

Research Article

A geometric morphometric study on geographical populations of *Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae) in some parts of Iran

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Abstract: Spotted Amber Ladybird, *Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae) is an important predator of aphids and some other insect pests. Since shape and size variation may affect biological characteristics of an insect, multivariate analysis of shape variations in four geographically isolated populations of *H. variegata* (Isfahan, Shahrekord, Shiraz, Yasuj) was performed based on eight landmarks of hind wing. According to the analysis, significant difference in weight matrices (as shape variable) was detected. The cluster analysis separated Isfahan population from other populations. According to ANOVA, we found significant difference in centroid size (as size variable), so that the two populations, Isfahan and Shahrekord, had larger wings than the two other populations. Distribution of 80 individuals based on partial warps on the relative warp axis placed Isfahan population in a separate group while the others had overlap and irregular distribution.

Keywords: Biological control, geometric morphometric, *Hippodamia variegata*, population

Introduction

The variegated ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is an Old World ladybird, occurring in the Palaearctic region including Iran. There is a wide variation in color patterns of *H. variegata* (Dobzhansky, 1933; Hodek, 1973) such that this species is now represented by many morphs in Iran (Biranvand *et al.*, 2013). Both the larvae and adults of *H. variegata* are important predators of aphids and some other plant pests in both sexes, and the females oviposit where aphids are abundant (Obrycki and Orr, 1990). Like most members of Coccinellidae, adult *H.*

variegata are able to exploit habitats where the prey exists and move on to new unexploited patches when local prey abundance dwindles (Obrycki and Orr, 1990). *H. variegata* has a wide distribution in different regions and like any animal species, geographical distances can affect its population structuring. Geographical distances lead to a decrease in gene flow between isolated populations, thus may underlie the process of population divergence and subsequent reproductive isolation and speciation (Menken and Rajiman, 1996). Beside genetic and reproductive properties, the morphological traits are also expected to differentially evolve in the isolated populations. Geometric morphometric take into account the geometry of configurations, provide a more detailed and precise information on small morphological differences among isolated populations (Rohlf and Marcus, 1993). These

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differences may affect biology of insects and the strategies followed by growers for biocontrol programs (Claridge *et al.*, 1997). There are a lot of reports dealing with detailed morphological differences among different populations of species in the order Coleoptera according to geometric morphometric method. For example, in *Phyllophaga hirticula* (Knoch) male and female genitalia were shown to be significantly more variable than the right elytron. female genitalia was significantly more variable than male genitalia (Polihronakis, 2006). The populations of *Erodiontes pfaundleri* (Schuster) had significant differences in pronotum size while populations of *E. aelleni* (Kaszab) were similar in pronotum size (Taravati *et al.* 2009). Adams and Funk (1997) found a remarkable shape variation between sexes and sympatric populations of Leaf beetle, *Neochlamisus bebbianae* (Brown) feeding on different host plants. Tatsuta *et al.* (2004) provided evidence for sexual dimorphism in size and shape in *Prosopocoilus inclinatus* (Motschulsky). More studies have investigated geometric morphometrics in many insect species (Kunkel, 1995; Adsavakulehai *et al.*, 1998; Haas and Tolley, 1998; Hoffman and Shirrifs, 2002; Sarafrazi *et al.*, 2004; Schachter-Broide *et al.*, 2004, Zahiri *et al.*, 2006; Mozaffarian *et al.*, 2007; Sadeghi *et al.*, 2009; Khiaban *et al.*, 2010; Fontoura and Morais, 2011; Lashkari *et al.*, 2013)

The Palearctic coccinellid species *H. variegata* is a widespread aphidophagous predator in many countries of the world (Natskova, 1973; Hamed *et al.*, 1975; Belikova and Kosaev, 1985; Gumovskaya, 1985; Nicoli *et al.*, 1995; Hodek and Honek, 1996). Also *H. variegata* is a widespread aphidophagous in Iran and the four provinces Isfahan, Chaharmahal-Bakhtiari, Fars, Kohgiluyeh-Boyerahmad (Fatemi, 1982; Ahmadi and Sarafrazi, 1993; Bagheri and Mossadegh, 1995; Saeedi, 1998) and is considered as an important agent in biological control of Aphids. Different geographical regions may cause morphological variations among their populations (Mayr and Ashlock, 1969). These morphological variations

also may affect some biological characteristics (Kim and McPherson, 1993) that might cause a considerable impact on biological control program strategies.

