

Effects of biological and environmental factors on sex ratio in *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of *Cydia pomonella* L. (Tortricidae)

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Abstract: The sex ratio in the natural population of most Hymenopteran parasitoids is 1 : 1. Females of these parasitoids, however, can regulate the sex of their offspring in response to environmental and biological factors. Under certain circumstances, this may lead to an overproduction of males, which can result in a very negative impact on any control program using these parasitoids. For these reasons, understanding the effect of these factors is very important. In this study, five biological and environmental factors known to affect sex ratio in parasitic Hymenoptera were investigated for *Ascogaster quadridentata* Wesmael, a parasitoid of *Cydia pomonella* L. Results showed that food, ambient temperature, and parasitoid age had significant effects on the sex ratio of the offspring of Hymenopteran parasitoids. The percentage of females decreased with lack of food, parasitoid age (when it became 10-days-old or older), and at low (15°C) and high (35°C) temperatures. The effect of host age and density, however, was not significant.

Key words: biological control, codling moth, percentage of females

Introduction

Ascogaster quadridentata Wesmael (Hymenoptera: Braconidae) is an egg-larval parasitoid of the codling moth *Cydia pomonella* L. (Tortricidae) (Brunner 1993). It is the most important parasitoid of codling moth (CM) egg-larval stages. *Ascogaster quadridentata* is the natural enemy with the highest potential in integrated pest management (IPM) programs against the codling moth pest. *Ascogaster quadridentata* is a strong flier and a good searcher. Its life cycle is synchronised with that of the codling moth (Brunner 1993). Adult females lay their eggs in the codling moth eggs and the parasitoid develops during the egg and larval stages of the host (Charmillot *et al.* 1997). Female-biased sex ratios are common in Hymenopteran parasitoids and many species adjust their sex ratio in response to environmental and ecological factors (Werren 1980; Godfray 1994). Female Hymenoptera can regulate the sex of their offspring as a result of their haploid genetic system in which fertilised eggs develop into (diploid) females and unfertilised eggs become (haploid) males. More often, the sex ratio in the natural population of Hymenopteran parasitoids is close to 1 : 1 (Harl and Brown 1970). However, it has been shown that sex ratio of hymenopteran parasitoids is affected by many biological and environmental factors, such as parasitoid age (Medeiros *et al.* 2006), host age (Uckan and Gulel 2002; Shuker *et al.* 2006; Bogdanovic *et al.* 2009), host density (Owen 1983; Werren 1987; Yu *et al.* 2003), temperature (Jalali *et al.*

1990; Biswas and Singh 1998; Fuester *et al.* 2003), and food availability (Hagley and Barber 1992; King 1993; Garcia-Medel *et al.* 2007). Over-production of males in this parasitoid is undesirable. Therefore, research is needed to determine which of these factors are responsible for the sex ratio allocation of *A. quadridentata*. The purpose of this work is to study the influence of some environmental and biological factors, particularly parasitoid age, host age, host density, temperature, and food on the *A. quadridentata* offspring sex ratio under laboratory conditions.

Materials and Methods

Insects

In these experiments, the CM were obtained from a colony that had been reared for over 80 generations on an artificial rearing diet (Mansour and Mohamad 2004). The colony originated from moths collected at several locations near the city of Damascus, Syria, in the summer of 1995. Plastic trays in which each contained about 800 g of larval diet were incubated under a 16L : 8D photoperiod, 27±2°C, and 75±5% relative humidity (RH). The emerged adults were placed in an oviposition cage similar to that reported by Proverbs and Logan (1970). In these cages, females deposit their eggs on wax paper sheet incases the oviposition cage. The parasitoid (*A. quadridentata*) colony originated from CM larvae collected from orchards in the apple production

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area north of Damascus in 2006. Parasitoid adults were maintained in plexiglass "parasitism containers" (40 × 30 × 20 cm) under the same environmental condition as the CM colony. Sheets of wax paper (12 × 16 cm) carrying CM eggs (egg sheets) were fastened, using small hooks, to the inside wall of the parasitism container. Twenty-four h later, the egg sheets were removed and placed above the plastic trays containing the CM rearing diet. Seven days later, the egg sheets were removed and the plastic trays were incubated under the same environmental conditions as for the CM colony. Four weeks later, the trays were transferred into a wooden cabinet to collect parasitoid adults.

