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Sexual dimorphism in the wing shape and size of the carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

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Abstract

The carob moth, *Ectomyelois ceratoniae* (Zeller), belongs to the family Pyralidae and the subfamily Phycitinae. In spite of some features of sexual dimorphism in size and shape in the order Lepidoptera and the mentioned family, it has not been recorded in this species. In the current study, sexual dimorphism in the wing shape and size of carob moth on four hosts (pomegranate, fig, pistachio and walnut) were detected using landmark- based geometric morphometric and analysis of partial warp scores and centroid sizes. The analysis showed significant wing shape differences (fore wing: P = 1.315E-011, hind wing: P = 1.168E-007) which was the same on all tested hosts. Geometric changes in the fore and hind wing of both sexes were illustrated. Analyses of size showed wings of the females are bigger than those of the males (fore wing: F = 23.19, P = 0.000; hind wing: F = 16.73, P = 0.000) on tested hosts and in spite of allometric growth in test specimens, significant shape differences are still remain in constant size.

Key words: sexual dimorphism, carob moth, *Ectomyelois ceratoniae*, geometric morphometric

چکیده

پروانه‌ی کرم گلوگاه انار، *Ectomyelois ceratoniae* (Zeller) ، به خانواده‌ی Pyralidae و زیر خانواده‌ی Phycitinae تعلق دارد. علیرغم وجود شواهدی دال بر وجود اختلاف شکل و اندازه در جنس‌های نر و ماده‌ی بسیاری از پروانه‌ها و همچنین در اعضای این خانواده، وجود چنین اختلافی تاکنون در گونه‌ی مورد بحث گزارش نشده است. در تحقیق حاضر، دوشکلی جنسی در اندازه و شکل بال پروانه‌ی کرم گلوگاه انار روی چهار میزبان انار، انجیر، پسته و گردو با استفاده از مرفومتریک هندسی و آنالیز partial warps و centroid sizes مورد بررسی قرار گرفت. نتایج تجزیه و تحلیل‌های فوق وجود اختلاف معنی‌دار در شکل بال پروانه‌های نر و ماده را نشان داد (بال جلو: P = 1.315E-011، بال عقب: P = 1.168E-007) و ثابت شد که این اختلاف در افراد روی میزبان‌های مورد آزمایش به یک میزان می‌باشد. سپس اختلافات هندسی شکل بال‌ها در نر و ماده به تصویر کشیده شد. آنالیزهای فاکتورهای مربوط به اندازه‌ی بال نشان داد که افراد ماده از اندازه‌ی بال بزرگتری نسبت به نرها برخوردار هستند (بال جلو: F = 23.19، P = 0.000؛ بال عقب: F = 16.73، P = 0.000). این اختلاف در جمعیت‌های روی میزبان‌های مختلف نیز وجود داشته و علیرغم وجود رشد آلومتریک، اختلاف معنی‌دار شکل بال در اندازه‌ی ثابت همچنان باقی می‌ماند.

واژگان کلیدی: دوشکلی جنسی، پروانه‌ی کرم گلوگاه انار، *Ectomyelois ceratoniae,*، مرفومتریک هندسی

Introduction

Sexual dimorphism is defined as the systematic difference in form between individuals of different sexes in the same species (Wikipedia contributors, 2006). Male and female differences have been studied extensively in several aspects such as physiological (Yurkiewicz, 1969; Porco *et al.*, 2004), biochemical, movement (Krasnov *et al.*, 2003), morphological and other traits. Most animal species exhibit phenotypic (size and shape) differences between males and females. Sexual dimorphism in size (SSD) has attracted the attentions of numerous biologists after Darwin (1871) and have been attempted to be explained in evolutionary biology (Webster, 1997). In insects, females are often larger than males. It is thought that the reason lies in the huge number of eggs that insects lay. A larger body size enables a female insect to lay more eggs (Wikipedia contributors, 2006). Longer lifespan is another advantage of large size; females live longer than males in most animals. Gender differences in lifespan and mortality rates have been recorded in two seed beetle species (Fox *et al.*, 2003). Color, development of some parts of the body such as horns in beetles (Emlen *et al.*, 2005), presence of sting in bees, the size of eyes (Land, 1989, 1990) and morphologic differences in some instars (Cook *et al.*, 2000) are some other sex specific differences.