Therefore the objective of the current study was to discover the morphological variations in wing shape and size in populations of *H. variegata* collected from four provinces of Iran.

Materials and Methods

A total of 80 adult females (20 specimens per population) were collected during August-September 2013 by sweeping net in alfalfa fields of four provinces, Isfahan (Isfahan; N 32/74243°, E 51/65322°, 1570 m), Chaharmahal-Bakhtiari (Shahrekord; N 32/30754°, E 50/87521°, 2070 m), Fars (Shiraz; N 29/617248°, E 52/543423°, 1486 m) and Kohgiluyeh-Boyerahmad (Yasuj; N 30/75191°, E 51/47580°, 1870m).

To avoid any problems related to asymmetry (Fink, 1990), only hind wings of the right side of females were detached and used for slide preparation. Digital images of wings were taken under a dissecting microscope using a CCD video camera (Sony, Dinolite 2). A total of eight landmarks were selected (Fig. 1). These landmarks were easily definable as homologous between forms that served at the reference points for measurements, which could then be measured both accurately and consistently within the depth of the field capabilities of available equipment. The geometric coordinates of each landmark were then deduced using software tpsDig2 v2.16 (Rohlf, 2010a).

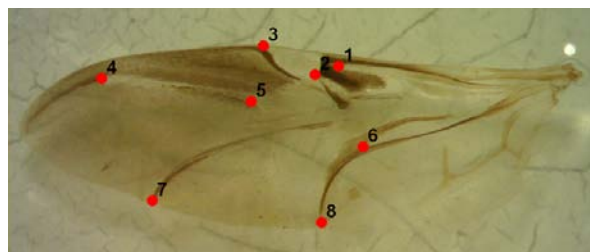


Figure 1 Distribution of eight landmarks in hind wing of *H. variegata*.

The raw coordinate data were aligned prior to analysis, then converted to shape variables (partial warps) using the software package tpsRelw v1.49 (Rohlf, 2010b). The Relative Warp Test was performed using tpsRelw software and the distribution of 80 individuals was determined on the basis of wing shape variables. Singular values analysis was performed for relative warp. The wings relative variations were determined using software tpsRegr v1.38 (Rohlf, 2011).

Weight matrices of partial warps were used for the cluster analysis using IBM SPSS Statistics software (version 19) and weight matrices with centroid sizes were used for the multivariate analysis. Comparison of wing size among different populations was also carried out.

Results

The Principal Components Analysis (PCA) showed shape variations in hind wing. The first component (PC1) had the most contribution (52.36%) in describing the variations (Table 1). Multivariate analysis of shape showed significant difference of weight matrices among the populations (Table 2). According to cluster analysis, the Isfahan population showed the most shape difference with others (Fig. 2). Results of the cluster analysis showed two clusters with the Isfahan population in one cluster and the others in the second one (Fig. 2). Distribution of 80 individuals based on partial warps on the RW axis based on the landmarks of hind wing, placed the Isfahan population in a separate group (Fig. 3) while the others had irregular distribution. The relative variations of wing were detected using the geometry coordinate of landmarks (Fig. 4). The positive extreme of wing shape variation along RW1 axis was more than negative extreme. In positive direct, apical lower part of wing was stretched toward inside and wing was smaller and was tucked and the landmarks 5 and 7 were close to each other. In negative direct, apical lower

part of wing was stretched toward outside and wing was stretched and the landmarks 5 and 7 were away from each other. It can be stated that the lower apical part of the wing is more variable than other parts and landmarks 5 and 7 contribute in describing the variation among populations. Analysis of variance of centroid size revealed significant differences among the populations (Table 3), so that according to Duncan test the Isfahan and Shahrekord populations were located in a unit statistical group and they had larger wing than Shiraz and Yasuj populations (Fig. 5).

