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Intercontinental effect on sexual shape dimorphism and allometric relationships in the beetle pest *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae)

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ABSTRACT

Hind wing shape variation was examined in 686 adult *Diabrotica virgifera virgifera* collected from maize plants in Europe and the USA Corn Belt, using geometric morphometric techniques. Sexual dimorphism at an Intercontinental scale was assessed using canonical variates analysis, a multivariate statistical method used to find the shape characters that best distinguish among groups of specimens. Our results showed that each of the populations of *D. v. virgifera* investigated in this study showed high levels of sex based hind wing shape dimorphism. In particular a stronger and more obvious pattern of hind wing shape variation was found in the USA than in Europe. These results support previous studies on *D. v. virgifera* wing shape that show that female *D. v. virgifera* have more elongated wings than males. These differences raise the question of whether sexual dimorphism may be modulated by natural selection.

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1. Introduction

One of the most interesting sources of phenotypic variation in animals and plants is sexual dimorphism, the study of which continues to be important in evolutionary biology (Andersson, 1994; Fairbairn and Preziosi, 1996; Fairbairn et al., 2007). Generally, females are on average larger than males, resulting in an adaptive advantage for females that includes increased fecundity and parental care (e.g. mole crickets, *Scapteris cusacletus* and *Scapteris cusvicinus*: Forrest (1987); flower longhorns, *Stenurella melanura*: Moller and Zamora-Muñoz (1997); *Drosophila melanogaster*: Reeve and Fairbairn (1999)). However, in some species males are longer in size but weigh less than females (e.g. Tenebrionidae (Coleoptera): Cepeda-Pizarro et al., 1996). Such variations in size and shape between the sexes and among closely related species calls into question the way in which sexual dimorphism is inferred and quantified. Over the past few decades there has been a shift towards more complex measurement techniques, such as geometric morphometrics, that are able to accurately quantify the shape and size of an organism rather than simply provide rudimentary measurements of mass and length (Gidaszewski et al., 2009; Hernández et al., 2010; Benítez et al., 2010, 2013a). Geometric morphometrics is especially useful in quantifying the differences in size and shape between the sexes of a species as demonstrated recently by Mikac et al. (2013) and Benítez et al. (2014) for the western corn rootworm *Diabrotica virgifera virgifera*. When investigating whether sexual dimorphism exists within a species it is important to also examine whether allometry also contributes to the sexual dimorphism found (Gidaszewski et al., 2009; Benítez et al., 2013b). Allometric studies are concerned with the relationship between organismal size and shape and numerous such studies have been conducted on both plants and animals, with the most relevant to this study being that of Mikac et al. (2013). The authors investigated sexual dimorphism and allometry in the beetle pest of maize (*Zea mays*), *D. v. virgifera* which they sampled from the US Corn Belt under the influence of crop-rotation resistance and continuous maize production. The cultural practice of crop rotation is one of several tactics used to manage *D. v. virgifera* in the USA and Europe (Naranjo, 1990; Levine et al., 2002). Mikac et al. (2013) only investigated sexual dimorphism and allometry in limited number of *D. v. virgifera* across a restricted spatial area and as such their results are specific to the locations (US Corn Belt) and situation (cultivation practise) under which the species were sampled. Given that *D. v. virgifera* is a pest of

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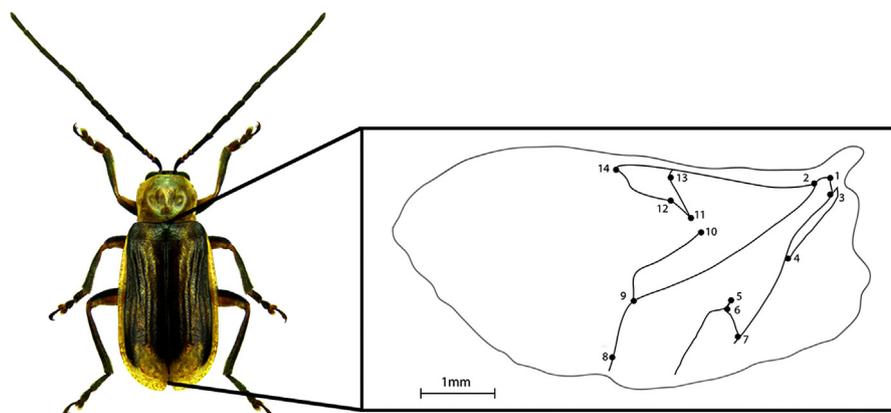
Fig. 1. Representation of the 14 morphological landmarks identified in the hind wings of *D. v. virgifera*.

