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GEOMETRIC MORPHOMETRIC STUDY ON GEOGRAPHIC DIMORPHISM OF CODING MOTH *CYDIA POMONELLA* (LEPIDOPTERA, TORTRICIDAE) FROM NORTH WEST OF IRAN

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Geometric Morphometric Study on Geographic Dimorphism of Codling Moth *Cydia pomonella* (Lepidoptera, Tortricidae) from North West of Iran. Khaghaninia S., Mohammadi S. A., Irani Nejad K. H., Zahiri R. — During years 2003–2004, nine geographical populations of codling moth *Cydia pomonella* (Linnaeus) from 4 north western provinces of Iran were collected. By preparing 575 images from fore wings and 564 from hind wings, a total of 15 and 11 landmarks were determined for fore and hind wings, respectively. With transforming of landmark's geometrical data into partial warp scores, 26 and 18 scores were obtained for fore and hind wings, respectively. Canonical correlation analysis (CCA) revealed significant correlation between environmental parameters and wing shape variables. Among environmental parameters, wind speed showed the highest correlation with wing shape variables whereas, the correlation between latitude, relative humidity as well as amount of precipitation and wing shape variables was low. Considering the effect of various environmental parameters on wing shape, wind speed was determined as important parameter affecting geographic dimorphism. Among the populations collected from different regions, two geographic population pairs; Meshkinshahr-Mahneshan and Zandjan-Khoramdareh were selected as representative of low and high windy regions, respectively. Relative warp analysis (RWA) of fore and hind wings shape variables in the areas with high and low wind showed shorter and wider fore wings as well as slender and narrower hind wings in populations from high windy regions compared with populations from low wind regions. Centroid size of fore and hind wings in high windy area populations were smaller compared with those from low windy ones as revealed by t-test. The results showed aerodynamic shape and small size of wings are as adapted traits for powerful flight and its control in high windy regions.

Key words: codling moth, *Cydia pomonella*, geometric morphometry, weather factors, morphological variation, geographic dimorphism.

Геометрически-морфометрическое исследование географического диморфизма яблонной плодожорки Cydia pomonella (Lepidoptera, Tortricidae) в северо-западном Иране. Кхаг-ханиниа С., Мохаммади С. А., Ирани-Неджад К. Н., Захири Р. — В течение 2003–2004 гг. были собраны экземпляры девяти географических популяций яблонной плодожорки *Cydia pomonella* (Linnaeus) в 4 северо-западных провинциях Ирана. Всего 15 и 11 меток передних и задних крыльев соответственно были определены при подготовке 575 изображений передних крыльев и 564 изображений задних крыльев. 26 показателей для передних крыльев и 18 — для задних были получены путем трансформации исходных геометрических данных методом частной деформации. Канонический корреляционный анализ обнаруживает значительную корреляцию между параметрами окружающей среды и вариабельностью формы крыльев. Из параметров окружающей среды отмечена наибольшая корреляция скорости ветра и вариабельности формы крыльев, тогда как корреляция между широтой, относительной влажностью, количеством осадков и изменчивостью формы крыла была низкой. Принимая во внимание влияние разнообразных параметров на форму крыла, скорость ветра была определена как важный фактор, действующий на географический диморфизм. На основании экземпляров, собранных в разных регионах как примеры регионов с сильными и слабыми ветрами, соответственно, были выбраны две пары географических популяций: Мешкиншахр-Махнешан и Зандьян-Кхорамдарех. Анализ относительного искажения формы передних и задних крыльев в областях с сильными и слабыми ветрами обнаружил более короткие и широкие передние крылья и более тонкие и узкие задние крылья в популяциях из регионов с более сильными ветрами по сравнению с представителями популяций из регионов с более слабыми ветрами. Значения центроидов передних и задних крыльев в популяциях из регионов с сильными ветрами были меньше, по сравнению с таковым из регионов со слабыми ветрами, как было проверено по критерию Стьюдента. Результаты показывают, что аэродинамическая форма и малый размер крыльев — адаптационные особенности для сильного полета и управления им в районах с сильными ветрами.

