Is parasitoid acceptance of different host species dynamic?

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Summary

1. Parasitoid acceptance of host individuals that differ in suitability is dynamic, varying with physiological state and experience. Female parasitoids with high egg loads and low life expectancy (i.e. time limited) are more willing to accept hosts with low suitability for progeny development than females with low egg loads and high life expectancy (i.e. egg limited). However, studies of dynamic acceptance behaviour in parasitoids have only considered high- vs. low-suitability host individuals within the same host species.

2. Here, we report the first results on whether acceptance of individuals from different host species that vary in suitability is also dynamic, using two aphid parasitoid species in the genus Aphelinus (Hymenoptera: Aphelinidae).

3. Stressors (starvation and age) and experience that increase time limitation did not affect the acceptance of low-suitability host species by two Aphelinus species with reciprocal specificities.

4. Oviposition in a high-suitability host species increased with egg load, but did not vary with egg load for females exposed to low-suitability host species. This is an unexpected pattern since it results in proportionally higher acceptance of low-suitability hosts with decreasing egg load.

5. The stability of behavioural host specificity in these parasitoids under very stressful conditions might be explained by (i) frequent transient egg limitation, (ii) higher fitness from egg resorption than oviposition or (iii) neural constraints on host recognition.

6. If neural constraints restrict the ability of these specialists to change their behaviour under stress, we predict that generalist species of Aphelinus should show greater dynamism in acceptance of low-suitability hosts. We are testing this prediction using Aphelinus species with very broad host ranges.

Key-words: Aphelinus, aphid, biological control, egg limitation, egg load, egg resorption, host specificity, neural constraints, time limitation

Introduction

Parasitoid acceptance of individual hosts within species is dynamic, varying with physiological state and experience. Female parasitoids with high egg loads and low future life expectancy (i.e. time limited) are more willing to accept hosts with low suitability for progeny development and survival; conversely, females with low egg loads and high life expectancy (i.e. egg limited) tend to reject low-suitability hosts (reviewed by Minkenberg, Tatar & Rosenheim 1992; Rosenheim & Heimpel 1994; Heimpel & Rosenheim 1998; Heimpel & Collier 1996; Papaj 2000). By rejecting low-suitability hosts when egg-limited and accepting them when time-limited, parasitoids can maximize their lifetime reproductive success (Mangel 1989; Heimpel, Mangel & Rosenheim 1998; Mangel & Heimpel 1998; Van Baalen 2000). To date, studies of dynamic acceptance behaviour in parasitoids have considered high- vs. low-suitability individuals within the same host species. Egg load and/or life expectancy have been shown to affect the acceptance of unparasitized vs. previously parasitized hosts (Roitberg et al. 1992; Roitberg et al. 1993; Fletcher, Hughes & Harvey 1994; Hughes, Harvey & Hubbard 1994; Sirot, Ploye & Bernstein 1997; Ueno 1999; Islam & Copland 2000) and small vs. large hosts (Rosenheim & Rosen 1991, 1992; Heimpel & Rosenheim 1995; Heimpel, Rosenheim & Mangel 1996).

Parasitoids having multiple host species that differ in suitability face an analogous situation, but dynamic host acceptance has not previously been studied at the
interspecific level. Parasitoids may attack host species that range in suitability from highly suitable to marginal or even unsuitable for development (Heimpel, Neuhauser & Hoogendoorn 2003), but under what conditions should low-suitability host species be accepted? Because this question is analogous to the one posed for low-suitability individuals within host species, one would expect that the answer would be the same: time-limited parasitoids should accept low-suitability species, but egg-limited parasitoids should reject low-suitability species. Several authors have suggested that this is the case (Roitberg 2000; Withers & Barton Browne 2004), and acceptance of low-suitability host species in the field has been interpreted as evidence for time limitation (Janssen 1989; Heimpel & Casas 2008).

In herbivorous insects, the hypothesis of broader host range for time-limited species enjoys some theoretical and empirical support (for review, see Mayhew 1997), including a comparative analysis by Jervis, Ferns & Boggs (2007) where shorter-lived species of Lepidoptera tended to have broader host ranges. However, the patterns for herbivores are mixed (Mayhew 1997), and closely related species with similar life histories may have very different host ranges (Oppenheim & Hopper 2010). Furthermore, while some herbivore species have shown increased oviposition on low-ranking hosts with increased age or egg load or low abundance of high-ranking hosts (Wilkund 1981; Singer 1983; Courtney, Chen & Gardner 1989), other species have not shown changes in oviposition under such conditions (Ives 1978; Tabashnik 1983). Experimental tests of the hypothesis that acceptance of host species varies in response to egg- vs. time-limitation have not been reported for parasitoids.

