A geometric morphometric study on populations of the Rice Stem Borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae) in northern Iran

by Reza Zahiri, Alimorad Sarafrazi, Latif Salehi and Joseph G. Kunkel

Abstract. Multivariate analysis of the morphological variation in six populations of the Rice Stem Borer, *Chilo suppressalis*, was performed using 15 and 10 landmarks selected from the fore- and hindwings respectively. The raw planar coordinate data were aligned using geometric and mathematical calculations in Kendall's shape space. Multivariate analysis was performed following transfer of the data to a linear Euclidean space, i.e. tangent space. 311 and 319 images of fore- and hindwings respectively were made using their geometric morphometric characters (26 in the forewings and 16 characters in the hindwings). The analysis showed a significant difference between the sexes and between the populations of Guilan and Mazandran provinces. A direct correlation was observed between morphological and geographic distance.

Kurzfassung. Die morphologische Variation innerhalb von sechs Populationen des Reisstengelbohrers, *Chilo suppressalis*, wurde mit Hilfe der multivarianten statistischen Analyse untersucht. Dazu wurden 15 Landmark-Punkte vom Vorder- und 10 Punkte vom Hinterflügel ausgewählt. Die Rohkoordinaten wurden mit Hilfe von geometrischen und mathematischen Berechnungen aufeinander angepasst. Die Daten wurden vor der multivarianten Analyse in einen linearen euklidischen Raum (Tangentenraum) überführt. Für die Analyse standen 311 Bilder von Vorder- und 319 Bilder von Hinterflügeln mit ihren geometrisch-morphologischen Parametern zur Verfügung (26 Parameter am Vorder- und 16 am Hinterflügel). Die Analyse brachte signifikante Unterschiede zwischen den Geschlechtern und zwischen den Populationen in Guilan und Mazandran zu Tage. Die morphologische Distanz ist direkt mit der geographischen Entfernung zwischen den einzelnen Populationen korreliert.

Key words. Geometric morphometrics, thin plate-spline, *Chilo suppressalis*, populations.

Introduction

The Rice Stem Borer (RSB), *Chilo suppressalis* Walker, is a key pest on rice that was first reported in Iran in the main rice-growing area of northern Iran (Tonekabon) (EBERT 1972). This pest is primarily controlled by the use chemicals that indirectly cause water pollution in the rivers that terminate in the Caspian Sea, causing environmental damage in the region. Observations on the pest behaviour show intraspecific variation in the damage to rice in different geographic locations. It is expected that the recognition of intraspecific variation, its nature and scope, may aid our understanding of the pest and allow us to predict the spatial and temporal occurrence of its problems, to devise effective management strategies, and to characterise RSB population responses to control measures. The purpose of this study is to estimate the relative importance of genetic and environmental (host plant) effects on the morphological characters of RSB in Iran.

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 compari-
son of shape similarities between conspecific populations (ADAMS & FUNK 1997, PAVLINOV
2001, ROHLF 1996, www.life.bio.sunysb.edu/morph). To gain information on intraspecific
variation in the RSB, this study searched for significant differences among populations of the
pest from six geographic locations using geometric morphometric techniques.

Material and methods

Collection of Specimens. Adult moths were collected during summer 2001 using light traps in
six regions spanning northern Iran: Amol (AML, N=70) and Tonekabon (TNK, N=45) in Mazan-
dran province, and Anzali (ANZ, N=100), Dehshal (DHS, N=100), Kiashahr (EDK, N=100) and
Rasht (RST, N=100) in Guilan province. From each population 30 individuals of each sex were
collected for preliminary analysis. Owing to the rarity of males, only females were examined
from some populations.