Table 1 Principal Component Analysis of wing shape in four populations (Isfahan, Shahrekord, Yasuj and Shiraz) of *H. variegata*. SV: source of variations, Cum: cumulative percent.

Component	SOV	Variance (%)	Cum (%)
1	0.4832	52.36	52.36
2	0.26237	15.44	67.79
3	0.1989	8.87	76.67
4	0.17237	6.66	83.33
5	0.15268	5.23	88.56
6	0.12198	3.34	91.89
7	0.11277	2.85	94.75
8	0.10268	2.36	97.11
9	0.06324	0.90	98.01
10	0.05678	0.72	98.73
11	0.05352	0.64	99.37
12	0.05292	0.63	100

SOV: Source of variations, Cum: Cumulative variance.

Table 2 Mutivariate analysis of weight matrices in wing shape of four populations (Isfahan, Shahrekord, Yasuj and Shiraz) of *H. variegata*.

Entries	Wilks' Lambda	Fs	Hypothesis df	Error df	p-value
Weight matrices	0.402	1.935	36	192.77	0.002

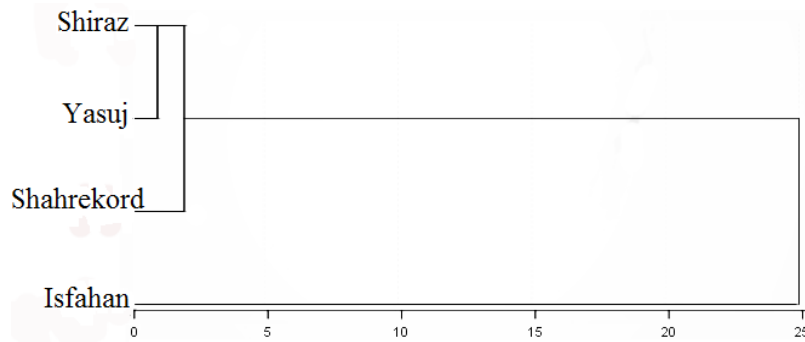


Figure 2 Dendrogram plotted by UPGMA method based on clustering of the generalized distance matrix of *H. variegata* populations

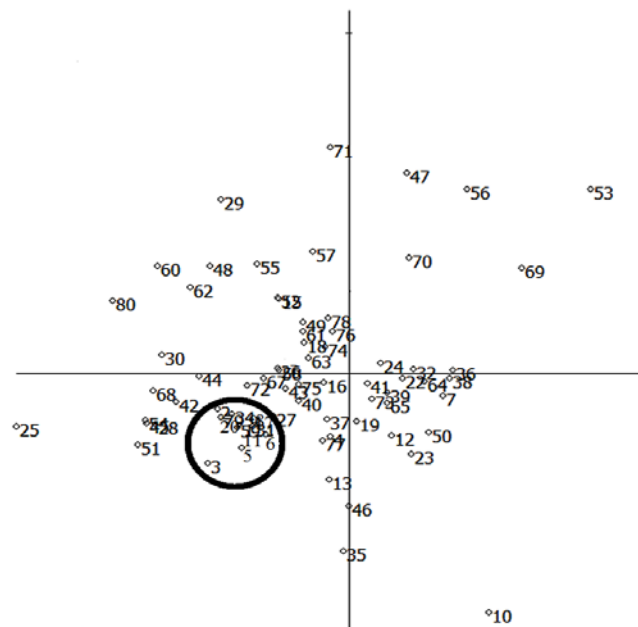


Figure 3 Distribution of *H. variegata* populations through RW1 and RW2 based on the landmarks, 1-20: Isfahan population, 21-40: Shahrekord population, 41-60: Shiraz population, 61-80: Yasuj population. Circle shows the Isfahan population.