Ключевые слова: яблонная плодожерка, Cydia pomonella, геометрическая морфометрия, погодные факторы, морфологическая вариация, географический диморфизм.

Introduction

More than half of the animal described species are insects (Groombridge, 1992). Therefore, understanding patterns and processes of biological diversification are central importance in evolutionary biology (DeVries et al., 1997). Study of phenotypic plasticity or biodiversity has attracted interest of most biologists ever since Darwin (Hood, 2000 and Bernardo et al., 2007). Morphological diversities including seasonal and geographic dimorphisms can be related to fitness and thus are a possible target of natural selection (Kemp, 2001 and Bernardo et al., 2007). Wing shape deformations have been important subject in studying of geographic forms of intra species evolution (Adams and Funk, 1997 and Kunkel, 2001).

Geometric morphometrics offers a new and powerful method for studying of intra species geographic dimorphism or biological forms (Hood, 2000). The shape of organism is very stable and has high heredity, therefore, study of overall shape similarity is known as an accurate way against quantitative estimations for investigating phylogeny as well as study of shape variability in biological forms (Bookstein, 1989; Rohlf, 1990). The population diversity of *Neochlamisus bebbianae* as well as *Chilo suppressalis* were studied by means of geometric morphometrics in north part of America and north part of Iran, respectively (Adams and Funk, 1997 and Zahiri et al. 2004).

Insect flight performance, dispersal and resource exploitation are pervasively affected by environmental parameters such as wind speed, temperature, relative humidity and elevation (Lambert, 1972, Epila, 1988, Pasek, 1988 and Jones, 2003). The foraging activity of pollinator insects in relation to weather factors like ambient temperature, relative humidity, wind speed and solar radiation was studied through various investigations and significant correlations were found between pollination activity and environmental factors (Szabo, 1980 and Narcis and Jordi, 2000).

The objectives of the present study were to study the correlation between environment parameters and wing shape variables as well as to estimate wing shape and size deformations in geographic codling moth populations from northwest of Iran.

Material and methods

Nine populations were collected from nine regions spanning north west of Iran: East Azarbayjan (four populations), West Azarbayjan (one population), Ardebil (one population) and Zandjan (three populations) during 2003 and 2004. The populations were collected from a number of sites within each region based on the span areas. To eliminate the effect of host in discrimination of populations, all the specimens were collected from only golden apple orchards. The specific specimens from each geographical population were randomly selected from moths pooled across collecting sites within a given region. Sampling of fifth instar larvae was carried out by single face cardboard fastened around the apple trees at 30 cm distance of ground.

For studying wing shape, the collected larvae were reared in rearing dishes separately for each region under laboratory conditions at 25°C, 60% relative humidity and 16/8 (light/dark) photoperiod. After unistitation of emerged adults, they were mounted by 00 mounting needles. Permanent slides of fore and hind wings were prepared following Borror et al. (1989) and transformed to digital images (table 1).

By selecting homologous spots as landmarks, 15 and 11 landmarks were chosen from fore and hind wings, respectively (fig. 1). Based on Bookstein's (1989) classification, all the landmarks were type I representing meeting points of wing veins and their attaching points to wing's margin.

Two-dimentional coordinates data for mentioned landmarks were performed from all the studied specimens using a Leica stereoscopic microscope, image analyzing system, and Tpsdig software (Rohlf, 1990). By transforming the row coordinate data into shape variables and partial warp scores, 26 and 18 scores were obtained for fore and hind wings, respectively. Canonical correlation analysis was performed to analyze correlation between climate parameters and shape variables of fore and hind wings of both sexes in geographical populations using STATISTICA 5.5 software (Moghaddam et al., 1994). Relative warp analysis (RWA)