Table 1

Measurement error Procrustes ANOVA for both centroid size and shape of *Diabrotica v. virgifera*, characterized by matching symmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless).

Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)
Centroid size							
Individual	0.003113	0.000107	29	0.1	1		
Side	0.000728	0.000728	1	0.71	0.407		
Ind × Side	0.02983	0.001029	29	662	<.0001		
Error 1	0.000093	0.000002	60				
Shape							
Individual	0.05169907	7.428E−05	696	1.15	0.03	14.91	<.0001
Side	0.00060088	2.5037E−05	24	0.39	0.997	0.86	0.3089
Ind × Side	0.04483652	6.442E−05	696	9.17	<.0001	11.81	<.0001
Error 1	0.01012083	7.0284E−06	1440				

maize in North America and Europe, the objective of this study was to use geometric morphometric analyses in a comparative morphology approach to determine whether shape dimorphism varies at an intercontinental scale and whether shape differences are sex based.

2. Materials and methods

2.1. Sample sites and data acquisition

A total of 686 adult *D. v. virgifera* were collected by hand from maize plants in July 2011 from four locations in Europe and five locations from the USA Corn Belt. All specimens were preserved in 70% ethanol and sex was determined through the examination of the abdominal apex prior to hind wing dissection (White, 1977). Left and right hind wings were removed from each individual and slide mounted using the fixing agent Euparal (Australian Entomological Supplies, Melbourne, Australia) based on standard

methods (Upton and Mantle, 2010) for subsequent morphometric analysis.

2.2. Multivariate analysis of shape

Geometric analyses exclusively considered variation in shape between the sexes and were performed using an image of the left and right wings of individuals taken by a Leica DFC295 digital camera (3 M Pixel) on a trinocular mount of a Leica MZ16a stereomicroscope and saved in JPEG format using the Leica Application Suite v3.8.0 (Leica Microsystems Limited, Switzerland). We digitized 14 landmarks (LMs: anatomical homologous points) on every image using the software tpsDig v2.10 (Rohlf, 2008) (Fig. 1). *X–Y* coordinates were obtained for all landmarks and the shape information was extracted using a full Procrustes fit (Rohlf and Slice, 1990; Dryden and Mardia, 1998). Procrustes superimposition is a procedure that removes the information of size, position and orientation to standardize each specimen according to centroid

Table 2

Percentages of total shape variation (first 10 PC scores) by principal component (PCA) using covariance matrices pooled within-sex (a) individual variation (allometric effects); (b) after correction by size (non-allometric effects).

	(a) Individual variation			(b) Corrected by size		
	Eigenvalues	% Variance	Cumulative %	Eigenvalues	% Variance	Cumulative %
1	0.00019334	25.111	25.111	0.00021343	26.113	26.113
2	0.00011774	15.292	40.402	0.00011832	14.477	40.59
3	0.0000844	10.961	51.364	0.00008849	10.827	51.417
4	0.00007717	10.023	61.387	0.00008192	10.023	61.44
5	0.00005724	7.434	68.821	0.00005995	7.335	68.775
6	0.0000456	5.923	74.744	0.00004901	5.996	74.771
7	0.00003217	4.178	78.923	0.00003529	4.318	79.089
8	0.00002391	3.106	82.028	0.00002422	2.964	82.053
9	0.00002121	2.755	84.783	0.00002187	2.676	84.729
10	0.00001679	2.181	86.964	0.0000184	2.251	86.98

Table 3
Results of the CVA analysis with Procrustes and Mahalanobis distances and the respective *P* values, for the respective male (M) and females (F) between continents.