Data Collection. Morphological characters were selected using a binocular dissecting micro-
scope. These were landmarks easily definable as homologous between forms that served as the
reference points for measurements, which could then be measured both accurately and consis-
tently within the depth of the field capabilities of the equipment available. Landmarks were meas-
ured from the right wing to avoid any problems due to asymmetry (FINK 1990). Both fore- and
hindwings were measured. Wings were first removed from the body and kept in alcohol until
processed. The scales on the wings were carefully removed with bleach. Both wings were placed
on a microscope slide and mounted in 2-3 drops of Canada balsam. All the selected morphologi-
cal structures were measured using the image analysis software, ASUS Live, which captures both
dimensions (x, y) of the forms under study. Characters to be measured were magnified to the
appropriate size with a dissecting microscope. Magnified images were recorded using a video
camera attached to the microscope and transferred to a video monitor linked to a digitizing card.
The raw morphological image was saved in bitmap format. Coordinates were taken from the
bitmap images using the digitizing program tpsDig (version 1.08). Fifteen and ten landmarks
were selected from the fore- and hindwings, respectively, for their capacity to define major ele-
ments of shape and for their reliability as homologous structures (Fig. 1a and 1b). A total of 311
fore- and 319 hindwing images respectively were analysed. The raw coordinate data were aligned
prior to analysis using the software package tpsRelw (version 1.15) to remove size and arbitrary
positioning effects of the specimens relative to the reference axis (ROHLF & MARCUS 1993).

An average shape or tangent configuration was computed as the average configuration of all
specimens. The specimens were aligned and scaled using superimposition methods. To make the
coordinate data interpretable, rotation, translation and scaling parameters were obtained to bring
all the specimens into a common coordinate system (ROHLF & MARCUS 1993). These parameters
were then used to superimpose the configurations. The rotational fitting options used were Gen-
eralized Least-Squares (GLS) *or* Generalized Resistant Fitting (GRF) (PAVLINOV 2001), depend-
ing upon the objective. These methods ensure that differences in the raw coordinates due to arbi-
trary positioning of the specimens to the reference axes of the digitizing device are corrected. All
landmarks were used to align the specimens according to a consensus configuration calculated by
the GLS fitting method. Consensus configurations of the fore- and hindwings were calculated for
all specimens using the superimposition method.

Variation between different groups of populations was analysed using tpsRelw or NTSYS-pc,
using partial warp scores for each specimen as variables in multivariate analyses of variance
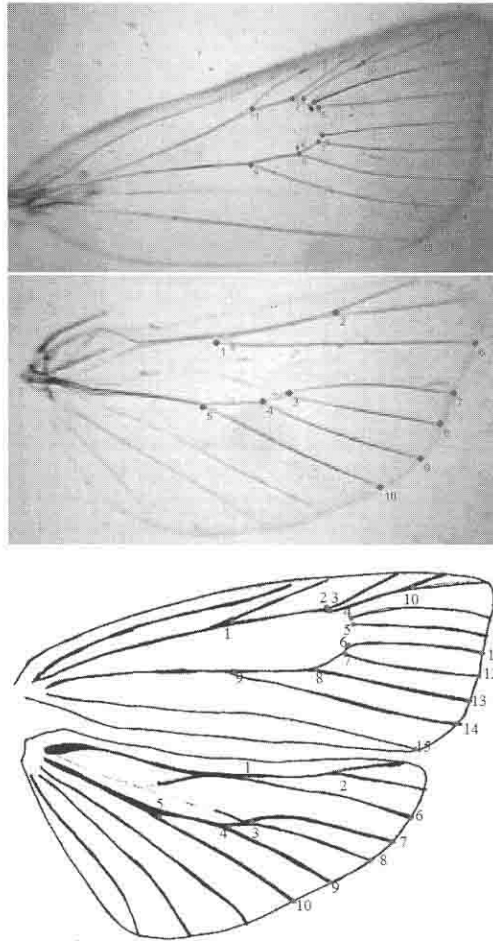(MANOVA). Differences between groups were examined using separate multivariate analyses.

Fig. 1. Selected landmarks on (a) the forewing and (b) the hindwing.

Analyses were also performed on males and females, together and separately, to establish any differences between the sexes. All these analyses assume that the data conform to a multivariate Gaussian model, normally distributed with homogeneous variances. However, the assumption of homoscedasticity has been found to be unrealistic when applied to biological data sets (LELE & RICHTSMEIRE 1991). The partial warps were illustrated graphically (tpsRelw and tpsRegr programs) as warping of the thin plate-spline grid (required to fit the test specimen to the starting form) or as vector diagrams, to assess shape differences at different geometric scales. TpsRegr was used to perform MANCOVAs of shape by regressing the partial warps (and uniform components if required) onto an independent variable (e.g. centroid size). ROHLF et al. (1996) ignore the heterogeneity of variance-covariance matrices in coordinate data taken from Old World Talpidae because of the small sample sizes relative to the number of variables. When small sample sizes are used, variation among variables may mask variation among groups. This study had similar

problems because the number of variables was large compared to the sample size, and minor deviations from normality may be expected.

