Evolutionary patterns of hind wing morphology in dung beetles (Coleoptera: Scarabaeinae)

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1. Introduction

Functional requirements linked with active flight have produced a wide variety of morphological solutions. Apparently this played a prominent role in the in the evolution of birds, pterosaurs, bats and last but not least pterygote insects. Especially in insects the acquisition of wings and active flight has triggered an unparalleled diversification, with a very rapid and successful radiation in the early Carboniferous (Grimaldi and Engel, 2005; Nachtigall, 2003; Wootton, 1981, 1992). Studying specific adaptations is important to understanding how natural selection has shaped trait values, and how constraints to adaptation may arise. The shape of wings can be a good predictor of adaptation to different selection pressures (Johansson et al., 2009). In birds, several studies have demonstrated that wing shape is affected by, for example, migration distance (Kaboli et al., 2007; Marchetti et al., 1995; Voelker, 2001), sexual selection (Hedenström and Möller, 1992; Stiles et al., 2009), and foraging strategies (Bullen and McKenzie, 2007; Kaboli et al., 2007). In insects, the value of wing features in taxonomic and phylogenetic studies has long been recognized. The use of wing features in systematics has usually been limited to the presence or absence of specific characters (e.g., Crowson, 1955, 1961, 1973; Dolin, 1975; Forbes, 1922; Scholtz, 1990), folding patterns (Fedorenko, 2009), morphology and function of the wing articulation and wing muscles (Snodgrass, 1935). In addition, the origin, evolution and succession of hind wing characters have been studied in detail (Kukalová-Peck and Lawrence, 1993, 2004; Fedorenko, 2009). The first investigation of the evolution and adaptations of wing shape using geometric morphometrics, a tool which does not only offer precise and accurate descriptions, but also serves the equally important purposes of visualization, interpretation and communication of results (Zelditch et al., 2004), has been carried out recently. Bai et al. (2011) analyzed morphological changes of dung beetle hind wings represented by 19 landmarks. However, whether wings as entities and their different regions are under the same selective pressures remained unclear and the picture of the evolutionary patterns of the different regions remained vague.

Hypotheses on hind wing features reflecting the evolution of the general body morphology and the suitability of wing characters for phylogenetic analyses were tested in Bai et al. (2011), but only the morphology of the hind wings as a whole represented by 19 landmarks was analyzed. In addition to that, in our present contribution, three specific regions are selected to represent regions of the hind wing of Scarabaeinae (Table s1), which were
demonstrated to be highly variable in Bai et al. (2011). We use both the geometric morphometrics and cladistic approaches (based on phylogenetic analyses from Bai et al. (2011)) to investigate the relationships among the morphological variations of entire hind wings and of three specific wing regions of 80 dung beetles species (Fig. 1A–C). Additionally, the detailed evolutionary patterns of the three regions and reconstructions of all ancestral forms (groundplan conditions) were computed by mapping the geometric morphometrics data onto the parsimony based phylogenetic tree.

2. Materials and methods

This study is based on exemplars of 80 species housed in the Institute of Zoology, Chinese Academy of Sciences. The specimens were examined and dissected using a LEICA MZ 12.5 dissecting microscope. Terms and terminology used in this study follow Kukalová-Peck and Lawrence (2004). Abbreviations for the name of tribe are:

OP: Onthophaginidae; CA: Canthoini; ON: Oniticellini; CO: Coprini; OT: Onitini (paraphyletic); GY: Gymnopoleurini; SC: Scarabaeinii; SI: Sisyphini; AT: Ateuchini.

