

Intraspecific morphological variation of the scorpionfly *Dicerapanorpa magna* (Chou) (Mecoptera: Panorpidae) based on geometric morphometric analysis of wings

Mei Liu¹, Na Ma^{1,2}, Bao-Zhen Hua^{1,3}

¹ State Key Laboratory of Crop Stress Biology for Arid Areas, Entomological Museum, Northwest A&F University, Yangling, Shaanxi 712100, China

² School of Agricultural Engineering, Henan Provincial Key Laboratory of Funiu Mountain Insect Biology, Nanyang Normal University, Nanyang, Henan 473061, China

³ E-mail: huabzh@nwafu.edu.cn

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Abstract

Wing shape variation was investigated between the sexes and among four populations of the scorpionfly *Dicerapanorpa magna* (Chou, 1981) endemic to the Qin-Ba Mountains area, China through the landmark-based geometric morphometric approach. The results show that sexual dimorphism exists both in wing size and shape in *D. magna*. Significant differences exist in female wing size and shape among *D. magna* populations. The possible reasons of the wing variation are discussed based on the divergence time of *D. magna* in combination with the tectonic and climatic events in the Qin-Ba Mountains during the late Miocene-Pleistocene period. Whether reproductive isolation exists between different populations needs further research.

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Introduction

Morphological variation is prevalent in wide-ranging species (Mayr, 1963) and is related to phenotypic plasticity, physiological response to environmental factors and adaptation to local environments and divergent selection, potentially leading to speciation through the evolution of reproductive barriers (McPeck, 1990;

Stillwell *et al.*, 2005; Neto *et al.*, 2013). Morphological variation provides a major source of characters and character states in traditional taxonomy (Michaux, 1989; McPeck, 1990). However, it is often difficult to determine whether variation is intraspecific or interspecific in taxonomic practice (Nedeljković *et al.*, 2013; Riedel *et al.*, 2013; Barão *et al.*, 2014).

Landmark-based geometric morphometrics is an effective tool to quantify and analyze the overall shape of biological structures based on two- or three-dimensional Cartesian coordinates of landmarks (Bookstein, 1991; Adams *et al.*, 2013). It has been widely applied in insect taxonomy and systematics to detect subtle morphological variation (Francuski *et al.*, 2009; Baracchi *et al.*, 2011; Neto *et al.*, 2013; Pepinelli *et al.*, 2013), which is often undetectable with classical morphometric methods (Zelditch *et al.*, 2004).

Insect wings are two-dimensional flattened structures that bear many homologous landmarks and are suitable for geometric morphometric analysis (Grodnitsky, 1999; Zelditch *et al.*, 2004). They have been successfully used to resolve taxonomic problems in closely related species groups (Palmer, 2004; Aytekin *et al.*, 2007; Baracchi *et al.*, 2011; Neto *et al.*, 2013; Barão *et al.*, 2014) and to discriminate populations within a species (Haas and Tolley, 1998; Pepinelli *et al.*, 2013).

The scorpionflies Panorpidae (Insecta: Mecoptera) usually live in large populations in moist forested habitats (Byers and Thornhill, 1983). Since the adults are relatively weak fliers (Thornhill, 1980; Byers and Thornhill, 1983), gene exchange between discrete populations is greatly limited, if not completely broken off. In this case, morphological variation is prevalent in Panorpidae, especially the wing markings and genitalia (Ward, 1979, 1983; Jones, 2010; Ma *et al.*, 2014).



Fig. 1. Localities of the populations analyzed: JLJ (Jialingjiang Source Forest Park, Shaanxi); TB (Mt. Taibai, Shaanxi); HDT (Huoditang Forest Farm, Shaanxi); NGS (Nangongshan National Forest Park, Shaanxi); BYS (Baiyunshan, Henan).

The Qin-Ba Mountains area is composed of the Qinling Mountains and Daba Mountains in central China (Liang, 2002). These two mountains are continuous and are divided by the Hanshui River and a series of basins. The Qinling Mountains are the prominent boundary between the Oriental and Palearctic Regions in China and are rich in the biodiversity of Mecoptera (Chou et al., 1980; Ying, 1994; Yuan et al., 2012).

Dicerapanorpa magna (Chou in Chou et al., 1981) was originally described based on a female holotype collected from Huoditang at the southern slope of the Qinling Mountains. Later, this scorpionfly species was found to be widely distributed in the Qin-Ba Mountains (Hua and Chou, 1997; Nie and Hua, 2004). Previous research reveals that individuals of *D. magna* from different areas exhibit variation both in external morphology and internal anatomy, including the number of female ovarioles and male salivary gland tubes (Hou and Hua, 2008; Ma et al., 2011). This led us to hypothesize whether these variations are intraspecific or interspecific.

