An analysis of seasonal dimorphism in codling moths, *Cydia pomonella*, from Iran using geometric morphometrics

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Abstract

Geometric morphometric methods, minimizing redundancy and allowing more powerful statistical tests of shape differences, represent an appropriate tool for differentiation of seasonal populations or closely related taxa. To study seasonal dimorphism, samples of summer and winter populations of the codling moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae), were collected during 2003 and 2004 in two regions of Iran, Mianeh and Salmas. 101 and 99 forewings, and 72 and 88 hindwings were dissected from individuals of Mianeh and Salmas populations respectively. The alignment of stereoscopic images yielded a total of 15 landmarks on the forewings and 11 landmarks on the hindwings, and the geometric transformation resulted in 26 and 18 partial warp scores for the fore- and hindwings respectively. The multivariate analysis of variance based on these variables revealed significant differences among seasonal forms, geographic populations and sexes. Relative warp analysis showed a good discrimination between seasonal forms, especially when using hindwing landmarks in females. Overall shape deformations indicated that fore- and hindwings of both sexes were wider in the winter generation compared to that of summer, especially in females. It appears that a wider wing enables the winter form to fly better and thus, increase the dispersal range, particularly in overwintering females searching for suitable oviposition sites during spring. In both sexes the winter generation also had smaller forewings and larger hindwings compared with the summer form. The aerodynamic shape of the wing in the winter form enables the moths to cope better with unpredictable environmental conditions, like strong wind and heavy rain early in the season. Furthermore, adults of winter form are generally darker than the summer generation. This is a beneficially adaptive trait enabling better absorption of solar radiation during early spring.

Key words: geometric morphometrics, phenotypic plasticity, seasonal dimorphism, codling moth, *Cydia pomonella*.

Introduction

The study of phenotypic plasticity has attracted the interest of biologists ever since Darwin (Hood, 2000; Bernardo *et al*., 2007). Morphological diversity in particular, including seasonal dimorphism, can be related to fitness and thus be a possible target of natural selection (Kemp, 2001; Bernardo *et al*., 2007). Wing shape changes have been an important subject in the study of seasonal forms evolution within species (Adams and Funk, 1997; Kunkel, 2001). For example, observations on seasonal dimorphism in the water strider, *Gerris paludum insularis* (Motschulsky), indicated that a short day photoperiod (12 h light) and low temperature (below 20 °C) resulted in more adults being brachypterous than under a long day photoperiod (14.5 h light) and high temperature (above 30 °C). Thus, this type of insect copes with high summer temperatures and solar radiation by macroptery (Harada and Taneda, 1989).

Currently, the study of phenotypic plasticity is expanding rapidly, with significant advances being made in the area of demonstrating adaptations such as geometric morphometric (Hood, 2000; Pavlinov, 2001). Shapes of organisms are very stable and have high heredity, therefore, the study of overall shape similarity is known as an accurate way for quantitative estimation of phylogeny and the evaluation of variability in biological form (Bookstein, 1989; Rohlf, 1990).

One group of animals that received considerable attention early at the beginning of the study on phenotypic plasticity is the order Lepidoptera, which demonstrated variation in both coloration and body size in response to differences in temperature, photoperiod, relative humidity and precipitation (Chaplin and Wells, 1982; Jones, 1992; Roskam and Brakefield, 1999; Kemp, 2000). Information concerning the influence of seasonal conditions on morphology is important to taxonomists if the range of variation has been underestimated, this may lead to errors in the construction of taxonomic keys to species (Bernardo *et al*., 2007). Phenotypic plasticity has been postulated to be both an inhibitor and a facilitator of phenotypic diversification (Schlichting and Pigliucci, 1998) also it permits organismal diversification within species, such as alternative morphs, without having to couple it to speciation. Moczek (2010) stated that phenotypic plasticity permits an increase in phenotypic diversity without a commensurate increase in species richness. Whitman and Agrawal (2009) noted that phenotypic plasticity, through its ecological effects, can facilitate evolutionary change and speciation. Plasticity is important because it is an encompassing model to understand life on earth, it can increase fitness, generate novelty, and facilitate evolution, it structures ecological communities, and it has numerous practical applications.