2.1. Taxa examined

Nine tribes (100% of Chinese scarabaeine tribes, 81.8% of world Scarabaeinae tribes), 26 genera (86.7% of all 30 Chinese scarabaeine genera, 11.0% of all 235 world scarabaeine genera), and a total of 79 species (including 23 type species, 23.2% of all Chinese Scarabaeinae species, 1.4% of world Scarabaeinae species) were included in the geometric morphometrics and phylogenetic analyses (Table s1). Every genus or subgenus is represented by 1 or 2 species. Aphodius denticulatus from Aphodinidae is chosen as outgroup in phylogenetic and morphometric analysis as Aphodiinae is considered as the sistergroup of Scarabaeinae (Browne and Scholtz, 1998; Hunt et al., 2007).

2.2. Morphometric protocols

Nineteen landmarks and 3 regions with outlines were selected based on homologous or corresponding criteria (Fig. 1A, C). The landmarks provide a nearly complete coverage of the morphological changes in the hind wings, even though the representation of the total variation of the most diverse regions (apical or medial field) is still difficult. The regions with outlines are selected as additional data.

Wings of all species were photographed using a Sony T9 camera, except A. denticulatus (taken from Kukalová-Peck and Lawrence, 1993). Images were entered in tps-UTILS 1.38 (Rohlf, 2006a) and Cartesian coordinates of landmarks were digitized with tps-DIG 2.05 (Rohlf, 2006b). Landmark configurations were scaled, translated and rotated against the consensus configuration using the GLS (Generalized least squares) Procrustes superimposition method (Bookstein, 1991). The coordinates were analyzed using tps-RELW 1.44 (Rohlf, 2006c) to calculate eigenvalues for each principal warp.

In the lift in insect forward flight is created by a flexible wedge-effect (Jin et al., 2007). Differences in vein shape lead to differential wing flexibility, which could influence flight behavior and create a competitive advantage. It is assumed that the three chosen regions (Fig. 1C, s1–6) precisely reflect the morphology of the apical or medial field, respectively, which play a significant role in flight activities. The first region, RRA (green area in Fig. 1C), is enclosed between the middle lines of RA3 and RA4. The second region, OR (blue area in Fig. 1C), lies between the middle line of RA4 and the external border of RP1. As RA4 and RP1 are very close in some cases, the outer border of RP1 is considered in the outline data acquisition of OR. The third region is CuA (purple area in Fig. 1C) between the middle line of MP1, MP2–MP1, and CuA. The medial fold is congruent with CuA. Outlines of regions were selected, reversed and exported in Photoshop CS4. Photographs were digitized with tps-DIG 2.05 (Rohlf, 2006b) using the outline tool. The outlines were then saved as XY coordinate resample in 50 landmarks. The following analytical processes are similar to those used for landmark data.

2.3. Aspect ratio

In aerodynamics, the aspect ratio of a wing is the length of the wing compared to the width (chord). A high aspect ratio indicates long, narrow wings, whereas a low aspect ratio indicates short, stubby wings. For most wings, the length of the chord varies along the wing so the aspect ratio (AR) is defined as the square of the wing planform divided by the area of the wing planform. The parameters of area, girth, length and width of the hind wings of Scarabaeinae were acquired in Photoshop CS4 and aspect ratios were calculated based on the formula shown in Table s2. Aspect ratio variations among tribes of Scarabaeinae were calculated by SPSS (Fig. 1D, Table s3).

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AR = \frac{b^2}{S}
\]

where \( b \) is the wing planform, and \( S \) is the area of the wing planform.

2.4. Disparity within tribes

Disparity is a measure of the amount of morphological variation in a group of samples, taking into account the volume of the hyper-dimensional morphospace occupied, the relative distances between samples, and the number of samples. The metric disparity score was computed from a PW scores matrix with the PW scores computed relative to the total mean. Using COV software (version 102, by Jean-Pierre Dujardin, http://www.mpl.ird.fr/morphometrics/cov/index.html), we then estimated the disparity of each tribe according to Zelditch et al. (2004) (Fig. 2B, D, E; Table s4). Standard errors (SE) were generated from 10 000 bootstrap pseudoreplicates. According to the number of bootstraps, COV generates random matrices, computes corresponding (random) metric disparity (MD) scores and differences among groups. For each comparison between two submatrices a confidence interval (CI) of the recorded differences were computed.