In this paper, different populations of *D. magna* collected from the Qin-Ba Mountains were examined

using landmark-based geometric morphometrics to quantify wing size and shape variations between the sexes and among allopatric populations.

Material and methods

Insect sampling

To assess intra- and interspecific variation of *D. magna*, the closely related species *D. baiyunshana* Zhong & Hua, 2013 was included in the analysis. Owing to limited sample size of *D. baiyunshana* males, interspecific analysis of wing variations was conducted only for females. Ten female specimens of *D. baiyunshana* were collected from Baiyunshan (BYS, 1300 m), Henan Province in 2001. Specimens of *D. magna* (76 males and 172 females) were collected from four regions in Shaanxi Province from June to August in 2013, including 24 males and 48 females from the Huoditang Forest Farm (HDT, 1500-1800 m), 22 males and 40 females from the Jialingjiang Source Forest Park (JLJ, 1500-1700 m), 8 males and 44 females from

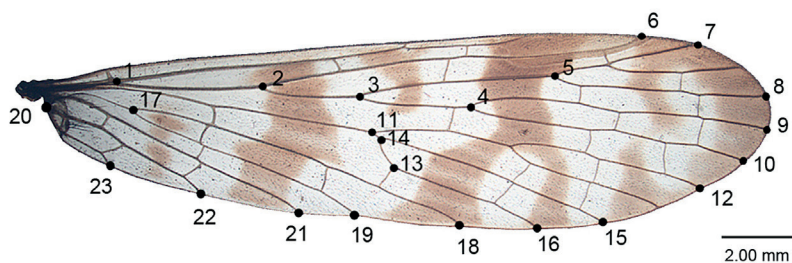


Fig. 2. Right forewing of *D. magna*, showing the landmarks used in this study.

Nangongshan National Forest Park (NGS, 1700–1800 m), and 22 males and 40 females from Mt. Taibai (TB, 2300–2500 m). The localities of populations analyzed are marked on the map (Fig. 1).

Geometric morphometric and statistical analyses

Right forewings were carefully removed from 75% alcohol-preserved specimens and temporarily mounted on micro-slides. Photographs with the same scale were taken with a digital camera attached to the Nikon SMZ1500 stereo zoom microscope (Nikon, Tokyo, Japan).

Twenty-three homologous landmarks (LMs) at vein intersections or terminations that could be reliably identified were selected (Fig. 2), and can be considered type I landmarks (Bookstein, 1991). Data for wing size and shape were obtained by positioning landmarks on digitized wings using tpsDig2 (Rohlf, 2004).

Measurement error. An analysis of measurement error was conducted on a subsample of 30 specimens of *D. magna*, which were chosen randomly and repeated 2 times to obtain landmark data. Procrustes ANOVA (analysis of variance) was performed for landmark data in MorphoJ v1.05c (Klingenberg and McIntyre, 1998; Klingenberg, 2011). All measurements were taken by the same person to reduce experimenter effect. The averaged error of wing centroid size did not exceed 0.18% of the total variation ($F = 0.01$, $P = 1.0000$), and 0.49% ($F = 0.12$, $P = 1.0000$) for wing shape variables. This means that the measurement error explained a negligible percentage of variance.

Size variation. Wing size variation was examined using centroid size (the square root of the sum of squared distance between each landmark and the wing centroid), which was uncorrelated with any shape variable and was not influenced by landmark variation (Bookstein, 1991, 1996). Centroid size was calculated using tpsRelw 1.44 (Rohlf, 2006) and tested for normality using the Shapiro-Wilk test. Leven's test was used to test homogeneity of the variance (Milliken and Johnson, 2009). One-way analysis of variance (ANOVA) was used to test the difference in centroid size between the sexes and among populations. A *post hoc* test (LSD test) after Bonferroni correction on centroid size defined pair-wise differences in centroid size of populations. All statistical analyses were performed in IBM SPSS statistics software version 19.0 for windows (IBM Corporation, 2010).

Shape variation. For wing shape variation, the 258 landmark configurations were scaled, translated, and

rotated against the consensus configuration using Generalized Procrustes Analysis (GPA) procedure to remove the nonshape effects of size, position and orientation (Rohlf and Slice, 1990; Dryden and Mardia, 1998). The resulted matrix (w ; 'weight matrix' *sensu* Rohlf *et al.*, 1996) was used for shape analysis. For a shape, a principal component analysis (PCA) was carried out to determine the explained percentage of each principal component (PC) of the total variation. The total shape variables were used for the multivariate analysis of variance (MANOVA) to test wing shape differences within and among species/population. Canonical variate analysis (CVA) and linear discriminant analysis (LDA) were used to discriminate populations and provide shape variations associated with canonical variates (CVs). The percentages of correct classification (hit ratio, HR *sensu* Gerard *et al.*, 2015; Huberty and Olejnik, 2006) based on a leave-one-out cross-validation procedure in LDA were used to evaluate the discriminatory power of the wing. The allometric effect or the change in shape associated with size differences was evaluated with a multivariate regression of shape variables onto size. Morphometric and statistical analyses were computed using the IMP series software (Sheets, 2012), MorphoJ v1.05c (Klingenberg, 2011) and R version 3.0.2 (R Core Team, 2013).

