


# Why resource history matters: age and oviposition history affect oviposition behaviour in exploiters of a mutualism

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**Abstract.** 1. Acceptance of hosts for oviposition is often hardwired in short-lived insects, but can be dynamic at the individual level due to variation in physiological state determinants such as ageing and prior oviposition. However, the effect of the oviposition history of resources together with time taken to accept less preferred hosts in ageing insects has rarely been investigated.

2. The time taken by parasitic fig wasps to accept resources with different oviposition histories was recorded in order to investigate the effect of wasp physiological state and resource oviposition history on oviposition behaviour. These wasps, which differ in life-history traits, oviposit at specific developmental stages of enclosed fig inflorescences called syconia.

3. Behavioural assays were performed with naive wasps and wasps aged with and without prior oviposition experience. Syconia at the same developmental stage but differing in oviposition history were offered in no-choice assays and the time taken to first oviposition attempt was recorded.

4. One short-lived pro-ovigenic galler species exhibited a decline with age in time taken to accept a syconium for oviposition. The exact timing of the transition from non-acceptance to acceptance of less preferred syconia was determined in terms of the proportion of elapsed life span at the transition; this occurred at 25% of elapsed life span.

5. Longer-lived parasitoids did not show any decline in specificity despite being aged for 50% of their life span. Therefore, host quality, trophic position, egg load and age may individually affect oviposition decisions or have interaction effects.

**Key words.** Ageing, egg load, fig wasps, host acceptance, host deprivation, host specificity.

## Introduction

In short-lived insects whose choices of oviposition sites affect offspring survival, the behaviours involved in host acceptance for oviposition may often be hardwired (Tierney, 1986; del Campo *et al.*, 2001; Damodaram *et al.*, 2014). However, response to host cues may be dynamic and influenced by extrinsic factors such as host availability, presence of conspecifics, predation risks (Bernays, 2001; Prokopy & Roitberg, 2001; Nomikou *et al.*, 2003; Schmitz, 2008) and/or intrinsic factors such as physiological state (Bell, 1990; Hopper *et al.*, 2013).

The presence of conspecifics at oviposition sites could indicate the suitability of hosts for oviposition, dilute predation risks, or indicate opportunities for mating (Prokopy & Duan, 1998;

Prokopy & Roitberg, 2001; Wertheim *et al.*, 2005), but they could also make the host less suitable due to possible resource limitation resulting in larval competition (Nufio & Papaj, 2001; Prokopy & Roitberg, 2001). The presence of other individuals at an oviposition site can thus affect the preference ranking of hosts, making them either more or less preferred. Oviposition decisions determined by conspecific presence are typically mediated by cues/signals associated with eggs, chemical footprints left by other individuals, or the act of oviposition itself, including probing and puncturing by an ovipositor (Hemphill *et al.*, 2001; Nufio & Papaj, 2001, 2004; Rostás & Wölfling, 2009; Yadav *et al.*, 2018). However, weak oviposition preference may also be favoured when certain factors such as rarity of preferred hosts, generalisation of acquired information and/or physiological constraints are at work (Mayhew, 1997; Vet *et al.*, 1998).

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The physiological state of an insect is the major intrinsic factor that contributes to variation in acceptance of a resource for oviposition. Such plasticity in oviposition could arise from factors such as age, feeding state, circadian rhythm, mating history and prior oviposition events (Gadenne *et al.*, 2001, 2016; Barrozo *et al.*, 2004; Saveer *et al.*, 2012). For an ovipositing female, plasticity in behavioural response to host cues could be adaptive in situations where the costs associated with being choosy are high (Jaumann & Snell-Rood, 2017). However, these responses are constrained by resource allocation among competing functions, and optimisation of this allocation forms the basis of life-history strategies (Ricklefs & Wikelski, 2002). Life-history traits such as life span and egg load can, therefore, influence physiological plasticity in an ovipositing female and affect oviposition behaviour as a consequence of egg limitation or time limitation at any given time point (Rosenheim *et al.*, 2008; Deas & Hunter, 2014). Within a species, an individual can be egg-limited (e.g. when host abundance is high) or time-limited (e.g. when it nears the end of its life span) and it may maximise reproductive success by balancing these two constraints, i.e. by accepting lower ranked hosts when time-limited and rejecting them when egg-limited (Heimpel *et al.*, 1998; Mangel & Heimpel, 1998).

