A geometric morphometric study on geographical Iranian populations of the pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

Multivariate analyses of the morphological variation in five geographical populations of the pod borer, *Helicoverpa armigera* (Hübner), was performed using 15 and 12 landmarks selected from the fore- and hindwings respectively. Using their geometric morphometric characters (26 in the forewings and 20 in the hindwings), 287 and 277 images of fore- and hindwings were made respectively. Analyses of size showed the wings of the females are bigger than those of the males (forewing: F = 3.98, P = 0.047; hindwing: F = 14.62, P = 0.000) on populations tested. The discriminant function analysis (DFA) of fore- and hindwings differentiated five geographical populations with 86.1% and 69.3%, respectively. The multivariate analysis of variance (MANOVA) of shape variables in fore- and hindwings indicated significant differences among populations as well as sexes (at least for one of the shape variables). Sex interaction within the populations was significant in fore- and hindwings, particularly based on the hindwing landmarks. The relative warp analysis (RWA) showed well-supported discrimination between sexes. The results showed significant difference between the sexes and also within the populations.

Key words: Geometric morphometric, sexual dimorphism, *Helicoverpa armigera*, geographical populations

چکیده

تصاویر بال جلو و عقب (به ترتیب دارای ۱۵ و ۱۲ لندمارک) ۵ جمعیت جغرافیایی پروانه‌ی کرم غـوزه‌ی پنبـه، *Helicoverpa armigera* (Hübner) از مناطق مختلف شمال و شمال غرب کشور مورد تجزیه و تحلیل چند متغیره قـرار گرفت. تعداد ۲۸۷ تصویر متعلق به بال جلو و ۲۷۷ تصویر متعلق به بال عقب با استفاده از ۲۶ متغیر ریخت‌سنجی هندسی در بال جلو و ۲۰ کاراکتر در بال عقب با یکدیگر مقایسه شدند. آنالیز فاکتورهای مربوطه نشان داد که بال در افراد ماده اندازه‌ی بزرگتری نسبت به افراد نـر دارد (بـال جلـو: F = ۳/۹۸، P = ۰/۰٤۷؛ بـال عقـب: F = ۱٤/٦۲، P = ۰/۰۰۰). تجزیـه‌ی تـابع تشخیص بال جلو و عقب، ۵ جمعیت جغرافیایی را به ترتیب به میزان ۸٦/۱ و ٦۹/۳ درصـد از یکـدیگر متمـایز نمـود. اثـر جمعیت، جنس و اثر متقابل جمعیت در جنس برای مجموع متغیرهای شکلی در بال جلو و بال عقب در سطح احتمال یـک درصد معنی‌دار بودند. بدین‌ترتیب بین جمعیت‌های مورد مطالعه و همچنین جنس نر و ماده حداقل از نظر یکی از متغیرهای شکلی تفاوت معنی‌دار وجود داشت. نتایج آزمون جنس‌ها معنی‌دار بود و در کلیه‌ی نتایج آزمون‌ها نر و ماده‌هـا از هـم جـدا شدند. نتایج آزمون بین جمعیت‌ها نیز معنی‌دار بود.

واژگان کلیدی: ریخت‌سنجی هندسی، دوشکلی جنسی، *Helicoverpa armigera*، جمعیت‌های جغرافیایی

Introduction

The pod borer, *Helicoverpa armigera* (Hübner), belongs to the family Noctuidae and subfamily Heliothinae, and is one of the key pests causing severe yield losses, infesting several crops such as cereals, pulses, cotton, vegetables and fruit crops as well as wild hosts

(Zalucki *et al*., 1986, 1994; Fitt, 1989). Ecological and physiological features like high fecundity, multi-voltinism, ability to migrate long distances, and diapauses during unfavorable conditions contribute for it's severity in different situations (Zalucki *et al*., 1986). This pest is primarily controlled by using chemicals that has several unfavorable side effects such as air, food and environmental pollutions. Observations on the pest behavior show interspecific variation in damage caused to several crops in different geographic locations. It is expected that the recognition of intraspecific variation and also its nature and scope may aid our knowledge to predict the spatial and temporal occurrence of the pest. It can improve our management strategies. This study is aimed to estimate the relative importance of genetic and environmental effects on the morphological characters of pod borer in Iran.