Results

Variation between D. magna and D. baiyunshana

The Shapiro-Wilk test revealed that centroid sizes of all populations show a normal distribution ($P > 0.05$). Leneve's test showed a significant homogeneity of variances ($F(1, 266) = 2.530$, $P = 0.113 > 0.05$) based on mean centroid sizes. A one-way ANOVA of mean centroid sizes of wings showed highly significant differences among all the analyzed populations ($F(8, 259) = 29.104$, $P = 0.000$). The result of LSD as a *post hoc* test after Bonferroni correction on centroid size showed that *D. baiyunshana* bears the largest centroid size (Fig. 3) and differs significantly from all *D. magna* populations studied ($P = 0.000$) (Table 1).

In order to discriminate taxa at the interspecific level, we used only the females to avoid the effect of sexual dimorphism. The twenty-three landmarks yield 42 shape variables, and accordingly 42 PCs. Each PC had progressively less variance, with PC1 (18.9%) and PC2 (15.9%) representing more than 34% of the total variance (Fig. 4). Most specimens from BYS are clustered

	HDT♂	JLJ♂	NGS♂	TB♂	HDT♀	JLJ♀	NGS♀	TB♀
JLJ♂	0.086							
NGS♂	0.247	0.145						
TB♂	0.206	0.654	0.075					
HDT♀	0.000*	0.000*	0.000*	0.000*				
JLJ♀	0.000*	0.000*	0.000*	0.000*	0.417			
NGS♀	0.000*	0.000*	0.000*	0.000*	0.000*	0.001*		
TB♀	0.000*	0.000*	0.000*	0.000*	0.859	0.344	0.003*	
BYS♀	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

Table 1. Pair-wise ANOVA on wing centroid size of *D. baiyunshana* (BYS) and *D. magna* populations (HDT, JLJ, NGS, and TB). * Significant differences, the adjustment of *P*-value after Bonferroni correction is < 0.006 .

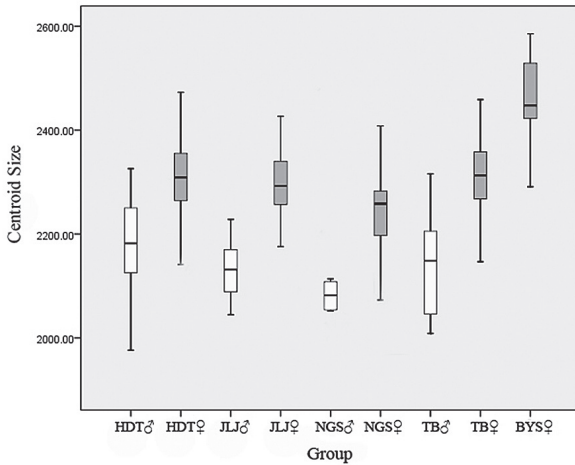


Fig. 3. Boxplot of centroid size of *D. baiyunshana* (BYS) and *D. magna* populations (HDT, JLJ, NGS, and TB). White (male), grey (female).

together with *D. magna* populations on the right of the scatter plots (Fig. 4). Shape variation associated with the first PC reflected a construction of distal and posterior margins of the wing and anterior furcation of veins Sc and R. The second PC represented an expansion of the anterior, distal and posterior margins of the wing, and a construction of the basal part of the wing (Fig. 4).

The shape analysis revealed that the shape variation exists between *D. baiyunshana* and four different populations of *D. magna* (MANOVA: $F(4, 177) = 3.68$; $P < 0.0001$). CV1 (40.3%, Wilks' $\lambda = 0.0372$; $P < 0.001$) and CV2 (27.3%, Wilks' $\lambda = 0.1183$; $P < 0.001$) accounted for 67.6% of total shape variation, clearly separating *D. baiyunshana* individuals from all *D. magna* specimens (Fig. 5). Shape changes associated with CV1 explained the most discrimination between species, representing a construction of anterior margin and an expansion of posterior margin of the wing. CV2 also involved in a construction of anterior margin of the wing (Fig. 5).