Interestingly, the relative ranking of preferences for different hosts may remain fixed, i.e. high-ranked hosts are always preferred, and less preferred hosts only become acceptable under stressful conditions such as host deprivation, as predicted by the hierarchy-threshold model (Courtney *et al.*, 1989). These changes in host acceptance threshold may vary between species and are time- and age-dependent phenomena (Hard, 1990; Browne & Withers, 2002; Defagó *et al.*, 2016) that have an important impact on the temporal profiles of host acceptance. Investigating the response of different species in stressful conditions such as deprivation of oviposition substrates or ageing can help to address several ecological and evolutionary questions such as differences in behavioural plasticity across species, potential trade-offs and limits on plasticity (Relyea, 2001). To our knowledge, there have been very few studies that have compared the effect of such stressful conditions across different species of insects (Sadeghi & Gilbert, 2000; Díaz-Fleischer & Aluja, 2003).

Figs (*Ficus*: Moraceae) host a community of specialist wasps that oviposit and breed within syconia (globular closed inflorescences; singular = syconium) and that also differ in life-history traits such as longevity, feeding status and initial egg load (Ghara & Borges, 2010). While these wasps are specialists on individual *Ficus* species, specificity for attraction to individual syconia for oviposition has been addressed only up to the level of the developmental stage of the syconium, e.g. pollen-receptive or interfloral stage (Proffitt *et al.*, 2007; Ranganathan *et al.*, 2010). The non-pollinating fig wasps (NPFWs) that arrive at different times during syconial development are either shorter-lived gallers that do not feed as adults in most fig–fig wasp systems (capital breeders) or longer-lived parasitoids that feed in the adult stage (income breeders) (Jousselin *et al.*, 2008; Ghara & Borges, 2010; Borges, 2015). With variation in life-history traits and high host specificity, fig wasps therefore provide an

excellent opportunity to understand the effect of physiology on oviposition host acceptance.

Non-pollinating fig wasps, unlike pollinating wasps, oviposit from the syconium exterior and distribute their eggs over a number of syconia (Weiblen, 2002). An NPFW female may, therefore, strategise the distribution of her eggs among a number of syconia that might differ in quality and oviposition history (prior oviposition in syconium), in order to optimise her lifetime fitness. Furthermore, NPFWs in the wild often form aggregations of conspecifics on a syconium when ovipositing (P. Yadav & R.M. Borges, pers. obs.). Whether this aggregation is a result of preference for host syconia exposed to oviposition by conspecifics and is mediated by cues left by ovipositing wasps, and whether this preference is affected by the physiological state of the wasp are questions that remain to be investigated. We investigate the effect of oviposition history, of ageing, and of host deprivation on syconium acceptance for oviposition in the highly species-specific community of NPFWs of the widely distributed tropical cluster fig *Ficus racemosa* Linn (Section: Sycomorus). This fig species has served as an excellent model system for questions in evolutionary and functional ecology (e.g. Ghara *et al.*, 2011; Krishnan & Borges, 2014; Yadav & Borges, 2017a, 2017b; Yadav *et al.*, 2018).

We asked the following questions:

- 1 Does the oviposition history of a syconium affect the time to oviposition by specialist NPFWs? Is the aggregation of NPFWs observed on individual syconia in the wild a result of preference for syconia that have been exposed to oviposition by conspecifics?
- 2 Does ageing differentially affect the time taken to accept a host syconium for oviposition in various NPFW species?
- 3 When does the behavioural transition from non-acceptance to acceptance of a less preferred oviposition host occur during the life span of an NPFW species?