By analyzing a population, a great genetic variability within the population or its subpopulations can be found (Warrit *et al*., 2006). Recent developments in landmark analysis of shape change using thin-plate spline (TPS) enable analysis of the spatial organization of shape changes to be made and permit comparison of shape similarities between conspecific populations (Rohlf *et al.*, 1996; Slice *et al*., 1996; Adams & Funk, 1997; Pavlinov, 2001).

A study on the *Aphis gossypii* Glover in Australia showed that it was phenotypically plastic and also it was proved that its morphology is affected far more strongly by its host plant via genetic differences among local populations (Wool & Hales, 1997). Mozaffarian *et al*. (2005) showed significant variation of wings among populations of *Ectomyelois ceratoniae* (Zeller) on fig trees in Iran using geometric morphometric approach.

Better understanding of the genetic differences of polyphagous pests such as *H. armigera* can be resulted from the study of its structure and population dynamics and also from its behavior and response to various selection pressures. To achieve more information on intraspecific variation in the pod borer, we searched for any significant difference among populations of the pest from five geographic locations using geometric morphometric techniques.

Materials and methods

Preparing specimens for study

The larvae were collected during summer 2007 – 2008 from several localities in Iran on tomato fields, *i.e.* Kermanshah province: Ravansar (K), Ardabil province: Parsabad (MT), East Azarbyjan province: Shabestar (SB), West Azarbyjan province: Shahindezh (SJ), and Golestan province: Gorgan (GT) (table 1). The larvae were reared to adulthood in laboratory

under experimental conditions of $70 \pm 5\%$ RH, $25 \pm 1°C$ with a photoperiod of L: D (16: 8 h). The scales of the wings were carefully removed using bleach. Microscopic slides were prepared for both wings by using Hoyer's medium. Morphological characters were examined using a stereo binocular dissecting microscope. The entire of the selected morphological structures were measured using the image analysis software, Leica MZ 7.5.

Geometric morphometrics and statistical analyses

Landmarks were opted on the right wings (both fore- and hindwings) to avoid any problems due to asymmetry. Fifteen landmarks on the forewing and 12 landmarks on the hindwing were chosen (fig. 1) and their Cartesian coordinates were digitized by tpsDig (Rohlf, 2003). Coordinates were taken from the Jpeg images using the digitizing program tpsDig (version 2.1). A total of 287 fore- and 277 hindwing images respectively were analyzed. The raw coordinate data were aligned prior to analysis using the software package tpsRelw (version 3.2) to remove size and arbitrary positioning effects of the specimens relative to the reference axis (Rohlf & Marcus, 1993).

Table 1. List and code of collecting sites and the number of male and female *H. armigera* on tomato.

Population	Code	Forewing			Hindwing		
		Female	**Male**	**Total**	**Female**	**Male**	**Total**
Parsabad	MT	41	23	44	30	36	66
Gorgan	GT	36	29	65	30	25	55
Ravansar	K	40	26	68	43	17	60
Shabestar	SB	18	22	40	29	19	48
Shahindezh	SJ	19	24	43	33	15	48
Total		158	129	287	165	113	277

An average shape or tangent configuration was computed as the average configuration of all specimens studied. The variation among different groups of populations was analyzed using tpsRelw or NTSYS - pc, and partial warp scores for each specimen as variables in multivariate analyses of variance (MANOVA). Relative warp analysis was performed and wings relative variations in two sexes were determined. For comparing overall wing size among all groups, we used the isometric estimator known as "Centroid size".