The expressed host ranges of parasitoids in the field have important implications for host–parasitoid community structure (Muller et al. 1999), for population dynamics of host–parasitoid systems in which a parasitoid attacks more than one host species (Hassell 2000; Heimpel, Neuhauser & Hoogendoorn 2003), and for biological control (Babendreier, Bigler & Kuhlmann 2006; Desneux et al. 2009). For biological control introductions, the impact of egg vs. time limitation on host selection has important consequences for the design of host-specificity tests and for predicting the risk to nontarget species (Barton Browne & Eastop 2004). In the system we studied, the two aphid species occur on different host plant species (and occasionally other legumes) or its overwintering host, Rhamnus species, whereas R. padi occurs only on grasses or its overwintering host, Prunus species (Blackman & Eastop 2006). Replacing an aphid species consistently while encountering no other species will eventually lead to egg resorption in Aphelinus, which decreases egg load but increases longevity, which in theory should make parasitoids less likely to accept low-suitability hosts.

Several factors may moderate egg- vs. time-limitation, these include egg load, egg production rate, longevity, searching behaviour, experience and the presence of host-feeding and egg resorption. Aphelinus females take a long time to handle their hosts (Collins, Ward & Dixon 1981; De Farias & Hopper 1999) and attack hosts that occur in colonies so that the time spent in each aphid colony can be long. Aphelinus species are small (Fig. 1d) and weak fliers with low dispersal rates (Fauvergue & Hopper 2009), searching primarily on foot (Fauvergue, Hopper & Antolin 1995), and their aphid hosts are patchily distributed (Dean 1974; Wu et al. 2004) so that transit times between colonies may be long. Aphelinus females feed on aphids for nutrients, which may be used for egg production or increased longevity (Rockwood 1917; Hartley 1922; Cate, Sauer & Eikenbary 1974; Papaj 2000; Wu & Heimpel 2007). Finally, Aphelinus females resorb eggs when deprived of aphids (Mackauer 1982; Bai & Mackauer 1990b; Le Ralec 1991).

In the field, aphid parasitoids typically encounter a single host species at a time, and to reproduce this situation, we measured responses to each aphid species separately. Upon encountering an aphid, a female parasitoid has three options: to oviposit, to host-feed or to move onto the next aphid or aphid colony. Within a colony or on the same host plant, the next aphid will likely be the same species, and in the system we studied, the two aphid species occur on different host plant species. Aphis glycines occurs only on Glycine species (and occasionally other legumes) or its overwintering host, Rhamnus species, whereas R. padi occurs only on grasses or its overwintering host, Prunus species (Blackman & Eastop 2006). Rejecting an aphid species consistently while encountering no other species will eventually lead to egg resorption in Aphelinus, which decreases egg load but increases longevity, which in theory should make parasitoids less likely to accept low-suitability hosts.

We expected that stressed Aphelinus females would more readily oviposit in low-suitability aphid species than would unstressed females. Furthermore, we expected that the increase in acceptance of low-suitability species would vary with egg load, such that females with many eggs would accept low-suitability species more readily than females with few eggs. However, our results contradicted both of
Fig. 1. Differentiation of parasitoid eggs at various stages using neutral red dye. The amount of staining varies with the state of the chorion: (a) immature eggs stain uniformly red because the chorion is impermeable, (b) mature eggs do not stain in the interior because the chorion is impermeable, (c) eggs being resorbed show interior staining because the chorion is becoming permeable again and also show clear areas where the cytoplasm has disappeared, and (d) adult female *Aphelinus rhamni* (adult photo by James B. Woolley).

these expectations: stressed females did not increase their rate of oviposition in low-suitability species, and egg load did not affect oviposition in low-suitability species. These patterns held for both parasitoid species and short and long periods of exposure to aphids. Aphid defensive behaviours did not affect the results.

**Materials and methods**

**INSECT SOURCES AND REARING CONDITIONS**

*Aphelinus rhamni* was collected as mummified *Aphis glycines* on *Rhamnus* species (*Rhamnaceae*) in Beijing, China, during 2005, and *A. kurdjumovi* (Kurdumov) was collected as mummified *Rhopalosiphum* species on wheat near Tbilisi, Republic of Georgia, during 2006. Cultures were established in the quarantine facility at the USDA-ARS, Beneficial Insect Introductions Research Unit, Newark, Delaware, USA. The cultures were divided into 4–6 subcultures, and each subculture was maintained with an adult population size >200 and sex ratio of 1:1 males/females. *Aphelinus rhamni* was reared on *Aphis glycines* on soybean, *Glycine max* (L.) (Fabaceae); *A. kurdjumovi* was reared on *Rhopalosiphum padi* on barley, *Hordeum vulgare* L. (Poaceae). Aphids for parasitoid rearing and experiments were from laboratory cultures at Newark, started from field collections in 1997 and 2008. All cultures were reared on appropriate host plant species in plant growth chambers at 20 °C, 50–70% relative humidity, 16:8 h (L:D) photoperiod. Vouchers are maintained at –20 °C in molecular-grade ethanol at the Beneficial Insect Introduction Research Unit, Newark, Delaware, USA. *Aphelinus rhamni* was recently described from the culture used in this study (Hopper et al. 2012); *Aphelinus kurdjumovii* was described by Kurdumov (1913), and the culture used in this study was included in a molecular phylogeny of species in the *Aphelinus varipes* complex (Heraty et al. 2007).