Preliminary statistical analysis was carried out in SPSS (version 9) and SigmaStat (version 2) statistical software to examine visually the distributions of values and to test for normality (SARA-FRAZI 2001). Stem and leaf plots and boxplots were used to identify outliers that were editing mistakes. The outliers found were then checked against a geometric vector diagram and the specimen from which the data were collected, to assess the accuracy of the landmark positions. If the outliers were obviously mistakes then they were omitted from the analysis. Anderson-Darling, Ryan-Joiner and Kolmogorov-Smirnov tests were used to assess and test data for normality.

A total of 26 shape variables (the matrix of partial warp scores) from forewings and 16 weight matrixes from hindwings were observed for the calculation of TPS functions in geometric analysis. After preliminary statistical standard analyses, such as normality tests and conformation to the multivariate Gaussian model, the number of forewing specimens (311) was reduced to 276 and the number of hindwings (319) to 300. Conventional MANOVA, principal component analysis (PCA), discriminant function analysis (DFA; DFA is used to assign an observation to one of a set of groups [SLICE et al. 1996]), canonical discriminant analysis (CDA; CDA represents a broad class of methods for assigning unclassified objects/specimens to previously defined groups [SLICE et al. 1996]), canonical variate analysis (CVA; CVA is a method of multivariate analysis in which the variation among groups is measured relative to the pooled within-group covariance matrix [SLICE et al. 1996]), and cluster analysis (CA; CA helps separate multivariate data into a series of hierarchically related sets [SLICE et al. 1996]) were used. Tests were used on populations that had a normal distribution. All analyses were carried out with the statistical packages STATGRAPH-ICS (version 5.1), MINITAB (version 11) and NTSYS-pc (version 2.02). Relative warp analysis (RWA; RWA represents principal components of shapes in space tangent to Kendall's shape space [SLICE et al. 1996]) was carried out to test for sexual dimorphism among RSB wing shapes.

Results

Males and females showed significant sexual dimorphism that involved multiple aspects of shape. PCA of forewings demonstrated nine principal components (PCs) with eigenvalues greater than 1%. The first principal component, PC1, accounted for 13.9% of variability and the first nine accounted for 68.83%. PCA of hindwings gave six PCs with eigenvalues greater than 1%. Here, PC1 accounted for 19.04% of variability and the first six accounted for 66.06%. RWA of fore- and hindwings also demonstrated significant sexual dimorphism in the wing shape of RSB moths (Fig. 2). The effect is seen more strongly in the forewings than in the hindwings (not shown).

DFA showed that four of the six discriminant functions were statistically significant at the 95% confidence level. DFA of forewings differentiated geographic populations in 53.3% of cases, i.e. among the 276 forewings, 147 specimens were placed correctly in one of the six geographic regions. For hindwings, DFA showed that the six populations were differentiated in 43% of cases, i.e. among 300 individual hindwings, 129 specimens were placed correctly. The morphological distances among forewings indicated that the eastern populations (Mazandran province) are more distant from western populations (Guilan province) and the nearest populations in Guilan province (DHL and EDK) had the least morphological distances (Tab. 1). The greatest morphological distance (6.142) was recorded between the TNK (36°49'N, 50°52'E) and EDK populations.

Given the substantial sexual dimorphism, CDA was carried out on females and males

Tab. 1. Morphological distances between geographic populations of Rice Stem Borer using weight matrix for fore wings (upper triangle) and hind wings (lower triangle) landmarks (for population codes see text).