Results

Males and females showed significant sexual dimorphism that involved multiple aspects of shape. Relative warp (= principal component) for forewings and hindwings demonstrated

16 and 5 principal components (PCs), respectively, with eigenvalues greater than 1%. The first principal component accounted for 26.73% and 30.99% of variability and the first 3 principal components accounted for 55.72% and 58.37% respectively.
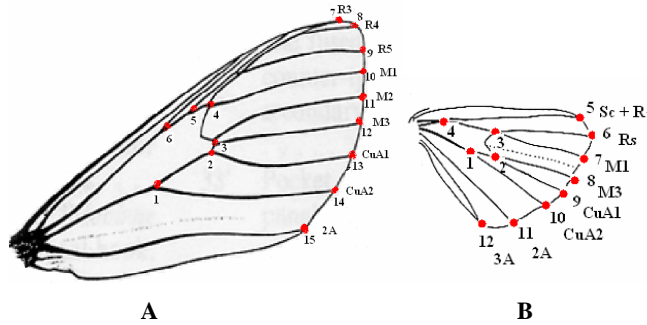

Figure 1. Distribution of landmarks in the wings of *H. armigera*. A, forewing: 1- beginning of CuA2, 2- beginning of CuA1, 3- beginning of M3, 4- junction of M1 and R5, 5- beginning of R4, 6- beginning of R2, 7- termination of R3, 8- termination of R4, 9- termination of R5, 10- termination of M1, 11- termination of M2, 12- termination of M3, 13- termination of CuA1, 14- termination of CuA2, 15- termination of 2A. B, hindwing: 1- beginning of CuA2, 2- junction of CuA1 and M3, 3- Junction of M1 and Rs, 4- beginning of Sc + R1, 5- termination of Sc + R1, 6- termination of Rs, 7- termination of M1, 8- termination of M3, 9- termination of CuA1, 10- termination of CuA2, 11- termination of 2A, 12- termination of 3A. Nomenclature of wing venation is burrowed from Matthews (1999).

The discriminant function analysis (DFA) in fore- and hindwings showed that four out of the six discriminant functions were statistically significant at 95% confidence level. The DFA of the forewings differentiated geographical populations in 86.1% of cases, *i.e* based on the 158 forewings studied, 136 specimens were placed correctly in one of the five geographical regions. For hindwings, DFA showed that the five populations were differentiated in 69.3% of cases, *i.e* among the 166 hindwings studied, 115 specimens were placed accurately.

The greatest morphological differences are 9.21 in forewings among the populations MT and SJ from Ardabil and West Azarbyjan provinces and 7.20 in hindwings among the populations K and GT from Kermanshah and Golestan provinces. The smallest morphological

distances are represented 4.34 in forewings between populations K and SB from Kermanshah and East Azarbyjan provinces and 4.87 in hindwings between populations SB and SJ from East Azarbyjan and West Azarbyjan provinces (table 2).

Table 2. Morphological distance within the geographical populations of *H. armigera* using weight matrix for forewings (upper triangles) and hindwings (lower triangles) landmarks. For abbreviations see the text.

Population	SJ	SB	K	MT	GT
GT	8.47	6.35	7.22	7.83	-
MT	9.21	6.38	7.62	-	7.15
K	7.03	4.34	-	6.01	7.20
SB	6.54	-	7.16	6.48	6.84
SJ	-	4.87	5.20	5.99	5.84

Given the substantial sexual dimorphism, canonical discriminant analysis (CDA) was carried out on females and males separately, both on forewings and hindwings. The CDA scatter plots of female forewings set apart the entire populations; however, the CDA illustrated some similarity between the populations "MT and GT" and the populations "K and SB". Moreover, the population SJ was differed from the other populations. The CDA results for male forewings imply that the populations "MT and GT" and the populations "SB and SJ" are very similar; nevertheless, the population K was differed from the other populations. The results from female hindwings showed as similar results as of the male forewings. The results for male hindwings imply that the populations "K, SB and SJ" are very similar but the populations MT and GT were differed from the other populations (fig. 2).

