

## The effect of choice and no-choice access on host (*Aphis fabae*) instars preference, in different ages and sizes of *Lysiphlebus fabarum* (Hymenoptera: Braconidae: Aphidiinae)

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### Abstract

The influence of parasitoids on population dynamics is affected by host instar preference. The preference pattern of host instars is not fixed and is modified by the availability and relative abundance of host instars, intensity of defensive behaviors in a particular instar, and also maternal effects of the female forager. In this study, host instar preference of the thelytokous strain of *Lysiphlebus fabarum* (Marshall) was studied in relation to progeny performance in choice and no-choice experiments, using the first, second, and fourth host instars of *Aphis fabae* (Hemiptera: Aphididae) as hosts. In addition, we investigated whether the maternal effects (age and size) influenced preference-performance associations. Based on the results, offsprings emerging from hosts that were parasitized as the first instar stage were smaller and took longer to develop than offsprings emerging from hosts that were parasitized as the second and fourth instars, irrespective of the age and size of their mothers. The results also showed that when females were offered different host instars under no-choice access, there was no preference for a specific instar, irrespective of the size and age of the female. However, in a choice situation, for the both age and size classes of the parasitoid mother, the number of mummies declined with increasing host instar, suggesting that host instar preference does not reflect offspring performance. For mass rearing of *L. fabarum*, it would be better if female foragers be introduced to lower density of first instar, under choice situation because of increasing offspring performance.

**Key words:** Aphididae, Hemiptera, Fitness, Maternal effect, progeny performance

### چکیده

اثر دسترسی انتخابی و غیرانتخابی روی ترجیح سن پورگی میزبان، در سنین و اندازه‌های مختلف زنبور *Lysiphlebus fabarum* (Hymenoptera: Braconidae: Aphidiinae) بگانه نجف پور، آرش راسخ و مهدی اسفندیاری

چگونگی تاثیر بارازیتوئیدها روی تغییرات جمعیت میزبان، تحت تاثیر ترجیح سن پورگی میزبان قرار دارد. این طرح ترجیح ثابت نبوده و همراه با فراوانی نسبی و میزان در دسترس بودن، تفاوت در شدت رفتارهای دفاعی و همچنین اثرات مادری زنبور کاوشگر تغییر می‌یابد. در این مطالعه ترجیح سن پورگی میزبان در استرین ماده‌زای زنبور پارازیتوئید *Lysiphlebus fabarum* (Marshall) در ارتباط با کارایی نتاج روی سنین اول، دوم و چهارم پورگی شته *Aphis fabae* (Hemiptera: Aphididae) در شرایط انتخابی و غیرانتخابی مطالعه شد. علاوه بر این اثرات مادری (سن و اندازه) روی ارتباطات کارایی ترجیح نتاج مورد مطالعه قرار گرفت. بر اساس نتایج، زنبورهای پرورش یافته روی پوره سن اول، صرف نظر از سن و اندازه‌ی مادر، کوچکتر بودند و طول دوره‌ی رشدی طولانی‌تری داشتند، نسبت به زنبورهایی که در پوره سن دوم یا چهارم پرورش یافته بودند. نتایج همچنین نشان داد که وقتی که زنبورها در شرایط غیرانتخابی به سنین مختلف میزبان دسترسی داشتند، صرف نظر از سن و اندازه، ترجیحی به سن پورگی خاصی نشان ندادند. با این اوصاف، در شرایط انتخابی، در هر دو کلاس سنی و اندازه‌ی زنبورهای مادر، تعداد مومیایی‌ها با افزایش سن پورگی میزبان کاهش یافت، که نشان دهنده عدم ارتباط بین ترجیح سن پورگی و کارایی نتاج می‌باشد. بر اساس نتایج، در پرورش انبوه زنبور پارازیتوئید *L. fabarum*، به منظور افزایش کارایی نتاج، بهتر خواهد بود که در شرایط انتخابی حداقل تراکم از پوره‌های سن اول در اختیار زنبورهای کاوشگر قرار گیرد.

واژگان کلیدی: Aphididae, Hemiptera, شایستگی، اثرات مادری، کارایی نتاج

### Introduction

One of the most important factors in evolutionary biology has been to identify and quantify traits that affect different fitness functions (Harvey, 2005). Some optimality models of host-parasitoid associations have argued that host quality (host size or age at parasitism) is as an important factor, as larger hosts are considered better because of their higher resources for developing larvae (Harvey *et al.*, 1994). In koinobiont parasitoid; which allows their hosts to continue development after parasitism (Cloutier & Mackauer, 1979; Mackauer, 1986), the predication of host quality is much more

complicated than idiobiont cases (Harvey, 2005) because it depends on the final size of the host when it is destroyed by the parasitoid (Harvey, 2005). Although the last instars and adult aphids have more nutritional content than the first and second instars, their resources may not always be available for the parasitoids (Sequeira & Mackauer, 1992b) because larger instars are able to display relatively higher physiological (Sequeira & Mackauer, 1992a) and physical defensive capacities (Chau & Mackauer, 2000; Rasekh *et al.*, 2010a).