The codling moth is the most important pest of apple orchards in Iran, case serious damage when the larvae

bores into a fruit and feeds in the core region (Pajac *et al*., 2012). This pest overwinters as non-feeding caterpillars in leaf litter or under loose flakes of bark and they pupate in the following spring (Pajac *et al*., 2012). This insect has three generations per year in Azerbaijan region- northern west of Iran (Radjabi, 1986). Adult moths first emerge (first generation) during midspring, usually around the period of full flower bloom. The adults of last generation occur in midsummer. Eighteen Km flight has been recorded for codling moth adults by various studies on its flight activity (Mani *et al*., 1995; Voigt, 1999).

The aim of the present study is to investigate seasonal differences in wing shape, size and colour variation in the codling moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae), and suggest adaptive explanations for the phenotypic plasticity of observed in this important orchard pest.

Materials and methods

The studied winter and summer forms were collected at the end of October and mid of July, respectively in two locations of Iran, Mianeh and Salmas during 2003 and 2004. Mianeh is a city in East Azerbaijan province located in 37°25'N 47°42'E, 1100 m asl and has warm and humid weather conditions and Salmas is a county in East Azerbaijan province located in 38°11'N 44°44'E, 1396 m asl and is a Mountainous region. Specimens were collected from a number of sites within each location. To exclude any host association effects, sampling was conducted only in commercial orchards on apple trees of the cultivar 'Golden Delicious'. Specimens from each population were randomly selected from moths pooled across collecting sites within a given region. Sampling of fifth instar larvae was carried out using single face cardboard fastened around the trees at 30cm distance of above ground. For each region, the adults used for wing shape study were obtained from the collected larvae maintaining under laboratory condition (25 °C, 60% relative humidity and 16/8 light/dark photoperiod). After emergence, the adults were anaesthetized using CO_2, killed using a cyanide bottle, then mounted on 00 mounting needles, kept in insect boxes (27 × 40 × 5 cm) at room conditions and air-dried. Immediately after killing and mounting, permanent slide preparations of dried fore- and hindwings were made according to Borror *et al*. (1989), and then digital images were taken using stereo microscope Nikon (SMZ 1000) connected to an image analysing system (table 1).

Homologous structures were selected as landmarks, i.e.

15 and 11 landmarks were chosen from fore- and hindwings, respectively (figure 1). Following the classification of Bookstein (1989), all the landmarks were of type I, representing either the meeting points of wing veins or the points where they reached the wing margin. Male and female moths were analysed separately to avoid any confounding effects caused by sexual dimorphism.

Landmark coordinates were estimated using a Leica stereo microscope (MZ 125) image analyzing system and the software tpsDig 1.3 (Thine Plate Spline Digitizing) (Rohlf, 1990). By the transformation of the coordinate data into shape variables, 26 and 18 partial warp scores were obtained for fore- and hindwings, respectively.

Multivariate analysis of variance (MANOVA) of these shape variables, for the fore- and hindwings of both sexes and both seasonal forms, was carried out using SPSS 14.0 software. Relative warp analysis (RWA) was performed and relative variation in the wings of the two seasonal forms determined using the software, tpsRelw 1.22 (Thine Plate Spline Relative Warp Analysis). By calculating the wing centroid size, the square root of the sum of squared distances of the set of landmarks from their centroid, of the summer and winter forms, a t-test was carried out with SPSS 14.0 to estimate the effects of seasonal conditions.

Results and discussion

The results of MANOVA considering all shape variables from fore- and hindwings indicated significant differences between locations, sexes and seasonal forms, as well as the interactions of each of the sources of shape variability with the exceptions of location × sex for forewing, and location × sex, sex × season, and location × sex × season for the hindwing (table 2).

To determine the contribution of both uniform and non-uniform components of shape explaining wing deformations, MANOVA was performed separately on each type of component (table 3). The results were similar to those of the analysis of all data and showed that wing deformation was stronger influenced by the non-uniform components than uniform components, especially in the hindwing., In a study of population variation in nine codling moth populations from northwest Iran, significant differences at the 1% probability level were found among populations and sexes for both types of variables (Khaghaninia *et al*., 2008). The authors determined non-uniform variables to be more effective in causing wing shape variation.