## Materials and methods

### Study system

Experiments were conducted on *F. racemosa* trees within the campus of the authors' institute. The phenology of *F. racemosa* can be divided into five stages (Galil & Eisikowitch, 1968; Ranganathan *et al.*, 2010): A, pre-pollination phase; B, pollen receptive phase; C, interfloral or seed and wasp development phase; D, wasp dispersal phase; and E, seed dispersal phase (Fig. 1). This fig has one specific pollinator species, *Ceratosolen fusciceps* Mayr (Agaonidae), whose progeny develop in some of the flowers at the expense of seeds. *Ficus racemosa* also hosts six specific NPFWs (*Sycophaga stratheni* Joseph, *Sycophaga testacea* Mayr, *Sycophaga fusca* Girault, *Sycophaga agraeensis* Joseph, *Apocrypta* sp. 2, *Apocrypta westwoodi* Grandi) that oviposit into syconia at different stages of syconial development (Ranganathan *et al.*, 2010) and differ in their oviposition windows, i.e. periods when the syconium is suitable for their oviposition (Fig. 1). *Sycophaga stratheni*, *S. testacea*, and *S. fusca* are pro-ovigenic (eclosing with all their eggs matured), short-lived and non-feeding, non-pollinating gallers that lay

their eggs inside empty (unoccupied) flowers, in floral primordia or in the syconium lumen (Ghara & Borges, 2010). *Apocrypta* sp. 2 and *A. westwoodi* are synovigenic (eclosing with only a fraction of their eggs matured), long-lived and feeding parasitoids that parasitise galls with different species specificity (Ghara & Borges, 2010; Yadav & Borges, 2017a). *Apocrypta* sp. 2 parasitises *S. fusca* and *S. testacea*, whereas *A. westwoodi* parasitises only *S. testacea* (Yadav & Borges, 2017a). Syconia and freshly emerging wasps for all experiments were collected between 10:00 and 12:00 hours. Most experiments were carried out with two species of NPFWs, the galler *S. fusca* and the parasitoid *Apocrypta* sp. 2. However, we also conducted some experiments with the relatively rarer galler *S. testacea* and parasitoid *A. westwoodi* whenever wasps were available.

As it is extremely difficult to determine the clutch size of females in this system, owing to the size and appearance of eggs (mildly opaque) against the background of plant tissue of similar transparency, we recorded the time taken to attempt oviposition in the offered host syconium as the behavioural assay parameter. Standard parameters such as patch residence time and giving up time during oviposition (Papaj *et al.*, 1989; Wang & Messing, 2003) that depend on the quantity of hosts available in a resource patch may not capture the actual behaviour of NPFWs. This is because the resource patch of the fig syconium, with ~2500 flowers inside, offers sufficient resources for many individuals to lay their full egg complement. The time taken to attempt the first oviposition is, therefore, more insightful for our experiments.

#### Acceptability of hosts with different oviposition histories by naive NPFWs

Acceptability of syconia with different oviposition histories was investigated for freshly eclosed naive (with no previous oviposition experience) galler and parasitoid wasps. Among gallers, experiments were performed only with the most abundant galler, *S. fusca*, whose arrival window for oviposition overlaps with that of the pollinator in pollen-receptive B-phase (Ghara & Borges, 2010; Ranganathan *et al.*, 2010). Two experimental conditions were provided for *S. fusca*: (a) pollen receptive-phase (B-phase) syconia unexposed to any wasps (control); and (b) B-phase syconia exposed to oviposition by conspecifics. Control syconia were generated by enclosing syconial bunches in wire-framed mesh bags on the tree at the primordia stage itself to prevent wasp oviposition, while syconia exposed to oviposition were generated by introducing wasps for oviposition into such enclosed syconia at the receptive B-stage. Syconia were collected from the experimental bunches on the fig tree not more than 30 min before the experiment. The latex exuding from the cut pedicel of the syconium was wiped off with tissue and the cut end was sealed with parafilm. The no-choice experiments (wasps were exposed to only one experimental condition at a time) were carried out under red light as insects are usually insensitive to red light, allowing observations without disturbance. The experimental setup had four syconia (collected from the same bunch) placed in a glass chamber. Four naive freshly eclosed *S. fusca* wasps were

released in the chamber. Wasps were observed for 30 min and the time spent on inspection before the first oviposition attempt by each wasp was recorded. The experiment was repeated six times.

