DE GRUYTER

Biologia **70**/9: 1228—1233, 2015
Section Zoology
DOI: 10.1515/biolog-2015-0137

A uni- and multivariate analysis approach to reveal sexual size dimorphism in Iranian populations of *Hypera postica* (Coleoptera: Curculionidae)

Ehsan Sanaei[1], Marjan Seiedy[1]* & Farzaneh Momtazi[2]

[1]*School of Biology and Center of Excellence in Phylogeny of living organisms, College of Science, University of Tehran, Iran; e-mail: ehsansanai@ut.ac.ir, mseyyedi@ut.ac.ir*

[2]*Iranian National Institute for Oceanography and Atmospheric Science (INIOAS), Tehran, Iran; e-mail: Momtazi.f@inio.ac.ir*

Abstract: Body size dimorphism between genders is a commonly observed phenomenon in insects, usually manifested in larger female body size. Sexual Size Dimorphism (SSD) varies from species to species, the degree and direction influenced by certain evolutionary pressures. Intraspecific variation in SSD may also occur between populations. The *Hypera postica* (Gyllenhal, 1813) is a well-known alfalfa plant pest that shows a degree of morphological divergence in its populations. The female alfalfa weevils are very fecund and have a larger body size compared to males. To improve our knowledge on magnitude and direction of SSD in alfalfa weevil, we studied 200 specimens of *H. postica* from four Iranian populations (Karaj1, Karaj2, Tuyserkan and Jovein). 10 morphological variables from three external anatomic parts (pronotum, elytra and rostrum) and 45 ratio characters were statistically analyzed in order to determine the amount of SSD in Iranian populations. In addition we investigated for morphological divergence pattern in mentioned populations. The results of this study show that a low degree of morphological divergence occurs in Iranian populations. Measured variables indicate that the SSD pattern of *H. postica* is compatible with the Rensch's rule, and is related to high fecundity of females and a lack of strong sexual selection. Also it is mentioned that the larger rostrum in females may correspond to its unique role in egg laying.

Key words: sexual size dimorphism; alfalfa weevil; discriminant analysis; fecundity selection; population divergence; Rensch's rule

Introduction

Lucerne, also known as alfalfa *Medicago sativa* L. (Fabacea) is a valuable economic crop comprising around 2.5% of total world crop cultivation (Živković et al. 2012). *Hypera postica* (Gyllenhal, 1813), generally known by its common name 'Alfalfa weevil' is a worldwide alfalfa pest that has a wide distribution in the Palearctic and North America. Both larvae and adults cause serious damage to stems, shoots, crown shoots and foliage of alfalfa (Radcliffe & Flanders 1998). In Iran, more than 60% of cultivated alfalfa is affected by *H. postica* (Khanjani & Pourmirza 2004).

Alfalfa weevil, a species of Palearctic origin (Hsiao 1993) is very varied in morphological and biological characters. North American populations are some of the best known populations of this pest. Since it was first introduced into United States about one century ago, three molecular strains have been identified (Hsiao 1993), and one strain (Egyptian strains) was erroneously elevated to other *Hypera* species, *Hypera brunneipennis* (Boheman, 1834) (Bundy et al. 2005). Although the American alfalfa weevil populations are mainly distinguished using molecular and ecological characteristics (Kuwata et al. 2005), they show some variation and even separation in morphological characters (Pienkowski et al. 1969). Comparative study of different aspects of populations is crucial to the understanding of the biogeography of *H. postica*, and the similarities and differences between populations could be important for accurate in the pest management.

The Iranian plateau was one of the first regions were alfalfa was cultivated in human history (Moradi-Vajargah et al. 2011). Even though it is possible the existing of *H. postica* in Iran related to historical time, it had been reported in Iran for the first time in 1937 (Karimpour 1994) and is considered an important pest for modern agriculture.