2.5. Correlation analysis

The matrix correspondence run in NTSYS-pc was based on the 2-way Mantel test (Mantel, 1967; Sokal, 1979). The number of permutations was set as 3000 for every run. The correlations of the general morphology and regions of the hind wings are based on the landmark and outline data (Table s5).

2.6. Reconstruction of ancestral forms of hind wing

Landmark data can be entered in Mesquite 2.72 (Maddison and Maddison, 2009) as a continuous matrix with multiple items. The ancestral forms are reconstructed by the Landmark Drawings module of the Rhetenor package. We linked the landmark data and the consensus tree (Fig. 3 from Bai et al., 2011) in the Mesquite
Fig. 1. Hind wing shape analysis of Scarabaeinae. (A): Description of the landmark positions (right hind wing of Copris lunaris (Linnaeus, 1758)), the terminology follows Kukalová-Peck and Lawrence (1993); (B): the positions of RP3+4, MP1, and MP2, green ellipse in (A); (C): description of the position of three regions of the hind wing for the outlines analysis; (D): aspect ratio variations among tribes of Scarabaeinae; (E): correlation analyses between the morphology of entire wings and selected specific regions; (F): comparisons of MD values of landmarks and outline data at the tribal level (Fig. 1A and B from Bai et al., 2011).
Fig. 2. Morphological variations of hind wings based on outline data (A, C, E). Relative warps computed from the data set of RRA, OR and CuA, plotted against one another to indicate positions of the relationships among species relative to one another and to the reference configuration (situated at the origin); (B, D, F): bar charts of Metric Disparity of outline data of RRA, OR and CuA; on the tribal level, standard error are generated from 10 000 bootstrap pseudoreplicates.
Fig. 3. Reconstruction of ancestral forms of the RRA region in hind wings of Scarabaeinae.
Fig. 4. Reconstruction of ancestral forms of the OR region in hind wings of Scarabaeinae.
Fig. 5. Reconstruction of ancestral forms of the CuA region in hind wings of Scarabaeinae.
environment. Then we ran the Landmark Drawings in the Analysis menu.

3. Results

3.1. Variations of regional morphology of hind wings based on outline data

The first two relative warps of the outlines of three regions, RRA, OR and CuA, account for 91.33%, 95.04% and 90.73% of the variations among the species, respectively. These were computed by a singular-value decomposition of the weight matrix (Rohlf, 1993). The first two relative warps were plotted to indicate variation along the two axes (Fig. 2A, C, E). The shape changes among different species implied by variation along the first two relative warp axes and shape changes are shown as deformations of the GLS (Generalized least squares) reference, using thin-plate splines (Fig. 2A, C, E). The splines, which show the deformation of the outlines in comparison to that of the reference, indicate the most significant deformation, as it is situated furthest from the origin. No tribe is significantly different from the others based on outline data of regions. The morphological variations in each tribe, reflected by the MD (Table s4, Fig. 2B, D, F), are compared with the first two relative warps of the outlines.

3.2. Co-variations of entire hind wings and regional wing morphology

The landmarks and all outline data do not show good correlations (Fig. 1E, Table s5) as the values of matrix correlation (r) are all lower than 0.5. This means they are statistically not significant at the 1% level (Lapointe and Legendre, 1992). In contrast, RRA is correlated well with OR (r = 0.42051), as they are located on the same vein, RA4. Apparently, none of the variations of the different wing regions reflects the morphological modification of the entire body or the entire hind wing. This is probably due to different forms of selective pressure on different body regions (see below).

3.3. Reconstruction of ancestral forms of hind wings of Scarabaeinae

By linking the landmark data and the consensus tree (Fig. 3 from Bai et al., 2011) resulting from PAUP* in the environment of Mesquite, the ancestral forms of all nodes were reconstructed. The ancestral (groundplan) hind wing morphology for tribes or basal nodes are shown in magnified splines, which are indicated in arrows in Figs. 3–5.