Population	TNK	RST	EDK	DHS	ANZ	AML
AML	3.905	5.178	4.833	4.777	4.261	-
ANZ	4.933	6.001	4.207	3.995	-	4.261
DHS	4.780	3.283	1.267	-	2.103	2.826
EDK	6.142	3.804	-	0.855	2.318	2.103
RST	5.140	-	1.395	1.318	1.407	2.871
TNK	-	4.470	3.290	3.444	4.933	3.159

Fig. 2. RWA of forewings of Rice Stem Borer demonstrating sexual dimorphism in wing shape. When individuals move toward female shape (1-175) in the left side grid insert, the wings elongate, but when individuals move toward male shape, the wing shape widens (in males, 176-311) in the right side grid insert. Relative warps 1 and 2 are plotted here showing individual positions on the two axes.

separately, both on forewings and hindwings. The CDA scatter plots of female forewings imply that the Mazandran populations (AML and TNK, less than 130 km apart) are very similar, that the western populations (Guilan province) are most dissimilar from eastern populations, and that the closest populations (EDK and DHS, less than 20 km apart) very distinctively overlap each other in similarity (Fig. 3). The ANZ population was clearly differentiated from the other populations for female forewings. The CDA results for male
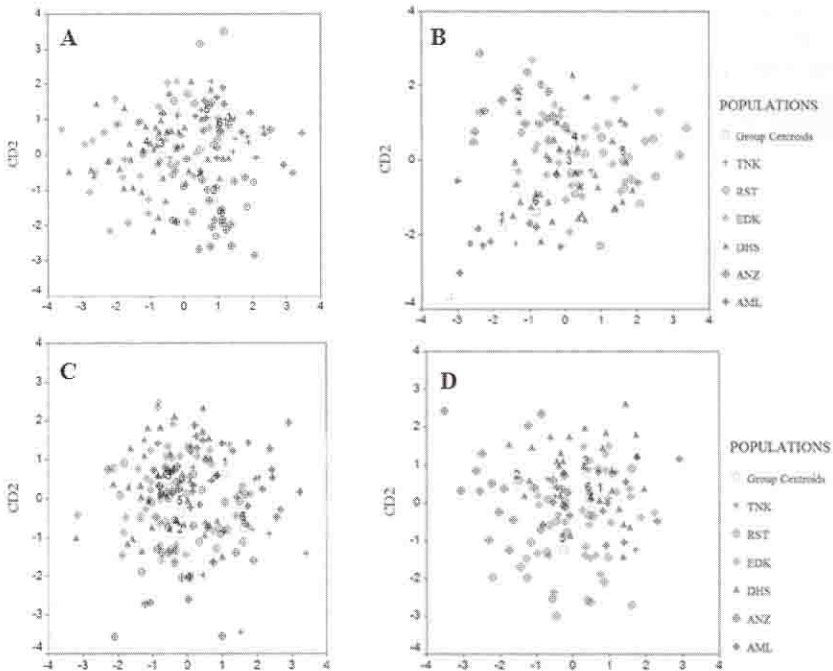
Fig. 3. Plot of CDA of fore and hindwings separately (for codes, see text). CDA scatter plots of (A) female forewings, (B) male forewings, (C) female hindwings, (D) male hindwings.

forewings showed similar results as for female forewings. The CDA results for female hind-wings were very similar to those for the forewings, namely that the ANZ population was the most differentiated of the five populations. The results from male hindwings illustrate that the ANZ and also RST populations are distinct from the other four populations (Fig. 3A-D).

The greatest morphological differences are 6.142 in forewings between populations TNK and EDK from Mazandran and Guilan provinces respectively (Tab. 1, upper triangle), and 4.933 in hindwings TNK and ANZ from Mazandran and Guilan provinces respectively (Tab. 1, lower triangle). The smallest morphological distances are represented by the same popula-tions (DHS and EDK) in fore- and hindwings (Tab. 1). These distances in general are consis-tent with the geographic isolation of the populations.

Results of the cluster analysis of forewings gave the same general results as DFA and CDA. Eastern female populations were placed in upper branches and western populations placed in lower branches (Fig. 4). However, males were not differentiated as clearly as fe-males probably because of the small number of TNK population specimens (n=3).

