J Pest Sci (2017) 90:649–658
DOI 10.1007/s10340-016-0798-8

ORIGINAL PAPER

Loss of host fidelity in highly inbred populations of the parasitoid wasp *Aphidius ervi* (Hymenoptera: Braconidae)

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Received: 11 March 2016 / Revised: 1 July 2016 / Accepted: 5 July 2016 / Published online: 2 August 2016

Abstract Inbreeding frequently reduces the fitness of organisms, but little is known about how this phenomenon can affect the biological control. Host fidelity provides an adaptive advantage to aphid parasitoids, allowing females to find their aphid host more quickly in heterogeneous environments. This trait is mediated by the learning of signals, mainly chemical cues emitted from the host in which parasitoids developed (natal). This article is aimed at studying whether host fidelity can be altered after many generations of inbreeding reproduction in caged laboratory populations, for which host preference and fitness parameters were measured in the parasitoid wasp *Aphidius ervi*. Also, the effect of the natal/non-natal hosts was studied, using parasitoids originated from the pea aphid (*Acyrthosiphon pisum*) and the grain aphid (*Sitobion avenae*). We observed a loss of host fidelity in the studied *A. ervi* populations, irrespective of their natal aphid host, which contrasts with previous reports showing preference for natal hosts in outbred laboratory populations. The loss of host fidelity is discussed in terms of the origin of populations; the sex ratio was strongly biased toward males and long-time maintenance under laboratory conditions. Our results highlight the need for controlling the genetic diversity of caged parasitoids before they are released into fields, as a long period of inbreeding could negatively affect the biological control.

Keywords Inbreeding · Loss of host fidelity · Host preference · Fitness · Biological control

Communicated by M. Traugott.

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Key message

- Highly inbred populations of *Aphidius ervi* showed no preferences for the natal host.
- Populations of *A. ervi* on both the natal and non-natal host showed similarly low fitness mostly due to the high proportion of offspring males.
- The loss of host fidelity can negatively impact the effectiveness of biological control.

Introduction

Inbreeding depression is caused by an increase in the homozygosity of individuals due to reproduction among relatives in small populations, which often leads to a fitness reduction in the offspring (Charlesworth and Willis 2009; Boivin et al. 2012; Tien et al. 2014). In Hymenoptera, arrhenotoky is the usual reproduction mode (Cook 1993), fertilized eggs giving rise to diploid females and non-fertilized eggs producing haploid males (He and Wang 2008). Interestingly, haplodiploid organisms could be less affected by inbreeding depression compared to diploids. This is because recessive deleterious alleles can be maintained in heterozygous individuals and thus not completely removed by purifying selection. Deleterious alleles in hemizygous males, however, are expected to be expressed and removed

by natural selection in haplodiploid species, thus reducing their frequency in populations (Antolin 1999; Henter 2003; Tien et al. 2014).

Parasitoid wasps are frequently used in biological control programs (Godfray 1994). Before being released, parasitoids are reared in small caged populations for several generations, which can increase the chance of fixation/extinction of some alleles. This proceeds through inbreeding followed by random drift, particularly when populations experience periodic reductions of their population sizes. When inbreeding causes the loss of sex alleles in populations of parasitoid wasps, this could result in two disadvantageous consequences for fitness: (1) a male biased sex ratio and (2) a reduced population growth rate (Stouthamer et al. 1992). These consequences could be critical for biological control, since only females are effective at parasitizing insect pests (Stouthamer et al. 1992).

The aphid parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) is a haplodiploid koinobiont solitary endoparasitoid native from Eurasia, which has been introduced in several regions including North America, South America, and Australia, mainly for controlling populations of the pea aphid *Acyrthosiphon pisum* (Harris) (Schwörer and Völkl 2001). The reproductive success of *A. ervi* parasitoids is partly determined by the ability to select a suitable aphid host, the oviposition being preferred on hosts from the same aphid/plant system from which females emerged (Henry et al. 2005, 2010). This preference for the natal host, also referred to as host fidelity, represents an important trait for *A. ervi* parasitoids searching for a suitable host in environments where many other potential host species can be available (Tumlinson et al. 1993; Henry et al. 2008). This preference would be learned during the pre-imaginal and emergence phases, being mediated mainly by chemical cues emitted by the interaction between the natal aphid host and its host plant (Storeck et al. 2000; Giunti et al. 2015).

Although there is much to be done regarding the mechanisms determining host fidelity, it is unknown how this trait is affected by the laboratory conditions under which populations are reared during several generations before they are released into fields. By studying fitness-related traits in highly inbred laboratory populations, we assessed the effects of inbreeding on host fidelity of the aphid parasitoid *A. ervi* and discussed how long-time caged rearing conditions may be detrimental for the efficacy of biological control.