Sexual Size Dimorphism (SSD) is a phenomenon that has appeared in most animals. The magnitude and direction of SSD show diversity within species and populations (Blanckenhorn et al. 2007; Blanckenhorn et al. 2006; Stillwell et al. 2010; Stillwell et al. 2007). The general pattern of SSD in insects is toward larger females

* Corresponding author

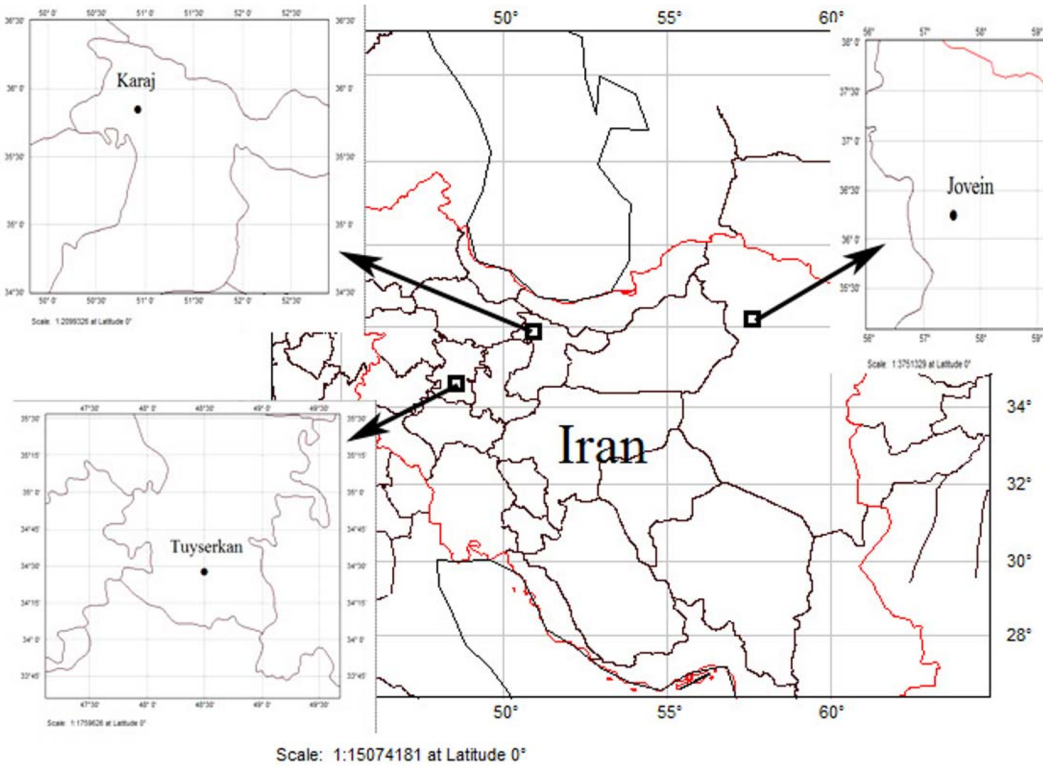

Fig. 1. Four stations: Karaj1 and Karaj2 (Alborz Province), Jovein (Razavi Khorasan Province) and Tuyserkan (Hamedan Province).

than males (Stillwell et al. 2014), and it is possible that different ecological conditions in different habitats influence development of sexes in each population or species (Stillwell et al. 2014). The first complete work on alfalfa weevil in America described males as being narrower than females (Titus 1909). Titus (1909) mentioned that alfalfa weevils vary from four millimeters long in the male and five in the female. There are some discriminative characters in alfalfa weevils for detecting sexes, like the degree of curvature of the last abdominal sternite in ventral view (Pienkowski et al. 1969), but the degree of SSD can be somewhat imprecise. Pienkowski et al. (1969) reported some differences in proportions of certain morphological characters (tibia, femur, elytra and pronotum) showing different sizes in males and females, and the degree of SSD differs again in American populations. Pienkowski et al. (1969) analyzed Duncan's multiple range test for 30 specimens of each sexes and significant differences between length of pronotum plus elytra, width of elytra, width of pronotum, length of front femur and wide of front femur among gender ($P < 0.005$) were found. Their investigation approved the Titus (1909) results about American populations and confirmed the direction of SSD towards larger females.

Awareness of population diversity and accurate identification of populations is a basic rule in designing efficient pest management (Menken & Ulenberg 1987). Iran is a vast country that contains various landscapes (Zehzad et al. 2002), and alfalfa is the most commonly grown forage crop of this region (Montazar & Sadeghi 2008). This crop has been cultivated in most areas, so a wide distribution for the alfalfa weevil was expected. Unfortunately, no previous comparative research has been carried out on the Iranian populations of *H. postica.* In this study, ten morphometric characters of pronotum, elytra and rostrum belonging to three Iranian populations of alfalfa weevil were measured and in addition, 45 ratios of metric characters were used in the dataset. The aim of this study is revealing magnitude and direction of SSD in Iranian populations of *H. postica* by precise statistical analysis. On the other hand, according to lack of information about Iranian populations of alfalfa weevil, we performed multivariate analysis in order to detect population divergence in morphological characters.