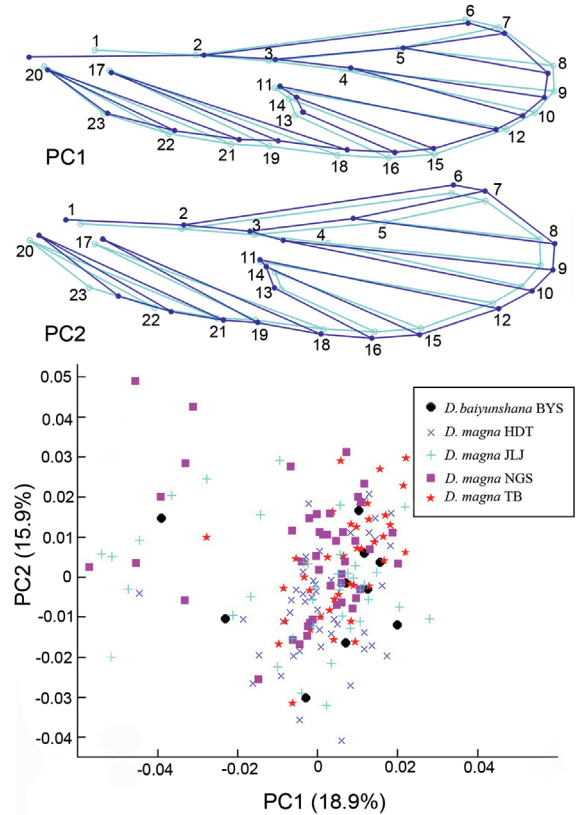


Fig. 4. Scatter plots of PC1 versus PC2 for *D. baiyunshana* (BYS) and *D. magna* populations (HDT, JLJ, NGS, and TB). Deformation grids illustrate the shape variations associated with PC1 and PC2. A dark blue wireframe graph (solid dots) is compared with the overall mean shape (bright blue, open dots).

The assignment by the cross-validation in LDA showed that 87% specimens were correctly assigned to their group (Table 2). Ten individuals of *D. baiyunshana* showed an HR of 100%. Four of HDT were misclassified, two being assigned to NGS and two to TB (HR 92%). Among 40 JLJ specimens, one was misclassified as HDT, three as NGS, and four as TB (93%). Among 44 NGS specimens, one was misclassified as

	BYS	HDT	JLJ	NGS	TB	HR%
BYS	10					100%
HDT		44	1	1	2	92%
JLJ			37		3	93%
NGS		2	3	37	2	84%
TB		2	6	4	28	70%

Table 2. Specimen assignment using the “leave-one-out” procedure in the LDA of *D. baiyunshana* (BYS) and *D. magna* populations (HDT, JLJ, NGS, and TB). Original groups are along the rows, predicted groups are along the columns. The hit ratio (HR%) is also given for each groups.

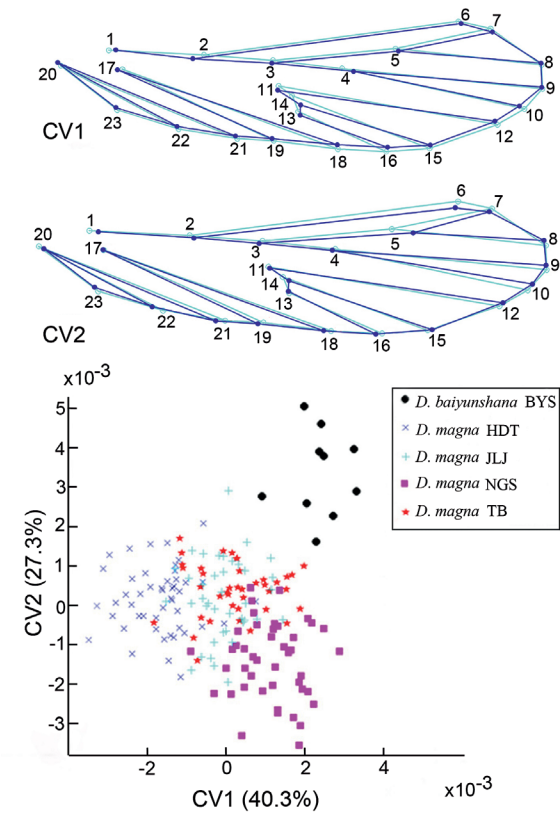


Fig. 5. CVA scatter plots of females of *D. baiyunshana* (BYS) and *D. magna* populations (HDT, JLJ, NGS, and TB). Shape changes associated with CV1 and CV2 are shown. A dark blue wireframe graph (solid dots) is compared with the overall mean shape (bright blue, open dots).