Table 1. Numbers of fore- and hindwing images sampled for each seasonal and geographic population of codling moth.

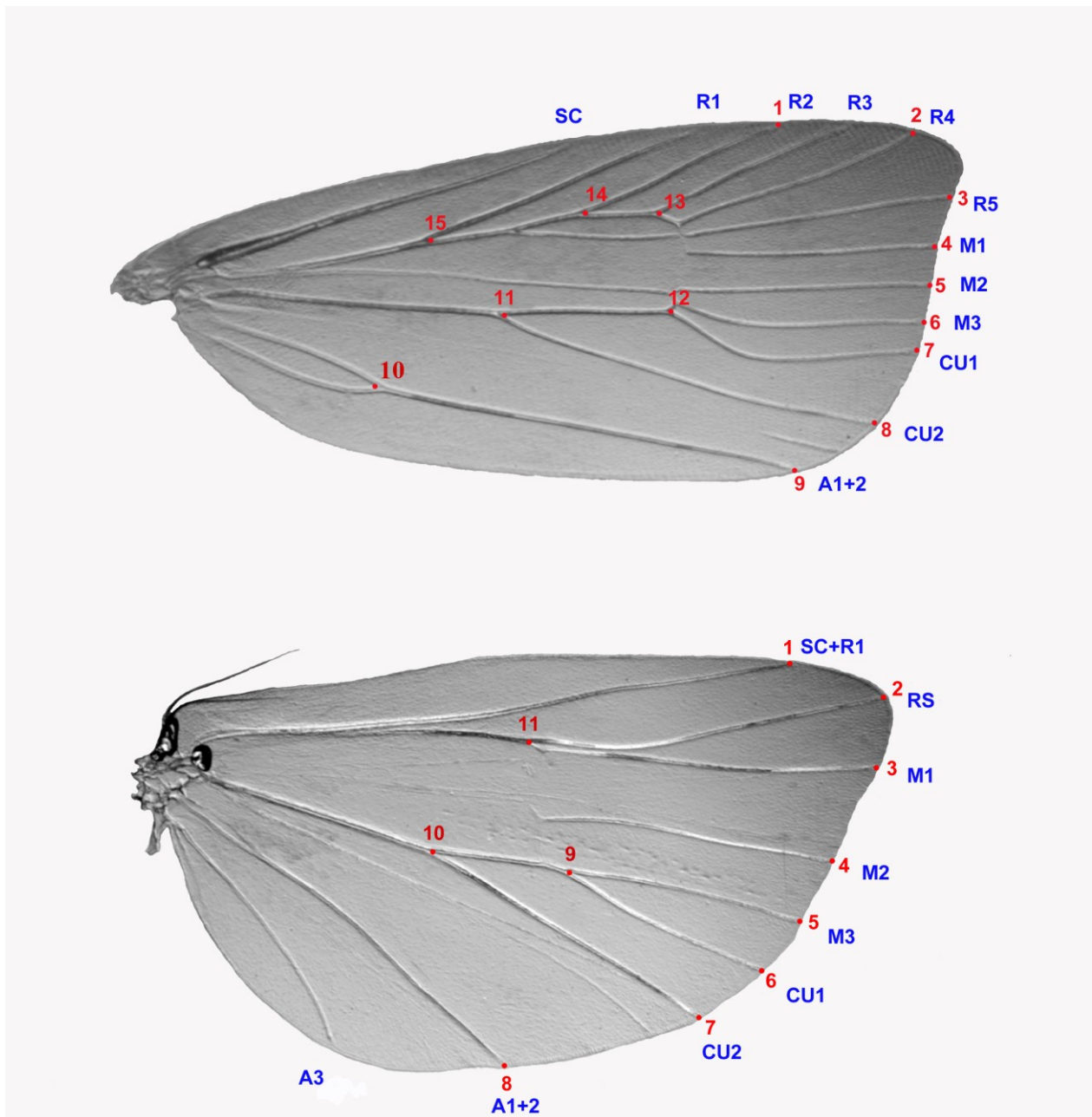| Population/Location	Forewings			Hindwings		
	Female	Male	Total	Female	Male	Total
Mianeh (summer)	27	27	54	19	18	37
Mianeh (winter)	24	27	51	21	21	42
Salmas (summer)	25	22	47	15	20	35
Salmas (winter)	22	26	48	21	25	46
Total	98	102	200	76	84	160

Figure 1. Representative fore- and hindwings of *C. pomonella* showing the positions of the landmarks.