The fitness of emerged progenies in different host instars may also be partially related to maternal effect. Mothers can match the phenotype of their offspring to changes in the local environment and that maternal effects can be effective means of buffering offspring from environmental stressors (Mousseau & Dingle, 1991). Although the exact mechanisms and the signals involved in the alteration of progeny phenotype in response to maternal experience remain unclear, the body size and age of the mother can be linked to the alteration of offspring fitness components (Rossiter, 1991; Ameri *et al.*, 2014). In relation to the age of females, Rasekh *et al.* (2010a) reported that the proportional time allocation to within-patch behaviors was unaffected by the age of the females while studying the asexual strain of *Lysiphlebus fabarum*. However, this factor did affect the overall patch residence time and numbers of parasitized hosts. Elderly females were less choosy than younger ones (Roitberg *et al.*, 1993) and with increasing age of females, the time availability and egg load decreased (Rosenheim & Hongkham, 1996) and led to an increase in their reproductive activity (Wajnberg, 2006).

To evaluate the role of individual factors proposed to influence host selection by female insects, the preference pattern of host instars have not been fixed and have been modified by the availability and relative abundance of different instars of host (Chow & Mackauer, 1991). van Alphen and Vet (1986) stated that host quality is one of several factors that determine oviposition decision in parasitoids, such that preferences for particular aphid instars may reflect behavioral interaction between that host instar and female forager (Gerling *et al.*, 1990), or it depends on the intensity of defensive behaviors in a particular instar (Kouamé & Mackauer, 1991; Chau & Mackauer, 2000). The pattern of preference is also in association with the physiological state of the female including her age, size, and previous reproductive experience (Weisser, 1994; Michaud & Mackauer, 1995). For relative size of female foragers, it may be effective in the intensity of hosts' defensive reactions (Losey & Denno, 1998; Ameri *et al.*, 2014) or physical constraint of females, such as ovipositor length (Gerling *et al.*, 1990). This means that larger females are more successful in attacking larger hosts (Chau & Mackauer, 2000).

The effect of choice or no-choice access (access to all host instars against exposure to a particular nymphal instar) on host instar preference is a topic that has received very little attention. It is expected that the access to all host instars affects host instar preference because it provides the opportunity to compare the different instars, in contrast to the case of exposure to a particular nymphal instar. In this study, we investigated whether size and age of the parasitoid mothers *L. fabarum* (Marshall) (Hymenoptera: Braconidae: Aphidiinae) influenced offspring performance (body size and developmental time) when reared in different host instars of *Aphis fabae* (Hemiptera: Aphididae) as hosts. In addition, we determined host instar preference of different size and age of female parasitoids in choice and no-choice experiments. According to the preference-performance hypothesis (PPH), female insects will evolve to oviposit on hosts conducive to offspring performance. The general assumption in the PPH is that differences in offspring performance produce a selection pressure resulting in mother preference. Body size has been traditionally considered more than other correlates of performance in parasitoids (Mackauer and Sequeira, 1993; Godfray 1994) because it can directly related to reproductive success by increasing their longevity, fecundity, and foraging efficiency (Bellows, 1985; Harvey *et al.*, 1994). Despite this we have also paid attention to development time that may vary with host quality.

*Lysiphlebus fabarum* is considered as the most important aphid parasitoids in northern Iran and central Europe. It attacks more than 70 aphid species (Stary, 1986; Rakhshani *et al.*, 2006), particularly the black bean aphid, *A. fabae*. Although both sexual (arrhenotokous) (Mahmoudi *et al.*, 2010; Mossadegh *et al.*, 2011) and asexual (thelytokous) (Rasekh *et al.*, 2011) strains of *L. fabarum* have been reported in Iran, the former appear to be more widely distributed. Aphid control in Iran relies heavily on pesticides in both field and greenhouse settings, whereas any availability of a new biological control agents such as *L. fabarum* would likely aid in reducing pesticide use. The results of this study can provide information regarding the mass rearing of this parasitoid wasp, for improving the offspring performance, and decreasing the cost of mass production.

## Materials and Methods

### Insect Colonies

A stock colony of black bean aphid, *A. fabae*, was established from bean fields in Zanjan Province, Iran, (36°40'N, 48°29'E). Moreover, the parasitoid *L. fabarum* were obtained from aphid mummies. The type of female reproduction (thelytokous) in this population is confirmed according to testes reported by Rasekh *et al.* (2011). The stock colony of *A. fabae* was maintained on potted broad bean, *Vicia fabae* L., grown in pots filled with fertilized sawdust and the parasitoid colony was maintained on *A. fabae* feeding on broad bean. All insects and experiments were maintained in growth chambers under the same climatic conditions: 21 ± 1°C, 65–75% RH, and a 16:8 (L:D) photoperiod.

### Synchronous cohorts of insects

Cohorts of black bean aphid were established on seedlings of bean plant in ventilated plastic cylinders (8 cm x 15 cm) by allowing 100 adult *A. fabae* to deposit nymphs on the plants. The adult aphids were removed after 8 h and the nymphs were left *in situ* to develop into the given nymphal instars. Preliminary observations revealed that, under these conditions, *A. fabae* require a mean of 1, 2.25, 4, and 5.75 days to molt to each successive instar stage, from first to fourth instars, respectively. Based on previous studies (Ameri *et al.* 2012, 2013) and our preliminary tests, wasps reared in the second and first instars obtained the highest and lowest fitness (based on body size and developmental time), respectively. In addition, the wasps reared in the fourth instar gained the intermediate fitness. To facilitate comparisons, the third instar was excluded in all experiments of this study.