	M – EUROPE Procrustes *P*-value	M – USA Procrustes *P*-value	F – EUROPE Procrustes *P*-value	M – EUROPE Mahalanobis *P*-value	M – USA Mahalanobis *P*-value	F – EUROPE Mahalanobis *P*-value
M – USA	0.0102 <0.0001			1.798 <0.0001		
F – EUROPE	0.0074 <0.0001	0.015 <0.0001		1.0321 <0.0001	2.2844 <0.0001	
F – USA	0.0076 <0.0001	0.0111 <0.0001	0.0078 <0.0001	1.546 <0.0001	1.6393 <0.0001	1.3346 <0.0001

size. The shape variation between males and females was analyzed using principal components analysis (PCA).

Sexual dimorphism associated with location (i.e. intercontinental differences) was assessed using canonical variates analysis (CVA), a multivariate statistical method used to find the shape characters that best distinguish among groups of specimens. The results were reported as Mahalanobis and Procrustes distances and the respective *P* values for these distances, after permutation tests (10 000 runs), were reported.

Sexual dimorphism associated with shape and size differences across all locations was assessed using an ANOVA on Procrustes transformed data (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002). Static allometry was then evaluated by a multivariate regression of shape, pooled within sex (Procrustes coordinates), based on centroid size. Finally, the predicted values were used as shape variables accounting for the allometric component of shape variation, and the residuals were used as the non allometric component of shape variation. All the aforementioned analyses were conducted using MorphoJ v1.05d (Klingenberg, 2011).

3. Results

Procrustes ANOVA for assessing the measurement error showed that the mean square for individual variation exceeded the measurement error (Table 1). The PCA of within sex variation showed that the majority of the shape variation was explained in a very few number of dimensions. The first three PCs accounted for 51.36% (PC1 = 25.11%; PC2 = 15.29%; PC3 = 10.96%) of the total hind wing shape variation and provided a reasonable approximation of the total amount of hind wing shape variation (Table 2). The remaining 21 PCs each accounted for no more than 10% of the total variation. After correction for size, a PCA of the residuals of the multivariate regression showed nearly the same eigenvalues as those for hind wing shape variation above, where the first three PCs accounted for 51.41% of the total variation (PC1 = 26.11%; PC2 = 14.47%; PC3 = 10.82%).

Similar to the PCA results, the CVA showed significant differences between males and females at an intercontinental level (Table 3 and Fig. 2). An ANOVA of Procrustes transformed data showed no significant difference in centroid size between sex (*P* = 0.325). However, a highly significant difference between sex was found for hind wing shape (*P* < 0.005) (Fig. 3). Finally, a slight but significant static allometric effect was found that accounted for only 27.76% of the total shape variation (*P* < 0.0001).