Table 2. Results of the multivariate analysis of variance for shape variables of fore- and hindwings of codling moth; values of specimens from Mianeh and Salmas were pooled.

		Forewing			Hindwing	
Source of variance	df	Wilks' lambda	F	df	Wilks' lambda	F
Location	26, 167	0.568	4.878^{**}	18, 135	0.642	4.189^{**}
Sex	26, 167	0.398	9.796^{**}	18, 135	0.18	34.172^{**}
Season	26, 167	0.619	3.96^{**}	18, 135	0.556	5.986^{**}
Location × sex	26, 167	0.811	1.499^{ns}	18, 135	0.839	1.434^{ns}
Location × season	26, 167	0.657	3.353^{**}	18, 135	0.72	2.92^{**}
Sex × season	26, 167	0.779	1.818^{*}	18, 135	0.876	1.063^{ns}
Location × sex × season	26, 167	0.773	1.881^{**}	18, 135	0.875	1.067^{ns}

ns: no significant difference; *: significant difference at 5% probability level; **: significant difference at 1% probability level.

Table 3. Multivariate analysis of variance for uniform and non-uniform shape variables of fore- and hindwings of codling moth.

Source of variance	Forewing			Hindwing		
	df	Wilks' lambda	F	df	Wilks' lambda	F
Uniform						
Location	2, 191	0.917	8.698**	2, 151	0.942	4.649*
Sex	2, 191	0.771	28.421**	2, 151	0.571	56.61**
Season	2, 191	0.923	8.009**	2, 151	0.976	1.851ns
Location × sex	2, 191	0.993	0.634ns	2, 151	0.974	2.044ns
Location × season	2, 191	0.861	15.407**	2, 151	0.984	1.22ns
Sex × season	2, 191	0.999	0.072ns	2, 151	0.993	0.513ns
Location × sex × season	2, 191	0.978	2.143ns	2, 151	0.958	1.298ns
Non-uniform						
Location	24, 169	0.576	5.175**	16, 137	0.725	3.243**
Sex	24, 169	0.429	9.357**	16, 137	0.19	36.405**
Season	24, 169	0.647	3.849**	16, 137	0.556	6.554**
Location × sex	24, 169	0.838	1.364ns	16, 137	0.872	1.257ns
Location × season	24, 169	0.685	3.242**	16, 137	0.72	3.322**
Sex × season	24, 169	0.789	1.882*	16, 137	0.885	1.214ns
Location × sex × season	24, 169	0.787	1.905*	16, 137	0.896	3.122*