To produce synchronous cohorts of wasps in small and large sizes (base on hind tibia length), a synchronous cohort of large females (2 days old) without prior experience with aphids were introduced to first or second instar aphids, respectively. Based on our preliminary tests, small and large wasps were reared from first and second instar hosts, respectively. After 8 h the female wasps were removed and the parasitized aphids were reared on potted bean seedlings. The emerged females were provisioned with droplets of diluted honey and water, and were used in tests in the second day of their life. To produce synchronous cohorts of wasps in different ages, a

synchronous cohort of large females (2 days old) was introduced into second instar aphid. The parasitized aphids were reared as described above. Two- and 4-day-old emerged progenies were used in the experiments as young and old females, respectively.

### Effect of maternal size and age in choice and no-choice access

The synchronous cohorts of small and large females were produced in as described above. In each female size, 2-day-old wasps (n = 30) were offered different host instars (first, second, or fourth), in a 1:5 ratio (1 wasp for each 5 aphids) or in a no-choice situation. After 8 h, the female wasps were removed and the parasitized aphids were reared on a bean seedling until mummies formed. The mummies were then transferred to Petri dishes (9 cm x 1 cm) to determine the developmental time of emerged progenies. To determine the body size of offspring, the newly emerged females were killed by exposure to alcohol vapors for three minutes. Then hind tibia length was photographed under a stereomicroscope equipped with a digital camera (Nikon Coolpix S10, Nikon Corporation, Tokyo, Japan) attached to a binocular microscope at 100x magnification and its length was measured with precision (0.003 mm), by ImageJ software. To determine the effect of maternal age, the synchronous cohort of large females in different ages, 2 (n = 30) or 4 (n = 30) days old, were individually introduced into each cohort of aphid instars as described above. All the cases described in different size experiment were also performed for these females in different ages to determine developmental time and hind tibia length of emerged progenies. Data from the maternal effects were analyzed using a factorial two-way ANOVA with female size/age and host instars (first, second, or fourth), as independent fixed factors.

### Comparison of host instars preference in choice and no-choice access

#### Mothers in different sizes

The synchronous cohort of small females was produced as described above. Two day old (n = 120) females were divided in two groups. In choice access, each female (n = 30) was introduced into experimental patches consisting of a leaf disk of broad bean on which 30 instars of *A. fabae* (10 aphids of each first,

second, and fourth instar) had been placed. In a preliminary experiment, females never parasitized less than that 10 aphid during the time available (8 h). In no-choice access, each female ( $n = 90$ ) was introduced into experimental patches consisting of a leaf disk of broad bean including 30 host instars (first, second, or fourth instars). In both accesses, the wasps were removed after 8 h, and the parasitized aphids were reared until mummies formed. These mummies were then transferred to Petri dishes (9 cm x 1 cm) to determine the emergence rate by dividing the number of emerged females to number of mummies. In choice access, the aphids of each instar ( $n = 10$ ) were separately reared in different bean seedlings. The same cohort of large females (2 days old) were also produced and used in host instar preference experiment as described above.

#### Mothers in different ages

The synchronous cohort of large females (two- or 4-day-old females) were used in this experiment using the same conditions described above in choice ( $n = 30$ ) and no-choice ( $n = 90$ ) access. Data from the host instars preference in no-choice experiment were analyzed for each female size/age using a one-way ANOVA with host instars as independent fixed factor. In all above tests, means were separated using the Tukey test ( $\alpha = 0.05$ ) when three treatments were compared. In choice tests, females were offered different host instars simultaneously. Therefore, the response variables (parasitism and emergence rates) on a specific instar occurred dependent of other instars and the Chi-square goodness of fit test was used with an expected exposure frequency of 0.333 for each specific instar. Data for emergence rate was arcsine-transformed before analysis. Allometric parameters of small and large females were compared by one-way ANOVA (SPSS 1998).

#### Results

##### The effect of maternal size and age

Offspring emerging from hosts that were parasitized at the first instar stage were smaller than offspring emerging from host that were parasitized at second and fourth instars, irrespective of the size of their mothers (Small females:  $F_{2,146} = 12.304$ ,  $P < 0.001$ ;  $N_1-N_2$ :  $P = 0.003$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P = 0.995$ ; Large females:  $F_{2,272} = 55.594$ ,  $P < 0.001$ ;  $N_1-N_2$ :  $P < 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P = 0.494$ ). At

the same host instars, the pairwise comparisons between the offspring with different mother sizes did not show any significant difference (Fig. 1a).