The CA of hindwings did not show as clear a separation as forewings. The geographically nearest populations (EDK and DHS), with less than 20 km separation, were placed closest together in all analyses. CVA of forewings in the eastern and western populations, in which CV1 is plotted against CV2, showed significant differences between populations (Figs. 5a, 5b).
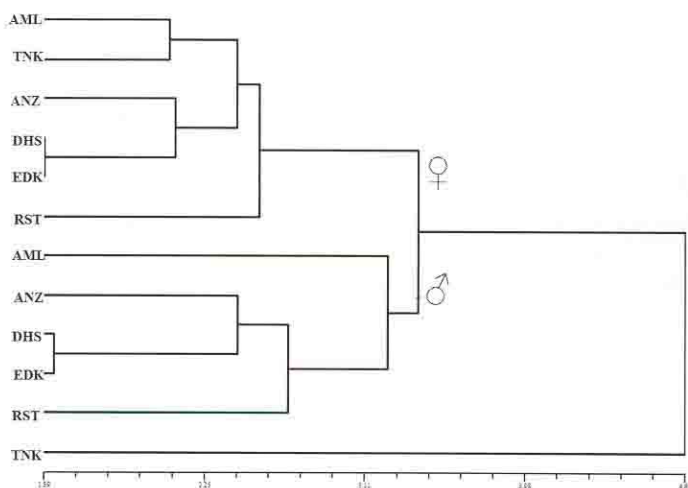
Fig. 4. Cluster Analysis of Rice Stem Borer forewings in Mazandran and Guilan populations
(for codes, see text).

Equivalent analyses of the hindwings did not separate the populations as clearly, but the results for the two Guilan populations (DHS and EDK) were the same for both fore- and hindwings. MANOVA tests showed there were significant differences between populations in both fore- and hindwings.

Discussion

The multivariate analyses of partial-warp scores of Rice Stem Borer (RSB) wing shapes demonstrated a significant difference among geographic populations. The alternative pheno-types may have allowed RSB 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 geo-graphic 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 ex-tensive and include geographic distance, dispersal capability, ecological specialization, phenological isolation, habitat patchiness, and the frequency and nature of extinc-tion/recolonization events.

The two main factors which may account for the observed variation of RSB are geo-graphic differences in habitats and different host races of rice. A consideration of the several rice cultivars commonly planted in the two main rice growing areas, i.e. Mazandran and Guilan, and the different geographic conditions of these two localities could in part explain the observed variation within and among the populations under study. For example, in Gui-lan cultivars Hashemi, Alikazemi, Binam, Tarom and Khazar are mostly planted, whereas in Mazandran the usual cultivars are Fajr, Sahel, Neda, Nemat and Tarom. Only the Tarom rice cultivar is common to both areas.
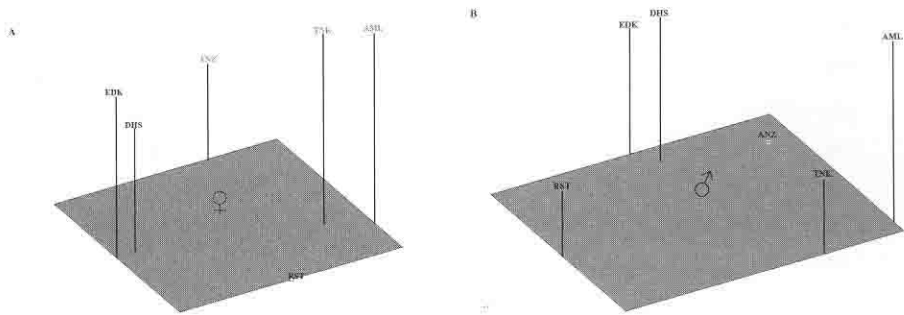
Fig. 5. CVA of forewings of Rice Stem Borer; (A) female (B) male (for code, see text).

A traditional morphometric study on numerous samples of aphids of the *Myzus persicae* group from different host-plants on four continents showed that aphids originating from Tobacco could be distinguished using a multivariate approach (BLACKMAN 1987). BLACK-MAN suggested that the different morphological traits of the Tobacco form were well stabilized and under polygenic control. A study of population variation in Sunn Pest, *Eurygaster integriceps*, using multivariate analysis of host plant-associated populations, revealed significant morphological differences between insects from wheat and barley, in both males and females (SARAFRAZI 2001). Likewise, a study of the aphid, *Aphis gossypii*, in Australia showed that it was phenotypically plastic and that its morphology is affected far more strongly by its host plant than by genetic differences among local populations (WOOL & HALES 1997).