ns: no significant difference; *: significant difference at 5% probability level; **: significant difference at 1% probability level.
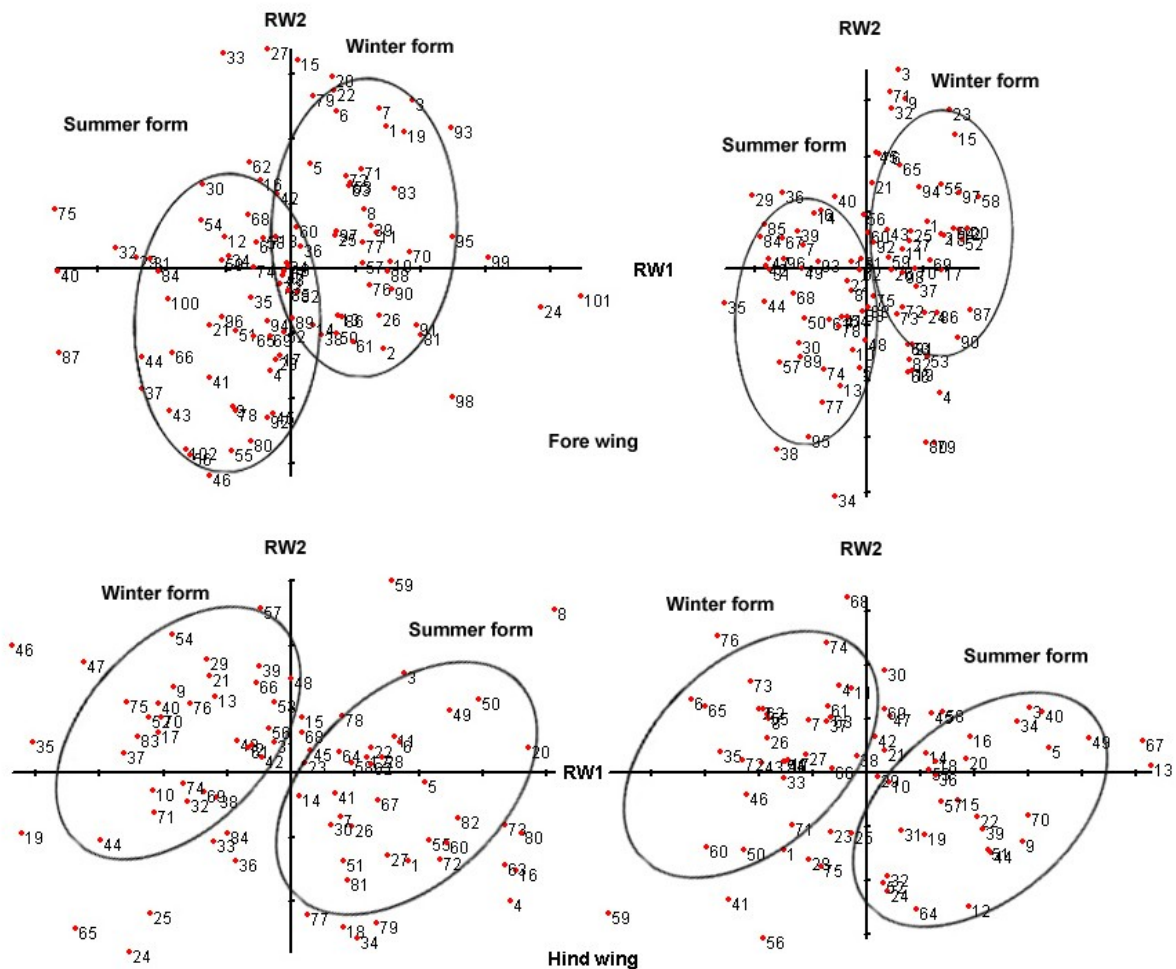