Materials and methods

Aphid and parasitoid rearing

In a previous study, Zepeda-Paulo et al. (2013) addressed the formation of host fidelity in *A. ervi*, finding that parasitoid females have a preference for natal hosts. In that study, *A. ervi* parasitoids were obtained from parasitized living aphids sampled from field populations of the pea aphid *Acyrthosiphon pisum* complex, including host races on alfalfa (APA) and pea (APP), and the grain aphid *Sitobion avenae* (Fabricius) on wheat and oat (SA). Aphids collected on legumes and cereals were then left to form mummies and emerge on broad bean *Vicia faba* (Linnaeus) and oat *Avena sativa (L.)*, respectively. Broad bean is the universal host favorable for all pea aphid biotypes in laboratory conditions (Peccoud et al. 2014), while oat is a cereal that does not have chemical defenses against aphids on which all grain aphid genotypes perform well (Figueroa et al. 2004; Niemeyer 2009). After species determination and sex identification (Starý 1995), the *A. ervi* individuals were caged in the same aphid/host race-plant system (APA, APP, and SA) from which they emerged. Experimental populations were founded using >300 individuals in a sex ratio near equality that is similar to the actual situation in the field (Zepeda-Paulo et al. 2015). Each population was maintained in the same aphid-plant systems for over 2 years (approximately 75 generations). To further reduce the genetic differences among the parasitoid individuals used for determining host preferences and measuring fitness, we randomly chose a single couple (one male and one female) from each inbred population to find new populations that were kept isolated until the end of the experiments (between 2 and 6 generations). All aphids and parasitoids were reared under controlled conditions that allowed their continued reproduction (20 ± 1 °C, 65 ± 10 % RH and D16/N8 photoperiod).

As aphids can carry some facultative endosymbiotic bacteria that can confer protection against the development of parasitoid larvae (e.g., *Hamiltonella defensa*) (Oliver et al. 2008), all aphid lineages were established from a single parthenogenetic individual, and then their progeny were checked for the presence of bacteria (Sepúlveda et al. 2016; Peccoud et al. 2013). Only bacteria-free aphid lineages were used for parasitoid rearing and experiments.

Parasitoid genotyping

Each parasitoid individual was genotyped at nine microsatellite loci (Ae01, Ae03, Ae06, Ae08, Ae16, Ae27, Ae29, Ae30, and Ae32) previously reported for *A. ervi* (Zepeda-Paulo et al. 2015). Due to the haplodiploid sex determination system in *A. ervi*, only diploid females were genotyped. The observed heterozygosity per locus was computed at the population level as a measure of the degree of inbreeding and calculated using the Microsoft EXCEL add-in GENALEX version 6.501 (Peakall and Smouse 2012). This was done in the original (Zepeda-Paulo et al. 2013) and newly established populations (after 75 generations), using 15 individuals per population.

Host preference

The behavior displayed by parasitoid females facing aphids is considered a good predictor of preference (Antolin et al. 2006). To determine host preference, virgin females from each parasitoid inbred line were individually separated and left to mate for 24 h with a male from the same lineage, providing water and 80 % diluted honey. Each mated female was transferred to an experimental arena (a modified 2-cm-diameter petri dish) containing one single wingless aphid from the second to third instar and a small piece of leaf from the plant where the aphid was feeding (i.e., bean for *A. pisum* and oat for *S. avenae*).

Host preference was measured through the observation of different behaviors performed by the focal parasitoid female, which were recorded and grouped into three categories according to their chronological order: (1) "recognition," the time to the first antennation and the frequency and proportion of time expended in antennations; (2) "handling," the frequency and proportion of time dedicated to oviposition attempts and handling time (the time between the first antennation and a successful oviposition), and (3) "oviposition," the acceptance of a host aphid measured as the time until the first successful oviposition (from the moment when the parasitoid touches the arena until the insertion of the ovipositor into the aphid's body for 1–2 s) (Völkl 1994; Weinbrenner and Völkl 2001).

The behavior displayed by each female parasitoid was observed through a stereoscopic microscope and recorded in EthoLog version 2.2.5 (Otoni 2000). Each female parasitoid was tested only once (i.e., until the first successful oviposition) and then removed from the experimental arena and kept for immediate use in fitness assays (see below). The oviposited aphid was separated and isolated for further recording the fitness variables. Then, another female parasitoid and aphid individual were introduced to a new experimental arena to repeat the test ($n = 20$ for each of the three parasitoid populations studied).

Fitness

The fitness is calculated based on the relative success of parasitoid larvae during their development inside the aphid host (Antolin et al. 2006). Ten wingless individual aphids from the second to third instar were offered to each parasitoid female from the previous host preference assay ($n = 10$ for each of the three parasitoid populations studied) waiting until all aphids were oviposited. Those aphids were then transferred to their host plant (bean or oat), where they were individually confined in a clip cage until the emergence of a new adult parasitoid. From these assays, the sex ratio, productivity, and development time were recorded. Sex ratio was computed as the proportion of males in the progeny and used because it is a good estimator of host adaptation in parasitoid insects (i.e., female parasitoids prefer laying fertilized eggs in high-quality hosts, which will give rise to females) (Godfray 1994). Productivity was measured as the average number of parasitoids emerging from all aphids that were effectively oviposited, while the development time was measured as the time from the oviposition to the emergence of an adult parasitoid.