Material and methods

During spring and summer 2013, a total of 200 specimens were collected by sweep netting, from six alfalfa farms (Fig. 1). Stations were located in environment of three cities in the Northern Provinces of Iran: 1 – Karaj, alfalfa farms of University of Tehran (Alborz Province); 2 – Karaj Shahriar road (Alborz Province); 3 – Jovein (Razavi Khorasan Province) and 4 – Tuyserkan (Hamedan Province). Specimens of Jovein stations were gathered from two farms that are located close to each other, as same as in Tuyserkan stations. Information on coordinates and elevation of stations, number and gender of specimens are given in Table 1. Specimens were killed using potassium cyanide and mounted on triangular cards. The images of individual specimens were provided by using a stereomicroscope (Hund, Model: Wiloskope) equipped with digital camera (3 megapixel resolution). Three main morphological characters of *H. postica* were chosen for taking pictures in dorsal view; pronotum, elytra and rostrum with magnification 2.5×, 1.5× and 2×,

Table 1. Information on stations: Location, coordinates and elevation, dates of collecting and number of males, females and total specimens were given. Specimens from station 3 and 4 collected from two nearby farms.

Station	Location	Coordinates	Elevation (m a.s.l.)	Collecting date	Males (*n*)	Females (*n*)	Total (*n*)
1	Karaj	35°48′04.6″N 50°57′39.6″E	1315	3 June 2013	17	31	48
2	Karaj	35°41′36.5″N 51°09′29.3″E	1193	3 June 2013	21	36	57
3	Jovein	35°38′10.3″N 57°24′14.2″E	1140	3 Aug. 2013	7	8	15
		36°41′30.4″N 57°20′56.5″E	1103		4	13	17
4	Tuyserkan	34°31′16.2″N 48°18′43.4″E	1657	14 Aug. 2013	6	7	13
		36°26′02.8″N 48°40′31.7″E	1712		29	21	50
	Total				84	116	200

Table 2. Description of measured variables in *Hypera postica*.

Length of pronotum (LP)	From mid anterior part of pronotum that is usually concave to the mid posterior part which is attached to scutellum. This area is usually distinguished by a bright colour line between the stripes of pronotum.
Width of pronotum (WP)	Maximum pronotal width (the longest line between the two lateral convex parts of pronotum).
Length of elytra (LE)	The line where the two elytra meet (elytral suture), from base of scutellum to apex of elytra; usually has been marked by elytral stripe.
Width of elytra (WE)	The line that measured at the middle of elytral disc.
Distance between elytra anterior margins (AE)	Distance between exterior parts of two terminal curvatures in anterior parts of elytra. This area is filled by width of pronotum base.
Distance between eyes (ED)	Located on the frons area of the head. Minimum frons width between eyes.
Width of rostrum base (WR)	In frontal view while rostrum leaving head part.
Width of rostrum at antennal insertion (AR)	The width line of rostrum in antennal insertion area (the line that crosses the antennal insertion)
Width of rostrum at apex of rostrum (MR)	The line exactly before the mouthparts appears in dorsal view of rostrum: this area may contain the clypeus (Davis 2011).
Length of rostrum (LR)	Measured in top view, in a position in which the base and apex at the same level. The perpendicular line between lines measuring distance between eyes and width of rostrum at mouthparts.

respectively. An image from a 1.0 mm scale was also captured by the camera at each magnification. The measurements were set as scale for measuring variables in tpsDig v2 software (Rohlf 2013).

In the current study, from three anatomic features, 10 measured variables (Fig. 2 and Table 2) and 45 ratio variables were used: 1: Length of pronotum (LP), 2: Width of pronotum (WP), 3: Length of elytra (LE), 4: Width of elytra (WE), 5: Distance between anterior margin of elytra (AE), 6: Distance between eyes (ED), 7: Width of rostrum base (WR), 8: Width of rostrum at antennal insertion (AR), 9: Width of rostrum at apex of rostrum (MR), 10: Length of rostrum (LR). Measurements are given in mm.