4. Discussion

4.1. Evolutionary trends of entire hind wings in Scarabaeinae

The observed variation of the entire hind wings among tribes is relatively low with respect to the value of MD based on landmark data (Fig. 4B in Bai et al., 2011), except in Ateuchini, which were only represented by two species in the analysis. Onthophagini is the largest tribe of Scarabaeinae (more than 1, 800 spp. in Onthophagus), but with the lowest degree of variation. This result fits with the relative stability in the general body morphology (with the exception of horns on the head and thorax) and the similar ecological niches of members of this tribe.

The aspect ratio (AR) is an important parameter from aerodynamics which can reflect the functions of a wing. Its variation among tribes of Scarabaeinae was not included in the analyses of Bai et al. (2011). Of course, several kinematic parameters, such as wing beat frequency, wing amplitudes, body mass and shape combined, etc., may play a role in the context of AR values. However, such data are presently not available and to acquire them within short time would be extremely difficult, if not impossible.

In birds it has been shown that the wings of migrating species have a higher aspect ratio with a longer and more slender shape compared to non-migrating species, suggesting that long-distance flight favors a different wing shape compared to that for flying short distances (Kaboli et al., 2007; Mönkkönen, 1995; Voelker, 2001). In insects, Johansson et al. (2009) found that migration affects the shape of both front and hind wings of dragonflies, and suggested that mate guarding behavior may also have an effect, especially on the front wing of dragonfly wings. This is in line with a theory predicting that a higher AR shape allows faster and energetically more efficient flight (Norberg, 1990). We compared the variation of the calculated AR (Table s2) on the tribal level (Fig. 1D) and found no significant differences among the dung beetles included in the evaluation. This is in accordance with the very limited migration of dung beetles compared for instance with dragonflies, which migrate several hundreds of kilometers to avoid temporary or seasonal droughts (Corbet, 1999; Wikelski et al., 2006). The minor AR differences among tribes may reflect the flight behavior of Scarabaeinae to a certain degree. AR of species of four currently recognized tribes, OT, ON, GY and SI (in fact ON is paraphyletic with respect to SI: Fig. 3), are higher than 4. Long, slender wings with a relatively high AR, tend to minimize induced power requirements — the power needed to create the air downwash that supports the insect’s weight and to overcome the drag resulting from the wing-tip vortices created in the process of lift generation. These four tribes are mainly day-active dung beetles, which may require relative long-distance flights from one dung pingle to another.

4.2. Evolutionary trends of hind wing regions in Scarabaeinae

The variations of the hind wing regions at the tribal level are relatively irregular in the value of MD based on outline data (Fig. 2B, D, F). For example AT, CA and SI are the tribes with the highest variation. This fits with the results of the reconstruction of ancestral forms of hind wings of Scarabaeinae. These three tribes show a very high variation in the related magnified splines compared with other reconstructed ancestral tribal conditions (Figs. 3–5).

Our results indicate that entire hind wings and their subregions are probably under different selective pressures. This is suggested by the evidence from the correlation analyses (Fig. 1E) and the comparisons of MD values of landmarks and outline data at the tribal level (Figs. 1F, 2B and 2D, 2F, Table s4). For example, Onitini is the group with the lowest degree of variation in the three regions based on outline data. In contrast to this, it has almost the highest variation based on landmark data. This may be due to the specific variation of the hind wings of the genera currently assigned to the paraphyletic Onitini, which apparently mainly is within the CuA region (for example, landmark 10, 13–16 in Fig. 1A), but not affecting the outline of CuA or other areas.

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Appendix A. Supplementary data

Supplementary data associated with this article (Tables S1–S5, Figs. S1–S6) can be found in the online version, at doi:10.1016/j.asd.2012.05.004.

References