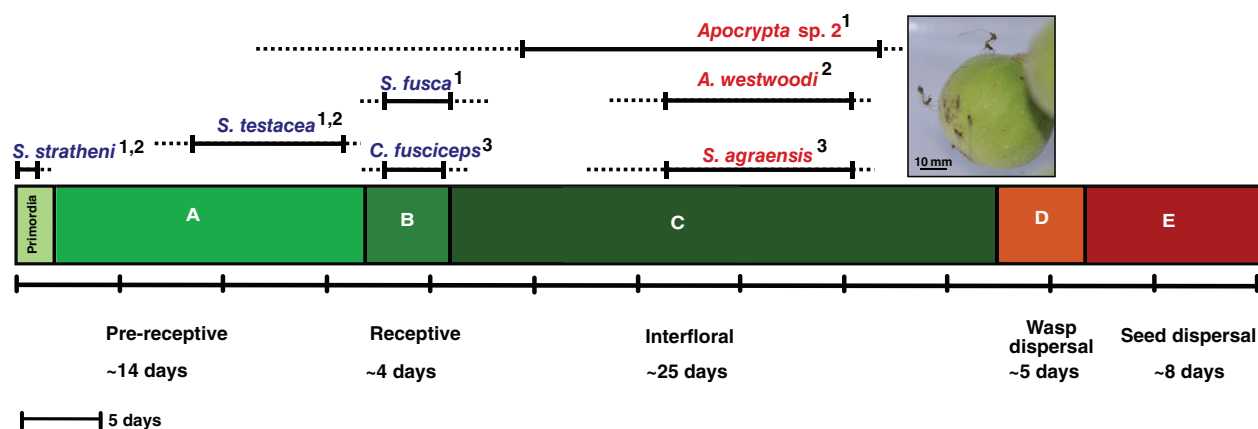
We then investigated the role of walking by conspecifics on the syconium surface (putative cues left by chemical footprints of possibly ovipositing wasps) in determining the oviposition choice of a naive *S. fusca* wasp. Three experimental conditions were set up with receptive-phase syconia: (a) syconia unexposed to any wasps (control – generated as described earlier); (b) syconia exposed to walking by *S. fusca* wasps with their ovipositor excised (only footprints); and (c) syconia exposed to *S. fusca* wasps with their ovipositor intact (oviposition-exposed). The exposure of syconia to wasps with or without ovipositors was allowed for 20 min on control syconia that had received no prior oviposition.

For the parasitoids, syconia containing different host galler larvae as oviposition substrates served as syconia with varying oviposition history. These syconia were generated by allowing only host gallers to oviposit into syconial bunches that were enclosed in mesh bags on fig trees, as described earlier. Specificity in parasitoid wasps for galler hosts is known for the *F. racemosa* system (Yadav & Borges, 2017a; Fig. 1), and this information guided the experiments. We accordingly tested freshly eclosed naive wasps of the parasitoid *Apocrypta* sp. 2 for differences in readiness to oviposit into different hosts by recording the time taken to attempt first oviposition.

#### Effect of ageing and host deprivation on host acceptance for oviposition

To investigate the effect of ageing and host deprivation on host acceptance, experiments were performed with aged individuals of different species (gallers *S. fusca* and *S. testacea*; parasitoids *A. westwoodi* and *Apocrypta* sp. 2). To age the wasps for varying periods of time, mature syconia were collected in wasp dispersal or D-phase and kept in vials covered with tissue to allow wasps to exit naturally. Female wasps were collected and transferred to separate vials (15 wasps per vial) where they were allowed to age for 3–5 days (gallers) and up to 10 days (parasitoids) as gallers and parasitoids differ in their longevities (life spans of gallers and parasitoids are ~6–8 days and ~15–25 days, respectively; Ghara & Borges, 2010). A cotton ball soaked in sucrose solution (10% w/v) was provided every day and the wasps were maintained under LD 12:12 h conditions at room temperature.

Galler *S. fusca* wasps were additionally subjected to two different treatments: (a) with oviposition opportunity on day 1; and (b) without any oviposition opportunity during ageing (thus preventing any decrease in egg load). For the galler species *S. testacea* (which specifically oviposits in pre-receptive syconia), the effect of ageing was tested by offering it non-target receptive phase syconia after ageing for 4 days without any oviposition opportunity. Aged wasps of both *Apocrypta* parasitoid species were offered syconia containing either non-host galler larvae or no galls to investigate the effect of ageing on readiness to oviposit.