Abbreviations of 45 ratios computed from original variable divided by each other are listed as following: 11: LP/WP, 12: LP/LE, 13:LP/WE, 14: LP/AE, 15: LP/ED, 16: LP/WR, 17: LP/AR, 18: LP/MR, 19: LP/LR, 20: WP/LE, 21: WP/WE, 22: WP/AE, 23: WP/ED, 24: WP/WR, 25: WP/AR, 26: WP/MR, 27: WP/LR, 28: LE/WE, 29: LE/AE, 30: LE/ED, 31: LE/WR, 32: LE/AR, 33: LE/MR, 34: LE/LR, 35: WE/AE, 36: WE/ED, 37: WE/WR, 38: WE/AR, 39: WE/MR, 40: WE/LR, 41: AE/ED, 42: AE/WR, 43: AE/AR, 44: AE/MR, 45: AE/LR, 46: ED/WR, 47: ED/AR, 48: ED/MR, 49: ED/LR, 50: WR/AR, 51: WR/MR, 52: WR/LR, 53:AR/MR, 54: AR/LR, 55:MR/LP.

Variables measured in tpsDig v2. Some characters were deformed or damaged during preparation. These were replaced by a mean of variables from each stations/gender. The data were entered into SPSS 19 (Brosius 2011) for statistical analysis. First, the descriptive statistics for each variable were done for each sex (Table 3). Test of normality was done between sexes for each measured variable. If a variable did not show normal distribution, we tried to normalize this data by computing its logarithm and used quantity of Log (the variable) or Ln (the variable) for analysis. For the univariate analysis T test for normal distribution variables and Mann-Whitney U for not-normal distribution variables between sexes were applied. For multivariate analyses, Discriminant Analysis and Principle Component Analysis (PCA) have been applied for sexes and stations.

Results

The results from the descriptive statistical analysis showed that mean for most variables was higher in females than males, showing that the direction of SSD trended towards larger females.

The P value for test of normality for variables in gender showed that only length of pronotum had a normal distribution for both male and female ($P = 0.2$). Other variables had P value less than 0.05 for males, females and even whole specimens (considered both males and females) ($P < 0.005$). The Log_{10} and Ln computed for other variables and test of normality was repeated for these but no normal distribution was observed. The result of t-test for pronotum length shows no significant

Table 3. Descriptive statistics of variables for each sex of *Hypera postica*.

	Male (84 specimens)					Female (116 specimens)				
Variable	Min	Max.	Mean	SD	Variance	Min	Max	Mean	SD	Variance
LP	0.92	1.33	1.17	0.089	0.006	0.95	1.57	1.19	0.090	0.008
WP	1.06	1.71	1.43	0.112	0.013	1.11	1.72	1.48	0.113	0.013
LE	2.89	4.30	3.38	0.264	0.070	2.52	4.05	3.44	0.287	0.082
WE	1.77	2.87	2.24	0.178	0.032	1.32	2.85	2.39	0.208	0.043
AE	1.11	1.63	1.35	0.096	0.009	1.12	3.07	1.44	0.182	0.033
ED	0.19	0.31	0.25	0.026	0.001	0.21	0.33	0.26	0.027	0.001
WR	0.22	0.38	0.30	0.025	0.001	0.25	0.36	0.31	0.023	0.001
AR	0.31	0.43	0.38	0.022	0.0005	0.31	0.42	0.37	0.019	0.0005
MR	0.21	0.33	0.27	0.022	0.0005	0.23	0.31	0.28	0.019	0.0005
LR	0.93	1.30	1.15	0.075	0.006	0.97	1.32	1.19	0.074	0.005