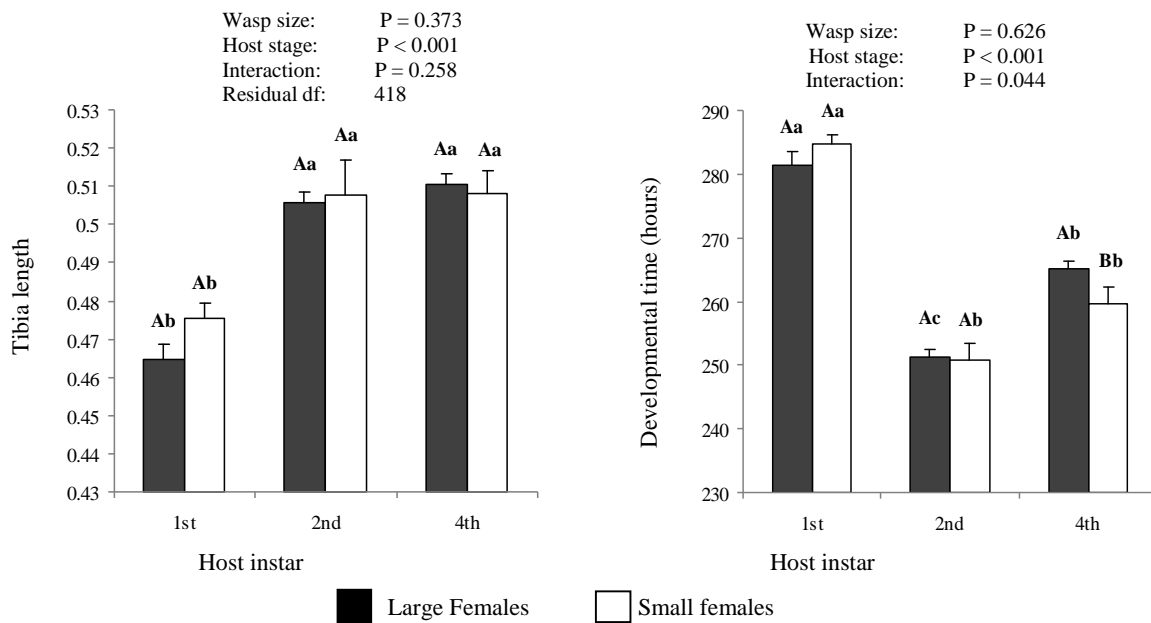
In large mothers, the longest and shortest developmental time of progenies occurred in the first and second instars, respectively ( $F_{2,315} = 90.637$ ,  $P < 0.001$ ;  $N_1-N_2$ :  $P < 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P < 0.001$ ). In small mothers, offspring emerging from hosts that were parasitized at the first instar stage took longer to develop than offspring emerging from the second and fourth instars ( $F_{2,179} = 59.96$ ,  $P < 0.001$ ;  $N_1-N_2$ :  $P < 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P = 0.112$ ). The size of females did not affect developmental time of progenies when they were reared in the same host instar, with the exception of the fourth host instar, in which faster development was observed in progenies of smaller mothers ( $F_{1,159} = 4.121$ ,  $P = 0.044$ ) (Fig. 1b).

In young and older wasp mothers, rearing of wasps in the first instar resulted in smaller progenies relative to other host instars (Young females:  $N_1-N_2$ :  $P < 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P = 0.494$ ; old females:  $N_1-N_2$ :  $P = 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P = 0.098$ ). At the same host instars, the pairwise comparisons between progenies emerging from mothers with different ages did not display any significant differences (Fig. 2a).

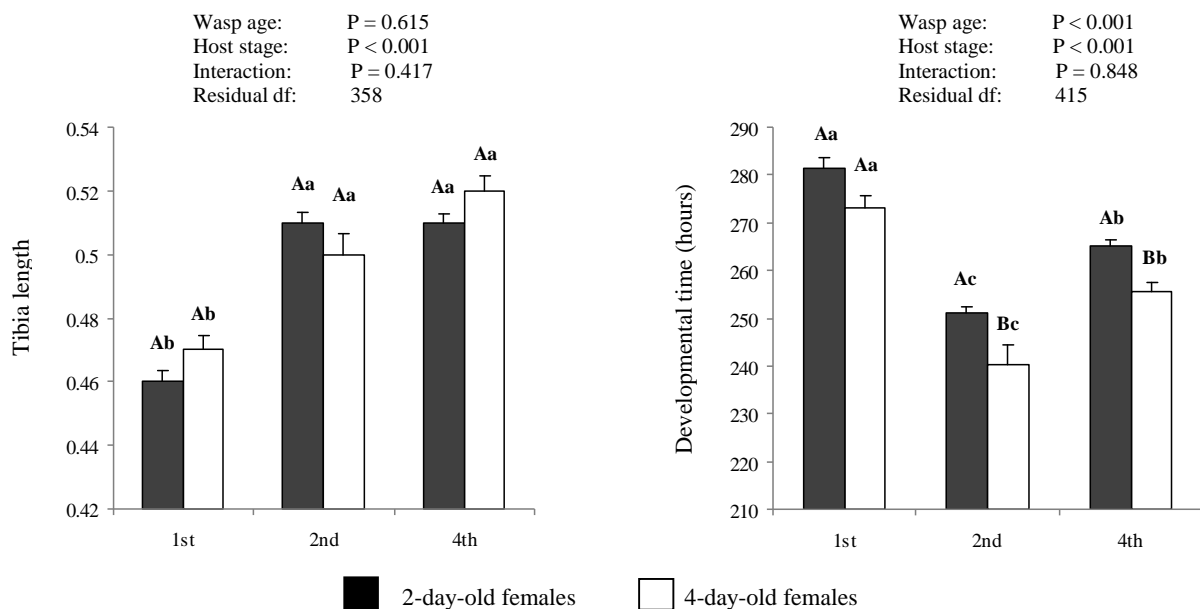
In both ages of wasp mothers, the longest and shortest developmental time of progenies occurred in the first and second instars, respectively (Young females:  $N_1-N_2$ :  $P < 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P < 0.001$ ; old females:  $N_1-N_2$ :  $P < 0.001$ ;  $N_1-N_4$ :  $P < 0.001$ ;  $N_2-N_4$ :  $P < 0.001$ ). The age of mothers affected the developmental time of progenies when they were reared in the second ( $F_{1,154} = 11.505$ ,  $P = 0.001$ ) and fourth instars ( $F_{1,148} = 20.749$ ,  $P < 0.001$ ). Faster development was observed in progenies of older mothers (Fig. 2b).