HDT and four as TB (HR 84%). Twelve of TB were misclassified, two being assigned to HDT, four to NGS, and six to TB (70%) (Table 2).

Sexual dimorphism of *D. magna*

The ANOVA analysis showed that the female wings bear larger centroid sizes than the males ($F(1, 246) =$

117.2; $P = 0.000$), and pair-wise ANOVA after Bonferoni correction on centroid size showed significant differences between the sexes of *D. magna* populations (Table 1), exhibiting sexual dimorphism of wing size in *D. magna* (Fig. 3).

In PCA, the first three PCs accounted for 54.49% (28.29% + 16.64% + 9.56%) of total shape variation, and provided a reasonable estimation of the total variation. The other PCs each accounted for no more than 6.7% of the variation. The scatter plots of PC1 and PC2 showed that the males cluster together on the left, whereas the females are on the right of the scatter plots, with a little overlap (Fig. 6). Shape variation along PC1 represented a construction of the distal part of the wing and anterior furcation of veins Sc and R. PC2 showed an elongation of the distal part of the wing, anterior furcation of Sc and R and a construction of anterior and posterior margins of the wing (Fig. 6).

Sexual dimorphism exists in wing shape of *D. magna* populations (MANOVA: $F(7, 240) = 5.686$; $P < 0.0001$). CV1 (43.54%, Wilks’ $\lambda = 0.0219$; $P < 0.001$) and CV2 (18.49%, Wilks’ $\lambda = 0.0789$; $P < 0.001$) accounted for 62% of the total shape variation. The males and females were clearly separated from each other in the CVA scatter plots (Fig. 7). Shape changes associated with CV1 illustrated the most of discrimination, including an elongation of the distal and basal parts of the wing, and posterior furcation of veins Sc and R. CV2 represented a construction of the anterior part of the wing (Fig. 7).

LDA provided assignment results and the percentage of correct classification (HR %) to discriminate the sexes of *D. magna* populations (Table 3). At the individual level, a well supported discrimination was given for 194 of the 248 males and females. In males, the hit ratio for assignment to the correct group was 71% (17 of 24) for HDT, 64% (14 of 22) for JLJ, 100% for NGS and 77% (17 of 22) for TB. In females, the hit ratio for assignment to the correct group was 81% (39 of 48) for HDT, 88% (35 of 40) for JLJ, 86% (38 of 44) for NGS, and 65% (26 of 40) for TB.

	HDT♂	JLJ♂	NGS♂	TB♂	HDT♀	JLJ♀	NGS♀	TB♀	HR%
HDT♂	17	6			1				71%
JLJ♂	1	14		4	1	1		1	64%
NGS♂			8						100%
TB♂	1	2	1	17				1	77%
HDT♀		1		1	39	2	3	2	81%
JLJ♀					1	35		4	88%
NGS♀					1	3	38	2	86%
TB♀					3	8	3	26	65%

Table 3. Specimen assignment in the LDA of sexes of *D. magna* populations (HDT, JLJ, NGS, and TB). Original groups are along the rows, predicted groups are along the columns. The hit ratio (HR%) is also given for each groups.

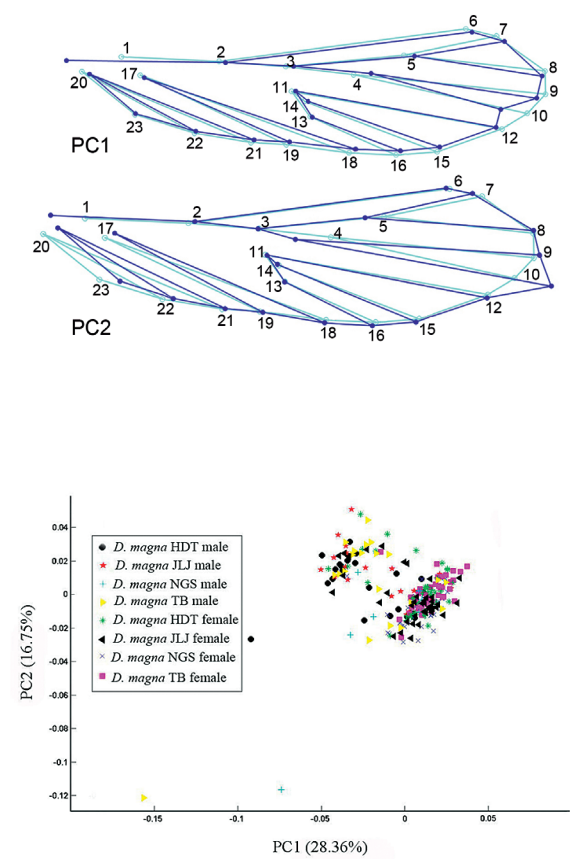


Fig. 6. Plots of the first two PCs (PC1 and PC2) obtained in the principal component analysis of the sexes of *D. magna* populations (HDT, JLJ, NGS, and TB). Wireframe graphs illustrate the shape variations associated with PC1 and PC2. A dark blue wireframe graph (solid dots) is compared with the overall mean shape (bright blue, open dots).