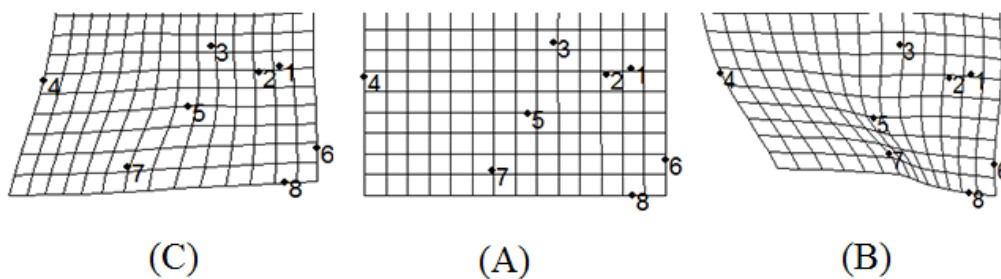


Figure 4 Wings relative variations in positive direct (B) and negative direct (C) compared to reference shape (A). Numbers show the landmarks.

Table 3 Analysis of variance of centroid size in wing size of four populations (Isfahan, Shahrekord, Yasuj and Shiraz) of *H. variegata*.

Source of variation	Mean Square	df	F	P-value
Centroid size	12182.164	3	8.883	0.0001
Error	1367.991	76		

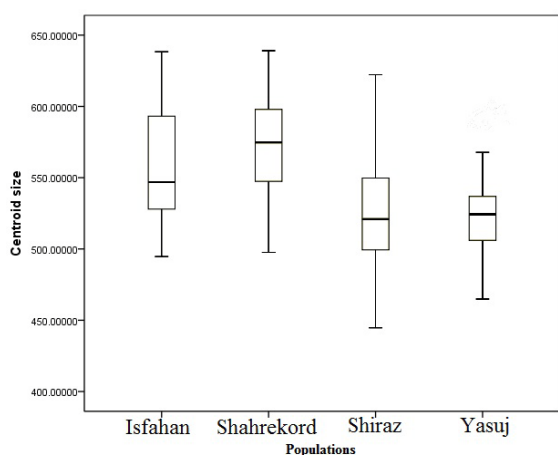


Figure 5 Boxplots of size for hind wing among the populations of *H. variegata*;

Discussion

The populations of different regions are expected to show different morphological, behavioral and genetic traits due to the reduced gene flow and differential evolutionary dynamics they experience as a result of geographical isolation (Menken and Rajiman 1996). In this study, a geometric morphometric method was used to evaluate the variations in size and shape of hind wing in four geographically isolated populations of *H. variegata*. The Multivariate analysis of wing shape demonstrated a significant difference among geographic populations. Other authors also found significant differences among their insect populations based on the landmarks of their wings in host associated populations of Carob moth *Ectomyelois ceratonia* (Zeller) (Mozaffarian et al., 2007), Pod Borer *Helicoverpa armigera* (Hubner) (Khiaban et al., 2010), Sunn pest *Eurygaster integriceps* Putton

(Sarafrazi et al., 2004), domestic and non-domestic populations of *Triatoma infestans* (Klug) (Schachter-broide et al., 2004), geographical populations of Rice Stem Borer, *Chilo suppressalis* Walker (Zahiri et al., 2006), Asian citrus psyllid, *Diaphorina citri* Kuwayama (Lashkari et al., 2013), *Drosophila lummei* Hackman (Haas and Tolley, 1998), *Drosophila serrata* Malloch (Hoffman and Shirrifs, 2002) and *Calopteryx splendens* (Harris, 1782) (Sadeghi et al., 2009).