##### Comparisons of choice and no-choice access Host instars preference in different size of the parasitoid females

The control experiment showed that the length of hind tibia of offspring emerging from first ( $0.44 \pm 0.005$ ) and second ( $0.49 \pm 0.004$ ) instars was significantly different ( $F_{1,223} = 44.8$ ,  $P < 0.001$ ). These females were used as small or large mothers in experiments.



**Fig. 1.** Mean ( $\pm$  SE) tibia length (a) and developmental time (b) of *Lysiphlebus fabarum* offspring emerging from hosts that were parasitized as different instars of *Aphis fabae*, by large and small mothers. Values bearing the same upper case letters were not significantly different (ANOVA,  $P = 0.05$ ) between different mother size, within a host instar. Values bearing the same lower case letters were not significantly different among host instars within a mother size (Tukey,  $P = 0.05$ ).

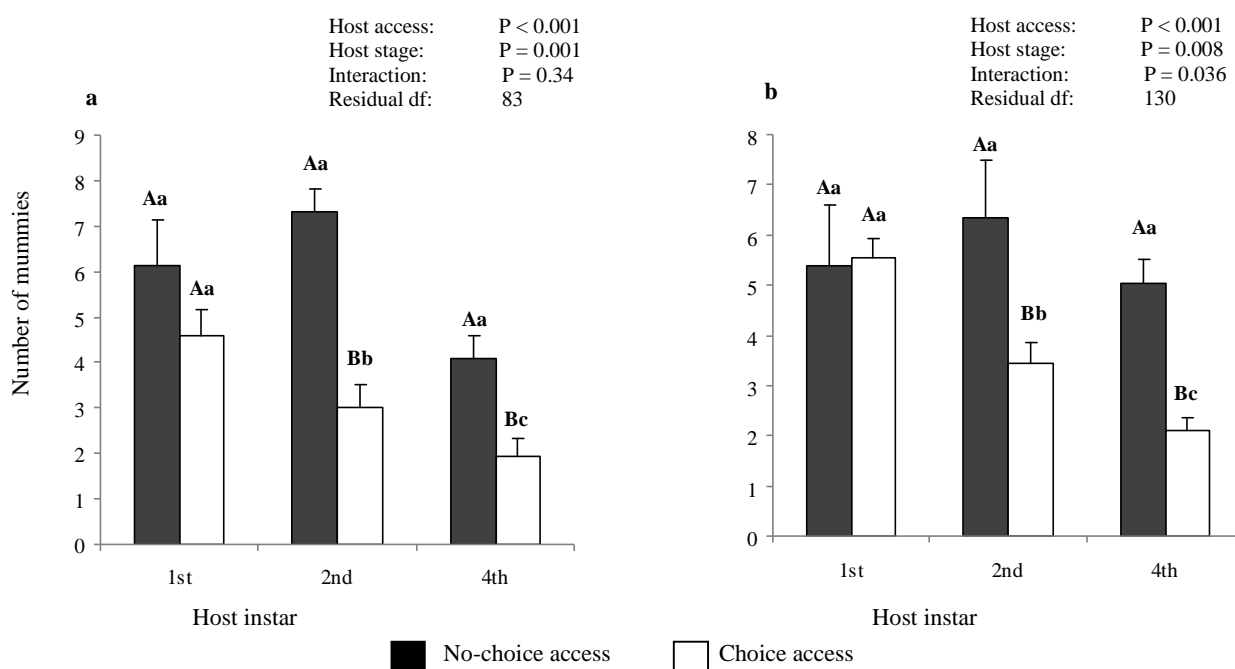


**Fig. 2.** Mean ( $\pm$  SE) tibia length (a) and developmental time (b) of *Lysiphlebus fabarum* offspring emerging from hosts that were parasitized as different instars of *Aphis fabae*, by two- and 4-day-old females. Values bearing the same upper case letters were not significantly different (ANOVA,  $P = 0.05$ ) between different mother age, within a host instar. Values bearing the same lower case letters were not significantly different among host instars within a mother age (Tukey,  $P = 0.05$ ).