Effects of parasitoid age

Four different age groups of the parasitoid adults (1, 5, 10, and 15 days old) were used in this experiment. For each age group, 4 transparent plastic trays (19 × 14 × 5 cm) with muslin covers were prepared (4 replicates). Ten pairs of the parasitoid (10 males and 10 females) were introduced into each tray and provided with a 75% honey solution as fine droplets on the inside walls of the tray. Egg sheets (18 × 13 cm) in which each carried about 250–300 CM eggs (1–24 h old) were placed in each tray, and the trays were incubated under the same condition as for the CM colony. The egg sheets were removed 24 h later and placed above the trays containing the CM artificial diet. One week later, the egg sheets were removed, examined under a stereomicroscope, the number of hatched eggs was recorded, and the percentage egg hatch was calculated. After removing the egg sheets, the trays were labeled appropriately, covered with muslin, and kept under standard conditions for CM rearing of adult emergence. The emerged parasitoid were collected daily, counted, sexed, and their number and sex were recorded.

Effects of host age

Four different age groups of host eggs (1–24, 25–49, 48–72, and 73–96 h old) were used in this study. For each age group, 4 egg sheets (18 × 13 cm), each carrying about 250–300 eggs each (4 replicates), were placed in a separate transparent plastic tray (19 × 14 × 5 cm). Ten pairs of 24–48 h old parasitoid adults were introduced to each tray. The trays were incubated under the same conditions mentioned before, for the CM colony, and supplied with a 75% honey solution, as before. The egg sheets were removed 24 h later and each placed above a tray containing the CM diet. Egg sheets were removed after 7 days and examined under a stereomicroscope. The number of hatched eggs was recorded, and the percentage of the hatch was calculated. The trays were labeled appropriately and incubated under the same conditions mentioned before, for about 4 weeks, for adult emergence. Emerging parasitoids were collected daily, counted, sexed, and their number and sex were recorded.

Effects of host density

Coddling moth egg sheets (18 × 13 cm) carrying 24–48 h old eggs, at 4 different densities (100, 200, 400, and 800

eggs) were prepared. For each density, 4 egg sheets were used (4 replicates), each in a transparent tray (19 × 14 × 5 cm). Ten pairs of 24–48 h old parasitoid adults were introduced into each tray and provided with a 75% honey solution, as before. Twenty-four h later, the sheets were removed, and each was placed above a tray containing CM diet. Seven days later, the egg sheets were removed and examined under a stereomicroscope. The percentage of the egg hatch was calculated. The trays were incubated under the same conditions for 4 weeks after which they were examined daily, emerging parasitoids were collected, counted, sexed, and their number and sex were recorded.

Effects of temperature

Five groups of parasitoids (24–48 h old), 40/group, were incubated under 5 different temperatures (15, 20, 25, 30, and 35±1°C). Each group consisted of 4 trays; 10 pairs/tray (10 males and 10 females). Each tray (replicate) was provided with a 75% honey solution, as before. One sheet carrying about 300–350 CM eggs, 24–48 h old, was introduced into each tray, and the trays were incubated under the same conditions as for the colony. The egg sheets were removed 24 h later and for one week were placed above the trays containing the CM diet, under same conditions. Following that, the egg sheets were removed, examined under a stereomicroscope, the number of hatched eggs was recorded, and the percent hatch calculated. After 4 weeks of incubation, the trays were examined daily, emerging adults were collected, counted, sexed, and their number and sex were recorded.

Effects of food

In this experiment, 4 trays with 24–48 h old *A. quadridentata* adults (10 pairs/tray) provided with a 75% honey solution were prepared. This experiment was repeated but without any source of nutrition. Egg sheets carrying about 300–350 CM eggs, 24–48 h old, were kept for 24 h, one in each tray. Following that, the egg sheets were placed above trays containing the CM diet and incubated under the same conditions as for CM rearing. The egg sheets were removed 7 days later, and examined under a stereomicroscope. The number of hatched eggs was recorded and the percent hatch calculated. Emerged parasitoid adults from the diet were collected daily, counted, sexed, and their number and sex were recorded.

Statistical analyses

Data from these experiments were subjected to analysis of variance (ANOVA). The means were separated by Fisher's protected least significant difference (LSD) test (StatView. Abacus Concepts Inc., Berkeley, Calif.).

