlf and Corti 2000). The scores were plotted onto the configuration defined by the first PLS axis of the head against the pronotum to visualize the correlation between them.

Results

Measurement error

The measurement error of the head and pronotum was estimated from the Procrustes ANOVA for each analysis (Table1). For the head, error caused by imaging and the digitizing error was 1.2% and 0.8% of the individual variation respectively. For the pronotum, the error caused by imaging and the digitizing error was 1.7% and 0.6% of the individual variation respectively. For both structure, the amount of measurement error is negligibly small compared to the source of variation dealt in the analysis. The intraspecific variation was 19.6% and 5.2% of the interspecific variation for head and pronotum respectively. Considering that the samples used in this Procrustes ANOVA are relatively related species of genus Carabus and therefore the amount of interspecific variation in this genus is considerably smaller than the family level interspecific variation. As a result, intraspecific variation is likely to be considerably small compared to the amount of variation within the entire family Carabidae. Therefore, a single individual for each species would be sufficient in this analysis.

Principal component analysis and the shape space

To see the positions of each species on the shape space, PC scores of each species were scattered on the shape space defined by the first two PC axes of the PCA performed on the original dataset (Figure3, 4). For the head shape, PC1 account for 37.1% of the total variance showing narrow elongated to stout short shape change. PC2 account for 21.6 % of the total variance with the eyes moving slightly forward and the landmark on the posterior end moving posteriorly. PC3 account for 10.8% of the total variance mainly with the eye size relative to the head size (Figure2). First and second PCs together take more than half of the total variance. For the pronotum shape, PC1 account for 43.9% of the total variance with the posterior part getting broader and short. PC2 account for 33.6% of the total variance with the anterior part getting broader and shorter (Figure2). First and second PCs take more than 70% of the total variance for the pronotum. From these results, the coordinate made by the first two PCs represent a satisfying fitting plane of the multidimensional shape space. The scatter plot on the shape space shows that there is a continuous variation along the PC axes where there is a large overlap in subfamily level with some exceptions. Cicindelinae had a relatively high value on PC1 axis and considerably small value of PC2 axis of the head showing their narrow head with large eyes which clusters them clearly separate from the main group. However, Cicindelinae were not clearly isolated in the shape space of the pronotum indicating extreme uniqueness only in their head shape. Scaritinae had a considerably small value of PC1 of the head indicating stout head and small eyes and labrum, which make them separate from the main group. Scaritinae were also clearly isolated in the pronotum shape space with extreme broadening and depression of the anterior part of the pronotum.

Principal components of independent contrasts

As expected, the PCA performed on the independent contrast residuals showed similar but slightly different patterns of shape change. Most part of the difference between the two PCAs was related to the degree of the shape change not the direction.