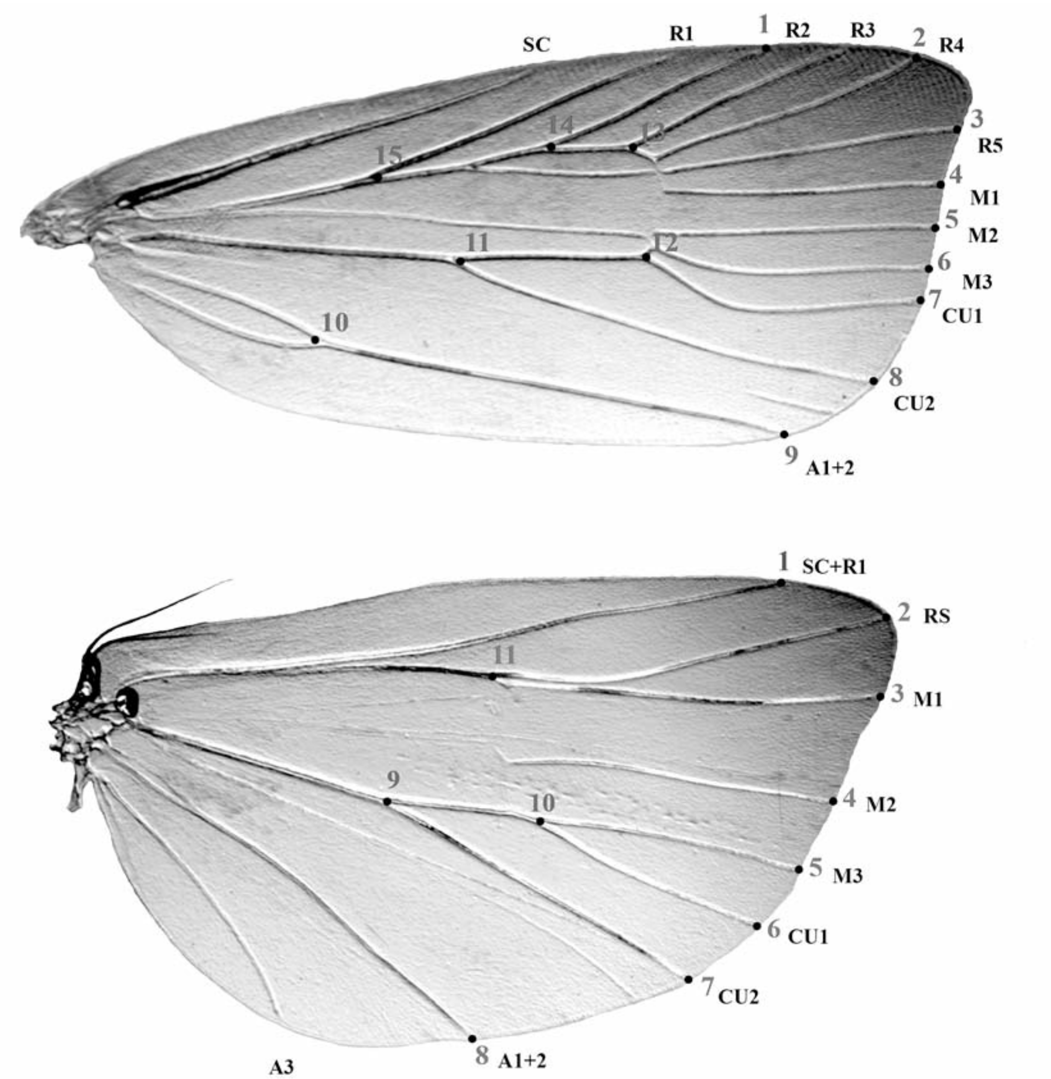

Fig. 1. Fore and hind wings of *Cydia pomonella* with landmarks' positions on them.

Рис. 1. Переднее и заднее крыло *Cydia pomonella* с положением опознавательных точек на них.