Results and Discussion

Data on the effects of *A. quadridentata* age on the sex ratio of its offspring are presented in table 1. The results clearly show that mean number of offspring changed in relation to parasitoid age. The changes between one-day-old

Table 1. Effect of the different ages of *Ascogaster quadridentata* on the sex ratio of its offspring

Parasitoid age [day]	Number of offspring/tray (mean ±SD)		
	male	female	% of female from total offspring/tray
1	51.8±6.3 a A	53.3±6.1 a A	50.7±4.4 A
5	54.2±5.8 a A	52.4±6.0 a A	49.2±4.2 A
10	66.6±7.8 a B	43.5±4.8 b B	39.5±3.8 B
15	72.7±8.2 a C	31.3±3.9 b C	30.1±2.9 C

The means followed by the same small letter in each line or the same capital letter in each column are not significantly different ($p < 0.05$)

and a five-day-old parasitoid adults were not significant ($p < 0.05$). However, the percentage of females decreased significantly ($p < 0.05$) when the age of the parasitoid increased to 10 and 15 days, respectively. This indicates that *A. quadridentata* adults, up to 5 days old, produced homogeneous offspring of both sexes, while the percentage of females was significantly lower when older parasitoids were used. Similar results were also reported for *Apan-tales galleriae* Wilkinson (Uckan and Gulel 2002), *Dinar-mus basalis* (Rond) (Damiens *et al.* 2003), *Glyptapantales flavicoxis* (Marsh) (Fuester *et al.* 2003), and *Bracon hebe-tor* Say (Gunduz and Gulel 2005). Most of these studies showed that male-biased sex ratio may be the result of unfertilised eggs because of a variety of factors, including physiological aging, sperm death, active sperm digestion by the female, and sperm depletion while stored in the spermathecae.

Table 2 presents data on the effects of age of CM eggs on sex ratio in *A. quadridentata*. The results show that host age did not significantly affect the sex ratio of the parasitoid offspring ($p < 0.05$); male to female ratios were about 1 : 1 in all treatments. Some previous studies related to the impact of host age on the sex ratio of the parasitoid offspring indicated that the age of the host, which does not lead to a change in the size and shape of the host, does not affect the sex ratio of the offspring of the parasitoid (Uckan and Gulel 2002; Shuker *et al.* 2006; Bogdanovic *et al.*

2009). Some of these studies also showed that parasitoids lay fertilised eggs (which produce females) in the larger hosts, while the parasitoids lay non-fertilised eggs (which produce males) in the smaller hosts. The above mentioned studies also showed that, in some cases, females suffered a greater mortality in smaller hosts, because they failed to reach pupal weight. Charnov *et al.* (1981) noted that, in general, parasitoids give homogeneous offspring of males and females when host eggs have a homogeneous size. This was also reported by Jarosik *et al.* (2003) when they studied the sex ratio of the *Aphidius colemani* Viereck that attack eggs of *Myzus persicae* Sulzer. It can be concluded, that the cause of the homogeneity of the number of males and females in the *A. quadridentata* offspring is due to the homogeneity of the host egg size (CM eggs). This may be due to the fact that *C. pomonella* was reared on an artificial diet under controlled conditions. These conditions may make the insect (CM) produce eggs of a homogeneous size (Howell 1981).

Table 3 presents data on the effects of host density on the sex ratio of the parasitoid offspring. The data indicate that host density did not significantly affect the sex ratio of the offspring of the parasitoid in all treatments; the male to female ratio was almost 1 : 1. These results are in general agreement with data reported by Owen (1983) about the relationship between the sex ratio of the *Asobara persimilis* (Prince) and the density of its host *Drosophila* sp.

Table 2. Effect of different ages of the host (*Cydia pomonella* eggs) on the sex ratio of *Ascogaster quadridentata* offspring

Host age [h]	Number of offspring/tray (mean ±SD)		
	male	female	% of female from total offspring/tray
1–24	48.9±5.1 a A	46.6±5.3 a A	48.8±4.4 A
25–48	47.9±5.4 a A	50.1±4.8 a A	51.1±5.2 A
49–72	46.3±6.2 a A	49.8±5.3 a A	51.8±4.8 A
73–96	45.4±4.4 a A	47.1±4.9 a A	50.9±6.0 A

The means followed by the same small letter in each line or the same capital letter in each column are not significantly different ($p < 0.05$)

Table 3. Effect of different densities of the host (*Cydia pomonella* eggs) on the sex ratio of *Ascogaster quadridentata* offspring

Host density	Number of offspring/tray (mean ±SD)		
	male	female	% of female from total offspring/tray
100	21.4±2.2 a A	19.9±1.8 a A	48.2±4.6 A
200	41.7±3.4 a B	39.9±3.8 a B	48.8±4.7 A
400	59.5±5.4 a C	61.9±5.6 a C	51.1±5.3 A
800	66.3±5.8 a D	67.7±6.2 a D	50.5±4.7 A