In no-choice test, counting the number of mummies formed showed that there was no preference for a specific instar in small ( $F_{2,29} = 2.13, P = 0.14$ ) and large ( $F_{2,49} = 0.64, P = 0.53$ ) parasitoid females while in choice test, the number of mummies declined with increasing host instar, and this pattern was the same for the both sizes of the parasitoid females (Small:  $\chi^2 = 20.99, P < 0.0001; N_1-N_2: \chi^2 = 5.84, P = 0.0157; N_1-N_4: \chi^2 = 19.36, P < 0.0001$ ; Large:  $\chi^2 = 49.39, P < 0.0001; N_1-$

$N_2: \chi^2 = 13.8, P = 0.0002; N_1-N_4: \chi^2 = 46.36, P < 0.0001$ ) (Fig. 3a).

When the number of mummies in choice and no-choice access was compared, in the second (Small females:  $F_{1,34} = 5.84, P = 0.02$ ; Large females:  $F_{1,44} = 7.18, P = 0.01$ ) and fourth instars (Small females:  $F_{1,32} = 10.78, P = 0.002$ ; Large females:  $F_{1,50} = 31.32, P < 0.001$ ) these differences were statistically significant (Fig. 3a,b).



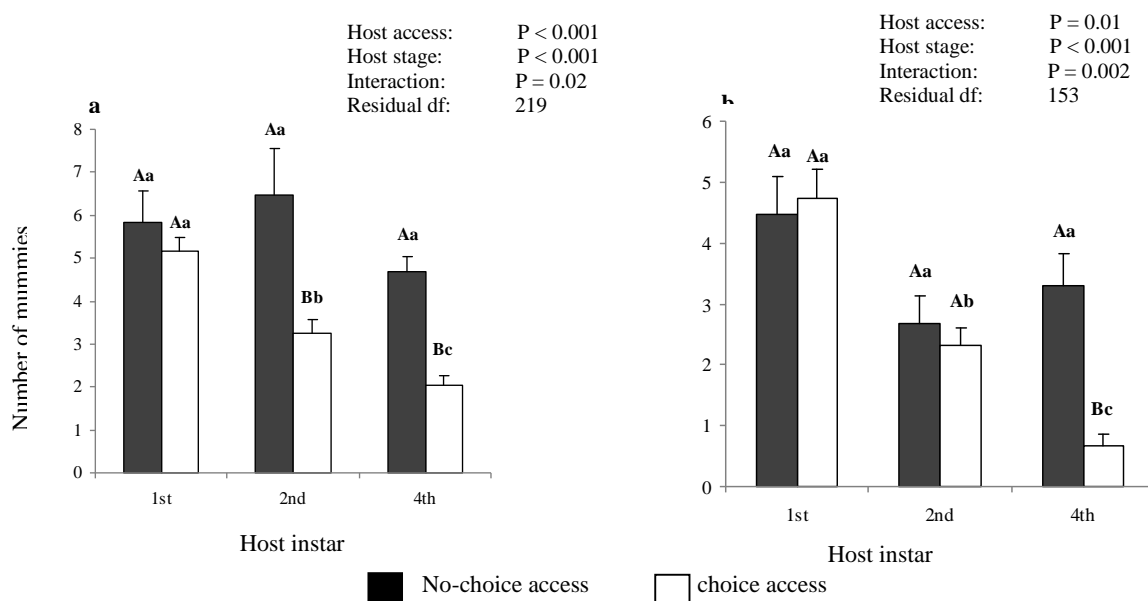
**Fig. 3.** Mean ( $\pm$  SE) number of mummies of small (a) and large (b) *Lysiphlebus fabarum* reared on *Aphis fabae* that had access into different host stages (first, second or fourth instars) by choice or no-choice. Values bearing the same upper case letters were not significantly different (ANOVA,  $P = 0.05$ ) between different method of host accesses, within a host instar. Values bearing the same lower case letters were not significantly different among host instars within a method of host access; choice (Chi-square,  $P = 0.05$ ) and no-choice (Tukey,  $P = 0.05$ ).

**Host instars preference in different age of the parasitoid females**

In no-choice test, counting the number of mummies formed showed that there was no preference for a specific instar, regardless of the age of parasitoid females (two-days:  $F_{2,75} = 1.34, P = 0.27$ ; four-days:  $F_{2,63} = 2.85, P = 0.06$ ). In choice test, the number of mummies declined with increasing host instar, and this pattern was the same for the both ages of the parasitoid mothers (Two-days old:  $\chi^2 = 32.95, P < 0.0001; N_1-N_2: \chi^2 = 9.34, P = 0.002; N_1-N_4: \chi^2 = 30.68, P < 0.0001$ ;

Four-days old:  $\chi^2 = 100.43, P < 0.0001; N_1-N_2: \chi^2 = 25, P < 0.0001; N_1-N_4: \chi^2 = 93, P < 0.0001$ ) (Fig. 4a, b).

In comparison between choice and no-choice accesses, the number of mummies was not significantly different in the first host instar (Two-days old:  $F_{1,69} = 0.92, P = 0.34$ ; four-days old:  $F_{1,50} = 0.12, P = 0.73$ ), but in the fourth instar (Two-day old:  $F_{1,84} = 41.29, P < 0.001$ ; four-day old:  $F_{1,52} = 27.60, P < 0.001$ ), more hosts were parasitized in no-choice access than choice access (Fig. 4a, b).