Table 1. Number of fore and hind wing images taken from various geographic populations of codling moth

Таблица 1. Количество изображений передних и задних крыльев яблонной плодожорки из различных географических популяций

Population	Fore wing			Hind wing		
	female	male	total	female	male	total
Mianeh	24	27	51	21	21	42
Salmas	22	26	48	21	25	46
Zandjan	34	31	65	32	30	62
Zunuz	27	27	54	40	29	69
Khoramdareh	31	18	49	31	24	55
Meshkin Shahr	14	22	36	16	22	38
Shabestar	31	23	54	31	28	59
Marageh	32	28	60	35	35	70
Mahneshan	29	28	57	23	28	51
Total	244	230	474	250	242	492

was carried out and wings relative variations in various geographic populations were determined by software tpsrelw. The wing centroid size of male and females of the studied populations were estimated and t-test was used to test the effect of geographical conditions on wing centriod size by SPSS 14.0.

Results and discussion

Two-way multivariate analysis of variance (MANOVA) based on all shape variables in fore and hind wings revealed indicated significant differences among populations as well as sexes at least for one the shape variable. The non-uniform variables were more effective in differentiation of geographical population especially due to hind wing compared with uniform one (Khaghaninia et al., 2008).

Mean annual weather data of studied areas are indicated in table 2. Canonical correlation analysis revealed significant correlation between fore wing shape variables and environment factors in both sexes except relative humidity and longitude in males (table 3). Among environment factors, wind speed showed highest correlation with wing shape variables in both sexes whereas relative humidity and elevation showed low correlations with environmental factors.

Correlation between environmental factors and fore wing shape variables as well as their effectiveness on fore wing deformations in females was greater than males. Various studies demonstrated longest flight capacity of female codling moth in individual flight as well as life time compared with male. This is in agree with more wing effectiveness and deformations of females in comparison to males (Mani et al., 1995, Schumacher et al., 1997, Dorn et al., 1999 and Voigt et al., 1999)

Table 2. Mean annual climate data of studied regions

Таблица 2. Среднегодовые климатические данные исследованных регионов

Sampling area	Longitude	Latitude	Temperature, °C	Relative humidity, %	Precipitation amount, mm	Elevation, m	Wind speed, km/h
Salmas	38.13	44.51	12.0	57	215.3	1337	6.111
Zunuz	38.45	45.75	11.1	56	415.1	1710	9.863
Marageh	37.24	46.16	12.9	49	322.4	1477.7	10.503
Mianeh	37.27	47.42	13.7	51	282.1	1110	8.711
Shabestar	38.11	45.41	-	-	-	1452	-
Mahneshan	36.46	47.40	14.6	48	275.7	1282	10.558
Zandjan	36.41	48.29	11.0	54	313.1	1663	7.191
Khoramdareh	36.11	49.11	11.9	51	301.1	1575	11.480
Meshkin shahr	38.23	47.40	10.7	60	383.9	1568.5	5.157

Table 3. Canonical correlation coefficient between environment parameters and shape variables of codling moth fore wing in both sexes

Таблица 3. Коэффициент канонической корреляции параметров окружающей среды и формы изменчивости передних крыльев яблонной плодожорки обоих полов

Climate parameters	Shape variables of female fore wing			Shape variables of male fore wing		
	Rc	P value	percentage	Rc	P value	percentage
Longitude	0.456	0.00891	4.03	0.369	0.347	4.48
Latitude	0.470	0.00371	5.23	0.461	0.0094	4.10
Temperature, °C	0.494	0.00067	4.06	0.440	0.029	3.09
Relative humidity, %	0.494	0.00068	5.70	0.378	0.282	4.30
Precipitation amount, mm	0.469	0.00383	5.52	0.518	0.00016	6.15
Elevation, m	0.520	0.000075	4.68	0.487	0.0017	5.30
Wind speed, km/h	0.719	0.000000	9.93	0.694	0.000000	8.31

Table 4. Canonical correlation coefficient between environment parameters and shape variables of codling moth hind wing in both sexes

Таблица 4. Коэффициент канонической корреляция параметров окружающей среды и формы изменчивости задних крыльев у яблонной плодожорки обоих полов