Statistical analysis

All data were analyzed in R 2.15.1 (R Core Team 2012). The frequency of antennations, oviposition attempts, and development time was adjusted to a generalized linear mixed model (GLMMs) (Bolker et al. 2009) using a Poisson distribution error and a log link function in the package *lme4* (Bates 2010). Due to the limited availability of female parasitoids, experiments were conducted separately for the three populations, considering the treatment (natal and non-natal host) as fixed factor and date as random factor. The proportion of time spent in antennations, proportion of time in oviposition attempts, productivity, and sex ratio were analyzed using a GLMM assuming a binomial error and a logit link function. The time to first antennation and time until the first successful oviposition were studied through a survival analysis using the *survival* package in R software (Kaplan-Meier estimates) (Therneau 1999). Handling time was analyzed using a generalized linear model (GLM) assuming a Gaussian distribution error. For models exhibiting overdispersion, a random factor was added at the individual level (Harrison 2014). Multiple comparisons were made withthe *multcomp* package (Hothorn et al. 2008).

Results

Heterozygosity in *Aphidius ervi* populations

After more than 75 generations of inbreeding reproduction, experimental parasitoid populations exhibited a similarly lower mean observed heterozygosity ($H_o = 0.26 \pm 0.08$) than founder populations ($H_o = 0.40 \pm 0.06$). Populations of *A. ervi* from the *A. pisum* alfalfa (APA) and pea races showed a H_o of 0.28 and 0.26, respectively, while the population from *S. avenae* (SA) exhibited a $H_o = 0.25$ (Table 1). In contrast, the mean H_o shortly after these parasitoid populations were established in the laboratory and used in the study published by Zepeda-Paulo et al. (2013) was 0.43, 0.41, and 0.36 for parasitoid populations from APA, APP, and SA, respectively.

Host preference of inbred parasitoid populations on natal and non-natal hosts

Recognition

The ability of parasitoids to identify their aphid hosts was compared when parasitoid females were faced to natal and non-natal hosts, finding significant differences in the "proportion of time spent in antennations" for parasitoids coming from APP ($\chi^2 = 6.8142$, df = 2, $P = 0.033$) and SA ($\chi^2 = 6.8$, df = 2, $P = 0.033$) populations, but not for APA ($\chi^2 = 0.1$, df = 2, $P = 0.921$). Parasitoids from APP took more time recognizing their natal aphid host (APP = 0.21 ± 0.03) than their non-natal SA host (0.10 ± 0.02), although no differences were found in the non-natal APA host (0.20 ± 0.04) (Fig. 1). Similarly, parasitoids from SA also took longer on their natal host (SA = 0.125 ± 0.02) compared to the non-natal APA (0.061 ± 0.01), but both did not differ significantly from the APP host (0.060 ± 0.01), while APA did not show differences among their host (APA = 0.16 ± 0.04, APP = 0.13 ± 0.02 and SA = 0.12 ± 0.02) (Fig. 1). Contrastingly, the "time to the first antennation" (APA: $\chi^2 = 1$, df = 2, $P = 0.596$; APP: $\chi^2 = 0.1$, df = 2, $P = 0.972$; SA: $\chi^2 = 0.8$, df = 2, $P = 0.667$) and the "frequency of antennations" (APA: $\chi^2 = 1.169$, df = 2, $P = 0.557$; APP: $\chi^2 = 4.6123$, df = 2, $P = 0.099$; SA: $\chi^2 = 2.6312$, df = 2, $P = 0.268$) did not vary significantly between natal and non-natal hosts for any of the tested parasitoid populations (Table 2).

Table 1 Observed heterozygosity (H_o) per locus in experimental populations of *Aphidius ervi*

Microsatellite locus	Generations under laboratory conditions					
	After 75 generations			After 6 generations		
	APA	APP	SA	APA	APP	SA
Ae 01	0.20	0.73	0.53	0.80	0.60	0.53
Ae 03	0.07	0.13	0.40	0.56	0.43	0.00
Ae 06	0.87	0.00	0.00	0.67	0.55	0.67
Ae 08	0.13	0.43	0.36	0.47	0.47	0.43
Ae 16	0.36	0.27	0.93	0.64	0.38	0.07
Ae 27	0.53	0.00	0.00	0.15	0.43	0.64
Ae 29	0.17	0.42	0.00	0.33	0.27	0.36
Ae 30	0.00	0.00	0.00	0.00	0.00	0.00
Ae 32	0.20	0.40	0.00	0.27	0.60	0.50
Mean H_o	0.28	0.26	0.25	0.43	0.41	0.36
Total mean H_o		0.26			0.40	

The values in the table show the observed heterozygosity (H_o) after 75 generations of laboratory rearing and during the first 6 generations (Zepeda-Paulo et al. 2013)

APA Acyrthosiphon pisum alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae*