Sexual dimorphism is frequently observed in Lepidoptera such as colour in Lycaenidae, elaboration of feelers bearing numerous sensory nerve endings, presence of scent-producing glands in Danaidae and even lack of wings in females in some species of Lymantriidae (Anonymous, 2004). Sexual dimorphism in wing patterns was recorded in some Lepidoptera (Yen *et al*., 2004; Yen *et al*., 2005). Frenulum in the hind wing shows a common difference in sexes in some Lepidoptera.

Sexual dimorphism in size and shape has not been reported in the carob moth, *Ectomyelois ceratoniae* (Zeller). Even the number of bristles in frenulum is the same (one) in both; as in other Phycitinae (Munroe & Solis, 1999). In this study, wing shape and size differences were detected in the male and female of the carob moth, using landmark-based geometric morphometric and analyses of partial warps and centroid sizes.

Geometric morphometrics is a relatively new method that allows better assessment of morphologic characteristics (Pretorius *et al*., 2006). This method has been used to show sexual dimorphism in mammals (Hood, 2000), turtles (Valenzuela *et al*., 2004), leaf beetle *Neochlamisus bebbianae* (Brown) (Adams & Funk, 1997), true bug *Panstrongylus geniculatus* (Latreille) (Jaramillo, 2002) etc.

Materials and methods

Preparing specimens for study: larvae of specimens were collected from infected fruits (pomegranate, pistachio, fig and walnut) and then reared to adulthood in the laboratory. Tested groups associated on any host, including male and female were collected in a same geographic region. In order to eliminate some limitations in further analyses, the number of specimens in any tested groups was chosen more than 2P-4 (which is equal to the number of elements of W matrix where P is the number of landmarks (Zelditch *et al.*, 2004) (table 1). Wing slides were prepared and captured by a dissecting microscope and a CCD video camera.

Table 1. Host plants, the number of any sex and codes for tested groups of the carob moth populations.

Host plant	Sex and number of the fore wing	Sex and number of the hind wing	Code (sex, host plant)
Pistachio	Female, 17	Female, 14	f, Pi
	Male, 17	Male, 14	m, Pi
Fig	Female, 17	Female, 14	f, Fi
	Male, 17	Male, 14	m, Fi
Pomegranate	Female, 17	Female, 14	f, Po
	Male, 17	Male, 14	m, Po
Walnut	Female, 17	Female, 14	f, Wa
	Male, 17	Male, 14	m, Wa

Geometric morphometric analysis: ten landmarks on the fore wing and seven landmarks on the hind wing were selected (fig. 1) and their Cartesian coordinates were digitized by tpsDig (Rohlf, 2003a). Landmark data have some information such as orientation, rotation and scale of any specimen. The non-shape information was held constant mathematically to remove non-shape variation (Rholf & Slice, 1990). Then all specimens were superimposed using generalized procrustes analysis (GPA), so that all homologous landmarks were located as close as possible (Rohlf & Slice, 1990). Points provided by aligned specimens were projected to tangent space of Kendall shape space (Kendall, 1984; Rohlf, 1999; Slice, 2001), therefore distances between specimens approximate the procrustes distance between the corresponding pairs of landmark configurations (Adams *et al.*, 2004). Then shape variables of geometric morphometric (partial warp scores) were generated by thin-plate spline equation (Bookstein, 1991). Such variables provide a quantification of overall shape that can be used in conventional statistical analysis, and preserve the geometry of anatomical structure, as well as present mean forms, shape attends and its covariation with other variables (Adams & Rohlf, 2000). Uniform components, which describe shape changes such as infinitive scale stretching or compression (Bookstein, 1996; Rohlf & Bookstein, 2003), were calculated by uniform equation (Bookstein, 1989, 1991, 1996). In this study, uniform components of shape variation were appended as additional columns (U1 and U2) in the matrix of partial warps (W matrix) suggested by Rohlf *et al.* (1996). Centroid sizes (the square root of the sum of squared distance of set of landmark from the centre of gravity or the square root of the sum of the variances of the landmarks about that centroid in x and y-directions) as a size measure of any specimen (Slice *et al.*, 1996) were calculated and used as variables in univariate statistical analysis for comparing the wing size of specimens (Adams & Funk, 1997). The above analyses were performed by tpsRelw (Rohlf, 2003b). Sexual dimorphism in wing shape could be shown graphically by tpsRegr (Rohlf, 2000).