**Fig. 1.** Wasp arrival sequence for oviposition across syconium development phases (A–E) in *Ficus racemosa* (adapted from Ranganathan *et al.*, 2010). Solid lines represent the top 50% of wasp arrival and dotted lines represent the rest. Gallers are marked in blue and parasitoids are in red. Numeric superscripts denote galler–parasitoid (host–parasite) pairs. Image in inset shows parasitoid *Apocryta* sp. 2 ovipositing into a syconium. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

#### Transition from non-acceptance to acceptance of unsuitable hosts for oviposition

The age at which the transition from high to low specificity of oviposition host acceptance occurred was examined in a no-choice experiment for the short-lived *S. fusca*. Unexposed receptive-phase syconia were collected from the experimental bunch on the fig tree not more than 30 min before the experiment, and were used as the unsuitable oviposition hosts (see results of acceptability experiments for justification). The procedure was as described in the first experiment. Four 2-day-old *S. fusca* wasps were released in the setup. Wasps were observed for 30 min and the time spent on inspection before the first oviposition attempt was recorded for each wasp. The experiment was repeated on subsequent days with different wasps, which were 3, 4 and 5 days old, respectively. The transition threshold experiment could not be performed with the parasitoid *Apocryta* sp. 2 (see ageing results for parasitoids for justification).

#### Statistical analysis

Response variables such as time taken to first oviposition attempt were examined for normality using the Shapiro–Wilk test. Student's *t*-tests were performed on normally distributed data and Kruskal–Wallis one-way ANOVAs were used to examine the difference between treatments in non-normally distributed data points. A Mann–Whitney *U*-test was used for pairwise comparisons between experimental conditions. All tests were carried out using RSTUDIO version 0.99.902 for Linux.

## Results

#### Acceptability of hosts with different oviposition histories by naïve NPFWs

The time taken to the first oviposition attempt by naïve *S. fusca* wasps on syconia exposed to conspecifics was

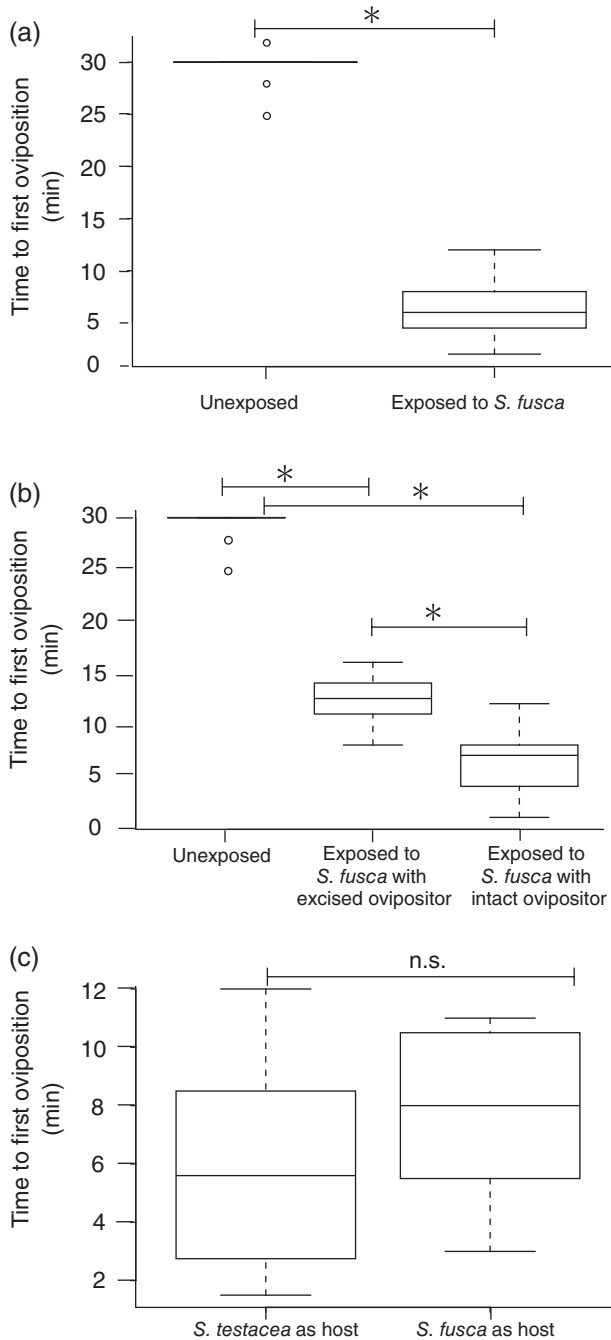
significantly lower than that on completely unexposed syconia (Kruskal–Wallis test,  $\chi_1^2 = 37.69$ ,  $n = 24$ ,  $P < 0.001$ , Fig. 2a). Wasps took longer than 30 min to attempt oviposition on the unexposed syconia; after this time period, experiments were aborted given that the volatile profile of syconia changes quickly after being excised from the tree and this change may additionally affect the results. We considered each wasp as an independent observation for statistical analysis and excluded the effect of conspecifics inside the arena for the following reasons. First, wasps that aggregate in the experiments in the laboratory do so on syconia previously exposed to conspecifics before syconia are placed in experimental setup. It is, therefore, more plausible that the phenomenon of aggregating on *S. fusca*-exposed syconia is the result of individual preference for syconia previously exposed to conspecifics rather than the result of being affected by the decisions of other wasps within the small experimental duration. Second, the experimental setup had just four wasps that made decisions to attempt oviposition within a couple of minutes of each other while, in the wild, often tens of wasps aggregate on single syconia. This suggests that four is a small enough number to ensure individual decision-making.