Handling

The handling behavior performed by female parasitoid wasps on natal and non-natal hosts showed significant differences in the frequency of oviposition attempts for the parasitoid population from APA ($\chi^2 = 9.1919$, df = 2, $P = 0.010$), but not for the other two tested populations (APP: $\chi^2 = 0.4818$, df = 2, $P = 0.786$; SA: $\chi^2 = 5.0819$, df = 2, $P = 0.079$). The *A. ervi* females from APA exhibited a significantly poorer oviposition attempts in the non-natal host, SA (0.35 ± 0.15) compared to the natal host APA (2.30 ± 0.65) and non-natal APP (1.90 ± 0.73) (Fig. 2). Also, the proportion of time invested in oviposition attempts showed by parasitoid wasps from APA were significantly less on the aphid SA (0.01 ± 0.007) ($\chi^2 = 9.3285$, df = 2, $P = 0.009$) compared to the other two aphid hosts offered (APA = 0.07 ± 0.02 and APP = 0.06 ± 0.02). Parasitoid populations from APP and SA showed no differences in the proportion of time between the natal and non-natal host (APP: $\chi^2 = 0.0445$, df = 2, $P = 0.978$; SA: $\chi^2 = 1.6373$, df = 2, $P = 0.441$) (Fig. 3). Finally, the total time of handling (the time between the first antennation and a successful oviposition) between natal and non-natal hosts showed no significant differences for any of the analyzed populations (APA: $\chi^2 = 1.4309$, df = 2, $P = 0.489$; APP: $\chi^2 = 2.5884$, df = 2, $P = 0.274$; SA: $\chi^2 = 2.3335$, df = 2, $P = 0.311$) (Table 2).

Oviposition

No statistical differences in the time to the first successful oviposition were found in any of the populations studied after comparing between natal and non-natal hosts (APA: $\chi^2 = 1.1$, df = 2, $P = 0.569$; APP: $\chi^2 = 0.4$, df = 2, $P = 0.835$; SA: $\chi^2 = 5.6$, df = 2, $P = 0.061$) (Table 2).

Fitness between natal and non-natal aphid hosts in *A. ervi* inbred lines

Sex ratio

The proportion of male parasitoids emerging in the immediately following offspring from the tested females was used to estimate the sex ratio. Parasitoids from APP produced significantly fewer males ($\chi^2 = 7.7563$, df = 2, $P = 0.021$) on their natal host (APP = 0.724 ± 0.099) than on the non-natal host APA (0.966 ± 0.059), but both did not differ from the SA host (0.856 ± 0.062) (Fig. 4). Other comparisons between parasitoid populations showed no significant differences for this variable (APA: $\chi^2 = 0.7443$, df = 2, $P = 0.689$; SA: $\chi^2 = 1.146$, df = 2, $P = 0.564$) (Fig. 4).

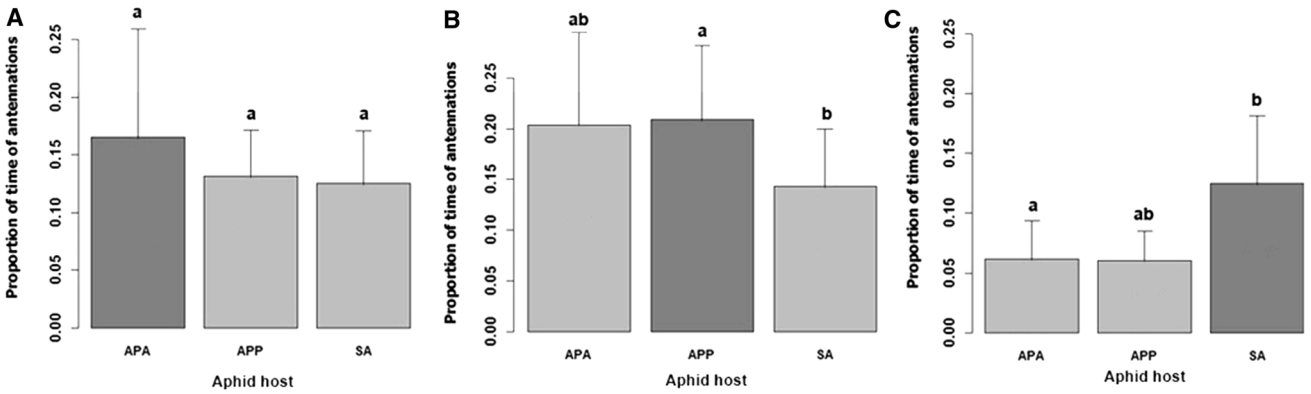

Fig. 1 Proportion of time expended in antennations (mean ± SE) in female parasitoids of *Aphidius ervi* tested on their natal (*dark bar*) and non-natal (*light bars*) hosts. *APA Acyrthosiphon pisum* alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae*. **a** Parasitoid population reared on *Acyrthosiphon pisum* alfalfa race; **b** Parasitoid population reared on *Acyrthosiphon pisum* pea race; **c** Parasitoid population reared on *Sitobion avenae*. *Different letters* over the *bars* indicate significant differences according to Tukey's HSD test ($P < 0.05$)

Table 2 Different components of the host preference measured in *Aphidius ervi* parasitoids reared on different aphids and tested on natal and non-natal hosts