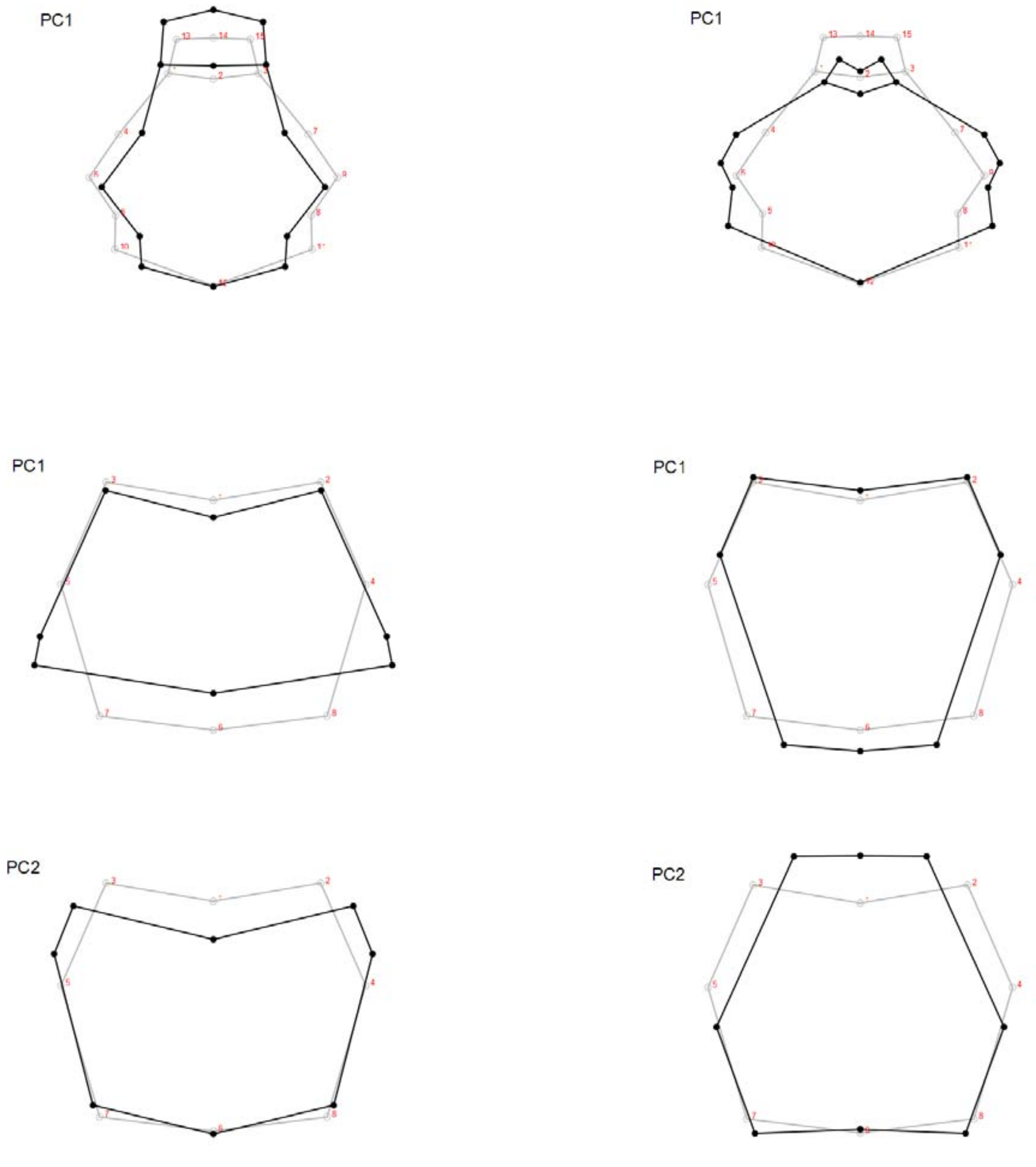

Figure2. The shape changes of head and pronotum

The dark lines show the extreme shape change in positive and negative direction of the PC shown above. The gray lines are the mean shape of head and pronotum respectively. The scale for each figure is; head PC1 (+0.2, -0.25), pronotum PC1 (+0.35, -0.2), pronotum PC2 (+0.2, -0.25).

