A GEOMETRIC MORPHOMETRIC STUDY ON THE HOST POPULATIONS OF THE POD BORER ,*HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) IN SOME PARTS OF IRAN

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ABSTRACT: The pod borer, *Helicoverpa armigera* (Hübner) is one of the key pests causing severe yield losses in several crops such as cereals, pulses, cotton, vegetables and fruit crops as well as wild hosts in Iran. In this study, shapes and sizes of wings were compared in populations on 4 host plants (cotton, tomato, corn and chickpea) using a land mark – based geometric morphometric method, analysis of partial warp scores and centroid sizes. The results showed significantly smaller wing size in populations on cotton and a significant host plant–associated shape difference among populations. Multivariate analysis of variance (MANOVA) of shape variables in forewings indicated significant differences among populations. Simple analysis of variance (ANOVA) indicated that the centroid size of cotton populations was significantly smaller than others. The analysis also showed a significant difference between the populations.

KEY WORDS: Geometric morphometric, Sexual dimorphism, Thin plate- spline, Pod borer, *Helicoverpa armigera,* Host populations

When an insect population has two or more host species, the possibility arises that gene flow is restricted between groups on different hosts that are subjected to divergent natural selection for host adaptations (Berlocher & Feder, 2002).

The ability of many of insect species to existence on diverse host plants is an useful strategy and adaptive advantage for their better survival in the ecosystem. In nature, polyphagous pests tend to be mono or oligophagic at the micro ecological level and their populations could be made up of individuals that are predominantly monophagous (Karowe, 1989). If host plant species produce different selective regimes to herbivorous insects, genetic variations and host plant–associated local adaptation may occur (Ruiz-Montoya et al., 2003).

The existence of host-associated populations has been demonstrated in several insect pests (Downie et al., 2001; Abdullahi et al., 2003; Sarafrazi et al., 2004; Mozaffarian et al., 2007). Polyphagous insects have many advantages and feed on different hosts providing different nutritional resources. The selective use among diverse resources may lead to the evolution of ecological specialization and adaptation (Berenbaum, 1996; Kawecki, 1997). The pod borer is migratory and is also a key pest on all continents (Feng et al., 2005). Hence polyphagy at the species level, as has been demonstrated in *H. armigera*, does not necessarily imply polyphagy at the individual level (Cunningham et al., 1999).

Host plants used by *H.armigera* have been recorded for India (60 cultivated and 67 wild plants) (Karim, 2000), Africa (Pearson, 1958), Australia (Zalucki et al., 1994), and New Zealand (Thanee, 1987).

H. armigera has high mobility and fecundity and has also shown great capacity to develop resistance to used different synthetic insecticides in its management (Armes et al., 1996; Kranthi, 1997). The versatility and adaptively of this species may be due to the presence of a strong genetic variability governing the behavior of *H. armigera* (Zhou et al., 2000; Scott et al., 2003) making it a serious pest on several crops.

A better understanding of the host populations differences of polyphagous pest like *H. armigera* can be very useful to understand the structure , population dynamics, their behavior and response to various selection pressures.

In our observation the relative abundance of *H. armigera* in chickpea and corn was much higher than in cotton and other host crops in North- west Iranian cotton ecosystems. To gain information on intraspecific variation in the pod borer, this study searched for significant differences among host populations of the pest using geometric morphometric techniques.

MATERIAL AND METHODS

During summer 2005-2006 Larve of *H.armigera* were collected from several provinces in Iran from North and North west of Iran on different crops such as Tomato, chickpea, Cotton, Corn and reared in laboratory (Table 1). Forewings were measured. 15 landmarks on the forewing were chosen (Figure 1), and their Cartesian coordinates were digitized by tpsDig (Rohlf, 2003a). A total of 134 forewing images 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 (Rolf & Marcus, 1993). The average shape or tangent configuration was computed as the average configuration of all specimens. Rotation, translation and scaling parameters were calculated in order to make the coordinate data interpretable and to bring all the images into a common coordinate system (Rohlf & Marcus, 1993). These parameters were then used to superimpose the configurations. The rotational fitting options used were generalised least-squares (GLS) (Pavlinov 2001).

Centroid sizes as a size measure of any specimen (Slice et al., 1996) were calculated and used as variables in univariate statistical analysis for comparing the size of specimens (Adams & Funk, 1997). Variation between different populations was analysed using tpsRelw or NTSYS-pc, using partial warp scores for each specimen as variables in multivariate analyses of variance (MANOVA).

Morphologic distances among test populations were computed and the result and distance matrixes were also subjected to cluster analysis by the unweighted pair group method to show similarity among test populations. To find any isometry in size variation between populations, analyses of allometry among known groups were performed. Statistical analyses were performed using NTSYS-pc (Rohlf, 1998) and SPSS 14.