Climate parameters	Shape variables of female fore wing			Shape variables of male fore wing		
	Rc	P Value	percentage	Rc	P Value	percentage
Longitude	0.528	0.000001	5.09	0.537	0.000001	4.58
Latitude	0.556	0.000000	5.79	0.552	0.000000	4.74
Temperature, °C	0.426	0.000378	4.49	0.453	0.000241	3.89
Relative humidity, %	0.433	0.000743	5.84	0.478	0.000028	5.75
Precipitation amount, mm	0.543	0.000013	5.48	0.545	0.000000	7.32
Elevation, m	0.454	0.000155	4.44	0.456	0.000184	5.24
Wind speed, km/h	0.661	0.000000	10.72	0.578	0.000000	9.42

The trend of correlations between environmental factors and hind wing shape variables were similar to those for fore wing as revealed by canonical correlation analysis, but the amount of correlation coefficients were higher except for elevation (table 4).

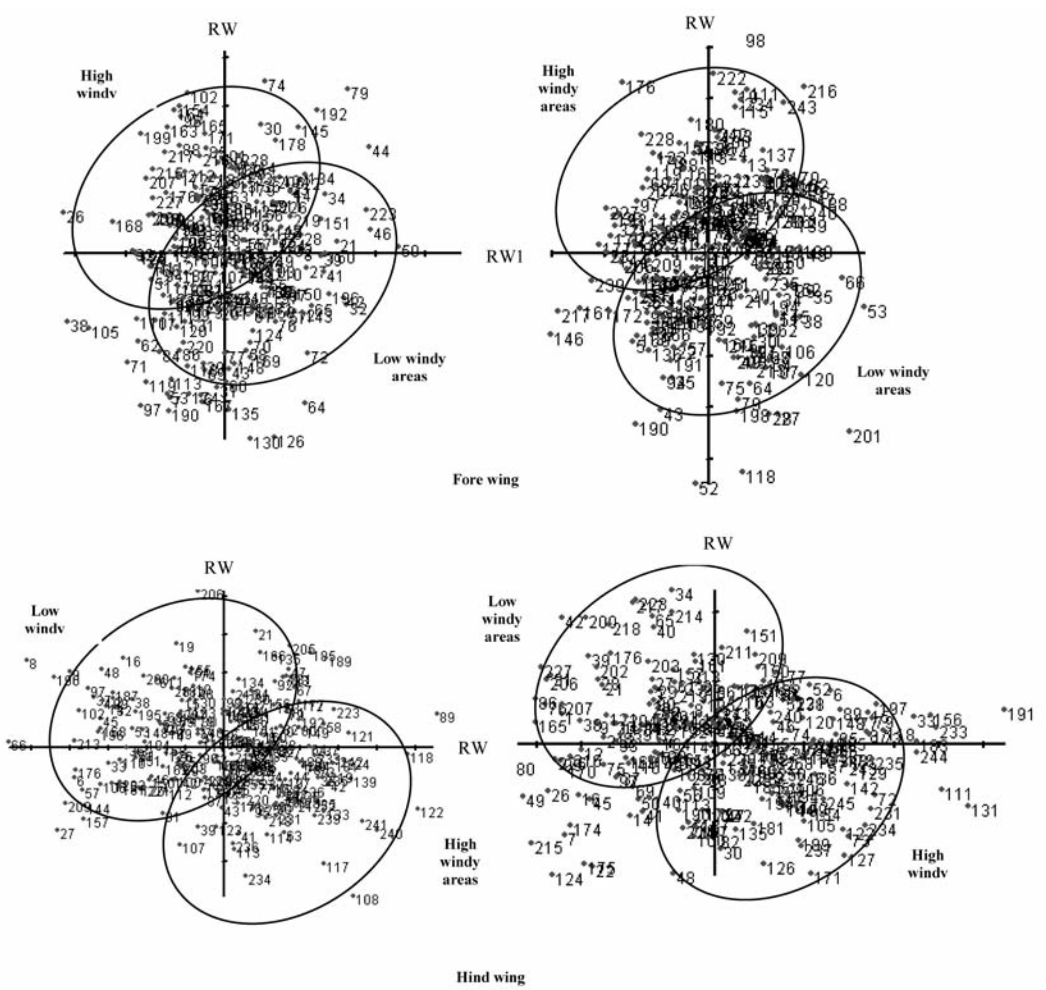

Fig. 2. Distribution of codling moth geographic populations through RWA by means of landmarks: females (right); males (left).