**DIFFERENCES IN PARASITISM BETWEEN APHID SPECIES**

To measure differences in parasitism between aphid species, we exposed individual, mated females of *A. rhamni* or *A. kurdjumovi* to one of two aphid species: *Aphis glycines* or *R. padi*. We put each female parasitoid in a cage (polystyrene, 10 cm diameter by 22 cm tall, with eight 2.5-cm holes in the sides and a 1.2-cm hole in the top covered with fine-mesh screening) enclosing the foliage of a potted plant of the appropriate species with 100 aphids of mixed instars of a single aphid species. Female parasitoids were removed after 24 h and were used only once. Because these parasitoids can parasitize a maximum of about 20 aphids in 24 h, this abundance of aphids and period of exposure allowed parasitoids to use their full egg complement. Furthermore, the density of aphids, amount of plant material and cage size meant that parasitoids were not limited by search rate.

We waited until the larval parasitoids killed and mummified their hosts before scoring parasitism. Therefore, we measured a combination of acceptance of aphid species for oviposition and suitability of aphid species for parasitoid survival. Ten days after exposure of aphids to parasitoids, we collected mummified aphids and held them for adult parasitoid emergence. After the adults emerged, we recorded the number of mummified aphids and the number and sex of adult parasitoids.

**ACCEPTANCE FOR OVIPOSITION AND APHID SUITABILITY**

To determine the relationship between acceptance for oviposition and aphid species suitability for parasitoid survival, we directly observed behaviour of virgin *A. rhamni* females with *Aphis glycines* vs. *R. padi* and measured oviposition and parasitoid progeny survival. We exposed each female parasitoid to 10 second-to-third instar aphids of a single species in small arenas (10 mm diameter by 5 mm height) on an excised leaf of the appropriate plant species: *G. max* for *Aphis glycines* and *H. vulgare* for *R. padi*. The arenas were formed using two microscope slides with vinyl foam weather-stripping glued to them; on one slide, the weather-stripping had a 10-mm-diameter hole bored in it, exposing the slide surface through which observations were made. A leaf freshly cut from a plant was clamped between the slides (weather-stripping against the leaf), and the leaf petiole placed in a 1% agar in a small vial to maintain turgor. We placed aphids in the arenas 0.5 h before beginning observations to allow them to settle and feed on the leaves. We used 2-day-old female parasitoids fed 1:1 honey/water solution. Females were exposed to 3 second-to-third
instar *Aphis glycines* for 0.5 h before being observed to allow host-feeding so that they would be less likely to host-feed during observations. We observed the behaviours of each female parasitoid and the aphids with which she was enclosed for 25 min under a zoom binocular stereoscope at 10–30× magnification and recorded these behaviours with a video camera, digital video tape recorder (for date and time stamping) and DVD recorder for archiving the recordings. Locations of insects and sequences of behaviours were noted on maps of the arenas during observations, and these were verified by reviewing the DVD recordings.

On emergence, aphids often present a potential host. *Aphelinus* females stop at about half their body length away from the aphid. Without touching the aphid, females move away from the aphid to count eggs laid, and thus do not oviposit in them. For each aphid that a parasitoid approached, we tabulated the outcome of the encounter (stung, host-fed, or rejected). If a female parasitoid stung one or more aphids, we either dissected all the aphids from a given observation period to determine whether eggs were laid, or we transferred all the aphids to their host plant and reared the aphids to determine the number of parasitoid progeny. Aphids were dissected in batches of five on glass microscope slides, and each batch was placed in a small drop of Ringer’s solution for 10 min under a zoom binocular stereoscope at 100× magnification. We dissected aphids at 40–100× magnification. We dissected aphids from half of the replicates and reared aphids from the other half of the replicates. Because dissections of aphids were destructive, we inferred survival by comparing mean numbers of aphids oviposited vs. mean numbers of aphids mummified.

**STRESSORS: NUTRITION AND AGE**

To test the effects of nutrition and age, female parasitoids of *A. rhamni* were kept in 5-ml vials individually without aphids and fed with either 4 µl 1:1 honey/water solution or 4 µl water renewed periodically; the females were checked daily for deaths. Adult *A. rhamni* females given nothing but water died quickly (mean life span = 2.5 days; maximum = 3 days), but females given 1:1 honey/water solution lived much longer (mean life span = 22 days; maximum = 41 days; see Results). To measure mature egg load and resorbed eggs, ovaries were dissected from subsets of 5–20 females at 1, 5, 10, 15 and 20 days for honey-fed females and at 0 and 2 days for water-fed females. The ovaries were placed in insect Ringers solution with neutral red dye. Mature eggs have intact chorions and thus do not stain, immature eggs and eggs being resorbed stain red, but immature eggs are uniformly stained, whereas eggs being resorbed have clear regions (Fig. 1a–c).

**BEHAVIOURAL OBSERVATIONS ON THE EFFECTS OF NUTRITION AND AGE ON OVIPOSITION IN LOW- VS. HIGH-SUITABILITY HOST SPECIES**

Using the methods described above for measuring host acceptance, we directly observed behaviour to test whether acceptance of low-suitability aphid species was affected by nutrition, age or egg load. We did two experiments with *A. rhamni*: one to test the effect of nutrition (2-day-old females fed water alone vs. honey-water) and egg load, and another to test the effect of age (1 day vs. 10 days postemergence females fed honey/water) and egg load. For *A. kurdjumovi*, we did one experiment on the effect of age (0–1 vs. 7–8 day postemergence females fed honey/water) and egg load. All females were unmated and had not been previously exposed to aphids. Because parasitoids were not exposed to aphids during ageing, age also affected experience so old parasitoids might perceive the environment as aphid-poor.