**Fig. 4.** Mean ( $\pm$  SE) number of mummies of young (a) and older (b) *Lysiphlebus fabarum* (4 day-old) reared on *Aphis fabae* that had access into different host stages (first, second or fourth instars) by choice or non-choice access. Values bearing the same upper case letters were not significantly different (ANOVA,  $P = 0.05$ ) between different method of host accesses, within a host instar. Values bearing the same lower case letters were not significantly different among host instars within a method of host access; choice (Chi-square,  $P = 0.05$ ) and no-choice (Tukey,  $P = 0.05$ ).

#### Survival of progeny in different size and age of the parasitoid mothers

In no-choice and choice tests, we observed no significant differences in survival of offsprings, when small/large (Fig. 5a, b) or two/four day old females (Fig. 5c, d) were reared in different host instars. Moreover, there was no significant difference when the emergence rate of offsprings was compared between choice and no-choice tests (Fig. 5a,b,c,d).

#### Discussion

For koinobiont parasitoids, the relationship between host size and progeny fitness is much more complicated than in the case with idiobionts because it reflects some selection pressures that occur less in the latter group of parasitoids. In the three most frequently reported empirical relationships of solitary koinobiont and host age, the second type (Sequeira & Mackauer, 1992a; Harvey & Strand, 2002; Harvey *et al.*, 2004) was exhibited by *L. fabarum* in which adult wasp size increased with host size. Moreover, the developmental time displayed a non-linear pattern such that the shortest development time occurred at the second stage, irrespective of the age and size of their mothers.

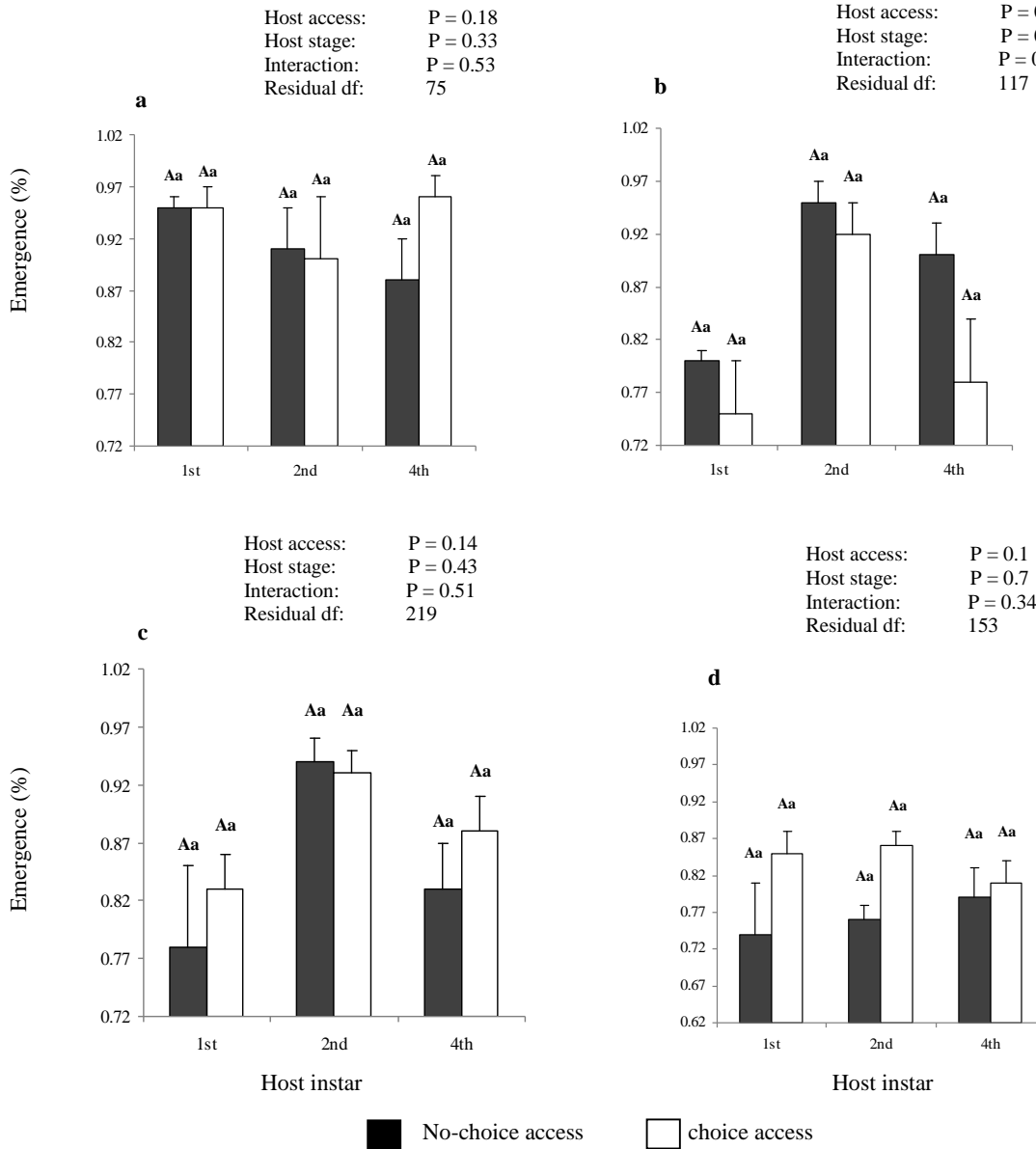
Based on the results, when *L. fabarum* females were offered different host instars separately (no-choice situation), a specific instar was not preferred, irrespective of the size and age of the females.