4. Discussion

Sex based differences in *D. v. virgifera* wing shape at an intercontinental scale were detected using geometric morphometrics. Each of the populations investigated in this study showed high levels of sex based shape dimorphism. Such sex based shape dimorphism is also evident in species of Diptera, Lepidopera and Hymenoptera (Bonduriansky, 2006; Gidaszewski et al., 2009; Benítez et al., 2011,
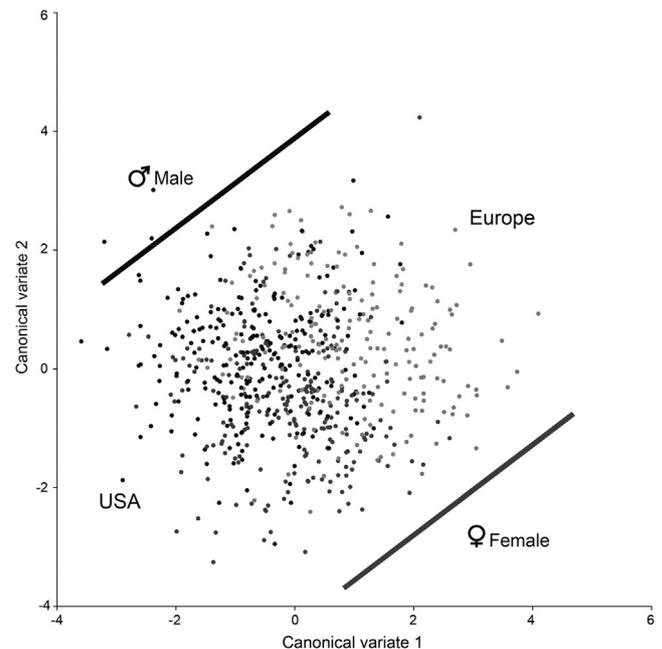
Fig. 2. Canonical variate analysis of wing shape variation using covariance matrices pooled within sex.

2013b). For *D. v. virgifera* this variation was most important in landmarks 1, 3, 7 and 14. These landmarks relate to the basal radial vein and are a key anatomical character used to distinguish different wing morphotypes (Benítez et al., 2011). Both in the USA and Europe female *D. v. virgifera* had more elongated wings than males. However, this pattern was stronger and more obvious in the USA than Europe. These results are supported by Mikac et al.
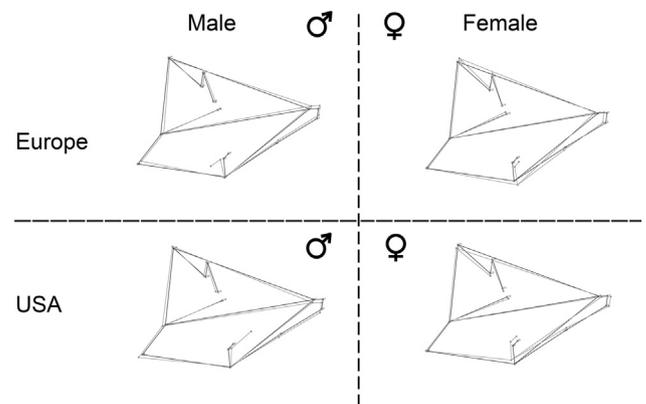
Fig. 3. Wireframe representation of the sexual shape dimorphism morphotypes found between continents and sex, the principal variation was found on the basal radial and cubital veins and on landmarks 3, 7 and 14.

(2013) for *D. v. virgifera* in the USA, where females were found to have more elongated wings than males. Also, these results are similar to that of Li et al. (2009, 2010) who showed that the elytra of female *D. v. virgifera* were larger than that of males; presumably the hind wings protected by the elytra would be of a comparable size also. Since elongated wings are considered to be found in the sex more involved in migratory movement, this study provides morphological evidence that most migration in the *D. v. virgifera* can be attributed to the females of this species. Also, as discussed in Mikac et al. (2013) it is thought that elongated wings are probably more aerodynamic and may also be useful for mated females that are known to engage in migratory flights over relatively long distances.

In contrast, male *D. v. virgifera* have less elongated wings. It is known that male *D. v. virgifera* mate with females almost immediately after they emerge and as such there is little reason for males to undertake long distance flights to find females (Spencer et al., 2009). This is in direct contrast to male potato tuber moths, *Tecia solanivora* that have more elongated wings compared to females of the same species, because males engage in long distance dispersal. Also, for male *D. v. virgifera* in large maize fields there are abundant food resources that do not require elongated migratory wings to access (e.g. Spencer et al., 2005). It is most likely that the sexual dimorphism in wing shape of *D. v. virgifera* may be explained by sex-specific dispersal requirements of this species. Future work will focus on combining population genetics and geometric morphometrics to gain a better understanding of how *D. v. virgifera* genotypes and phenotypes have changes over space and time in their endemic and expanded range in the USA and Europe.

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