Рис. 2. Распространение географических популяций яблонной плодожорки согласно анализу относительных отклонений с помощью меток: самки (справа); самцы (слева).

The influence of wind speed on taking off, landing, recognition of flight trout and its control was repetitively reported. In high windy areas or at windy times, the flying insects accumulate leeward of windbreaks as compared with unsheltered sites (Epila, 1988; Pasek, 1988). Lambert (1972) by studying the effect of wind speed and temperature on locusts flight found that insect's flight was inhibited at conditions with high wind speed and the temperature below 19°C.

The discrimination of different geographical populations based on RWA using fore and hind wings shape variables were shown in figure 2. Due to various direct and indirect effects of environmental factors on wing shape deformations, incomplete discrimination between geographical populations was observed. Because of the highest correlation between wind speed among environmental factors, the fore and hind wings deformations were studied on two pair populations.

RWA based on fore and hind wings landmarks of Meshkinshahr (Mes.) and Mahneshan's (Mah.) populations as candidate of low and high windy areas, respectively revealed well discrimination especially for hind wing (fig. 3). The discrimination pat-

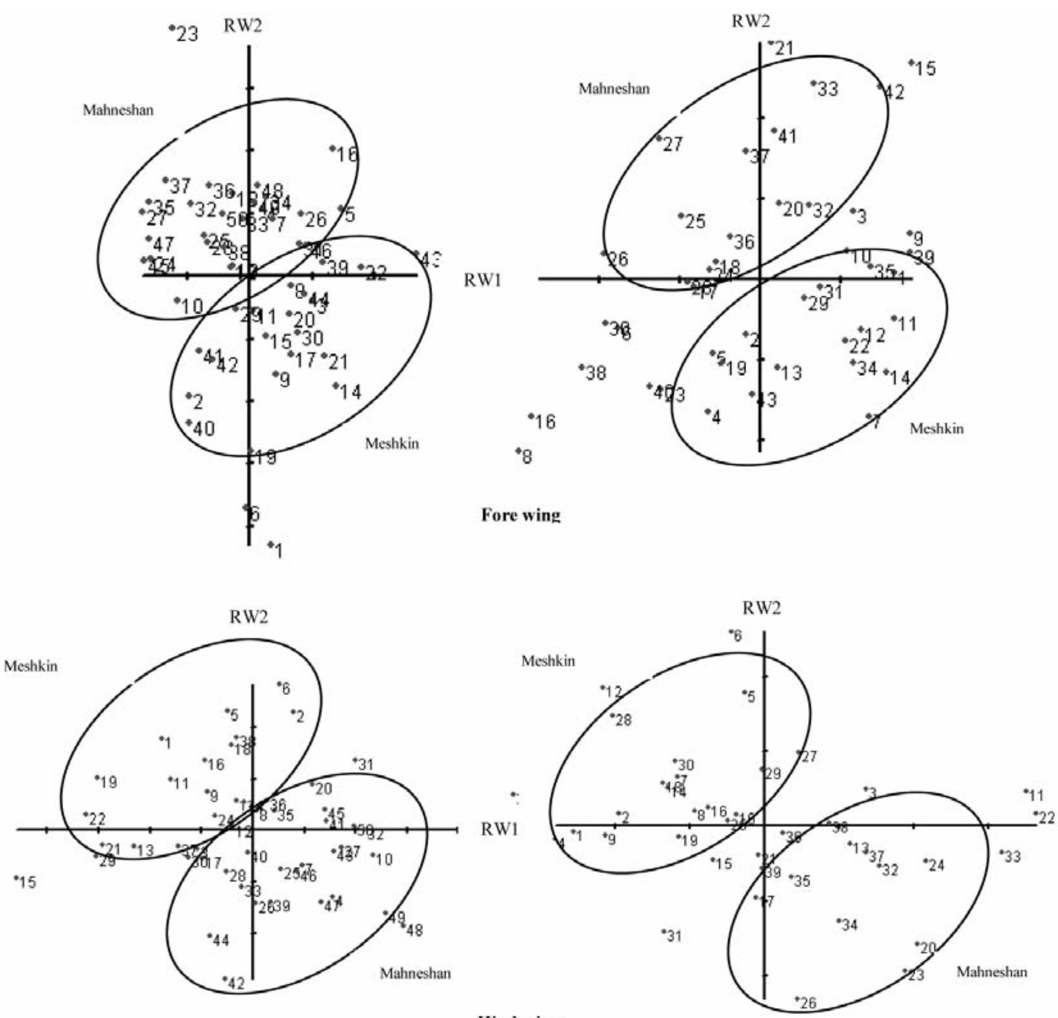

Fig. 3. Distribution of Meshkin Shahr and Mahneshan's codling moth populations through RWA by means of landmarks: females (right); males (left).

Рис. 3. Распространение популяций яблонной плодожорки из популяций Мешкин Шахр и Махнешан через RWA с помощью меток: самки (справа); самцы (слева).

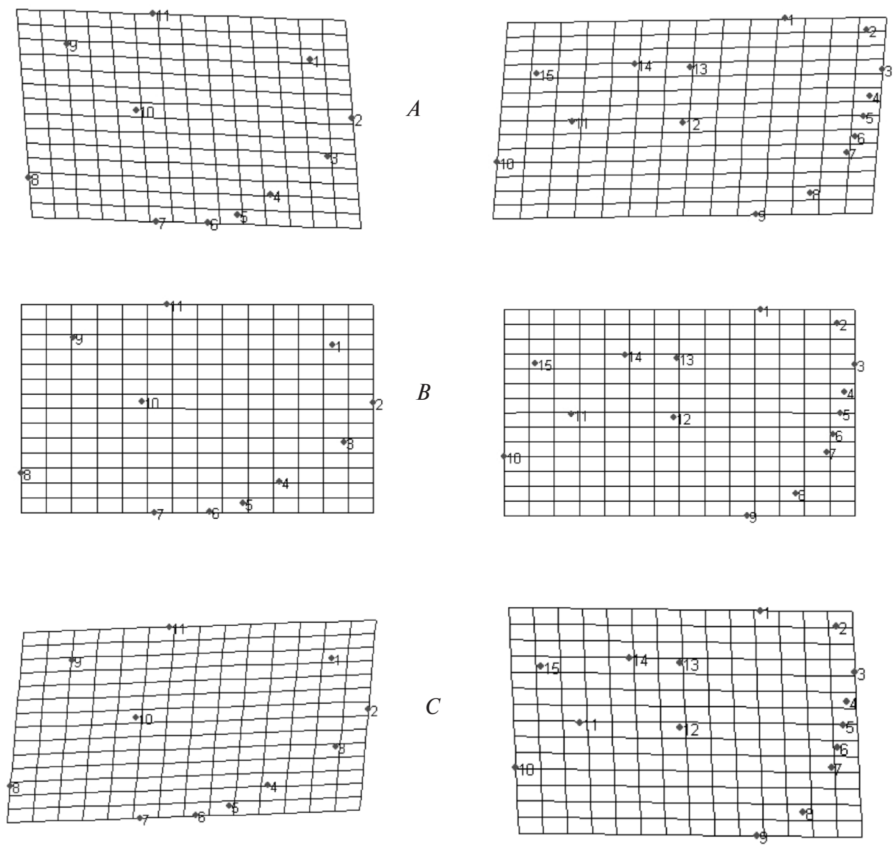

Fig. 4. Overall deformations in fore wing's (right) and hind wing's (left) shape of codling moth: *A* — population of Meshkin Shahr; *B* — reference shape; *C* — population of Mahneshan, Numbers show the landmarks.