According to the wing relative variations, it seems that the lower apical part of the wing is more variable than other parts of the wing and has a more important role in population differentiation (Fig. 5). Our results are in line with Lashkari et al. (2013). Differences among the studied populations suggest that wing characteristics could be valuable in the preliminary assessment of population structure in Iran. Like any other species, the distribution of *H. variegata* is not uniform in a geographic range. Even, laboratory-reared populations of *H. variegata* show molecular differences compared with natural populations (Krafsur et al., 1996). *H. variegata* like other lady beetles is very sensitive to changes in the environment (Iperti, 1999) which can be one of the reasons for the results of this study (existence of difference and wing variations among populations). Different temperature, altitude, moisture and such environmental factors could affect the population morphs. Results of the cluster analysis showed that the Isfahan population was separated from the others. It could be suggested that Isfahan population had the least gene flow with the others because of being located between central mountains and eastern Zagros (Rokh and Dena mountains) as geographical barriers, planting different varieties of alfalfa and different climatic conditions.

The results of this study provide evidence for wing shape variation of *H. variegata* in different agricultural ecosystems which could result from genetic and behavioral changes in this ladybird (Mayr and Ashlock, 1969). According to importance of *H. variegata* in biological control, findings of this study (wing variations, proof of

difference among the populations and relationship among the populations) can affect directly augmentation and conservation in biological control programs. Actually, these morphological variations can imply some biological differences (Kim and McPherson, 1993) that in this case, biological differences may appear in some biological characters such as search rate, hunting power, distribution, attack rate etc. which could have a considerable impact on biological control program. Molecular and behavioral studies will be needed as supplementary studies to verify and confirm these findings.

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مطالعه ژئومتریکی مورفومتریکی جمعیت‌های جغرافیایی کفشدوزک *Hippodamia variegata* (Coleoptera: Coccinellidae) در برخی مناطق ایران

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چکیده: کفشدوزک کهربایی، (*Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae)) شکارگر مهم شته‌ها و برخی آفات دیگر گیاهی است. از آنجایی که تنوع شکل و اندازه ممکن است روی خصوصیات بیولوژیکی تأثیر بگذارد، تنوع شکل و اندازه بین جمعیت‌های *H. variegata* (به‌عنوان عامل مهم در کنترل بیولوژیک) میتواند بر روی برنامه‌های کنترل بیولوژیک این گونه تأثیر بگذارد. آزمون تجزیه واریانس چند متغیره شکل در چهار جمعیت جغرافیایی کفشدوزک *H. variegata* (اصفهان، شهرکرد، شیراز، یاسوج) براساس هشت لندمارک بال عقب انجام شد. براساس این آزمون اختلاف معنی‌دار ماتریس‌های وزنی متغیرهای شکلی بین جمعیت‌ها مشاهده شد. آزمون تجزیه خوشه‌های نشان داد جمعیت اصفهان از سایر جمعیت‌ها کاملاً جدا میشود. براساس آزمون تجزیه واریانس یکطرفه، اختلاف معنی‌داری در مجموع مربعات متغیرهای شکلی (به‌عنوان متغیر اندازه) بین جمعیت‌ها دیده شد، به‌گونه‌ای که جمعیت‌های اصفهان و شهرکرد بزرگتر از سایر جمعیت‌ها بودند. پراکنش ۸۰ فرد براساس متغیرهای شکلی روی محور RW نشان داد جمعیت اصفهان در گروهی مجزا نسبت به سایر جمعیت‌ها قرار می‌گیرد. درحالی‌که سایر جمعیت‌ها با یکدیگر همپوشانی داشته و پراکنش نامنظمی داشتند.

واژگان کلیدی: کنترل بیولوژیک، ژئومتریکی مورفومتریکی، *Hippodamia variegata*، جمعیت