The means followed by the same small letter in each line or the same capital letter in each column are not significantly different ($p < 0.05$)

Table 4. Effect of different temperature degrees on the sex ratio of *Ascogaster quadridentata* offspring

Temperature [±1°C]	Number of offspring/tray (mean ±SD)		
	male	female	% of female from total offspring/tray
15	26.1±3.2 a A	18.8±2.9 b A	41.8±3.8 A
20	51.6±6.2 a B	49.9±5.7 a B	49.2±4.3 B
25	52.4±5.8 a B	51.6±6.3 a B	49.6±4.5 B
30	39.9±3.8 a C	41.3±4.1 a C	50.8±5.3 B
35	11.2±1.2 a D	5.40±0.6 b D	32.5±2.9 C

The means followed by the same small letter in each line or the same capital letter in each column are not significantly different ($p < 0.05$)

Table 5. Effect of food on the sex ratio of *Ascogaster quadridentata* offspring

Food situation	Number of offspring/tray (mean ±SD)		
	male	female	% of female from total offspring/tray
Without food	28.7±1.8 a A	20.9±1.2 b A	42.2±3.1 A
With food	54.3±6.1 a B	52.7±5.3 a B	49.3±5.5 B

The means followed by the same small letter in each line or the same capital letter in each column are not significantly different ($p < 0.05$)

Similar results were also reported by Yu *et al.* (2003) regarding *B. hebetor* and its host *Plodia interpunctella* (Hubner). In the study by Werren (1987), that high density of the parasitoid and low density of the host may lead the females in some species to lay most of their fertilised eggs in the larger individuals of the host (to produce females), and unfertilised eggs in the smaller individuals (to produce males).

Results on the effects of temperature on the sex ratio of the *A. quadridentata* offspring are presented in table 4. The results show there was a significant difference ($p < 0.05$) between the mean number of males and females at 15 and 35°C; sex ratio was skewed in favor of males. From this experiment, we conclude that *A. quadridentata* homogeneous offspring of males and females were produced at temperatures which ranged between 20 and 30°C. Males outnumbered females when the temperature dropped to 15°C or increased to 35°C. These results indicate that the appropriate temperature for the parasitoid to exercise its activity in mating and parasitisation is between 20 and 30°C. At these temperatures, reproduction was high and the sex ratio was homogeneous. To the contrary, low (15°C) and high (35°C) temperatures may have prevented a proportion of the parasitoid individuals from mating. This may lead the females to produce unfertilised eggs, and consequently, to produce more males than females. Low and high temperatures cause a reduction in the activity of parasitism, or may cause the death of some parents (Fuester *et al.* 2003). This leads to lower numbers resulting from the offspring of the parasitoid. These findings are consistent with the results reported by Kfir and Luck (1979) on the parasitoids *Aphytis melinus* DeBach and *A. lingnanensis* (Compere). Similar results were also reported by Jalali *et al.* (1990) for *Cotesia marginiventris* (Cresson), and by Biswas and Singh (1998) for *Lysiphlebia mirzai* (Aphididae), and by Fuester *et al.* (2003) for *Glyptapanteles flavicoxis* (Marsh).

Table 5 shows that lack of food for *A. quadridentata* caused a significant reduction in the number of female

offspring compared with male offspring ($p < 0.05$). The mean number of males and females was 28.7 and 20.9/ /tray, respectively, and there were 42.2% females. When the parasitoid was provided with food, however, the mean number of males become equal to that of females ($p < 0.05$) and % of female reached 49.3. This means the sex ratio returned to homogeneity and became almost 1 : 1. On the other hand, the results presented in the same table show that lack of food caused a significant reduction in the mean number of individuals of both sexes of the offspring compared to when food was available. The lack of food for the parasitoid may have caused a reduction in activity and reduction in its mating ability. Such a reduction may then lead to a high percentage of females producing unfertilised eggs – resulting in a high percentage of males. Lack of food may also cause a high parasitoid death-rate leading to a decrease in the number of parasitoid offspring. These results are similar to those reported by Hagley and Barber (1992) for *Pholetesor ornigis* (Weed), and results reported by King (1993) for many species of Hymenopteran parasitoids, and results reported by Garcia-Medel *et al.* (2007) for fruit fly parasitoids.

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