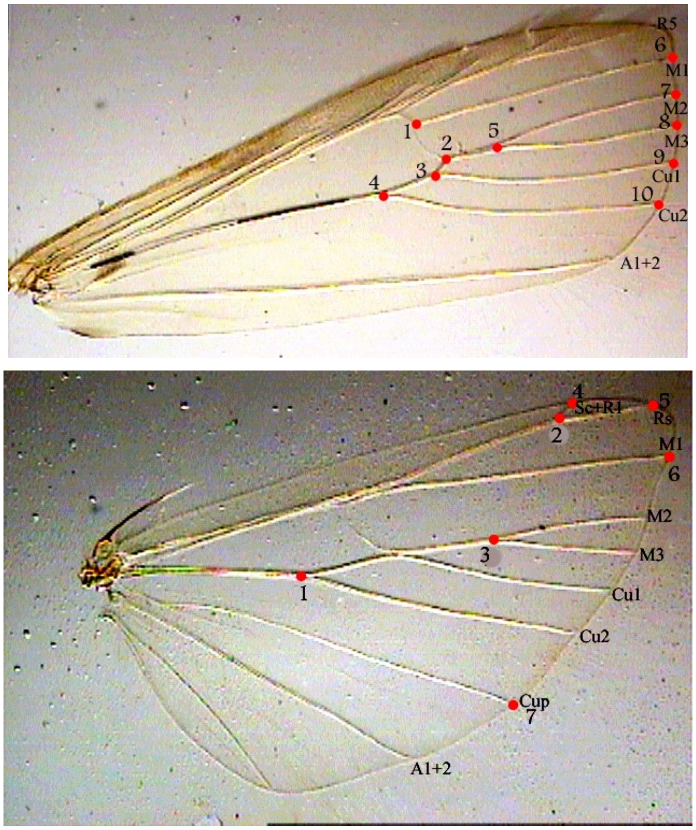


Figure 1. Landmarks used on the fore and hind wings of *E. ceratoniae.* Terminology follows that of Borror *et al.* (1989).

Statistical analysis: two-way MANOVAs were designed for both fore and hind wings to detect any significant wing shape differences among sexes and test populations and their interaction. Sexual dimorphism in size was detected by comparing centroid sizes of female and male associated with any host plant together and separately. Then regressions of variables of shape on variables of size and a MANCOVA were designed to detect any allometric growth and separate allometric trajectories. Statistical analyses were performed using NTSYS-pc (Rohlf, 1998) and MINITAB (Minitab Inc., 2000).

Results

According to the results of two-way MANOVA (table 2), there was a significant difference in the wing shape of the two sexes of carob moth. Since the interaction term is not significant, therefore the sexual dimorphism is the same in all tested host plants.

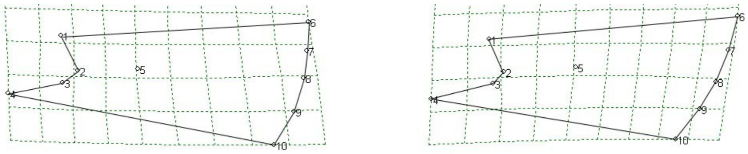
Table 2. Two-way MANOVA on Wmatrix of host plant associated populations of *E. ceratoniae* in Iran.