To estimate allometric effect, multivariate regression of shape variable (CV1) was used on centroid size. The results show that the allometric effect accounted for 16.91% of the total shape variance ($P < 0.0001$).

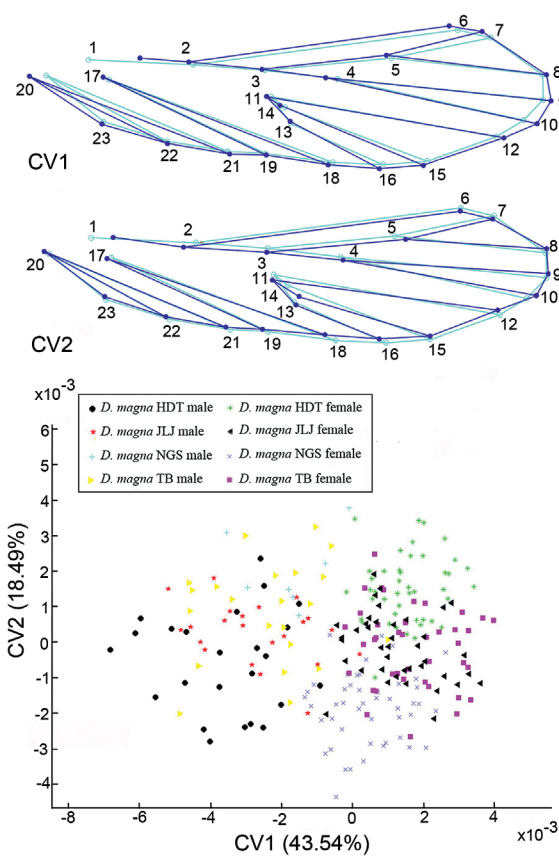


Fig. 7. Scatter plots showing scores on the first two canonical variables for the sexes of *D. magna* specimens. Wireframe graphs illustrate the shape changes associated with CV1 and CV2. A dark blue wireframe graph (solid dots) is compared with the overall mean shape (bright blue, open dots).

Because of the wing sexual dimorphism of *D. magna*, the following analyses were conducted separately on the males and females from four localities, respectively.

	HDT	JLJ	NGS	TB	HR%
HDT	43	1	1	3	90%
JLJ	0	37	0	3	93%
NGS	1	1	40	2	91%
TB	2	4	3	31	78%

Table 4. Specimen assignment in the LDA of only the females in *D. magna* populations (HDT, JLJ, NGS, and TB). Original groups are along the rows, predicted groups are along the columns. The hit ratio (HR%) is also given for each groups.

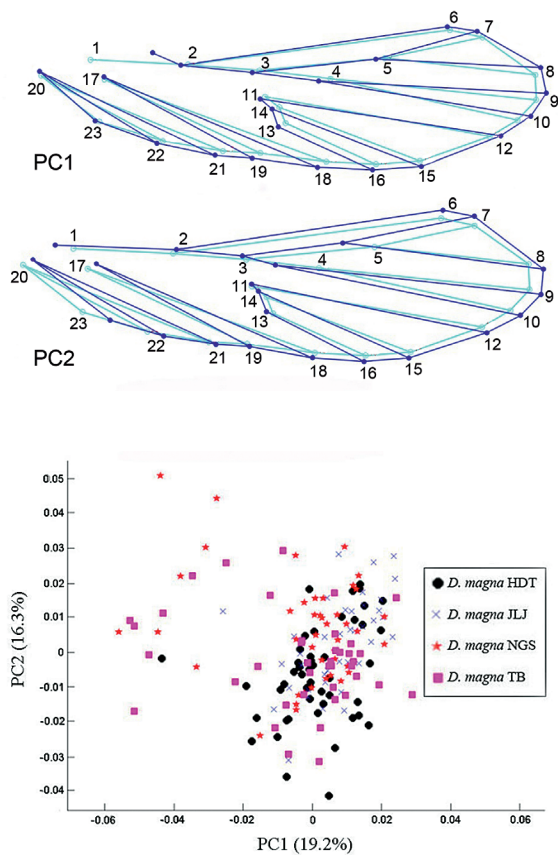


Fig. 8. Scatter plots showing scores on the first two PCs and deformed grids along each PC for females of *D. magna* populations. A dark blue wireframe graph (solid dots) is compared with the overall mean shape (bright blue, open dots).