The results yielded from the cluster analyses of the forewings and hindwings were not able to differentiate the female and male populations clearly. The results for "GT and MT" populations and "SB and SJ" in male forewings and female hindwings were the same (fig. 3). The canonical variate analysis (CVA) of forewings and hindwings, in which CV1 is plotted against CV2, showed significant differences among the populations examined (fig. 4).

Simple analysis of variance (ANOVA) of fore- and hindwings centroid size of both sexes showed that both wings of females are significantly larger than of the ones for males (table 3). The multivariate analysis of variance (MANOVA) of all shape variables in both fore- and hindwings indicated significant differences at 1% probability among populations as well as sexes (at least for one shape variable). Sex interaction within the populations was significant in both wings (table 4).
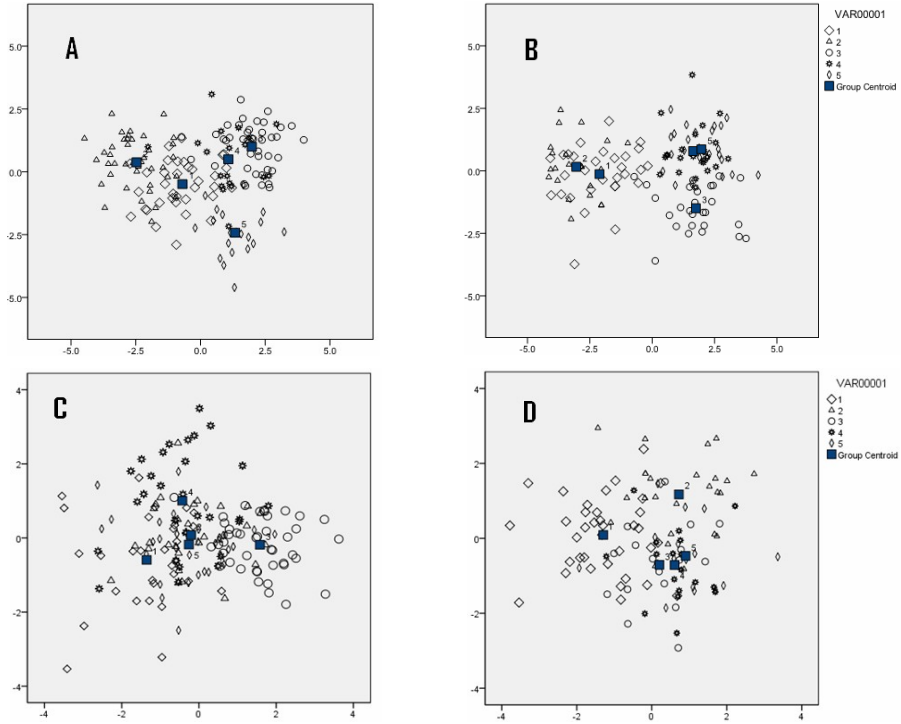
Figure 2. The CDA scatter plots of fore- and hindwings of *H. armigera*. A, female forewings, B, male forewings, C, female hindwings, D, male hindwings. For abbreviations see the text.
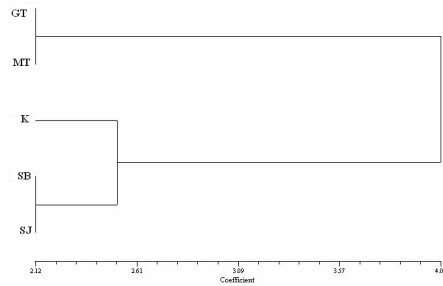