Wing	**Source**	**Wilks' Lambda**	**Prob.**
Fore wing	Host plant	0.21603252	1.474E-022**
	Sex	0.58319883	1.315E-011**
	Interaction	0.67893928	0.6401
Hind wing	Host plant	0.22697036	3.203E-022**
	Sex	0.67635244	1.168E-007**
	Interaction	0.72413700	0.4348

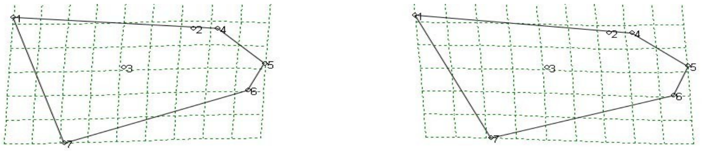
** Significant at $P < 0.01$

Wings in females are wider than those in males, veins M2 and M3 in the fore wing are longer in females and landmark 1 and landmark 3 in the hind wing are closer to each other than those in males. There are some other changes in the position of other landmarks as shown in figure 2.

Size comparisons showed significant differences between wing size of female and male (fore wing: $F = 23.19$, $P = 0.000$; hind wing: $F = 16.73$, $P = 0.000$). Similar comparisons of wing size between sexes on different host plants separately showed larger sizes of female than male in any host plant (fig. 3).

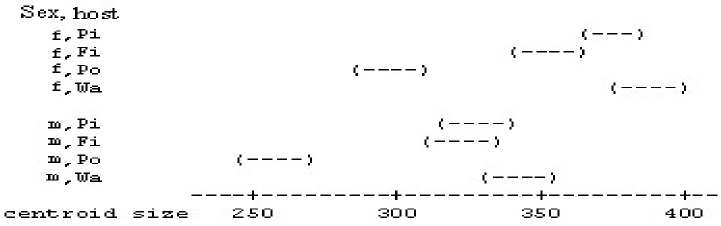


Fore wing, female Fore wing, male



Hind wing, female Hind wing, male

Figure 2. Predicted shape differences of fore and hind wings in the female and male of *E. ceratoniae* on test host plants.



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Sex, host
   f, Pi                                              (---)
   f, Fi                                         (----)
   f, Po                          (----)
   f, Wa                                                (----)

   m, Pi                                    (----)
   m, Fi                                   (----)
   m, Po                  (----)
   m, Wa                                       (----)
                     ----+---------+---------+---------+--
centroid size          250       300       350       400
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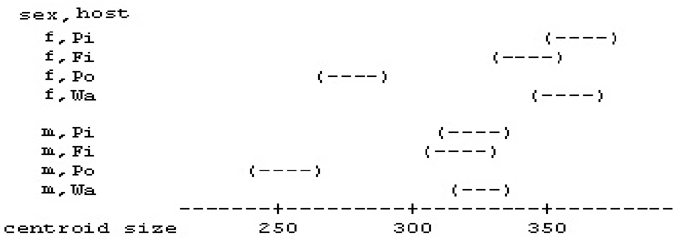
a) Fore wing



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sex, host
   f, Pi                                           (----)
   f, Fi                                       (----)
   f, Po                         (----)
   f, Wa                                          (----)

   m, Pi                                  (----)
   m, Fi                                 (----)
   m, Po                   (----)
   m, Wa                                   (---)
                 -------+---------+---------+---------
centroid size         250       300       350
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b) Hind wing

Figure 3. Comparison of size in host plant associated populations in the both sexes of *E. ceratoniae* in Iran. See table 1 for codes.

Allometric analysis showed allometric growth among female and male individuals (table 3a). Allometric slopes in female and male populations did not vary significantly (table 3b) but there were still significant differences between shapes of wings when size was held constant (table 3c). Therefore, the wing shape of females and males vary in parallel and separate allometric trajectories.

Table 3. Allometry significant tests: allometric growth (a), comparing allometric slope (b) and comparing shape in constant size (c) in the female and male of *E. ceratoniae*.