As in the acceptance/suitability experiment, we exposed female parasitoids to 10 second-to-third instar aphids of a single species in small arenas (10 mm diameter by 5 mm height) on excised leaves of the appropriate plant species. For aphids that were approached, we recorded the outcome of each encounter (stung, host-fed, rejected) and the presence of aphid defence behaviours (kicking, bucking, rotating, walking away, or cornicular secretion). From these data, we tabulated the number of aphids approached, stung and fed upon. We calculated aphid defence as the number of aphids defending themselves weighted by the frequency of each defence behaviour when approached by female parasitoids. We removed female parasitoids after the observation period, dissected their ovaries in Ringer’s solution mixed with neutral red stain and counted the number of mature eggs. In these experiments, we dissected all the aphids to count eggs laid, and thus, we did not measure suitability.

**LONGER EXPERIMENT ON EFFECTS OF AGE ON PARASITISM OF HIGH- VS. LOW-SUITABILITY HOST SPECIES**

Female parasitoids may behave differently after encountering low-vs. high-suitability host species for longer periods than the short behavioural observations above. Therefore, we compared parasitism by 1- to 2-day-old vs. 11- to 12-day-old *A. rhamni* females in a 24-h experiment with high- vs. low-suitability host species. We used the same protocol as that for measuring differences in parasitism. As with the 25-min observations, parasitoids were not exposed to aphids during ageing so older females might perceive the environment as aphid-poor.

**DESIGN STRUCTURE AND STATISTICAL ANALYSIS**

The experiments were designed as randomized complete-blocks: location within rearing chamber was the blocking factor for 24-h exposure experiments, and date was the blocking factor for direct observation experiments. For each analysis, block was included in the model, but was rarely significant so we do not report block effects here. Because egg load, the number of aphids oviposited, the number of adult parasitoids and the weighted aphid defence behaviour had nonnormal distributions with variances proportional to means, we used generalized linear models with appropriate distributions (e.g. negative binomial or Poisson) for the dependent variable to test for the effects of model factors (GLIMMIX procedure in SAS/STAT Version 9.2, copyright 2002–2006, SAS Institute Inc., Cary, NC, USA). We chose the appropriate distribution using the residual deviance divided by degrees of freedom, which should be about 1 for models that fit well (Littell et al. 1996). For the figures, least square means and standard errors were converted back to the original distributions, using the appropriate inverse link functions.

For the experiment on parasitism, we tested the effect of aphid species on number parasitized (mummified) and on adult sex ratio (proportion males). For the experiment on the relationship between acceptance for oviposition and aphid suitability, we tested the effect of aphid species on the number aphids in which females oviposited and the numbers of adult offspring. For the direct behavioural observation experiments, we tested the effect of stressor (nutrition or age), aphid species, egg load and the interaction of stressor and aphid species on the number of aphids in which
females oviposited, the number of aphids stung and the amount of aphid defence behaviour. For the 24-h cage experiments, we tested the effects of stressor (age), aphid species, egg load and the interaction of stressor and aphid species on the number of parasitoid progeny. We also tested the interaction between egg load and other factors having significant main effects, and where the interaction was significant, we tested for significant regression slopes using t-tests estimated under the generalized linear model. Where means comparisons were needed, we used stepdown simulations of probabilities, corrected for multiple comparisons of least squares means.

Results

DIFFERENCES IN PARASITISM BETWEEN APHID SPECIES

The patterns in parasitism of *Aphis glycines* and *R. padi* by *A. rhamni* vs. *A. kurdjumovi* were mirror images (Fig. 2). *Aphelinus rhamni* produced fivefold more parasitized (mummified) aphids on *Aphis glycines* than on *R. padi* ($F = 29.7$; d.f. $= 1,50$; $P < 0.001$), whereas *A. kurdjumovi* produced fourfold more parasitized aphids on *R. padi* than on *Aphis glycines* ($F = 13.2$; d.f. $= 1,34$; $P < 0.001$). Sex ratio of *A. rhamni* progeny did not differ between *Aphis glycines* and *R. padi* (0.54 ± 0.09 vs. 0.47 ± 0.17 proportion males, respectively; $F = 0.1$; d.f. $= 1,35$; $P = 0.73$), suggesting that females did not manipulate sex ratio based on host suitability.

APHID SUITABILITY VS. ACCEPTANCE FOR OVIPOSITION

*Rhopalosiphum padi* is a low-suitability host for *A. rhamni*, producing twofold fewer adults per egg laid than *Aphis glycines*, a high-suitability host in which survival was 100% (Fig. 3). Young, well-fed *A. rhamni* females approached *R. padi* less often than *Aphis glycines* ($F = 8.8$; d.f. $= 1,38$; $P = 0.005$), stung them less often ($F = 8.8$; d.f. $= 1,38$; $P = 0.005$), oviposited in them less often ($F = 8.6$; d.f. $= 1,18$; $P < 0.01$) and produced fewer adult progeny ($F = 11.3$; d.f. $= 1,18$; $P = 0.004$). This pattern suggests that *A. rhamni* recognizes that *R. padi* has lower suitability than *Aphis glycines*.