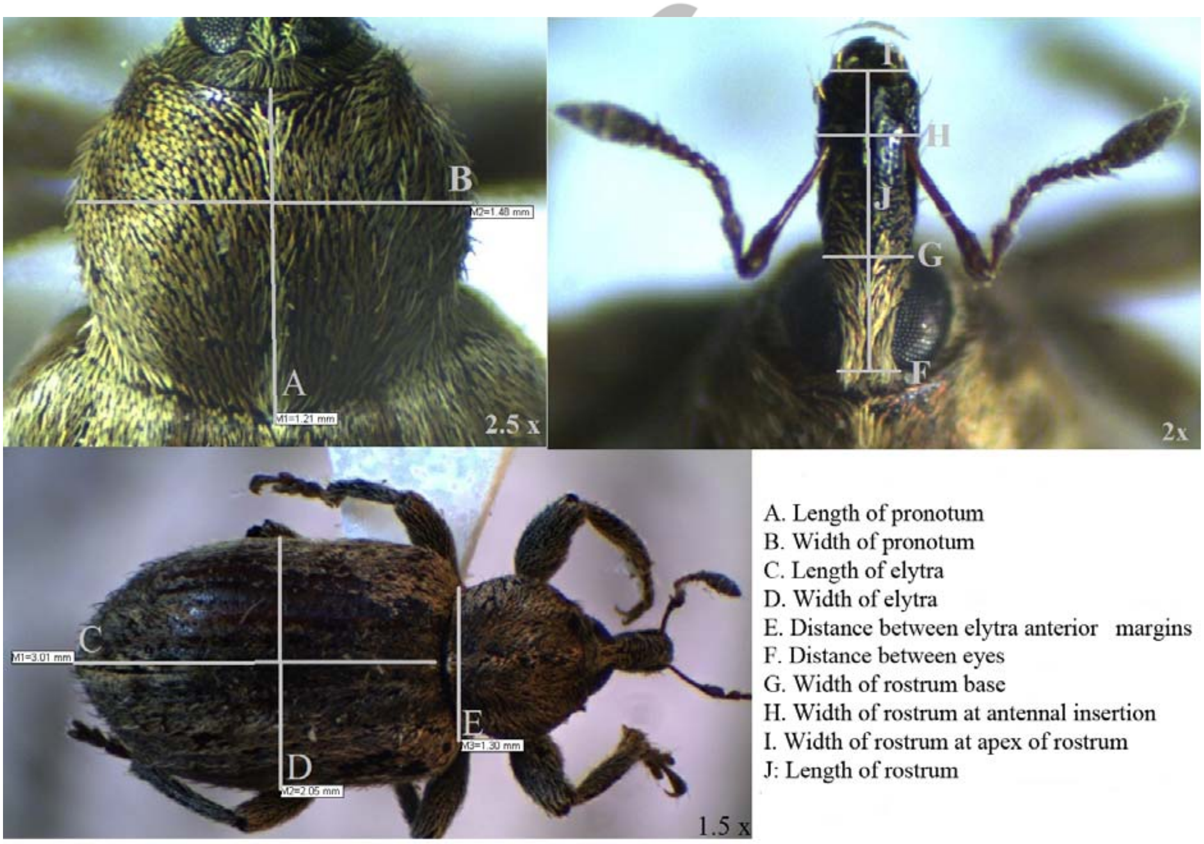

Fig. 2. Measured variables from pronotum, elytra and rostrum of *Hypera postica*.

difference ($P > 0.5$). Mann-Whitney U-Test for abnormal characters revealed significant differences between sexes in all characters ($P > 0.005$), except of rostrum width at the antennal scrobe ($P = 0.184$).

In multivariate analysis, discriminant analysis correctly classified 83% of the total specimens into the male and female groups. Separation of the sexes was also calculated by discriminant analysis in each station. The analysis for stations, namely Karaj1, Karaj2, Jovein and Tuyserkan correctly classified gender by 81.3%, 98.2%, 100% and 76.2%, respectively.

Discriminant analysis of variables between stations was done for males and females independently. From three canonical discriminant functions, the first two functions were chosen for both, males and females. Male and female specimens were correctly, 70.9% and 76.3%, classified to stations, respectively. Of total specimens (male and female) 62.5% were correctly classified. The scatter plot of the first two discriminant functions for males and females between stations is shown in Fig. 3.

PCA analysis calculated 10 components from 55 variables. Combination of none of the components was able to separate sexes or stations.

Discussion

In this study, 200 individuals of *Hypera postica* from four stations located in three cities of Iran were analyzed using a morphological approach in order to find out SSD direction and magnitude in each station. Also possibility of their population divergence in Iran is discussed. Most measured variables show significant differ-