Considering these two factors (geographic conditions and the cultivars), the phenotype of each individual could be the result of an interaction between its genotype and its environment. The geographic and climatic conditions in the two provinces are different: for example, the annual rainfall in Guilan is 1036 mm, while in Mazandran it is only 723 mm. Geographic conditions interact with the phenotype and genotype of organisms (ARMBRUSTER et al. 2001). These authors demonstrated that populations of the Pitcher-Plant Mosquito, *Wyeomyia smithii*, from northern latitudes (49°N) produced larger eggs than populations from southern (30°N) latitudes. Indeed, geographic variation of species seems to be the rule rather than the exception and it can be maintained, for example, by non-overlapping distributions of an insect species and all of its host plants, by genetic variation in insects or host plants, or a geographically variable natural enemy fauna (BALLABENI et al. 2003).

CDA analysis of female forewings provides discriminant measures which imply that the Mazandran populations (AML; 36°28'N, 52°28'E and TNK; 36°49'N, 50°52'E) are hard to differentiate, while western populations (Guilan province) are easily differentiable from eastern populations, and that the closest populations (EDK and DHS) are overlapping and most difficult to discriminate from one another. The ANZ population was clearly distinct from the others based on female forewings. The CDA results based on male forewings showed the same results, the two most similar populations being EDK and DHS (with geographic coordinates 37°23'N, 50°04'E and 37°19'N, 50°02'E, respectively). Mazandran populations were differentiated from Guilan populations, and the ANZ population was again very distinct. Documentation on other insects shows contrasting results, for example a Clus-

ter Analysis (CA) of the moth, *Lymantria dispar,* carried out by HILLIS et al. (1995) showed that North American populations clustered together irrespective of where or when they were collected. These authors also showed that contiguous populations within and between adjacent years retain similarity within that pool of populations, whether from North America, Europe (Slovakia and Austria) or Asia.

Dispersal of RSB could happen in two ways, first by dispersal of adults from one location to another, or in rice chaff, which is a commonly used livestock feed and is frequently transported from one locality to another. RSB hibernates in rice stems, and so the latter mechanism would strongly assist the pest to move among locations based on cultural and commercial patterns of distribution of the chaff product.

The CDA on female hindwings produced results very similar to those on forewings. The ANZ population is most differentiated from the remaining five populations. The results for male hindwings also illustrate that both the ANZ and the RST ($37°13'N$, $49°38'E$) populations are distinctly separated from the other four populations, and other methods show the same pattern.

The geographically most distant populations are also the most morphologically distant. The scatter plots of CVA in RSB illustrated that the greatest morphological distance was observed between the population TNK, the first to be reported in Iran, and the other more distant populations from Guilan and Mazandran provinces. In the forewings the greatest morphological distance (6.142) was recorded between TNK ($36°49'N$, $50°52'E$) and EDK ($37°23'N$, $50°04'E$) populations and in hindwings the greatest distance (4.933) was between TNK and ANZ ($37°28'N$, $49°27'E$). Natural selection, working through temperature, latitude and longitude, altitude, humidity and other environmental conditions, could also influence the evolution of this trait over the climatic gradient in northwestern Iran. MERRILL & DENNO (1998) illustrated that isolation by distance (IBD) slopes did not vary with mobility, but that intercepts increased with mobility, and, alternatively, that IBD slopes varied with dispersal ability. They also found that from tens of kilometres to more than 1,000 km, IBD is weak in sedentary and highly mobile species, but pronounced in moderately mobile species. Developmental instability in the face of environmental change may lead to alternative phenotypes in alternative environments and may give the appearance of adaptiveness (SPITZE 1996). However, only if at least some of the adapted alternative phenotypes can be demonstrated to have enhanced fitness in their respective environments can such plasticity be termed adaptive phenotypic plasticity (SPITZE 1996).