**Fig. 2.** Differences in parasitism (mummified aphids) between aphid species for *Aphelinus rhamni* and *Aphelinus kurdjumovi*. Error bars are standard errors of the means.

**Fig. 3.** Acceptance behaviour and survival of *Aphelinus rhamni* on *Aphis glycines* vs. *Rhopalosiphum padi*. Error bars are standard errors of the means. Progeny per wasp egg is the ratio of the mean number of adult wasps per mean number of aphids oviposited. Observation duration was 25 min.

**Fig. 4.** Longevity and egg load of *Aphelinus rhamni* in the laboratory. Error bars are standard errors of the means. Common letters indicate significant differences, whereas different letters indicate significant differences.

LONGEVITY AND EGG LOAD IN *APHELINUS RHAMNI*

Female *A. rhamni* lived 22 ± 0.75 days ($n = 165$) as adults, with a maximum age at death of 41 days (Fig. 4). For parasitoids fed honey/water but deprived of aphids, the number of mature eggs declined ($F = 39.3$; d.f. $= 4,214$; $P < 0.0001$), but the number of eggs being resorbed increased with time after emergence ($F = 44.4$; d.f. $= 4,75$; $P < 0.0001$). The decline in mature eggs was 0.5 egg per day, and at 20 days post-emergence, females had almost no mature eggs. At one day after emergence, female *A. rhamni* had a median remaining lifetime of 23 ± 2 days and a mature egg load of 9.7 ± 0.9, whereas at 10 days, they had a median remaining lifetime of 15 ± 1 days and a mature egg load of 5.2 ± 0.4. To avoid parasitoids with egg loads that were too low to measure oviposition, we used females that were 1 vs. 10 days old to test the effect of age on host acceptance.

EFFECT OF NUTRITION ON OVIPosition BY APHELINUS RHAMNI IN LOW- VS. HIGH-SUITABILITY HOST SPECIES

Starved *A. rhamni* females persisted in ovipositing more in the high-suitability aphid species than in the low-suitability aphid species (Fig. 5a): *A. rhamni* females oviposited twice as often in *Aphis glycines* as in *R. padi* \((F = 7.2;\text{ d.f.} = 1.71;\ P < 0.009)\), and the difference in oviposition between aphid species did not vary with nutrition \((F = 0.4;\text{ d.f.} = 1.71;\ P = 0.51)\). Also, 65\% of *A. rhamni* females oviposited in one or more *Aphis glycines*, but only 45\% oviposited in one or more *R. padi*. An additional 16\% of *A. rhamni* females host-fed on one or more aphids, but did not oviposit in any aphids. Starved and fed females laid the same numbers of eggs \((0.9 \pm 0.2;\ F = 0.0;\text{ d.f.} = 1.71;\ P = 0.99)\).

Oviposition increased somewhat with egg load, but the main effect was not significant \((F = 3.0;\text{ d.f.} = 1.71;\ P = 0.13)\). Egg load interacted with aphid species \((F = 5.9;\text{ d.f.} = 2.89;\ P = 0.004)\) such that the increase in oviposition was restricted to parasitoids exposed to *Aphis glycines* \((t = 3.0;\text{ d.f.} = 89;\ P = 0.004)\), and oviposition in *R. padi* did not vary with egg load \((t = 0.7;\text{ d.f.} = 89;\ P = 0.51)\) (Fig. 6a). The outcome was that the difference in acceptance of low- vs. high-suitability host species decreased as eggs became more limiting, which is the opposite of the pattern expected when parasitoids become choosier as eggs become more limiting.

At the start of observations, 2-day-old fed females carried about two more eggs than 2-day-old starved females \((10.1 \pm 0.7\text{ vs. } 8.2 \pm 0.6\text{ eggs, respectively};\ F = 4.8;\text{ d.f.} = 1.74;\ P = 0.03)\). By the end of observations, starved and fed females had ample unlaid eggs, although fed females still carried about two more eggs than starved females \((8.8 \pm 0.6\text{ vs. } 7.1 \pm 0.5\text{, respectively};\ F = 4.9;\text{ d.f.} = 1.74;\ P = 0.03)\).

Higher oviposition in *Aphis glycines* did not result from stinging this species more often: female parasitoids stung *R. padi* at the same rate as *Aphis glycines* \((3.4 \pm 0.4\text{ vs. } 3.9 \pm 0.3\text{ aphids, respectively};\ F = 1.1;\text{ d.f.} = 1.78;\ P = 0.29)\), although they oviposited in *Aphis glycines* twice as often. This result shows that *A. rhamni* females reject...
R. padi twice as often as Aphis glycines after ovipositor insertion, which is consistent with previous evidence that Aphelinus females assess host quality with their ovipositor (Bai & Mackauer 1990a). Stinging did not vary with nutrition ($F = 0.91$; d.f. = 1.78; $P = 0.91$) or egg load ($F = 1.0$; d.f. = 1.78; $P = 0.32$). However, nutrition and aphid species interacted in their effect on numbers of aphids stung ($F = 4.3$; d.f. = 1.78; $P = 0.04$). Honey-fed parasitoids stung Aphis glycines more often than water-fed parasitoids, but stinging of R. padi did not vary with parasitoid nutrition, which matches the pattern seen in oviposition.