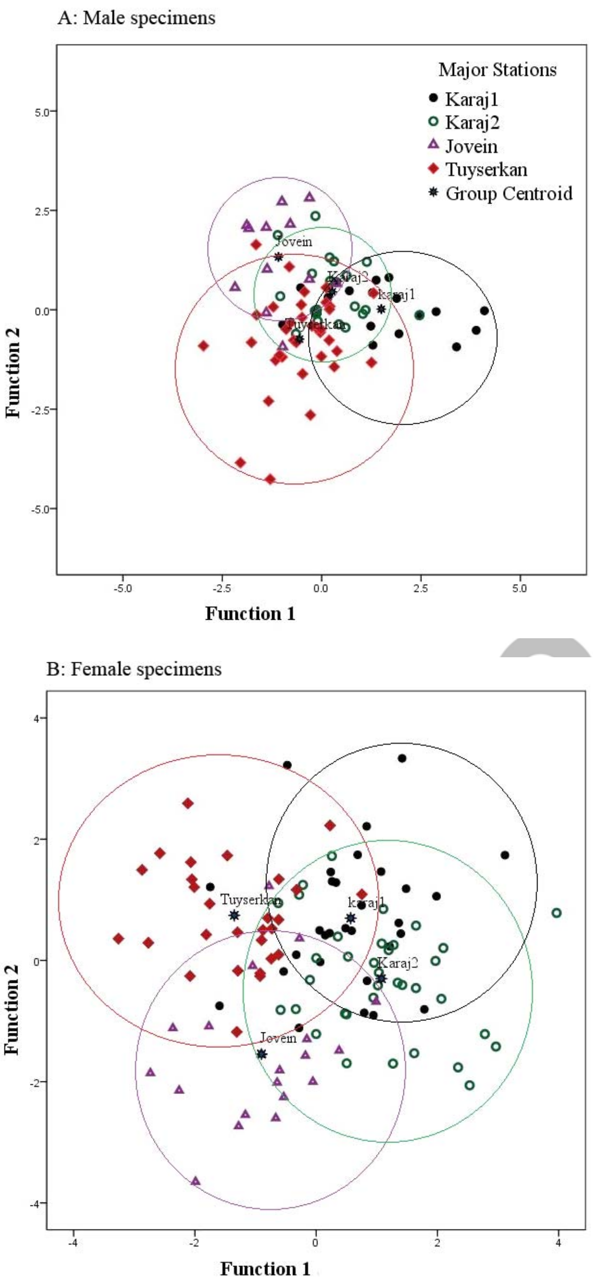

Fig. 3. Discriminant scatter plot between stations. A: 70.9% of *H. postica* male specimens are correctly classified to stations, B: 76.3% of female specimens are correctly classified to stations.

ences between sexes. Length of pronotum and width of rostrum at antennal insertion were two variables that did not show any significant sexual differences in univariate analysis. When these two variables were discarded from the discriminant analysis, there was no increase in the degree of separation. Therefore, there is no enough evidence to consider these variables in evaluation of SSD. Descriptive statistical analysis showed that females have larger pronotum and elytra in both width and length, the mean of three elytral characters in the female was slightly greater than in male. Females have a longer and slightly wider rostrum, especially at the apex of rostrum. Also the distance between eyes is greater in females.

The amount and direction of SSD related to ecological characters of organisms is widely observed in the animal kingdom (Fairbairn 1997). In this research, females were found to be slightly larger. Fecundity selection is the general evolutionary hypothesis used to explain SSD when females are larger (Fairbairn & Preziosi 1994). Increasing size in females is related to their ability to receive resources and produce more offspring (Liao et al. 2013) so larger female size is selected. Also Rensch's rule indicates that SSD increases in species where males are larger and decreases in species where females are the larger sex (Rensch 1950).

Due to their size, length and width of elytra are the best characters to indicate whole body size. These two characters are greater in females, therefore extra body size may allow them to accumulate more resources and lay more eggs. Even though longer rostrum is usually responsible for digging the holes in steams for laying eggs in weevils (Oberprieler et al. 2007). A longer and wider rostrum may help the females to consume more alfalfa, again selected for by fecundity selection. There is no evidence for strong sexual selection in the alfalfa weevil (Lecato & Pienkowski 1970). Previous sexual dimorphism studies on American populations of *H. postica* indicated that females were the larger sex in respect to body proportions (Pienkowski et al. 1969; Titus 1909). For Iranian populations in the current study, the direction of SSD (females were the larger sex) in elytra and rostrum was confirmed, but it did not strongly separate males and females because they show an overlap in measurement of variables; it is not possible to distinguish males and females using body size alone. Slightly larger females in the alfalfa weevil may support Rensch's rule, but more comparative studies should be done in closely related species to confirm this hypothesis for *H. postica.*

Because of low magnitude size differences in sexes, lack of evidence for strong sexual selection and highly fecund behavior in mature females (Coles & Day 1977), we suggest that: fecundity selection is more effective than sexual selection in *H. postica,* leading to slightly larger females. It seems, one more evolutionary reason for SSD in the rostrum, is related to its special function in females. Adult females are highly fecund, laying almost 4,200 eggs during life (Coles & Day 1977). Females chew stems in order to lay eggs inside them (Litsinger & Apple 1973). According to Bland (1984), mouthpart sensilla and mandibles show no differences between sexes and therefore here, the ability to make a hole in stems and clean their inside may be related to a stronger and larger rostrum appears in females.