Parasitoid population	Treatment	Time to the first antennation	Frequency of antennations	Handling time	Time to the first successful oviposition
A. pisum alfalfa race	APA	**15.85 ± 0.11**	**1.6 ± 0.28**	**16.72 ± 8.88**	**15.85 ± 0.11**
	APP	15.52 ± 0.11	2.2 ± 0.65	12.34 ± 5.54	15.52 ± 0.11
	SA	22.10 ± 0.11	3.9 ± 2.27	6.00 ± 1.63	22.10 ± 0.11
A. pisum pea race	APA	12.12 ± 0.11	7.5 ± 3.3	28.02 ± 14.95	19.40 ± 0.11
	APP	**7.43 ± 0.11**	**14.3 ± 6.5**	**65.61 ± 28.46**	**36.56 ± 0.11**
	SA	8.97 ± 0.11	5.15 ± 2.8	22.58 ± 14.59	17.93 ± 0.11
S. avenae	APA	10.56 ± 0.11	5.7 ± 1.2	129.12 ± 41.10	90.79 ± 0.11
	APP	26.90 ± 0.11	6.2 ± 1.2	131.84 ± 38.51	162.8 ± 0.11
	SA	**58.59 ± 0.11**	**3.8 ± 0.6**	**72.38 ± 15.97**	**74.66 ± 0.11**

The "Time to the first antennation" and "Time to the first successful oviposition" are the Kaplan-Meier estimators for the survival function

Data are expressed as the mean ± SE

The host preference and components of fitness that *A. ervi* parasitoids performed in the same host from they emerged (natal host) are in bold

APA Acyrthosiphon pisum alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae*

Productivity

The proportion of parasitoids emerging from effectively oviposited aphids showed no significant differences between natal and non-natal hosts for any of the three populations assayed (APA: $\chi^2 = 0.3836$, df = 2, $P = 0.826$; APP: $\chi^2 = 0.5336$, df = 2, $P = 0.766$; SA: $\chi^2 = 1.3814$, df = 2, $P = 0.501$) (Table 3).

Development time

Determined as the average number of days since parasitoids laid their eggs until the emergence of new parasitoids, this variable ranged between 15 and 18 days (Table 2). The analysis, however, showed no significant differences between natal and non-natal hosts for any of the populations studied (APA: $\chi^2 = 0.6392$, df = 2, $P = 0.726$; APP: $\chi^2 = 0.3528$, df = 2, $P = 0.838$; SA: $\chi^2 = 1.2051$, df = 2, $P = 0.547$) (Table 3).

Discussion

Inbreeding usually has negative effects on fitness-related traits in animals (Charlesworth and Willis 2009), and parasitoid wasps are not the exception (Luna and Hawkins 2004; Vayssade et al. 2014). The importance of inbreeding depression in these organisms underlies in their role as agents of biological control (Schwörer and Völkl 2001). In the present work, we studied the behavioral and life-history changes that occurred between parasitoids established straight from field samples (Zepeda-Paulo et al. 2013) and

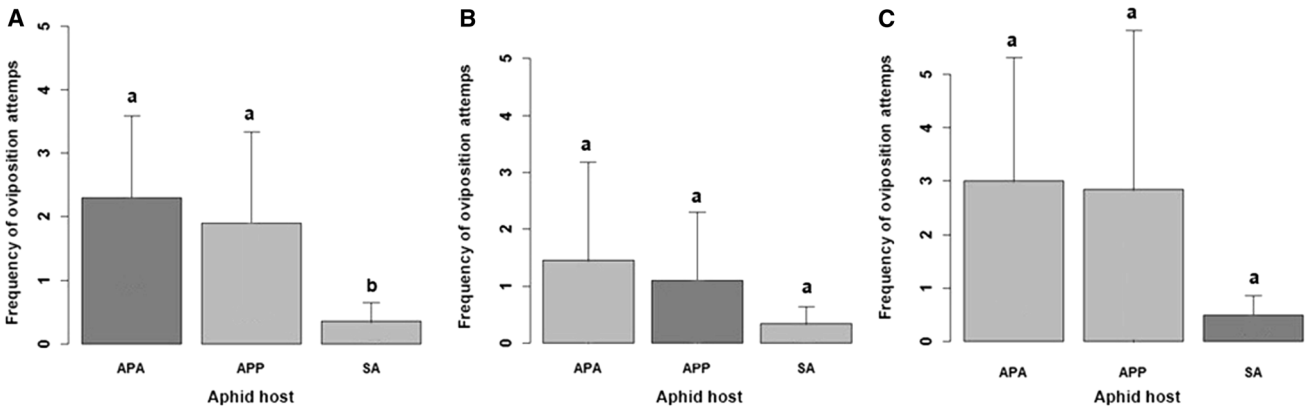

Fig. 2 Frequency of oviposition attempts (mean ± SE) of *Aphidius ervi* parasitoids tested on their natal (*dark bar*) and non-natal (*light bars*) hosts. *APA Acyrthosiphon pisum* alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae*. **a** Parasitoid population reared on *Acyrthosiphon pisum* alfalfa race; **b** Parasitoid population reared on *Acyrthosiphon pisum* pea race; **c** Parasitoid population reared on *Sitobion avenae*. *Different letters* over the *bars* indicate significant differences according to Tukey's HSD test ($P < 0.05$)