Although oviposition rates differed twofold, the amount of defence behaviour did not differ between aphid species ($F = 0.91$; d.f. = 1.78; $P = 0.34$). Honey-fed parasitoids elicited less defence behaviour than water-fed parasitoids ($F = 4.5$; d.f. = 4.78; $P = 0.04$), although these parasitoids oviposited at the same rate. Together these results show that the amount of defence behaviour cannot explain patterns in oviposition.

**EFFECT OF AGE ON OVIPOSITION BY APHELINUS RHAMNI IN THE LOW- VS. HIGH-SUITABILITY HOST SPECIES**

Ten-day-old honey-fed A. rhamni females persisted in ovipositing more in the high-suitability aphid species than in the low-suitability aphid species (Fig. 5b): A. rhamni females oviposited twice as often in Aphis glycines as in R. padi ($F = 12.3$; d.f. = 1.55; $P = 0.0009$), and this difference did not vary with age ($F = 0.1$; d.f. = 1.55; $P = 0.72$). Also $78\%$ of A. rhamni oviposited in one or more Aphis glycines, but only $34\%$ oviposited in one or more R. padi. An additional $5\%$ of A. rhamni females host-fed on one or more aphids, but did not oviposit in any aphids. Young and old females laid about the same number of eggs ($0.9 \pm 0.2$ vs. $0.7 \pm 0.1$ eggs, respectively; $F = 0.7$; d.f. = 1.55; $P = 0.39$).

Oviposition increased with egg load, although as in the nutrition experiment, the main effect was not quite significant ($F = 3.8$; d.f. = 1.55; $P = 0.06$). Egg load interacted with aphid species ($F = 7.8$; d.f. = 2.76; $P = 0.0008$), such that the increase in oviposition was restricted to parasitoids exposed to Aphis glycines ($t = 3.8$; d.f. = 76; $P = 0.0003$); oviposition in R. padi did not vary with egg load ($t = 1.3$; d.f. = 76; $P = 0.19$) (Fig. 6b). As in the nutrition experiment, these results give a pattern that is the opposite of what one would expect if female parasitoids became choosier as eggs became more limiting.

At the start of observations, young females carried more eggs than older females ($9.5 \pm 0.9$ vs. $6.2 \pm 0.6$, respectively; $F = 10.0$; d.f. = 1.77; $P = 0.002$), and by the end of observations, both young and old females still had eggs they had not oviposited ($8.0 \pm 0.8$ and $5.3 \pm 0.6$, respectively; $F = 8.6$; d.f. = 1.77; $P < 0.005$).

The higher oviposition in Aphis glycines was partly a result of stinging this aphid species more often. Unlike the nutrition experiment, where Aphis glycines and R. padi were stung equally often, female parasitoids in this experiment stung Aphis glycines more often than they did R. padi ($5.6 \pm 0.5$ vs. $4.4 \pm 0.4$ aphids stung, respectively; $F = 4.8$; d.f. = 1.55; $P = 0.03$). However, this difference gives only 1.3-fold more stings in Aphis glycines, whereas oviposition was 2.5-fold greater in Aphis glycines than in R. padi. Thus, as in the nutrition experiment, A. rhamni females reject R. padi twice as often as A. glycines after ovipositor insertion. Stinging did not vary with age ($F = 0.42$; d.f. = 1.55; $P = 0.52$) or egg load ($F = 0.8$; d.f. = 1.55; $P = 0.39$), nor did age interact with aphid species in its effect on numbers of aphids stung ($F = 1.2$; d.f. = 1.55; $P = 0.29$).

As in the nutrition experiment, differences in aphid defence cannot explain differences in oviposition between species: although Aphis glycines defended itself as much as R. padi ($2.3 \pm 0.3$ vs. $2.0 \pm 0.2$ defensive behaviours per approach, respectively; $F = 0.6$; d.f. = 1.55; $P = 0.44$), it received over twice as many eggs.