The population differences in RSB in this study were based on wing shape in both sexes. Defining appropriate morphological characters in Lepidoptera, COMSTOCK (1893) illustrated the importance of the wings for taxonomic purposes. Investigations of insect wings is of current interest in studying shape change and the ability to discriminate shape differences between the sexes, populations and species (ZHOU et al. 1985, KUNKEL 2001).

This study also demonstrated the capacity of thin-plate spline (TPS) analysis (shape analysis) to characterise morphological variation between the sexes and detect subtle morphological evidence of biological species that may have eluded the biologist relying on meristic characters (ADAMS & FUNK 1997). Study of phenotypic plasticity using multivariate analysis in the Australian cotton aphid also showed some evidence indicating that geographic samples of *Aphis gossypii* may differ (genetically) in shape rather than in size (WOOL & HALES 1997). The results of TPS analysis also showed there was a significant difference between males and females of RSB. The results of RWA of fore- and hindwings were the same and indicated that when individuals moved toward females along the RW1, the wing shape is transformed into elongation and enlargement but when moved away from the female, the

wing shape transformed toward widening (in males). It also shows in the hind wings in which, when moved in direction of female shape in thin plate-splines (TPS), the intersection landmarks moved toward the interior and resulted in elongation, but in the opposite direction the wing shape moved to widen the wings in males. Comparison of centroid sizes showed that RSB females are significantly larger than males. Such female-based sexual size dimorphism is commonly observed in invertebrate taxa (ADAMS & FUNK 1997). Sexual dimorphism in shape attributable to relatively larger female abdomens could be the result of selection on increased female fecundity (ADAMS & FUNK 1997). ADAMS & FUNK (1997) found similar results in a chrysomelid leaf beetle, *Neochlamisus bebbianae*, in parts of North America.

The results of this study provide evidence for the causes of shape 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). An understanding of evolutionary change occurring in agricultural systems could facilitate the formulation and implementation of innovative strategies of pest management (GOULD 1988). If ecologically important characters are genetically variable within populations, they will generally evolve in a way that increases the average fitness of individuals and the mean phenotype will change over time (VIA 1990). Moreover, to understand the process of evolution, it is important to go beyond the simple documentation of genetic variability (for example, the identification of biotypes) to the estimation of the magnitude of genetic variation within and between populations (DIEHL & BUSH 1984).

This TPS analysis provides evidence of phenetic differentiation among RSB populations. However, host-association studies using cross-infection rice cultivars with RSB populations would be necessary to assess the correlation of observed population shapes with the different RSB populations versus the host rice cultivars, to evaluate natural enemy parasitism on local populations, and to investigate the probable pheromone races of RSB populations. Since the enigma of genetic diversity and genome-phenome organisation and evolution in nature has been fruitfully explored by using modern molecular techniques (NEVO 2001), molecular work now needs to be done to confirm and add to the results obtained here.

References

ADAMS, D. C. & D. J. FUNK (1997): Morphometric inferences on sibling species and sexual dimorphism in *Neochlamisus bebbianae* leaf beetles: multivariate applications of the thin-plate spline. – Systematic Biology 46: 180–194.

ARMBRUSTER, P., W. E. BRADSHAW, K. RUEGG & M. HOLZAPFEL (2001): Geographic variation and the evolution of reproductive allocation in the pitcher-plant mosquito, *Wyeomyia smithi*. – Evolution 55: 439–444.

BALLABENI, P., K. GOTTHARD, A. KAYUMBA & M. RAHIER (2003): Local adaptation and ecological genetics of host-plant specialization in a leaf beetle. – Oikos 101: 70–78.

BLACKMAN, R. L. (1987): Morphological discrimination of a tobacco-feeding form from *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (*Nectarosiphon*) species. – Bulletin of Entomological Research 77: 713–730.

COMSTOCK, J. H. (1893): Evolution and Taxonomy. An essay on the application of the theory of natural selection in the classification of animals and plants, illustrated by a study of the evolution of the wings of insects. – Extract from The Wilder Quarter-Century Book, Ithaca, NY.

DIEHL, S. R. & G. L. BUSH (1984): An evolutionary and applied perspective of insect biotypes. – Annual Review of Entomology 29: 471–504.