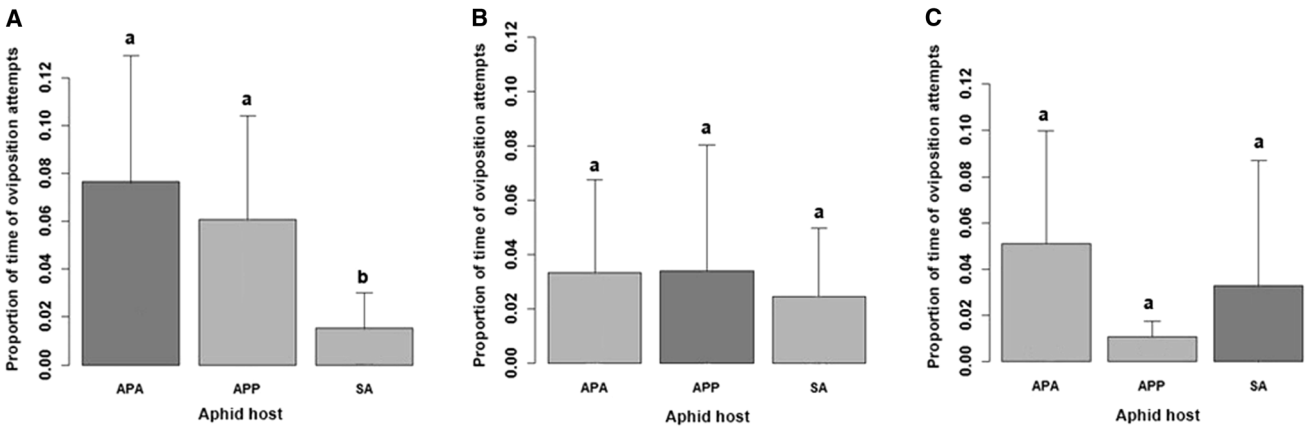

Fig. 3 Proportion of time invested in oviposition attempts (mean ± SE) of *Aphidius ervi* parasitoids tested on their natal (*dark bar*) and non-natal (*light bars*) hosts. *APA Acyrthosiphon pisum* alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae*. **a** Parasitoid population reared on *Acyrthosiphon pisum* alfalfa race; **b** Parasitoid population reared on *Acyrthosiphon pisum* pea race; **c** Parasitoid population reared on *Sitobion avenae*. *Different letters* over the *bars* indicate significant differences according to Tukey's HSD test ($P < 0.05$)

parasitoids after 75 generations under laboratory conditions. Next, we discussed whether caged rearing and inbreeding may produce a loss of host fidelity, which could have important consequences for the "quality" of parasitoids after mass rearing leading to failures in biological control programs (Van Lenteren 2003).

Loss of host fidelity in highly inbred populations of *A. ervi*

No evidence of preference for the natal host by female parasitoids was found in our experiments. Indeed, the host preference traits measured (i.e., the proportion of time invested in antennations, frequency, and proportion of time invested in oviposition attempts) were only slightly but not significantly different between natal and non-natal hosts (e.g., parasitoid population from APA shows lesser proportion of time of antennations on SA and the population from SA had more antennations on SA). This result contrasts with those previously reported by Daza-Bustamante et al. (2002) and Zepeda-Paulo et al. (2013), who observed that populations of *A. ervi* lay their eggs faster on their natal hosts. As we used the populations established by Zepeda-Paulo et al. (2013) to find our experimental populations, the effects of sampling and rearing conditions on host preference are comparable.

The time until the first oviposition reflects how efficiently a parasitoid oviposits on its respective host; if there is a preference for the natal host, then this should lead to a more rapid acceptance, because the parasitoid should recognize both visual and chemical cues from its natal host faster than those coming from other hosts because of

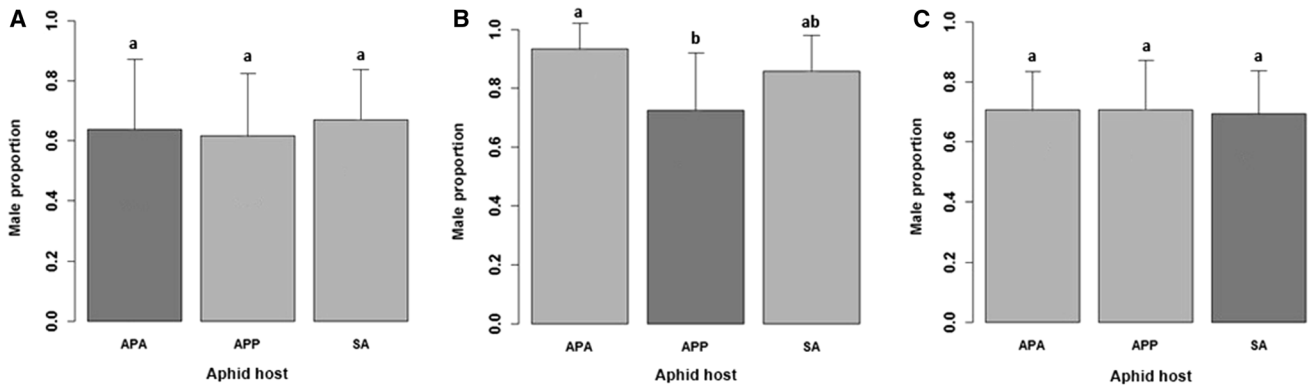

Fig. 4 Sex ratio expressed as the proportion of males (mean ± SE) emerging from aphids parasitized by females of *Aphidius ervi* tested on their natal (*dark bar*) and non-natal (*light bars*) hosts. *APA Acyrthosiphon pisum* alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae* treatment. **a** Parasitoid population reared on *Acyrthosiphon pisum* alfalfa race; **b** Parasitoid population reared on *Acyrthosiphon pisum* pea race; **c** Parasitoid population reared on *Sitobion avenae*. *Different letters* over the *bars* indicate significant differences according to Tukey's HSD test ($P < 0.05$)