**EFFECT OF AGE ON OVIPOSITION BY APHELINUS KURDJUMOVI IN LOW- VS. HIGH-SUITABILITY HOST SPECIES**

For A. kurdjumovi, the acceptance of aphid species was reversed from A. rhamni: A. kurdjumovi oviposited over twice as often in R. padi than in Aphis glycines ($F = 8.0$; d.f. = 1.47; $P = 0.007$; Fig. 5c). Oviposition did not vary with age ($F = 0.1$; d.f. = 1.47; $P = 0.75$), nor did aphid species interact with age in its effect on oviposition ($F = 0.9$; d.f. = 1.47; $P = 0.35$). Less than $22\%$ of A. kurdjumovi oviposited in one or more Aphis glycines, but $44\%$ oviposited in one or more R. padi. An additional $6\%$ of A. kurdjumovi females host-fed on one or more aphids, but did not oviposit in any aphids. The effect of egg load on oviposition was marginal for A. kurdjumovi ($F = 2.9$; d.f. = 1.47; $P = 0.10$), and unlike for A. rhamni, egg load did not interact with aphid species in its effect on oviposition ($F = 1.2$; d.f. = 2.68; $P = 0.32$). Also unlike A. rhamni, young females of A. kurdjumovi had lower egg loads than older females at the start of observations ($11.2 \pm 0.8$ vs. $14.4 \pm 1.0$, respectively; $F = 6.7$; d.f. = 1.69; $P = 0.01$), but by the end of observations, both young and old females had eggs they had not oviposited ($10.6 \pm 0.8$ and $13.8 \pm 1.0$, respectively; $F = 6.4$; d.f. = 1.70; $P = 0.01$).

Higher oviposition in R. padi was correlated with stinging this aphid species more often. Female A. kurdjumovi stung R. padi twice as often as Aphis glycines ($5.1 \pm 0.5$ vs. $2.4 \pm 0.3$ stung, respectively; $F = 20.8$; d.f. = 1.48; $P < 0.0001$) and oviposited in R. padi over twice as often; thus, increased oviposition could be explained mostly by increased stinging. Stinging did not vary with parasitoid age ($F = 0.03$; d.f. = 1.48; $P = 0.87$) or egg load ($F = 0.1$; d.f. = 1.48; $P = 0.74$), nor did age interact with aphid species in its effect on stinging ($F = 0.5$; d.f. = 1.48; $P = 0.48$).
Differences in aphid defence cannot explain differences in oviposition between species because *R. padi* defended itself as much *Aphis glycines* \((2.5 \pm 0.3 \text{ vs. } 2.6 \pm 0.3)\) defensive behaviours per approach, respectively; \(F = 0.2; \text{d.f.} = 1.54; \text{P} = 0.64)\), yet received over twice as many eggs.

**Longer Experiment on Effects of Age on Parasitism of High- vs. Low-Suitability Host Species by *Aphelinus rhamni***

When *A. rhamni* females were exposed to the aphids for 24 h, more adult parasitoid progeny were produced on the high-suitability aphid species than on the low-suitability aphid species, regardless of female parasitoid age (Fig. 7). There were 16-fold more progeny from *Aphis glycines* than from *R. padi* \((F = 36.7; \text{d.f.} = 1.32; \text{P} < 0.0001)\). Number of progeny did not vary with parasitoid age \((F = 0.6 \text{ d.f.} = 1.32; \text{P} = 0.45)\), nor did aphid species and age interact in their effect on number of progeny \((F = 0.1; \text{d.f.} = 1.32; \text{P} = 0.74)\). Almost 90% of *A. rhamni* produced one or more mummified *Aphis glycines*, but only 35% produced one or more mummified *R. padi*. Some of the difference in numbers of progeny do not arose from differences in progeny survival, but the difference in progeny survival is twofold (Fig. 3) so the remaining eightfold difference in number of progeny must have arisen from the difference in host acceptance, rather than difference in host suitability.

**Discussion**

Stressors that decreased life expectancy did not increase oviposition by *A. rhamni* in a low-suitability host species. Egg load was lower in stressed females, because they resorbed eggs in response to starvation and ageing, and changes in life expectancy and egg load may have counter-balanced one another. However, the decrease in egg load was small relative to the decrease in life expectancy for starved females, which carried about three-quarters the egg load of honey-fed females, but would die the day following our assays. Furthermore, although oviposition increased with egg load for *A. rhamni* females exposed to the high-suitability aphid species, oviposition did not vary with egg load for females exposed to the low-suitability aphid species. The outcome of this pattern was that females became effectively less choosy as egg load decreased, rather than becoming more choosy, as theory predicts. Besides having lower life expectancy, older females were kept without aphids as they aged so they experienced a more aphid-poor environment than did young parasitoids. We expected that this experience would also tend to make oviposition in low-suitability aphid species more frequent, yet it did not. *Aphelinus kurdjumovii* showed the same pattern: acceptance of a low-suitability host species did not increase with age. Here, egg load increased with age, but still choosiness did not decrease. There are studies where the effects of egg load and experience on oviposition have been decoupled (Rosenheim & Rosen 1991) and where the effects of age and experience of oviposition have been separately tested (Jenner et al. 2012). However, independent tests of age, experience and egg load are quite difficult, especially in synovigenic parasitoid species that host-feed and resorb eggs.

In any case, our results suggest that host specificity in these *Aphelinus* species is robust and unlikely to expand under conditions of time limitation. This is in contrast to some results with herbivorous insects (Wiklund 1981; Singer 1983; Courtney, Chen & Gardner 1989), although other studies of herbivores showed similar stability in oviposition behaviour (Ives 1978; Tabashnik 1983).