Рис. 4. Всесторонняя деформация в форме задних крыльев (слева) и передних крыльев (справа) яблонной плодожорки: *A* — популяция из Мешкин-Шахр; *B* — стандартная форма; *C* — популяция из Махнешан, номера показывают опознава-тельные точки.

terns of these populations were different in view point of fore and hind wings landmarks.

Overall wings shape deformation compared with reference picture is showed in figure 4. The results showed shorter and wider fore wing and also slender and narrower hind wing in Mah. compared with Mes. population. Because of higher windy condition in Mah. in comparison with Mes. (mean wind speed of 10.558 and 5.157 km/h, respec-

Table 5. T-test results of codling moth wing centroid size in Meshkin Shahr and Mahneshan populations

Таблица 5. Результаты проверки размера центроида крыла у яблонной плодожорки из популяций Мешкин Шахр и Махнешан по критерию Стьюдента

Wing	Sex	Number		Centroid size		T
		Meshkin shah	Mahneshanr	Meshkin shah	Mahneshanr	
Fore	Female	14	29	3.40×10^{-2}	3.13×10^{-2}	1.024^{ns}
	Male	22	28	3.16×10^{-2}	3.11×10^{-2}	0.0005^{ns}
Hind	Female	16	23	4.11×10^{-2}	3.85×10^{-2}	0.952^{ns}
	Male	22	28	3.89×10^{-2}	3.41×10^{-2}	5.226^{ns}

Note. ns — no significant difference.

Примечание. ns — нет значительных отличий.

Table 6. T-test results of codling moth wing centroid size in Zandjan and Khoramdareh populations

Таблица 6. Результаты проверки размера центроида крыла в популяциях Зандьяна и Кхорамдарех по критерию Стьюдента

Wing	Sex	Number		Centroid size		T
		Meshkin shah	Mahneshanr	Meshkin shah	Mahneshanr	
Fore	Female	34	31	3.39×10^{-2}	3.30×10^{-2}	0.0028^{ns}
	Male	31	18	3.31×10^{-2}	3.28×10^{-2}	0.0002^{ns}
Hind	Female	32	31	4.68×10^{-2}	3.92×10^{-2}	8.043^{ns}
	Male	30	24	3.04×10^{-2}	3.84×10^{-2}	0.521^{ns}

Note. ns — no significant difference.

Примечание. ns — нет значительных отличий.

tively) presence of this adapted aerodynamic shape of fore and hind wings in Mah. population is predictable. Aerodynamic shaped wings enabled insects to powerful flight and well its control in high windy areas (Epila, 1988; Pasek, 1988). Discrimination of Zandjan (Zan.) and Khoramdareh (Kho.) populations as well as their wing shape deformations based on RWA were similar to Mes. and Mah. populations with reduced degree because of lower differences at wind speed between Zan. and Kho. areas (with wind speed of 7.191 and 11.480 Km/h, respectively) compared with of Mes. and Mah. areas.

In high windy areas (Mah. and Kho.), centroid size of fore and hind wings were smaller than those in low windy ones (Mes. and Mah.) but the differences were not significant as revealed by t-test. (table 5, 6). Insect's small wing is an adapted characteristic for orientation and controlling flight in windy regions (Kemp, 2001). Wing centroid size differences between Mes. and Mah. were more than of Zan. and Kho. in agreement with their differences at wind speed.

Sadeghi et al. (2005) studied population variation of codling moth in Zanjan and Khorasan regions. They reported smaller wing in Zanjan Population compared with Khorasan population. Increased longitude and decreased latitude (colder and more humid condition) in Zanjan than of Khorasan could be the main reasons of these differences. Alipanah et al. (1994) found elevation as affecting factor on wing size and limited generations and opportunity for better feeding were known as factors contributing to bigger wing in elevated regions.

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