Wing size variation among populations of *D. magna*

In males, the ANOVA on centroid sizes of wings does not differ significantly among the four populations

($F(3, 72) = 2.589$; $P = 0.059$) and no *post-hoc* LSD pair-wise comparisons were significant on centroid sizes (Table 1). However, the males from NGS have a smaller centroid size than other populations (Fig. 3). In females, a remarkable difference was found among the four populations (ANOVA: $F(3, 168) = 8.569$; $P = 0.000$). The LSD test after Bonferroni correction on centroid sizes shows that NGS has the smallest centroid size ($P < 0.006$), differing significantly from other *D. magna* populations. No significant difference was found among the other three populations (PHDT/JLJ = 0.417; PHDT/TB = 0.859; PJLJ/TB = 0.344) (Table 1, Fig. 3).

Wing shape variation among populations of *D. magna*

In males, wing shape does not show significant difference among the four populations (MANOVA: $F(3, 72) = 1.37$; $P = 0.089 > 0.05$).

In females, more than 35% of the wing shape variation was described in PC1 and PC2 (Fig. 8). The scatter plots from PC1 (19.2%) and PC2 (16.3%) show a high degree of overlap and cluster of individuals from different localities. Most specimens clustered together on the right of scatter plots, only one specimen from HDT, a few individuals from NGS and TB were on the left of scatter plots (Fig. 8). The greatest shape variation associated with PC1 primarily involved in the elongation of the distal part of the wing, the expansion of the posterior margin of the wing and the furcation of veins Sc and R. PC2 represented an elongation of the anterior and distal parts of the wing and an expansion of the posterior margin of the wing (Fig. 8).

Significant shape differences in female wings were found among the four geographic populations (MANOVA: $F(3, 168) = 3.95$, $P = 0.0058 < 0.05$). The two axes of CVA scatter plots, CV1 (52.6%, Wilks' $\lambda = 0.0843$; $P < 0.0000$) and CV2 (33.7%, Wilks' $\lambda = 0.2668$; $P < 0.001$), show that TB partly overlaps with NGS, HDT, and JLJ at the centre of the scatter plots. Overall, individuals from the same population were clustered together and populations from different localities were placed close to each other, with a low degree of overlap

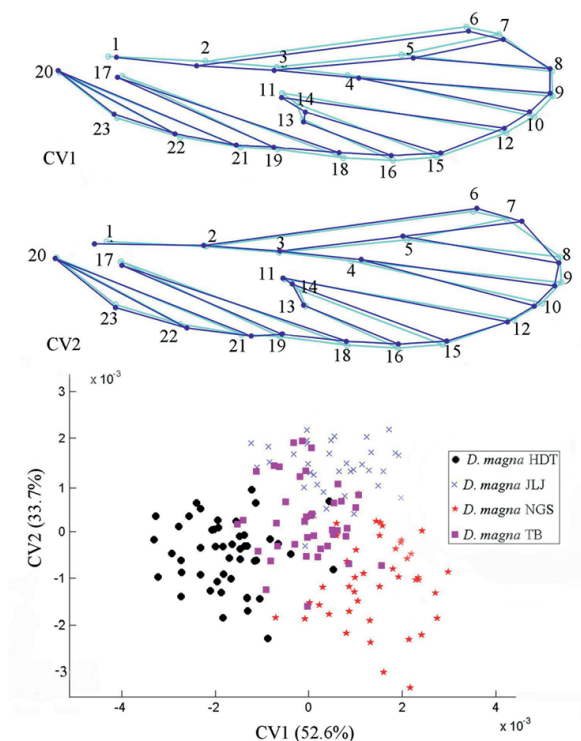


Fig. 9. Scatter plots from the CVA showing shape differentiation in females of *D. magna* populations. Shape changes associated with CV1 and CV2 are shown. A dark blue wireframe graph (solid dots) is compared with the overall mean shape (bright blue, open dots).

(Fig. 9). Shape changes associated with CV1 represented a construction of the anterior and posterior margins of the wing. CV2 reflected a construction of the distal part of the wing (Fig. 9).

The females of the four *D. magna* populations were well separated from each other in the LDA, indicating that the wing shape has a certain inter-population discrimination power. The assignment by the leave-one-out cross-validation showed that 88% of the females were correctly assigned to their groups (Table 4). The percentage of correct classification (HR%) was 90% (43 of 48) for HDT, 93% (37 of 40) for JLJ, 91% (40 of 44) for NGS, and 78% (31 of 40) for TB (Table 4).