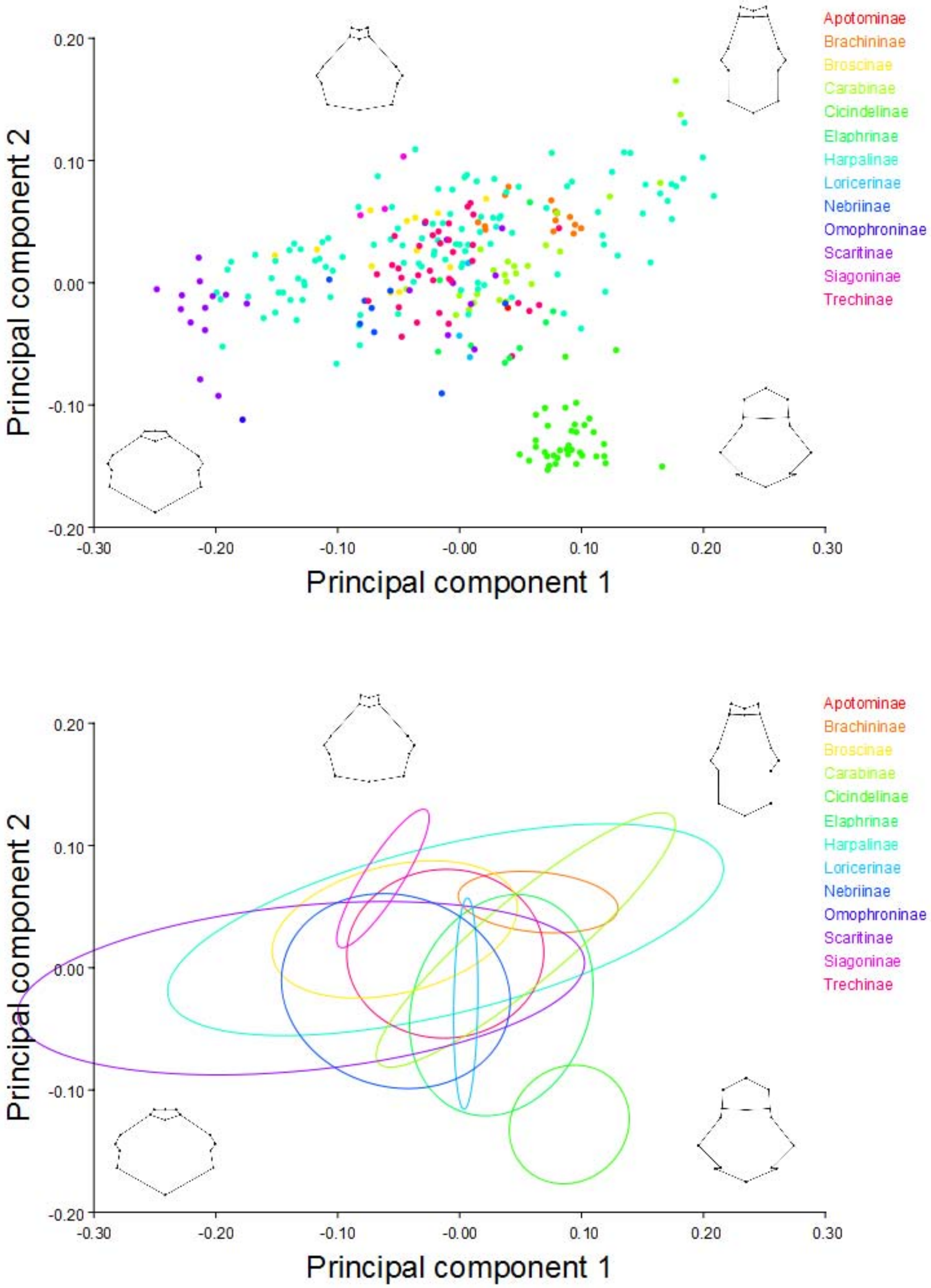

Figure3. Head shape space

A. PC scores of each species are plotted against the PC coordinates. Species in the same subfamily are in the same color. The four shapes represent the extreme shaped species.

B. 90% confidence ellipses of PC scores. Color of ellipses corresponds to the subfamilies written alongside. The four shapes are the same as figure A.