However, in a choice situation, the pattern of host preference completely changed, such that the host instar preference was progressively decreased with host age, and this pattern was the same for both the age and size classes of the parasitoid females. Changes in patterns of host instar preference have been previously reported in some parasitoids when females were given a choice. For example, Sharma and Subba Rao demonstrated that preference was shifted from first instar to second or older stages when *Aphidius smithi* females had access into all host instars (Mackauer, 1983). The preference changed from third instar to equal preference for second, third, and fourth instars when all instars simultaneously were introduced into *Proan pequodorum* Viereck (Sequeira & Mackauer, 1987).

Considering the difference in the pattern of preference of *L. fabarum* females between a choice and no-choice situation, it seems that the first instar were more preferred in choice access due to the lower level of their defensive behaviors. In this situation, females have an opportunity to compare the intensity of defensive behaviors of different host instars. When females were offered different host instars separately (no-choice situation), this comparison was not made. Therefore, even exposure to fourth instar with the highest defensive behaviors did not decrease the rate of parasitism. It is anticipated that females are able to

parasitize the first instar aphids easier with lower energy consumption compared to females that forage

on the older instars; an issue that can affect their longevity and their total fecundity.



**Fig. 5.** Mean ( $\pm$  SE) emergence rate of different sizes [(a) small, (b) large] and different ages [(c) two-days old, (d) four-days old] *Lysiphlebus fabarum* reared on *Aphis fabae* that had access into different host stages (first, second or fourth instars) by choice or no-choice. Values bearing the same upper case letters were not significantly different (ANOVA,  $P = 0.05$ ) between different method of host accesses, within a host instar. Values bearing the same lower case letters were not significantly different among host instars within a method of host access; choice (Chi-square,  $P = 0.05$ ) and no-choice (Tukey,  $P = 0.05$ ).

The PPH states that female insects will evolve to oviposit on hosts conducive to offspring performance (Gripenberg *et al.*, 2010). In this study, female preference and offspring performance appear uncoupled for both the age and size classes of the parasitoid mothers. Offspring emerging from hosts that

were parasitized as the first instar stage were smaller and took longer to develop than offspring emerging from hosts that were parasitized as second and fourth instars, irrespective of the age and size of their mothers. This suggests that host instar preference does not reflect offspring performance. In accordance with



our results, numerous studies on phytophagous insects report cases where female preference and performance seems maladaptive or where the relationship was weak (Valladares & Lawton, 1991; Underwood, 1994; Fritz *et al.*, 2000). Nevertheless, in some cases, female choice seems clearly adaptive with offspring performance (Gripenberg *et al.*, 2010).

Several evolutionary and ecological considerations have explained apparent mismatches between mother choice and offspring performance (Thompson, 1988; Craig & Itami, 2008). A noticeable factor to favor a strong link between preference and performance is the aggregation of offspring. It seems that selecting high-quality hosts may be more important for females laying their eggs in clutches than for females distributing their eggs individually as a risk-spreading strategy (Mangel, 1987; Gripenberg *et al.*, 2010). The PPH was not supported in *L. fabarum*, which is a species that lay their eggs singly. Adult females feeding could also be assumed as a factor proposed in the association between preference and performance. When adult females have the potential to feed, it makes them less dependent on food resources acquired at previous stages (e.g. Wheeler, 1996). In addition, for adult females that consume different resources from their offspring, it makes them selfish at the expense of the performance of their offspring (e.g. Scheirs & De Bruyn, 2002; Janz *et al.*, 2005). In *L. fabarum*, where both issues exist (adults feed and consume from honeydew as a different resource from offspring), the conflict between parent and offspring seems justifiable. The association between adult feeding and preference-performance phenomenon in

*L. fabarum* which exhibits ant-like antennation to solicit honeydew directly from host aphids (Rasekh *et al.*, 2010b), could be more complicated when the frequency and size of dedicated honeydew droplets depends on the host instar.

In aphidiinae wasps, the second and third host instars are preferred in most cases (Kant *et al.*, 2008), but the first instar or adult aphids may also be appropriate (Volkl & Mackauer, 1996; Chau & Mackauer, 2000). In accordance with our results, the preference of first instar has also been reported in some species of aphidiinae parasitoids (Hofsvang & Hagvar, 1986; Gerling *et al.* 1990; Kouamé & Mackauer, 1991; Chau & Mackauer, 2000). This could be related to less defensive behavior of first instar individuals than other larger instars (Kouamé & Mackauer, 1991; Chau & Mackauer, 2000); an issue that has also been reasoned as the preference of smaller host instars (Singh & sinha, 1982).

The results of this study can provide some information to improve the mass rearing of *L. fabarum*. In this connection, it is recommended that female wasps have access to appropriate stage of host aphids (2<sup>nd</sup> instar) under no-choice situation, because of increasing offspring performance, although, it is difficult to produce a synchronous cohort of 2<sup>nd</sup> instar on a large-scale, the point that can be the subject of future studies.

#### Acknowledgements

We thank Shahid Chamran University of Ahvaz for providing financial support for this research.

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Accepted: 15 May 2016

Received: 12 September 2016