Figure 2. Distribution of summer and winter forms of codling moth found by RWA of landmarks in the Mianeh and Salmas populations; females (right), males (left).
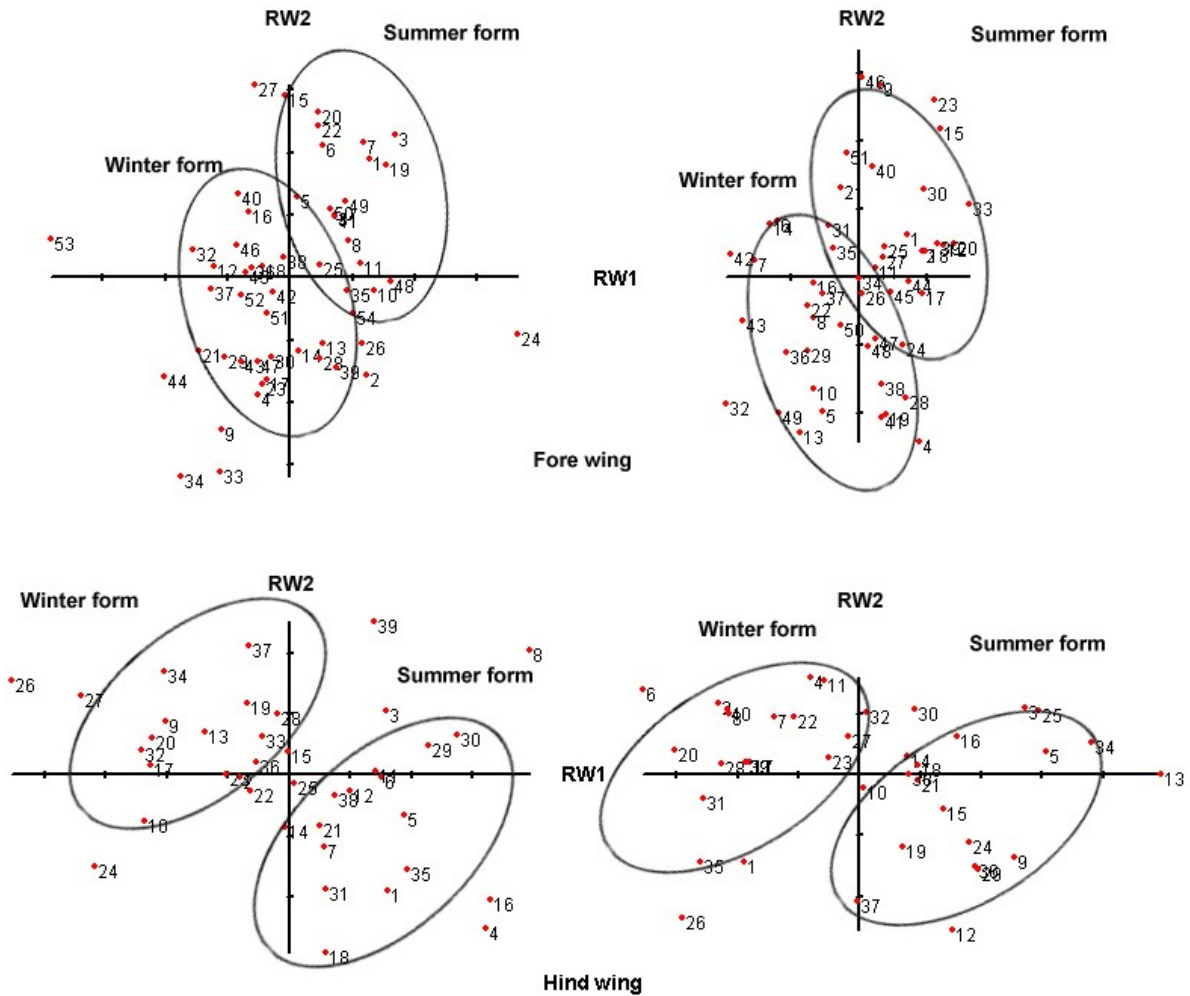
Figure 3. Distribution of summer and winter forms of codling moth found by RWA of landmarks in the Salmas population; females (right), males (left).

The discrimination of different seasonal forms of codling moth based on RWA using fore- and hindwing shape variables is shown at figure 2. The discrimination pattern shows that discrimination of seasonal forms was more distinct in females than in males, especially for hindwing, which is in agreement with the significant differences found in the interaction between sex × season, especially when based on non-uniform variables.

The discrimination patterns of seasonal forms of codling moth based on RWA of fore- and hindwing shape variables at different geographic locations, Salmas and Mianeh, are shown in figure 3 and figure 4 respectively. A significant location × season interaction is confirmed by the improved discrimination of seasonal forms in the Salmas population compared with the Mianeh population. As the geographic data of sampling sites show, Salmas located in mountainous area and the environmental conditions are more different between summer and winter than to Mianeh region.

Overall wing shape deformations of the fore- and hindwings in the two seasonal forms, and a reference picture for comparison, are shown in figure 5. The re-

sults show a stretching of the forewing from the posterior basal region distally towards the wing tip (apex) in the winter form and a stretching in the reverse direction, from the anterior basal region distally towards the tornus, in the summer form. The stretching of the hindwings is less than that in the forewings and is in the opposite direction in both generations, i.e. the elongation in the forewings of the winter generation is in the same direction as that in the hindwings of the summer generation. The overall effect is that wings in the winter form are wider and shorter compared with the summer form, which enhances the dispersal ability of this generation early in the spring (Epila, 1988; Pasek, 1988; Kemp, 2001). Dorn *et al*. (1999) and Lambert (1972) demonstrated that females are more effective than males in dispersing in spring, findings that are confirmed by the results of our RWA analysis, which showed that the influence of seasonal conditions on wing deformation is greater in females than males.