RESULTS

Altogether, 134 forewing images were analyzed. PCA of forewing data found three principal components (PCs) with eigenvalues greater than 1%. The first principal component, PC1, accounted for 46.399% of variability, and the 2 accounted for 85.14%. An ordination plot of PCA (Fig. 2) shows that the first principal component separated the cotton and tomato populations from the other populations. DFA found three discriminant functions that were statistically significant at the 95% confidence level. DFA of forewings differentiated geographic populations in 72.2% of cases, i.e., among the 134 forewings, 97 specimens were placed correctly in one of the four regions. The morphological distances among forewings was greatest between the tomato population of shahindej and the cotton populations of cotton and corn population of Khodaafarin. The two closest populations (23.546) were chickpea population of sardasht and corn population of khodaafarin (Table 2).

A DFA scatter plot of populations implies that the cotton population was very dissimilar from others (Fig. 3). The cotton population was clearly differentiated from the other populations. The cluster analysis gave the same general results as did the DFA. The closest populations (chickpea and corn), were placed closest together in all analyses.

CVA plots of CV1 against CV2 also showed significant differences between all populations (Fig,6).

Comparing centroid sizes of host plant associated populations showed significant differences between them and in all comparisons cotton associated populations had smaller wings than other host plant populations (Figure 4).

ANOVA test on centroid size found significantly different populations among the four populations in the study areas in general(Table 3).

Regression of shape on size in the above comparisons did not show significant allometric growth between host plant-associated populations (Table 4).

DISCUSSION

Cluster analyses of morphologic distances showed that wing shape within populations feeding on cotton is more dissimilar than those feeding on other host plants. And within populations feeding on corn and chickpea are more similar together. Comparing centroid sizes showed cotton associated populations had smaller wings than other host plant populations. A study of genetic variability of the bollworm, *Helicoverpa armigera*, occurring on different host plants showed that cotton stood out as unique in one cluster while the insects collected and reared on all other hosts grouped separately (Subramanian & Mohankumar, 2006). The bollworm, *H. armigera* inflicts severe damage on cotton worldwide. However, laboratory studies on the relative host preferences of *H. armigera* for cotton revealed that cotton was the host of lowest relative preference. However in areas of intense cotton cultivation a very high percentage of local pod borer populations may feed exclusively on cotton at certain times of the growing season (Gould 1998). our results showed the same results and may confirm association between molecular and geometric morphometric works particularly in the cotton population. Smallest size in cotton population confirm that cotton has lowest preference. The larger size of moths on hosts other than cotton showed that some host plants such as tomato, corn and chickpea can provide for increased stored nutritional reserves by larvae that may result in more successful over-wintering and higher fecundity in adults. The scatter plots of CVA in *H.armigera* confirmed that the greatest morphological distance was observed between the cotton and tomato population.

Multivariate analyses of partial-warp scores of the wing shapes of the pod borer *H.armigera* demonstrated significant differences among host populations. The existence of *H.armigera* in different phenotypes may therefore have allowed survival in a variety of geographic conditions. Because many different selective pressures can be hypothesized to explain host plant specialization, it is easy to predict that the evolutionary process will be strongly dependent upon geographic variation in insect-plant interactions (Ballabeni et al. 2003), but evidence of this is seldom documented. The observed variation in *H.armigera* is probably linked to geographic differences in habitats and different altitude and enviromental conditions, as has been observed in other species.

A geometric morphometic study on the host plant-associated population variation of carob moth, *Ectomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae), showed significantly smaller wing size in populations on pomegranate and a significant host plant-associated shape difference among populations as a consequence of allometric growth (Mozaffarian, et al 2007). The phenotype of each individual could therefore be the result of an interaction between its genotype and its environment, related to different geographic, altitude and climatological conditions.

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Table 1. List and code of collecting sites and host plants, and the number of forewing female Pod borer.

Province	**Population**	**Host**	**code**	**Fore wing Female**
Golestan	Gorgan	cotton	GC	15
Ardabil	Khodafarin	corn	KF	30
West Azarbyjan	Sardasht	chickpea	SD	66
West Azarbyjan	Shahindej	tomato	SJ	20
	Total			134

Table 2. Square of morphological distances between four host populations of *Helicoverpa armigera*.

	Squared Euclidean Distance			
	1	2	3	4
1	.000	54.739	49.506	64.179
2	54.739	.000	23.546	62.770
3	49.506	23.546	.000	57.260
4	64.179	62.770	57.260	.000

This is a dissimilarity matrix

Table 3. One way ANOVA of Centroid Size in female fore wings.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	239380.7	3	79793.567	14.969	.000
Within Groups	692969.3	130	5330.533		
Total	932350.0	133			

Table 4. Regression of shape on size in host populations of *Helicoverpa armigera* in Iran.