Wing	**(a) Wilks' Lambda**	**(a) Prob.**	**(b) Wilks' Lambda**	**(b) Prob.**	**(c) Wilks' Lambda**	**(c) Prob.**
Fore wing	0.27992978	2.257E-034**	0.88027012	0.2144	0.64168668	8.501E-009**
Hind wing	0.37518052	4.586E-023**	0.85554500	0.0255	0.73857483	1.722E-005**

** Significant at $P < 0.01$

Discussion

In the most of the dioecious animal species, females and males have different sizes. In fact, the body size of males and females is determined by forces acting for survival and reproduction (Arak, 1988). Sexual dimorphism in size and shape has not ever been recorded in *E. ceratoniae* previously. Our analysis showed that females of *E. ceratoniae* have larger wing size than that of the males. Larger wing size in females may be the result of larger body size. The phenomenon of larger body size is commonly observed in arthropods e.g. dung fly (Kraushaar & Blanckenhorn, 2001), vinegar fly (Harisson & Cooper, 2003), some seed beetles (Fox *et al.*, 2003), true bug giant water strider (Tseng & Rowe, 1999), a whitefly *Bemisia afer* (Priesner & Hosny) (Maruthi *et al.*, 2004) and most spider species (Schneider & Lubin, 1998). There are logical basis in evolutionary terms to sexual dimorphism based on size. The female is the originator and in some cases, protector of the next generation of the species; so her functions are likely to be carried out more efficiently by a larger size (Preston-Mafham & Preston-Mafham, 1989). According to the results shown in figure 3, the wing sizes of females on any host are larger than those of the males on the same host but there are overlaps between different sex individuals on different hosts, which may occur because of different nutrition during larval feeding. Maruthi *et al.* (2004) believed in spite of a singificant difference between the body size of male and female of *B. afer*, overlaps in their body sizes, however, makes sex assignment based on size alone unreliable.

Our geometric morphometric analyses in the current study showed significant wing shape sexual differneces in *E. ceratoniae* graphically (fig. 2). Wing shape sexual dimorphism is a common feature in several insect species. The phenomenon of brachyptery or wing reduction is a well known example in this criterion. Although Haas & Tolley (1998) did not find any substantial sexual dimorphism in the wing size and shape of *Drosophila lummei* Hackman (Dip.: Drosophilidae), there are various records which show sexual dimorphism in appearance of wings in insects such as wing patterns in zygaenids (Yen *et al.*, 2005), a pyraloid, the genus *Austromusotima* Yen & Solis (Lep.: Crambidae) (Yen *et al.*, 2004) and colour pattern in Libellulidae (Borror *et al.*, 1989). Kunkel & Bettencourt (2001) showed a significant wing shape difference in the male and female of *Drosophila melanogaster* Meigen (Dip.: Drosophilidae), and it was also shown in *Chilo suppressalis* (Walker) (Lep.: Pyralidae) by Zahiri *et al.* (2004). The function and origin of secondary sexual differences including the role of sexual selection are not clear, especially among the invertebrates (Adams & Greenwood, 1983). McLechlan (1986) referred to the wing shape sexual dimorphism in *Chironomus imicola* Kieffer (Dip.: Chironomidae) and believe due to different roles of adult males and females, flight might be expected to be sexually dimorphic in this species. According to our two way MANOVA, sexual dimorphism in the wing shape of carob moth is the same in individuals on the four tested hosts. Sarafrazi *et al.* (2004) showed sexual dimorphism in *Eurygaster integriceps* Puton (Hem.: Scutelleridae) on wheat while there was not such difference in the same species on barley. Gilchrist *et al.* (2000) showed constant gender related shape differences of wing shape among populations of *D. melanogaster* and suggested that the gender differences represent a developmental constraint on wing shape which can also be true in carob moth populations. Further multivariate analyses in this study showed different and parallel allometric trajectories for males and females. It can be concluded that environmental conditions such as different larval nutrition may cause different degrees of growth in adults of the carob moth and produce large males or small females in different hosts, but there are still significant difference in the wing shape of males and females. Hence, the male and female groups with the same wing size have still significant different wing shapes.

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