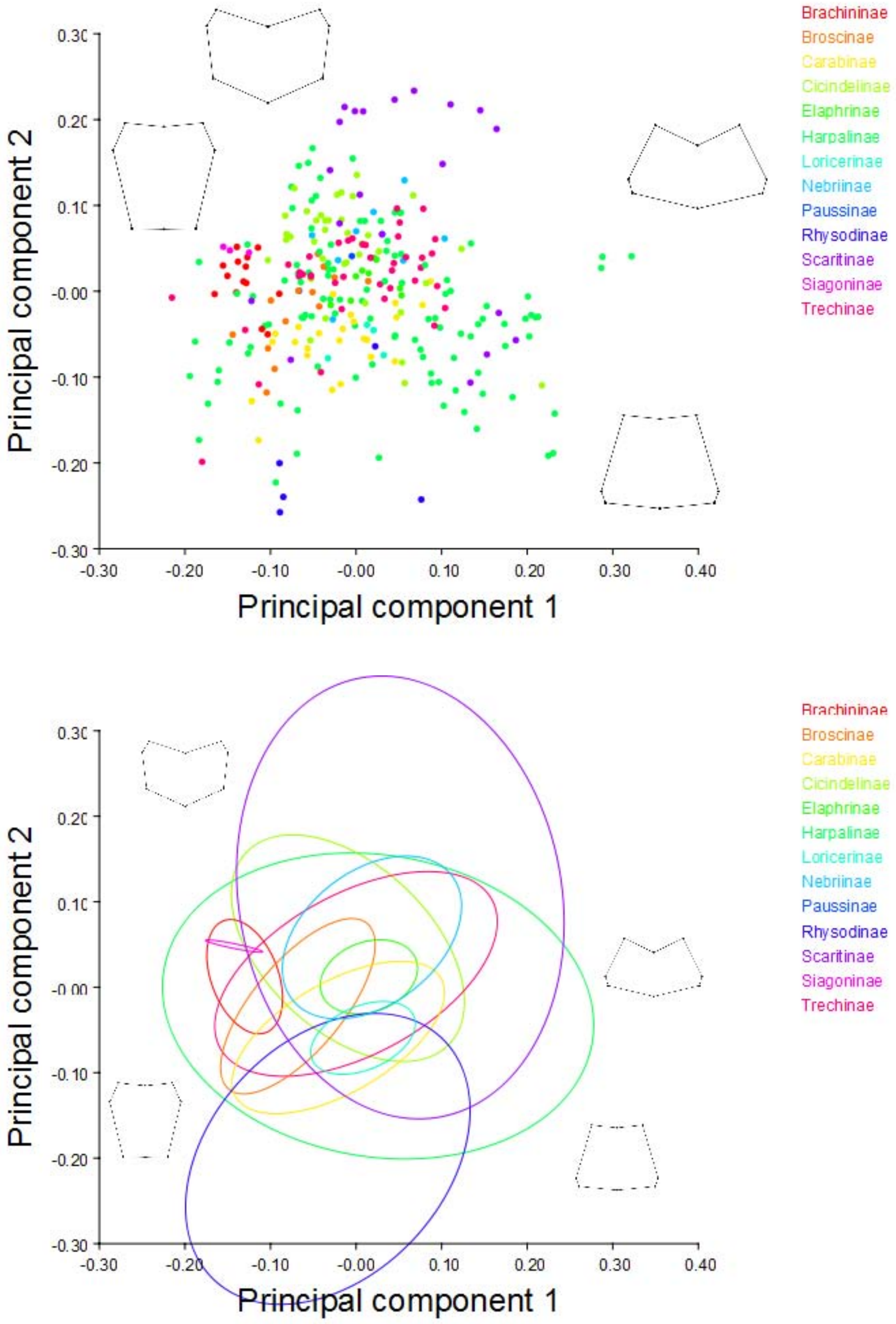

Figure4. Pronotum shape change

A. PC scores of each species are plotted against the PC coordinates. Species in the same subfamily are in the same color. The four shapes represent the extreme shaped species.

B. 90% confidence ellipses of PC scores. Color of ellipses corresponds to the subfamilies written alongside. The four shapes are the same as figure A.

Evolutionary allometry

Evolutionary allometry focuses on character covariation with size among several phylogenetic lineages that shares a common ancestor (Klingenberg 1996). In the analysis of the head, there was a significant evolutionary allometry ($P<0.0001$) which showed a small eye relative to the head and elongated labrum in large species (Figure5). However, the allometry only described 2.8% of the total variance and the associated shape change differed from the main PCs of PCA analysis. The pronotum also showed a relatively significant evolutionary allometry (P=0.0137), however, the amount of variation is small (1.3% of the total variation) and the associated shape change is subtle. Therefore, the effect of evolutionary allometry is significant but negligibly small.

Covariation of head and pronotum

Partial least squares (PLS) analysis was performed on the independent contrasts of head against the pronotum to examine the covariation between these structures (Figure6). Large proportion of the covariance was contained in the first pair of PLS axes (93.4%) so we took into account only this pair of PLS axes in subsequent discussions. The RV correlation coefficient was relatively low but the correlation was significant (RV=0.14, $P<0.0001$). The shape change associated with the first pair of PLS axes is slenderized and elongated head corresponding to slenderized and elongated pronotum or the other way around with stout head and stout pronotum.