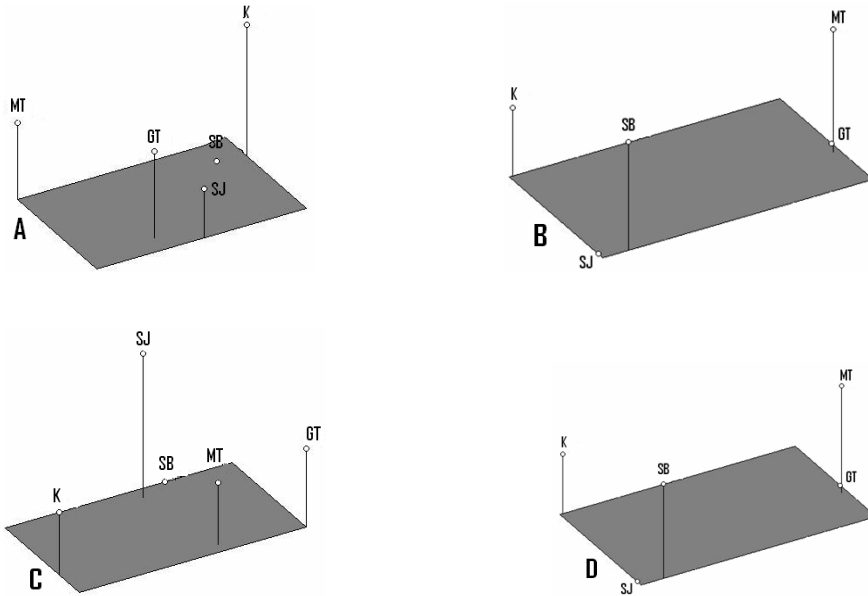**Figure 3.** Cluster analysis of male forewings of geographical populations *H. armigera*.

Figure 4. The CVA of forewings and hindwings of *H. armigera*. A, female forewings, (B) Male forewings, (C) female hindwings, (D) male forewings.

Table 3. One way ANOVA of centroid size in the female and male for fore- and hindwings of *H. armigera*.

No. Female	Mean	No. Male	Mean	F	Probability	Wing
Forewing	158	1291.85	130	1274.38	3.98	0.047
Hindwing	165	1175.04	112	1143.21	14.62	0.000

Table 4. Two-way MANOVA for shape variables of fore- and hindwings of *H. armigera* in different geographical populations.

Source	Forewing					Hindwing				
	Wilks` λ	df1	df2	Fs	P	Wilks` λ	df1	df2	Fs	P
Population	0.091	104	1006.24	8.04	0.000	3.482	80	980.750	9.56	0.000
Sex	0.679	26	253	0.78	0.000	.565	20	248	9.56	0.000
Interaction	0.338	104	1006.24	3.04	0.000	.507	80	98.750	2.30	0.000

The regression of shape on size in the female populations showed significant allometric growth in geographical populations. Furthermore, the relative warp analysis (RWA) of fore- and hindwings demonstrated significant sexual dimorphism in the wing shape of pod borer

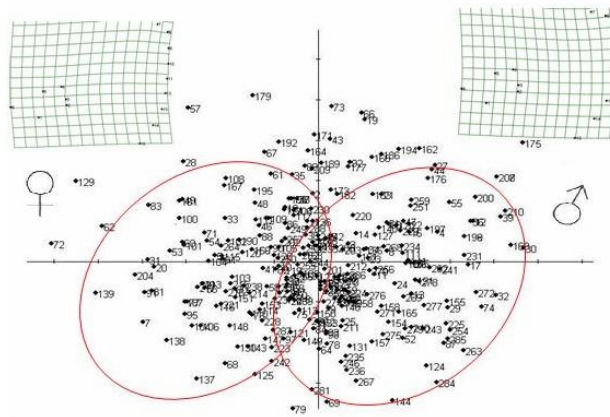moths (fig. 5). The effect is seen more strangle in the hindwings than in the forewings (not shown).

Figure 5. The RWA of forewings of *H. armigera* demonstrating dimorphism in wing shape. Whenever the individuals move toward female shape (1-158) in the left side grid insert, the wings elongate, but whenever they move toward male shape, the wing shape widens (in males, 159-288) in the right side grid insert. Relative warps 1 and 2 are plotted here showing individual positions on two axes.