Wing	**Sex**	**Wilks` λ**	**Fs**	**df1**	**df2**	**Probability**
Forewing	**Female**	**0.76775022**	**1.513**	**22**	**110**	**0.0842**

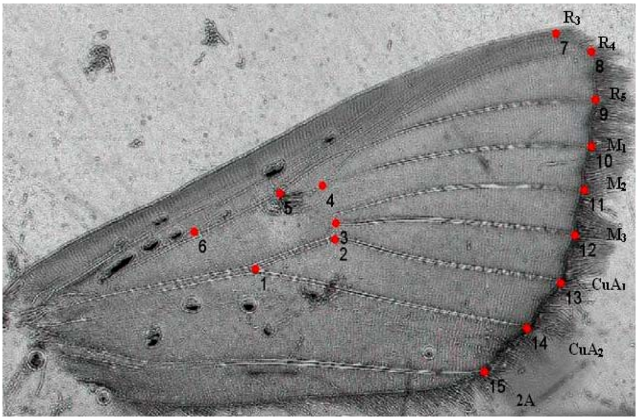

Figure 1. Distribution of landmarks on forewing of *Helicoverpa armigera*.

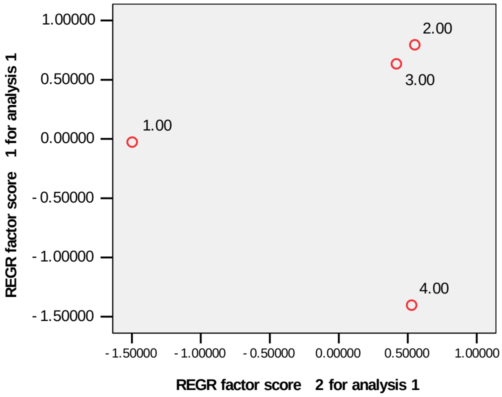

Fig. 2. Ordination plot for four populations in principal of component analysis (PCA) 1-GC, 2-KF, 3-SD, 4-SJ

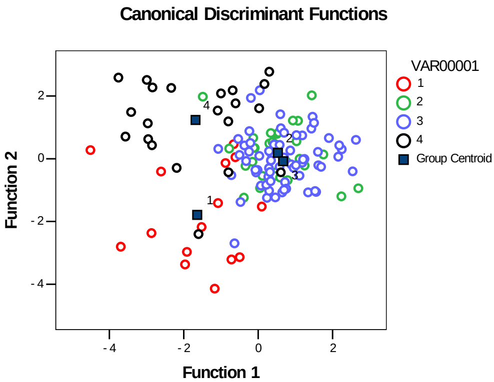

Fig. 3. Plot of discriminant function analysis (DFA) forewing shapes 1-GC, 2-KF, 3-SD, 4-SJ

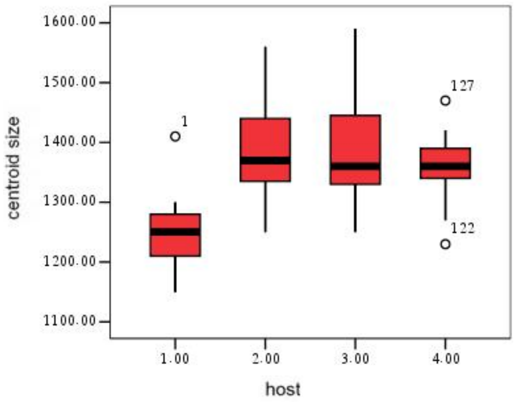

Figure 4. Comparing size of forewings between populations on different host plants. 1- cotton, 2- corn, 3- chickpea, 4- tomato

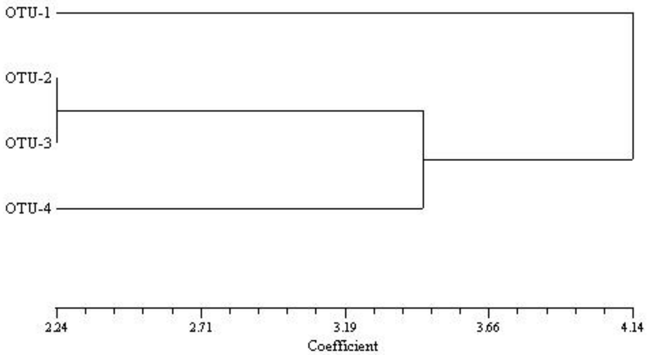

Figure 5. Cluster Analysis of Pod borer forewings 1-GC, 2-KF, 3-SD, 4-SJ

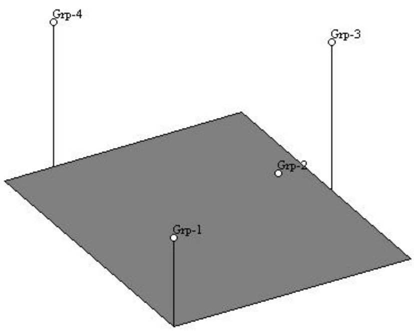

Figure 6. CVA of female forewings of pod borer 1-GC, 2-KF, 3-SD, 4-SJ