Discussion

The shape diversity of head and pronotum in family Carabidae

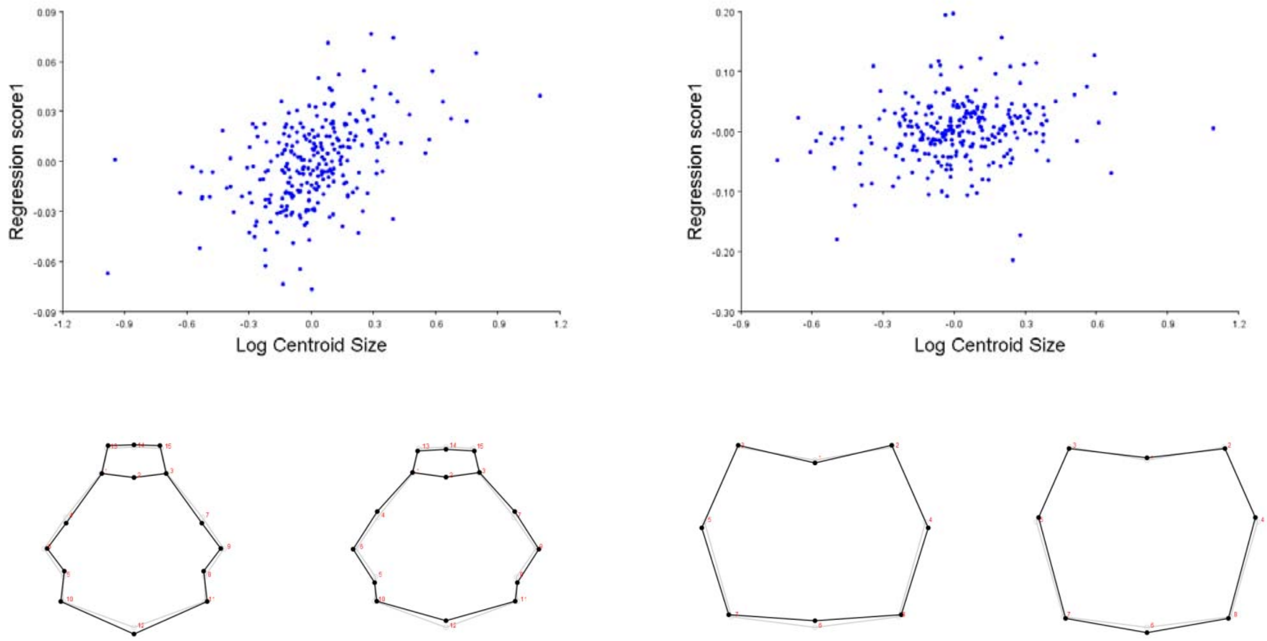

Figure5. Evolutionary allometry

PC scores of evolutionary allometry of head (left) and pronotum (right). Regression score 1 is the shape change that corresponds most with log transformed centroid size. The associated shape changes are shown below. Gray lines are the mean shape and dark lines show the shape change (From the left, head +1.0, head -1.0, pronotum +1.0, pronotum -1.0).

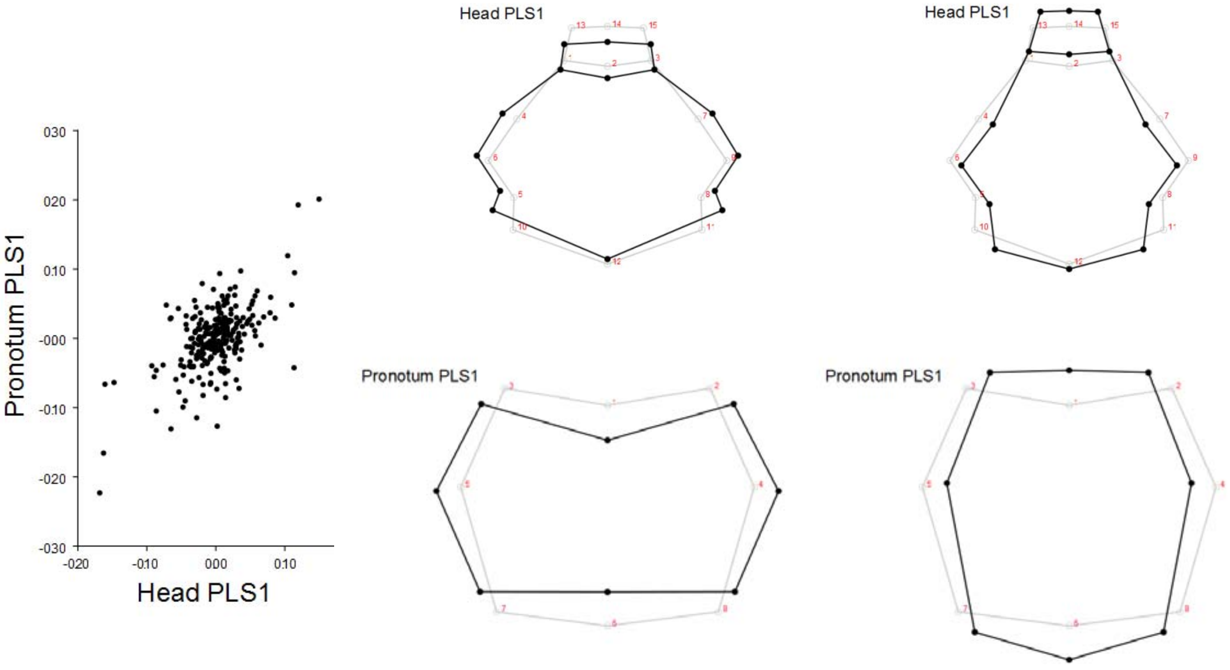

Figure6. Shape correlation of head and pronotum

A. Independent contrasts are plotted against the first PLS axis of head and pronotum.

B. Dark lines show the positive and negative extreme of independent contrasts in the direction of PLS axis (head +0.15, -0.15; pronotum +0.2, -0.2). Gray lines are the mean shape of independent contrasts.