The centroid sizes of the fore- and hindwings of the winter form were significantly smaller and larger, respectively, compared with those of the summer form as
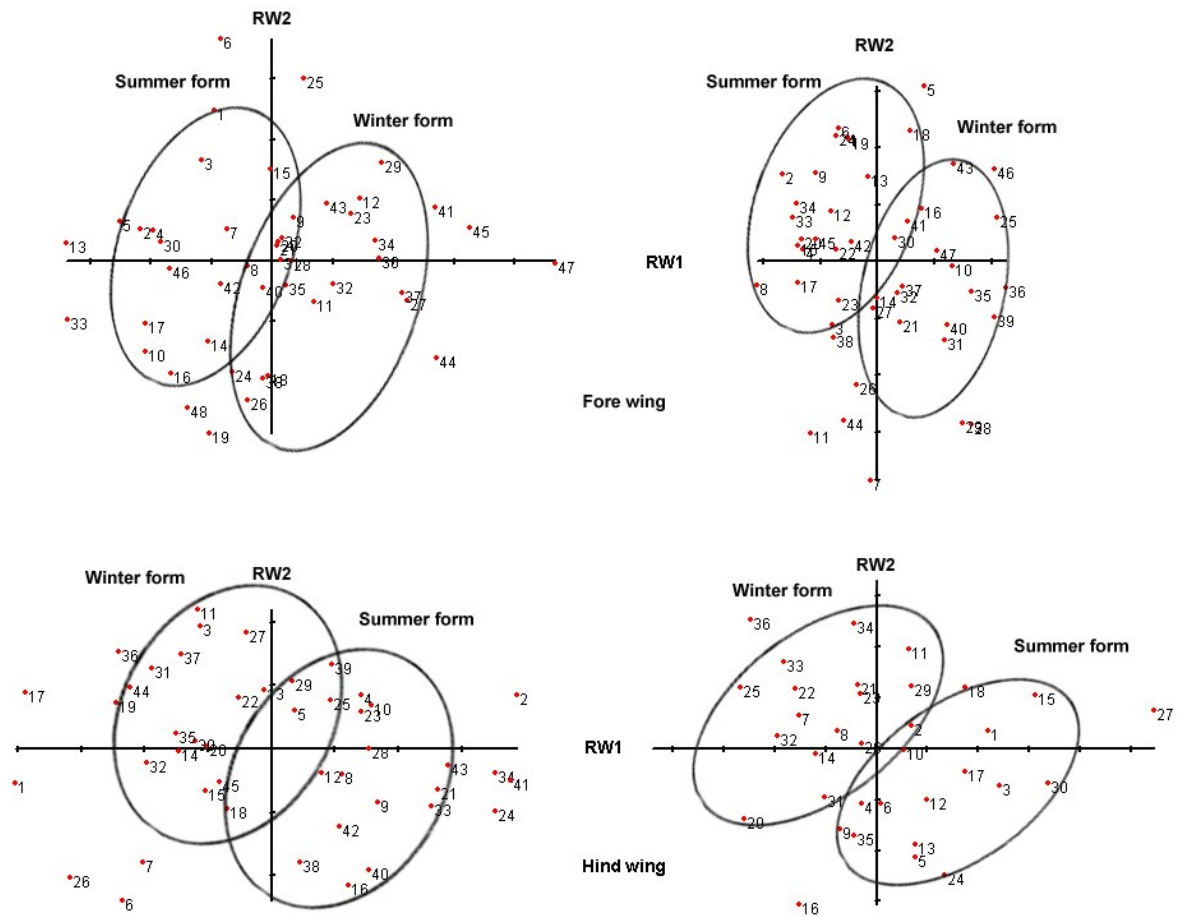
Figure 4. Distribution of summer and winter forms of codling moth found by RWA of landmarks in the Mianeh population; females (right), males (left).
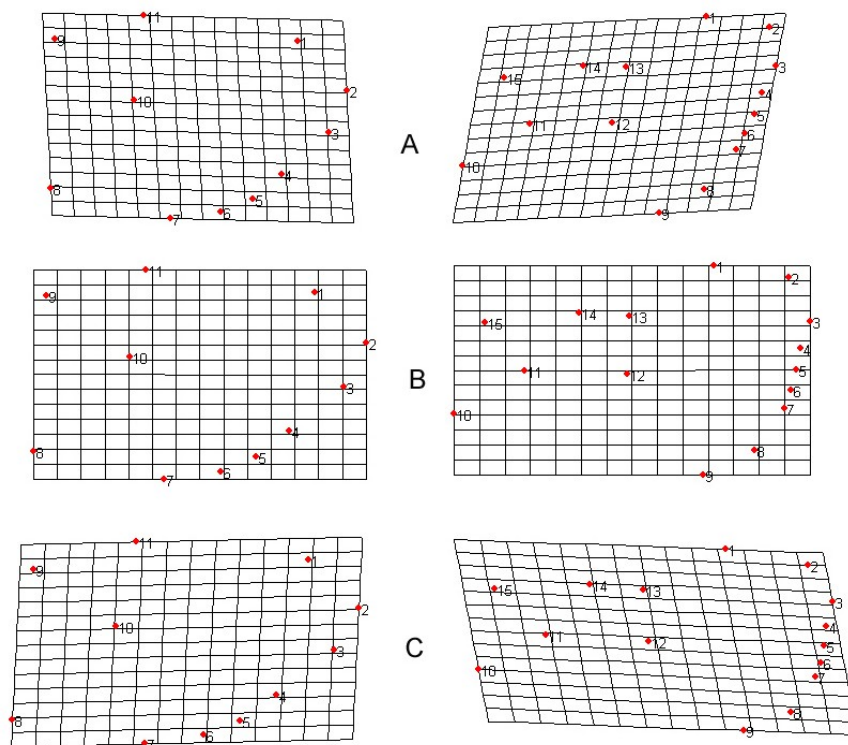