Table 3 Different components of the fitness measured in *Aphidius ervi* parasitoids reared on different aphids and tested on natal and non-natal hosts

Parasitoid population	Treatment	Productivity	Development time
A. pisum alfalfa race	**APA**	**0.74 ± 0.1**	**15.9 ± 0.23**
	APP	0.63 ± 0.1	16.0 ± 0.29
	SA	0.63 ± 0.06	17.2 ± 0.32
A. pisum pea race	APA	0.70 ± 0.05	15.0 ± 0.21
	APP	**0.74 ± 0.08**	**15.0 ± 0.29**
	SA	0.63 ± 0.07	15.9 ± 0.25
Sitobion avenae	APA	0.67 ± 0.06	17.2 ± 0.53
	APP	0.55 ± 0.08	16.6 ± 0.16
	SA	**0.50 ± 0.06**	**18.6 ± 0.27**

Data are expressed as the mean ± SE

The host preference and components of fitness that *A. ervi* parasitoids performed in the same host from they emerged (natal host) are in bold

APA Acyrthosiphon pisum alfalfa race, *APP Acyrthosiphon pisum* pea race, *SA Sitobion avenae*

associative learning during its embryo and larval development, metamorphosis, and emergence (Giunti et al. 2015). Thus, it should be expected that a parasitoid should take a shorter time to the first successful oviposition on the natal host. However, the maintenance of relatively small caged populations for long periods of time can cause significant changes in the behavior, physiology, and life-history traits because of founder effects, genetic drift, and inbreeding depression (Roush 1990; Van Lenteren et al. 2003). Most introduced natural enemies for biological control programs undergo significant bottlenecks because of the small samples that have been commonly used in these introductions, beside the effects of quarantine. This quarantine procedure should favor the inbreeding and consequently reduces the genetic diversity of populations even more (Unruh et al. 1983). This has been studied for *A. ervi* in Chile, where bottlenecks were estimated and the loss of genetic diversity measured and compared to a source population in France (Zepeda-Paulo et al. 2015). Further inbreeding and loss of diversity can follow the isolation of parasitoid populations in rearing chambers. Indeed, it is predicted that under repeated inbreeding, as experienced by caged populations tested in our study, haplodiploid organisms will gather a genetic load for sex-linked traits (limited only to females), which can cause serious detriments to the fertility, host finding ability, and sex ratio (Werren 1993).

Some authors, however, discuss that haplodiploid organisms are less likely to suffer from the effects of inbreeding because of their lower effective mutation rate compared to diploids (Werren 1993). Although we cannot rule out the purging effect that haploid males can have on the frequency of deleterious and lethal mutations, the occurrence and impact of deleterious mutations on host finding traits could be faster in caged populations as males cannot fly away and disperse and due to deleterious mutations are accumulated in sex-linked traits (Henter 2003).

Environmental and biological factors influencing the sex ratio in *A. ervi* parasitoids

Parameters related to fitness reflect the success of parasitoids on a certain host (Antolin et al. 2006). In our results, parameters of fitness remained almost unaltered between natal and non-natal hosts, except for the sex ratio. Differences were observed in a single parasitoid population from *A. pisum* (APP), which showed a significantly higher number of males in the non-natal APA compared to their natal APP host. Despite this, it is noteworthy that the sex

ratio in the offspring was strongly male-biased in all populations. It has been reported that inbreeding in parasitoid insects can strongly increase the proportion of males in the offspring (Luna and Hawkins 2004; Vayssade et al. 2014). This is more evident when our results are compared with those reported by Zepeda-Paulo et al. (2013), in which the proportion of males ranged between 0.47 and 0.75 during the first generations, while 75 generations later this proportion increased to 0.61–0.96 (Fig. 4).

Parasitoid wasps reproduce by arrhenotoky; mated females can adjust the proportion of fertilized eggs during oviposition (Ode et al. 1997). This strategy allows parasitoid females to assess the quality of the aphid host before the oviposition fertilized eggs, which are more expensive from an evolutionary point of view. The sex in parasitoid wasps is determined by the single locus complementary sex determination or sl-CSD model (Vayssade et al. 2014). In this model, the sex does not depend on the number of chromosome sets as in other insects, but on the heterozygosity at a single locus (Cook 1993). Hence, CSD hemizygous non-fertilized eggs will develop as haploid males while heterozygous fertilized eggs will originate diploid females. Interestingly, diploid males can also be developed from fertilized eggs, particularly under inbreeding conditions (Cook and Crozier 1995). As homozygosity is expected to increase under inbreeding, the CSD alleles can be fixed or lost because of random drift, causing unpredictable impacts on the frequency of offspring males (Keller and Waller 2002). In our study, we detected a drop in the observed heterozygosity at nine microsatellite loci (from 0.40 to 0.26 after 75 inbred generations) (Table 1), which can anticipate the fate of sex alleles (i.e., a higher number of offspring males due to homozygous eggs). Indeed, females of the braconid parasitoid *Habrobracon hebetor* (Say) mated with their progeny accounted for over 50 % of males in their diploid offspring (Torvik 1931). As a consequence, the population growth rate may be reduced by the occurrence of diploid males, as some fertilized eggs will develop as males that may die during development or become sterile (Stouthamer et al. 1992). This phenomenon is often referred to as a form of inbreeding depression (Vayssade et al. 2014), which could account for the high proportion of males we observed in all the caged parasitoid populations studied.