The shape space of both head and pronotum shows that there are still characteristic tendency in each clade although there is a large amount of overlap in the middle area (Figure3, 4). Carabinae and Broscinae showed a strong variation in the direction of PC1 for the head. For the pronotum, only Carabinae showed a different direction of shape variation; wide posterior part with slender anterior part, slender posterior part with wide anterior part. As a result of this unique shape variation of Carabinae, this subfamily and subfamily Cicindelinae is separated from the other species in the Canonical Variate Analysis (CVA).

For both head and pronotum, subfamily Harpalinae is wide spread covering almost the whole area made by others (Figure3, 4). Harpalinae is the most species rich clade in family Carabidae. Within Harpalinae, each genus constructed an independent cluster making a considerably clear separation. This means that subfamily Harpalinae alone is highly diverse in morphology, almost the same level of variation of the whole family. Most of only a few numbers of herbivores are included in this clade indicating their diversity in shape.

Focusing on the head shape space, Cicindelinae makes a cluster clearly isolated from other clades (Figure3, 4). This clade has a bizarre shape with extremely large eyes and wide, pointed labrum (Forsythe 1987). Cicindelinae, which is known as tiger beetles, are carnivorous with large mandibles and has relatively long legs, running around rapidly to catch their prey (Forsythe 1987). In addition, species of this clade can fly properly by their developed wings. However, shape of the pronotum, which might be affected by flying ability, did not show clear difference from other clades.

Although phylogenetic studies show different results depending on authors, tiger beetles were thought to be an out group of Carabidae from morphological phylogenetics.

Another clade that shows distinctive shape characters is subfamily Scaritinae (Figure3, 4). There seems to be two types of Scaritinae species in terms of head and pronotum morphology. One type has general shape characters but the other type including genus Scarites has extremely stout head and wide pronotum with enlarged posterior part. These species are specialized in digging holes in sandy soil. Stout and flattened head makes advantage by producing enough power to dig into soil (Forsythe 1991).

Phylogenetic signal and independent contrasts

Mapping the shape change onto a phylogeny and extracting independent contrasts has a subtle but significant influence to the subsequent analysis in a dataset with 300 species included, even if it is an unresolved phylogeny with related species under genus level clustered in a polytomy.

Phylogenetic signal exists although there is high divergence and overlaps in the shape space. However, many other factors should influence the form of ground beetles (Barton et al 2011). Especially when more than two different factors appear in the same clade, shape variation that phylogeny does not account may appear (Barton et al 2011, Claude et al 2003).

Evolutionary allometry

The variation due to size was subtle but species in same clade tend to be similar in size. Especially large species were Scaritines and especially small species were Trechinae. The small body size of Trechinae species maybe due to their underground or troglobite lifestyle. Some of the Trechinae lost their compound eyes by adapting to their underground life. Large species showed small eyes relative to the size of their head in contradiction to small species which their eyes cover large part of their head, indicating that they might share a common eye size regardless of their body size.

Shape correlation between head and pronotum

There was a significant shape correlation between head and pronotum associated with stout-slender shape changes (Figure6). In the studies of snail eating genus Carabus, there was a significant shape correlation of the whole body in the stout-slender direction. Species inserting their heads into snail shells had slender body form and on the other hand species crushing snail shells had stout body form to gain enough power. The crossed individual of these two different types of species showed intermediate shaped beetles (Konuma and Chiba 2007). Similar shape correlation was seen considering the whole family. From this, it can be presumed that bodies of ground beetles change simultaneously adapting to their highly diverse lifestyle as a whole. For instance, diet can be considered as one of the factors controlling their shape. Depending on what they eat, structure of the mandible and attached muscle influences the shape of head and pronotum. Another source of body shape change is habitat and locomotion. Ground dwelling species showed relatively stout shape. On the other hand, species walking around freely had wide variation of shape. Furthermore, flight ability might be affecting the pronotum shape (Zerm and Adis 2002). However, it is difficult to classify flight ability because some species show both flyable and flightless phenotype. By

Comparing and applying different types of factors as classifications into the shape analysis, we can reveal the evolutionary processes of how the shapes are formed.

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