Figure 5. Overall deformations in forewing (right) and hindwing (left) shape of codling moths *C. pomonella*: (A) winter generation, (B) reference shape, (C) summer generation. Numbers refer to the landmarks in figure 1.

Table 4. Differences in codling moth wing centroid sizes between seasonal forms of the Salmas population corresponding t-test statistics.

	Sex	Examined number/sample size		Centroid size (dimensionless)		t
		Summer form	Winter form	Summer form	Winter form	
Forewing	Female	25	22	3.58×10^{-2}	3.32×10^{-2}	20.621^{*}
	Male	22	26	3.47×10^{-2}	3.28×10^{-2}	0.762^{ns}
Hindwing	Female	15	21	4.38×10^{-2}	4.48×10^{-2}	26.296^{*}
	Male	20	25	3.34×10^{-2}	3.93×10^{-2}	8.655^{ns}

ns: no significant difference; *: significant difference at 5% probability level.

revealed by the t-test. The influence of environmental factors on wing size variation in females was greater than in males (table 4). Other studies have demonstrated the major contribution of females to dispersal and geographical range coverage, especially early in the year and before oviposition (Lambert, 1972; Dorn *et al*., 1999; Voigt, 1999). Short and small forewings, and wide and large hindwings, give a more aerodynamic wing shape to the winter form, resulting in an adaptive phenotype that makes them resistant/robust to more extreme and unpredictable weather conditions in early spring, such as strong wind and rainfall (Epila, 1988; Pasek, 1988; Kemp, 2001).

Adults produced by overwintering larvae were generally darker than those of the summer generations. Since overwintering moths begin reproductive activity in the spring, when ambient temperatures are relatively low, dark wing markings may be thermally beneficial at these times. That effect is commonly known for numerous insect species (Watt, 1968; Kingsolver, 1995). However, overheating, even for short periods, has been shown to reduce survivorship and fecundity in temperate region species, so light wing colour would help alleviate such overheating in summer forms (Kingsolver and Watt, 1983). Phenotypic plasticity, the ability of a single genotype to give rise to different phenotypes depending upon perceived environmental signals, allows the insects to deal adaptively with unpredictable environmental conditions (Kemp, 2001).

Although phenotypic plasticity in Lepidoptera has been extensively studied, there still remain examples of striking plasticity that are functionally obscure (Gotthard and Nylin, 1995). Our study suggests an explanation for a case showing clear adaptive grounds (shape, size and colour of wing) to expect plasticity of a given nature within a species, but where plasticity had never been investigated and the underlying cause is not immediately obvious.

Acknowledgements

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