Quality of habitats (Benbow 2008), latitude (Stillwell et al. 2007, 2008) and seasonality (Stillwell et al. 2007) affected SSD in other populations of alfalfa weevil. Suitable ecological conditions that are related directly to quality and quantity of nutrition increased magnitude of SSD in insects (Stillwell et al. 2014). Although many variables control the amount of SSD in populations, we suggest that in this case study, more pronounced SSD observed in the Jovein population (showing 100% sex divergence in discriminant analysis) is related to food availability due to ecological conditions, and also lower altitude (1140 m and 1103 m a.s.l.). Karaj2 populations had 98.2% sex divergence in 1193 m altitude, and lower divergence in Karaj1 (81%) happened in higher altitude (1315 m). Less pronounced SSD observed in station with higher altitude; Tuyserkan population with 76.2% divergence in 1657–1712 m altitude. Therefore, in higher altitude the variably of SSD is lower.

Discriminant analysis of variables in stations for male, female and total specimens showed almost the same results. The PCA analysis also failed to separate stations. There was a weak separation between stations using the morphological variables used in this study. Transportation of alfalfa products by human activity may play a role in gene flow between populations, and decrease the level of separation between stations. To resolve certain differences between Iranian populations of *H. postica,* attention to molecular and ecological aspects and also geometric morphometric methods may be useful.

Acknowledgements

We are grateful to Dr. Alireza Sari for his immense technical help in statistical analysis and revising the version of the manuscript. We appreciate Dr. Milosz Mazur, Dr. Peter Sprick and Dr. Jirislav Skuhrovec for reviewing the manuscript. Special thanks also to Dr. Nico M. Franz for his comments. We want to acknowledge Dr. Hassan Rahimian for his generosity in providing a suitable stereomicroscope and camera for use in this research. This study was part of the Master's Thesis of first author, supported by the University of Tehran.

References

Benbow M. 2008. Role of larval sexual dimorphism, biased sex ratios, and habitat on the energetics of a tropical Chironomid. Environ. Entomol. **37:** 1162–1173. DOI: 10.1603/0046-225X(2008)37[1162:ROLSDB]2.0.CO;2

Blanckenhorn W.U., Meier R. & Teder T. 2007. Rensch's rule in insects: patterns among and within species: Sex, size, and gender roles, pp. 60–70. In: Fairbairn D.J., Blackenhorn W.U. & Székely T. (eds), Evolutionary Studies of Sexual Size Dimorphism, Oxford University Press Inc, NewYork. ISBN: 9780199208784

Blanckenhorn W.U., Stillwell R.C., Young K.A., Fox C.W. & Ashton K.G. 2006. When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? Evolution **60:** 2004–2011. DOI: 10.1111/j.0014-3820.2006.tb01838.x

Brosius F. 2011. SPSS. Hüthig Jehle Rehm.

Bundy C.S., Smith P.F., English L.M., Sutton D. & Hanson S. 2005. Strain distribution of alfalfa weevil (Coleoptera: Curculionidae) in an intergrade zone. J. Econ. Entomol. **98:** 2028–2032. DOI: http://dx.doi.org/10.1093/jee/98.6.2028

Coles L. & Day W. 1977. The fecundity of *Hypera postica* from three locations in the eastern United States. Environ. Entomol. **6:** 211–212.

Fairbairn D.J. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. Annu. Rev. Ecol. Syst. **28:** 659–687.

Fairbairn D.J. & Preziosi R.F. 1994. Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis.* Am. Nat. **144:** 101–118.

Hsiao T.H. 1993. Geographic and genetic variation among alfalfa weevil strains, pp. 311–324. In: Kim K.C. & McPheron B.A. (eds), Evolution of Insect Pests: Patterns of Variation, John Wiley & Sons Inc, New York, 496 pp. ISBN: 978-0-471-60077-0.