To avoid the complexity of progeny sex, we used only unmated females in the stressor experiments; thus, all eggs were unfertilized and therefore male. Mated females would have the additional choice of whether to lay eggs that were fertilized (female) or unfertilized (male). Theory suggest that when the effects of host quality on fitness differ between the sexes, female parasitoids should manipulate fertilization of their eggs so that the sex most affected by host quality is laid in high-quality hosts (Charnov et al. 1981; Boivin 2012). Although experimental studies have given mixed results concerning this prediction (Hopper 1986; King 1993; Wyckhuys et al. 2008), one might expect that unmated *Aphelinus* females would be more willing to lay male eggs in low-suitability hosts if males are less affected by host quality. Unmated females persisted in accepting high-quality hosts over low-quality hosts, even when stressed. Progeny sex ratio did not differ between *Aphis glycines* and *R. padi* when we measured parasitism of these species by mated *A. rhamni*. This suggests that this parasitoid species does not manipulate sex ratio in response to differences in the suitability of these aphids, perhaps because the effects of the suitability differences are the same for males and females.

Regardless of stress, some *A. rhamni* females laid more eggs on the low-suitability host than most *A. rhamni* females: 9% of females laid at least as many eggs in *R. padi*...
as the mean number of eggs laid in *Aphis glycines*. This suggests that there may be genetic variation in *A. rhamni* for acceptance of *R. padi*. However, no *A. rhamni* females produced as many mummies on *R. padi* as the mean number produced on *Aphis glycines*, indicating that the willingness to accept *R. padi* for oviposition is not correlated with increased survival in *R. padi*. Nonetheless, selection on *A. rhamni* for parasitism of *R. padi* might produce a shift in host specificity that stress did not, and we are currently testing whether there is such a response to selection.

At least three hypotheses may explain the stability in behavioural host specificity of these parasitoids: (i) a history of frequent transient egg limitation, (ii) greater benefits of resorbing eggs than ovipositing them in low-suitability host species and (iii) neural constraints on host recognition. Frequent transient egg limitation would make eggs more valuable than if parasitoids were never egg-limited and thus make females less likely to lay them in low-suitability host species – it would effectively put them in a constant state of near-egg-limitation. In support of frequent transient egg limitation, *Aphelinus* species have maximum egg loads of 10–25 (Hartley 1922; Bai & Mackauer 1990b; Wu & Heimpel 2007), but colony sizes of the aphids which they parasitize often exceed these values (Mueller, Blommers & Mols 1992). Although *Aphelinus* females can produce more eggs, this takes time (about 1 day to reproduce the maximum egg load), so these parasitoids are likely to suffer transient egg limitation in the field, like other aphelids.

Resorbing eggs to provide nutrients for survival may confer greater fitness than ovipositing in low-suitability host species. *Aphelinus* females have three options when they encounter an aphid: oviposit, host-feed or reject. If females reject hosts for oviposition but accept them for host-feeding, this can increase their egg load and also their life expectancy. However, we found no effect of egg load or life expectancy on acceptance of low-quality hosts. If females reject all aphids encountered, they will eventually start resorbing their eggs. Whether resorbing eggs confers greater fitness than ovipositing or host-feeding depends on the relative value of low- vs. high-suitability host species, on the effect of each resorbed egg on longevity, and on the likelihood that this increase in longevity results in finding and ovipositing in high-suitability host species. The latter depends on the long-term distribution and abundance of host species of various qualities.

Infrequent oviposition in low-suitability host species may result from neural constraints on recognition, rather than decisions involving egg vs. time limitation. Neural constraints are limitations of nervous systems that restrict the rate of information processing (Dusenbury 1992), and such constraints may provide the proximate mechanism that limits host range (Bernays & Wcislo 1994). Insects with broad host ranges appear to be less efficient at correctly accepting or rejecting hosts or may simply take longer to decide whether to accept a host. The ability of adult females to select the most suitable hosts depends on accurate host assessment, and generalist herbivores in several insect orders seem to perform poorly at this task compared with specialists (Janz & Nylin 1997; Bernays 1998; Bernays & Funk 1999; Egan & Funk 2006). Specialists appear to have sacrificed broad host ranges to concentrate on efficient processing of cues from a limited set of hosts (Bernays 2001). Although there is no published research on neural constraints on host range in parasitoids, the lack of increased oviposition in low-suitability host species in the face of strong time limitation suggests that neural constraints may affect the behaviour of these specialist species of *Aphelinus*. There is ample evidence for learning in parasitoids (Dukas 2008), but our evidence suggests that the oviposition behaviour of these *Aphelinus* species is innate and fixed. If neural constraints do restrict the ability of these specialist species to change their behaviour under stress, we predict that generalist species of *Aphelinus* should show greater dynamism in acceptance of low-suitability hosts. We are testing this prediction using *Aphelinus* species with very broad host ranges.

Whatever the mechanism, our finding of inflexible host specificity in these parasitoids species has implications for their use in biological control of aphids. *Aphelinus rhamni* is a candidate for introduction against the Asian soybean aphid, *Aphis glycines*, in North America (Ragsdale et al. 2011). Our results bode well for the reliability of host-specificity testing of *Aphelinus* species that are candidates for biological control introductions because they suggest that the host range will not broaden in response to time limitation (Roitberg 2000; Withers & Barton Browne 2004).

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Parasitoid acceptance of host species


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