Discussion

Sexual dimorphism is a common phenomenon in many insect taxa and is considered one of the most interesting sources of phenotypic variation (Fairbairn

and Preziosi, 1976). Sexual dimorphism in wing morphology may be an adaptive response to flight and behavioral activities, as in dipterans and parasitoids (McLachlan, 1986; Gidaszewski *et al.*, 2009; Benítez *et al.*, 2013). In *D. magna* the wing size and shape differ significantly between the sexes, with the centroid size of female wings remarkably larger than the male's, indicating that a female-biased sexual size dimorphism exists, in accordance with approximately 80% of investigated insect species (Honěk, 1993; Teder and Tammaru, 2005). This kind of sexual size dimorphism is primarily ascribed to the strong fecundity advantage of larger females (Allen *et al.*, 2011) and the sensitivity to environmental conditions of females (Teder and Tammaru, 2005). In addition, small size of males may be related with protandry (Jarošík and Honek, 2007), because the faster-developed males can increase the frequency and success of mating or increase the opportunity to access preferred territories (Allen *et al.*, 2011). Significant difference in wing shape is also present between the sexes of *D. magna* and allometry is an important component of sexual shape dimorphism in the wings. The difference is considered to be associated with flight behavior (Gilchrist, 1990; Gidaszewski *et al.*, 2009; Allen *et al.*, 2011). In general, male insects usually bear shorter and narrower wings because of the fast-beating frequency for courtship and mating, whereas female insects have evolved longer and broader wings to allow them much more easily to find oviposition sites with heavier metasomas carrying eggs (Gilchrist, 1990; Honěk, 1993; Blanckenhorn, 2000; Gidaszewski *et al.*, 2009).

Speciation is an evolutionary process by which new species arise and is considered one of the main ways by which organisms adapt to exploit the diversity of environments available to them (White, 1978; Singh, 2012). Rapid parapatric speciation on the time scale of a few hundred to a few thousand generations is plausible without the need of complete geographic isolation, even when neighboring populations exchange several individuals each generation (Gavrilets *et al.*, 2000). In the present study, wing morphology of *D. baiyunshana* differs significantly from that of *D. magna*, supporting the previous research that the former is an evolutionarily independent entity (Zhong and Hua, 2013). In the geographic distribution aspect, *D. baiyunshana* is located in the hinterland of the Funiu Mountains continuously overlapping with *D. magna* from eastern Qinling Mountains in a small region.

The variation of wing morphology reflects the evolutionary history with a potential influence of environ-

mental factors, mainly climatic changes (Velzen *et al.*, 2013; Perrard *et al.*, 2014) and geological events (McCulloch *et al.*, 2009). In the present study, significant wing size and shape variations were found only in the females of three *D. magna* populations from the Qinling Mountains (HDT, JLJ, and TB) and one from the Daba Mountains (NGS). The Qinling Mountains, the biogeographical boundary between the Oriental and Palearctic Regions in central China, also the boundary of the north warm temperate and the north subtropical zones, has been modified greatly in topography under the influence of the East Asian monsoon climate due to the rapid uplift of the Qinghai-Tibetan Plateau during the late Miocene (An *et al.*, 2001; Zhang *et al.*, 2006; Dong *et al.*, 2011; Jorge *et al.*, 2011). The Daba Mountains arose in the Indosinian-Yanshanian orogenies of mid-late Jurassic period, and fixed in the Himalayan orogenic belt during the Miocene-Pleistocene (Wang *et al.*, 2004). Along with the uplift of the Qin-Ba Mountains, the climate of the north Qinling Mountains has gradually changed from subtropics to temperate type (Ying, 1994; Wang and Yan, 2011). The Mecoptera were recorded as early as the Lower Permian and were very prosperous from late Permian to whole Mesozoic era based on fossil records (Byers and Thornhill, 1983; Grimaldi and Engel, 2005; Sun *et al.*, 2007). *D. magna* is endemic to the Qin-Ba mountain regions and evolved approximately during the late Miocene at 7.5 million years ago (Hu *et al.*, 2015). It has experienced dramatic tectonic uplift during the late Miocene-late Pleistocene in the Qin-Ba Mountains. Due to its weak flight ability, the dispersal of *D. magna* is severely limited even by a narrow zone of unsuitable habitat, such as the Hanshui River and the series of basins between the Qinling Mountains and Daba Mountains, since the scorpionflies live in the mountain area at high elevations (above 1300 m) and are unable to tolerate the high temperature at the low elevations, so that it is almost impossible for them to pass the Hanshui River under natural conditions. In other words, the Hanshui River is likely a strong geographical barrier to restrict the dispersal of the scorpionflies, thus preventing the gene flow between populations. However, whether reproductive isolation is leading allopatric speciation in different geographic populations need further research.

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