EBERT, G. (1972): Der Reisstengelbohrer *Chilo suppressalis* Walker (Lep.: Pyralidae), ein für Iran neuer Schädling [in German and Farsi]. – Entomologie et Phytopathologie Appliquées [Applied Entomology and Phytopathology] 35: 1–25.

FINK, W. L. (1990): Data acquisition for morphometric analysis in systematic biology. p. 9–19. In: J. F. ROHLF & F. L. BOOKSTEIN (Eds.), Proceedings of the Michigan Morphometrics Workshop. – Special Publication No. 2, Museum of Zoology, University of Michigan.

GOULD, F. (1988): Genetic engineering, integrated pest management and the evolution of pests. – Trends in Ecology and Evolution 3: 15–18.

HILLIS, D. M., C. MORITZ & J. G. KUNKEL (1995): Clusters and discrimination of gypsy moth populations based on wing venation. – www.bio.umass.edu/biology/kunkel/lymantria_disc.html.

KUNKEL, J. G. (2001): Shape Changes in Biology, D'Arcy Thompson's classic fish transformation. – www.bio.umass.edu/biology/kunkel.

LELE, S. & J. T. RICHTSMEIER (1991): Euclidean distance matrix analysis: A coordinate free approach for comparing biological shapes using landmark data. – American Journal of Physical Anthropology 86: 415–428.

MERRILL, A. P. & R. F. DENNO (1998): The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. – The American Naturalist 152: 428–446.

MORPHOMETRICS SUNY STONY BROOK (2002): Introduction to multivariate statistics and geometrics morphometrics. – www.life.bio.sunysb.edu/morph.

NEVO, E. (2001): Evolution of genome-phenome diversity under environmental stress. – www.pnas.org/cgi/doi/10.1073/pnas.101109298.

PAVLINOV, I. Y. (2001): Geometric morphometrics, a new analytical approach to comparison of digitized images. – Zoological Journal of Moscow 79: 1–27.

ROHLF, F. J. (1990): Morphometrics. – Annual Review of Ecology, Evolution and Systematics 21: 299–316.

ROHLF, F. J., A. LOY & M. CORTI (1996): Morphometric analysis of Old World Talpidae (Mammalia, Insectivora) using partial-warp scores. – Systematic Biology 45: 344–362.

ROHLF, F. J. & L. F. MARCUS (1993): A revolution in morphometrics. – Trends in Ecology and Evolution 3: 129–132.

SARAFRAZI, A. M. (2001): Molecular and morphological structures and host associations of the Iranian Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutelleridae) populations. – Ph.D Thesis at University of Wales, Cardiff.

SLICE, D. E., F. L. BOOKSTEIN, L. F. MARCUS & F. J. ROHLF (1996): A Glossary for geometric morphometrics. – http://life.bio.sunysb.edu/morph.

SPITZE, K. & T. D. SADLER (1996): Evolution of a generalist genotype: multivariate analysis of the adaptiveness of phenotypic plasticity. – American Naturalist 148:108–123.

VIA, S. (1990): Ecological genetics and host adaption in herbivorous insects: The experimental study of evolution in natural and agricultural systems. – Annual Review of Entomology 35: 421-446.

WOOL, D. & D. HALES (1997): Phenotypic plasticity in Australian cotton aphid (Homoptera: Aphididae): Host plant effects on morphological variation. – Annals of the Entomological Society of America 90: 316–328.

ZHOU, Y. H., L. B. LING & F. J. ROHLF (1985): Automatic description of the venation of mosquito wings from digitized images. – Systematic Zoology 34: 346–358.

Authors' addresses: Reza Zahiri and Alimorad Sarafrazi, Insect Taxonomy Research Department (ITRD), Plant Pests and Diseases Research Institute (PPDRI), P.O. Box 1454, Tehran 19395, Iran. – Latif Salehi, Department of Plant Protection, University of Guilan, Rasht 3179, Iran. – Joseph G. Kunkel, Department of Biology, University of Massachusetts at Amherst, MA 01003 USA. – Email contact: zahiri@ppdri.ac.ir.