Discussion

Our results showed that the female pod borers have larger wing than that of the males. Larger wing might be attributable to relatively larger body size. The phenomenon of larger body size is commonly observed. Similar conclusions were obtained through separately investigations on *Neochlamisus bebbianae* (Brown) and *Chilo suppressalis* (Walker) by Adams & Funk (1997). Zahiri *et al.* (2006) showed significant difference between sexes in six populations of rice stem borer, *C. suppressalis*, in northern Iran. Mozaffarian *et al.* (2007) showed significant wing shape differences in carob moth, *Ectomyelois ceratoniae* (Zeller). Their analyses of size showed that the female wings are larger than those of the males. Alipanah *et al.* (2004) and Khaghaninia *et al.* (2008) showed comparable results on *Cydia pomonella* (L.).

Mendes *et al*. (2007) showed the geometric morphometric facts of forewings are extremely powerful and informative to assess biodiversity within inter-population variability of *Nannotrigona testaceicornis* Lepeleteir, (Hymenoptera, Meliponini). We found the best cluster that could explain variations in male forewings and female hindwings populations.

The CDA results for male forewings did not show similar results as for female forewings. The scatter plots of CVA in forewings of pod borer illustrated that the greatest morphological distance was observed between the populations "MT and SJ" and "GT and SJ"; because, the SJ has annual mean temperature (2.3 ∘C), and highest geographical height (1765 m) as compared with the other populations. The smallest morphological distances are represented 4.34 between populations K and SB (table 2), with similar altitude of 1362.7 m and 1362 m and also annual mean temperature of 8 ∘C and 6.8 ∘C, respectively. In hindwings, the greatest morphological distance was observed between the populations GT and K. Possibly, their geographical distance might have effect on this, since the K population has the maximum geographical distance (more than 900 Km) as compared to the other populations. It seems that geographical distance, by itself, is insufficient for isolating adjacent populations except natural barriers that may play a role in such segregation. Temperature, latitude and longitude, altitude, humidity and other environmental conditions could also influence on the evolution of populations.

The multivariate analyses of partial-warp scores of fore- and hindwing shapes demonstrated a significant difference among geographic populations. The alternative phenotypes may have allowed pod borer populations to persist in a variety of geographic conditions. The large numbers of selective pressures that have been put forward to explain host plant specialization suggest that the evolutionary process will be strongly dependent upon geographic variation in insect-plant interactions (Ballabeni *et al*., 2003). Merrill & Denno (1998) listed the factors that have been hypothesized to influence gene flow, which are extensive and include geographic distance, dispersal capability, ecological specialization, phonological isolation, habitat patchiness, and the frequency and nature of extinction recolonization events.

The two main factors that may account for the observed variation of pod borer are geographic differences in habitats and different host. A consideration of the several cultivars commonly planted in growing areas and the different geographic conditions of these localities could in part explain the observed variation within and among the populations under study. Dispersal of pod borer could happen, by dispersal of adults, from one location to another

location. Depending on the climatic conditions, 2 to 11 generations annually have been reported. The wide geographic distribution over the world shows that *H. armigera* can establish in regions with (seasonal changes from) tropical climates (*i.e.* Africa, tropical Asia) to regions with a cooler temperate climate (*i.e.* Mediterranean area of the EU). In regions with a cooler temperate climate, *H. armigera* overwinters in a diapause stage (Kurban *et al*., 2005). Feng *et al*. (2005) stated that gene flow was high because of large-scale migration of populations. The *H. armigera* can move very easily due to natural migration. Pedgley (1985) showed that *H. armigera* migrates up to 1000 km to reach Britain and other parts of Europe from sources in southern Europe and northern Africa.

The result showed significant differences at 1% probability among the populations examined and also within their sexes for both type of variables. The non-uniform variables were more effective in sex population interaction differences especially in the hindwing in comparison with the uniform one. The results of this study provide evidence for the causes of shapes evolution or evolution in general in agricultural ecosystems that may be useful in an applied context because it is evolution that could produce genetic change in pest populations in response to management efforts, leading to their eventual failure of control (Via, 1990). This tps analysis provides evidence of phonetic differentiation among pod borer populations. However, host association studies using cross-infection with pod borer populations to assess the correlation of observed population shapes with the different pod borer populations is still under study.

Acknowledgements

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