Sex ratio can also be affected by the effective population size (*Ne*), as lower *Ne* can rapidly increase the homozygosity in small laboratory colonies of *A. ervi* (Unruh et al. 1983). In our experiments, the number of sex alleles was expected to be low, as we started with a single couple to raise each population before the assays, which may have accelerated the decrease of heterozygosity. Indeed, it has been shown that the *Ne* can be lower than the actual population size in *A. ervi*, indicating that not all females and males contribute to the next generation (Unruh et al. 1983). In addition, genes in males are inherited from their mothers, which can further increase the homozygosity (Boulton et al. 2015). Other biological factors that can limit the number of female offspring thus affecting the sex ratio include the spermatic depletion and the age of females. Males of parasitoid wasps can copulate more than once depending on the species, a phenomenon that can provoke the sperm stock to be depleted from their seminal vesicles, causing no longer transfer of sperm during copulation (Damiens and Boivin 2006). On the other hand, older females of *A. ervi* produce more males, which can also reduce the production of female offspring (He and Wang 2008).

The origin of experimental populations and the loss of host fidelity in *A. ervi* parasitoids

Natural selection is the primary evolutionary force shaping populations of aphid parasitoids in nature, mainly acting on the ability of individuals to find their resources (de Rijk et al. 2013). But selection may be weakened because of smaller *Ne*, lower genetic diversity, and the absence of those pressures that operate in nature. Hence, random drift under continued laboratory conditions should be much stronger, leading to the random loss of ecologically important traits (van Lenteren 2003; Boivin et al. 2012; Grenier and De Clercq 2003). As each experimental population arose from a single couple (one female and one male) randomly chosen from the populations used by Zepeda-Paulo et al. (2013), this may have acted as a strong founder effect that profoundly reduced the genetic diversity, making populations more prone to the loss of host fidelity. This idea is supported by the fact that after 75 generations female wasps exhibited shorter times to the first successful oviposition (from 15.52 to 162.08 s) than wasps recently established in the laboratory (38.54–355.08 s) (Zepeda-Paulo et al. 2013). Therefore, it seems that inbred parasitoids are rapidly accepting aphids making no true host choice (i.e., they lost their host preference behavior). Further studies on the molecular base of host fidelity can shed light on how the detection and learning of specific cues from different aphid/plant systems may control the decision-making process in parasitoid wasps (i.e., oviposition on the grain aphid or the pea aphid).

Mass rearing of aphid parasitoids and the success of biological control

More than 125 species of natural enemies are commercially available for biological control worldwide (van Lenteren 2003). About 600 companies in the USA and 200 in Europe produce and distribute biocontrol agents (Boivin et al. 2012). The success of biological control using parasitoids depends, among others, on how efficiently the parasitoids are mass reared so they can maintain their quality for

detecting and ovipositing target species after their release into fields (Gandolfi et al. 2003; Boivin et al. 2012).

The sex ratio appears to be an interesting aspect to consider during parasitoid rearing for biological control (Stouthamer et al. 1992). In haplodiploid Hymenoptera, the sex ratio is generally biased to females, which allows a greater population growth rate (Boivin et al. 2012). Under inbreeding conditions, however, the sex ratio is male biased (Salin et al. 2004; Zhou et al. 2007; Vayssade et al. 2014). Similarly, mass rearing can significantly and rapidly deteriorate host searching behavior and host fidelity because of inbreeding (Geden et al. 1992). But despite the importance of host fidelity in parasitism, little is known about the effects of mass rearing on the success of biological control.

Our work highlights the importance of considering some evolutionary drivers such as founder effect (each population originated from a single founder couple), random drift (small laboratory populations), and inbreeding depression (sex ratios biased to males) during mass rearing of biocontrol agents, as they may accelerate the loss of adaptive genetic variation involved in the formation of host fidelity.

Author contribution statement

DAS and CCF conceived and designed the research. DAS conducted the experiments. DAS, BL, FZP, CCR, and CCF analyzed the data. DAS and CCF wrote the manuscript. All authors read and approved the manuscript.

Acknowledgments The authors thank Angélica González, Gabriel Ballesteros, and Sebastián Ortíz for helping us in aphid and parasitoid rearing and data analysis and with advice on parasitoid biology and species determinations. DAS thanks to Iniciativa Científica Milenio grant NC120027 for a fellowship. Also, we thank the valuable comments made by anonymous referees.

Funding This study was funded by FONDECYT (Grant Number 1130483 to CCF).

Compliance with ethical standards

Conflict of Interest Authors declare no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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