Karimpour Y. 1994. Studies on the effect of different insecticides on alfalfa weevil (*Hypera postica*) and Etrimfos residues in green alfalfa, Vol. MSc: Tarbiat Moddares University, Tehran, Iran, 102 pp.

Khanjani M. & Pourmirza A.A. 2004. A comparison of various control methods of alfalfa weevil, *Hypera postica* (Col: Curculionidae) in Hamadan. J. Entomol. Soc. Iran **1:** 67–81.

Kuwata R., Tokuda M., Yamaguchi D. & Yukawa J. 2005. Coexistence of two mitochondrial DNA haplotypes in Japanese populations of *Hypera postica* (Col., Curculionidae). J. Appl. Entomol. **129:** 191–197. DOI: 10.1111/j.1439-0418.2005.00954.x

Lecato G.L. & Pienkowski R.L. 1970. Sexual responsiveness of the male alfalfa weevil, *Hypera postica*, as affected by prior contact with other alfalfa weevils. Entomol. Exp. Appl. **13:** 462–466.

Liao W.B., Zeng Y., Zhou C.Q. & Jehle R. 2013. Sexual size dimorphism in anurans fails to obey Rensch's rule. Front. Zool. **10:** 10. DOI: 10.1186/1742-9994-10-10

Litsinger J.A. & Apple J.W. 1973, Oviposition of the alfalfa weevil in Wisconsin. Ann. Entomol. Soc. Am. **66:** 17–20.

Menken S. & Ulenberg S.A. 1987. Biochemical characters in agricultural entomology. Agric. Zool. Rev. **2:** 305–360.

Montazar A. & Sadeghi M. 2008. Effects of applied water and sprinkler irrigation uniformity on alfalfa growth and hay yield. Agric. Water Manage. **95:** 1279–1287. DOI: 10.1016/j.agwat.2008.05.005

Moradi-Vajargah M., Golizadeh A., Rafiee-Dastjerdi H., Zalucki M.P., Hassanpour M. & Naseri B. 2011. Population density and spatial distribution pattern of *Hypera postica* (Coleoptera: Curculionidae) in Ardabil, Iran. Not. Bot. Horti Agrobot. Cluj-Napoca **39:** 42–48.

Pienkowski R.L., Hsieh F.K. & Lecato G.L. 1969. Sexual dimorphism and morphonietric differences in the eastern, western, and Egyptian alfalfa weevils. Ann. Entomol. Soc. Am. **62:** 1268–1269.

Radcliffe E.B. & Flanders K.L. 1998. Biological control of alfalfa weevil in North America. Integr. Pest Manage. Rev. **3:** 225–242. DOI: 10.1023/A:1009611219360

Rensch B. 1950. Die Abhängigkeit der relativen Sexualdifferenz von der Körpergrösse. Bonn. Zool. Beitr. **1:** 58–69.

Rohlf F.J. 2013. tpsDig. State University of New York, Stony Brook, New York.

Stillwell R.C., Blanckenhorn W.U., Teder T., Davidowitz G. & Fox C.W. 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. Annu. Rev. Entomol. **55:** 227–245. DOI: 10.1146/annurev-ento-112408-085500

Stillwell R.C., Daws A. & Davidowitz G. 2014. The ontogeny of sexual size dimorphism of a moth: when do males and females grow apart? PloS One **9:** e106548. DOI: 10.1371/journal.pone.0106548

Stillwell R.C., Morse G.E. & Fox C.W. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. Am. Nat. **170:** 358–369. DOI: 10.1086/520118

Stillwell R.C., Moya-Laraño J. & Fox C.W. 2008. Selection does not favor larger body size at lower temperature in a seed-feeding beetle. Evolution **62:** 2534–2544. DOI: 10.1111/j.1558-5646.2008.00467.x

Titus E. 1909. The alfalfa leaf-weevil. J. Econ. Entomol. **2:** 148–154.

Zehzad B., Kiabi B.H. & Madjnoonian H. 2002. The natural areas and landscape of Iran: an overview. Zool. Middle East **26:** 7–10. DOI: 10.1080/09397140.2002.10637915

Živković B., Radović J., Sokolović D., Šiler B., Banjanac T. & Štrbanović R. 2012. Assessment of genetic diversity among alfalfa (*Medicago sativa*) genotypes by morphometry, seed storage proteins and RAPD analysis. Ind. Crops Prod. **40:** 285–291. DOI: 10.1016/j.indcrop.2012.03.027

Received April 13, 2015
Accepted July 16, 2015