Naïve *S. fusca* wasps took a significantly shorter time to attempt oviposition into syconia exposed to conspecific wasps with excised ovipositors than into unexposed syconia (Kruskal–Wallis test,  $\chi_2^2 = 67.28$ ,  $n = 26$ ,  $P < 0.001$ , Fig. 2b), indicating the putative role of chemical footprints. The treatments were significantly different from each other.

Freshly eclosed naïve *Apocryta* sp. 2 parasitoids did not exhibit any preference between syconia containing different galler hosts (independent *t*-test,  $t_1 = 11.1$ ,  $n = 15$ ,  $P = 0.14$ , Fig. 2c).

#### Effect of ageing and host deprivation on host acceptance for oviposition

The relative ranking of acceptance time of aged wasps for differently treated syconia, i.e. unexposed syconia, those exposed



**Fig. 2.** Acceptance of different hosts by naive wasps. Time of acceptance of: (a) receptive-phase syconia with different oviposition histories offered to *Sycophaga fusca*; (b) syconia exposed to *S. fusca* with intact and excised ovipositors offered to *S. fusca* (unexposed versus excised ovipositors,  $W = 676$ ,  $n = 26$ ,  $P < 0.001$ ; excised versus intact ovipositors,  $W = 644$ ,  $n = 26$ ,  $P < 0.001$ ; unexposed versus intact ovipositors,  $W = 676$ ,  $n = 26$ ,  $P < 0.001$ ); (c) syconia carrying different galler larvae as hosts offered to freshly eclosed *Apocrypta* sp. 2 parasitoids. Box plots show median (central line), first and third quartiles, and minimum and maximum values; unfilled circles represent outliers. \*, significant difference at  $\alpha = 0.05$ ; n.s., non-significant.

to conspecific wasps with intact or excised ovipositors, was the same as for naive wasps. Wasps took the longest to oviposit into unexposed syconia, followed by exposed syconia (Kruskal–Wallis test,  $\chi^2_2 = 34.59$ ,  $n = 16$ ,  $P < 0.0001$ ); yet, the amount of time taken to attempt oviposition was significantly shorter in each treatment for 3-day-old wasps than for naive wasps (Fig. 3a).

*Sycophaga fusca*, when aged for 3–5 days, also accepted pre-receptive as well as post-receptive syconia (non-target phases) for oviposition within  $\sim 10$  min, similar to naive wasps on receptive-phase syconia (Fig. 3b,  $n = 20$  wasps for pre-receptive syconia,  $n = 24$  for receptive syconia,  $n = 38$  for post-receptive syconia). When aged *S. fusca* wasps were allowed to oviposit during the ageing period, the decrease in acceptance time of unexposed syconia and syconia of the inappropriate phase, which was seen earlier in aged wasps, was lost (Table 1).

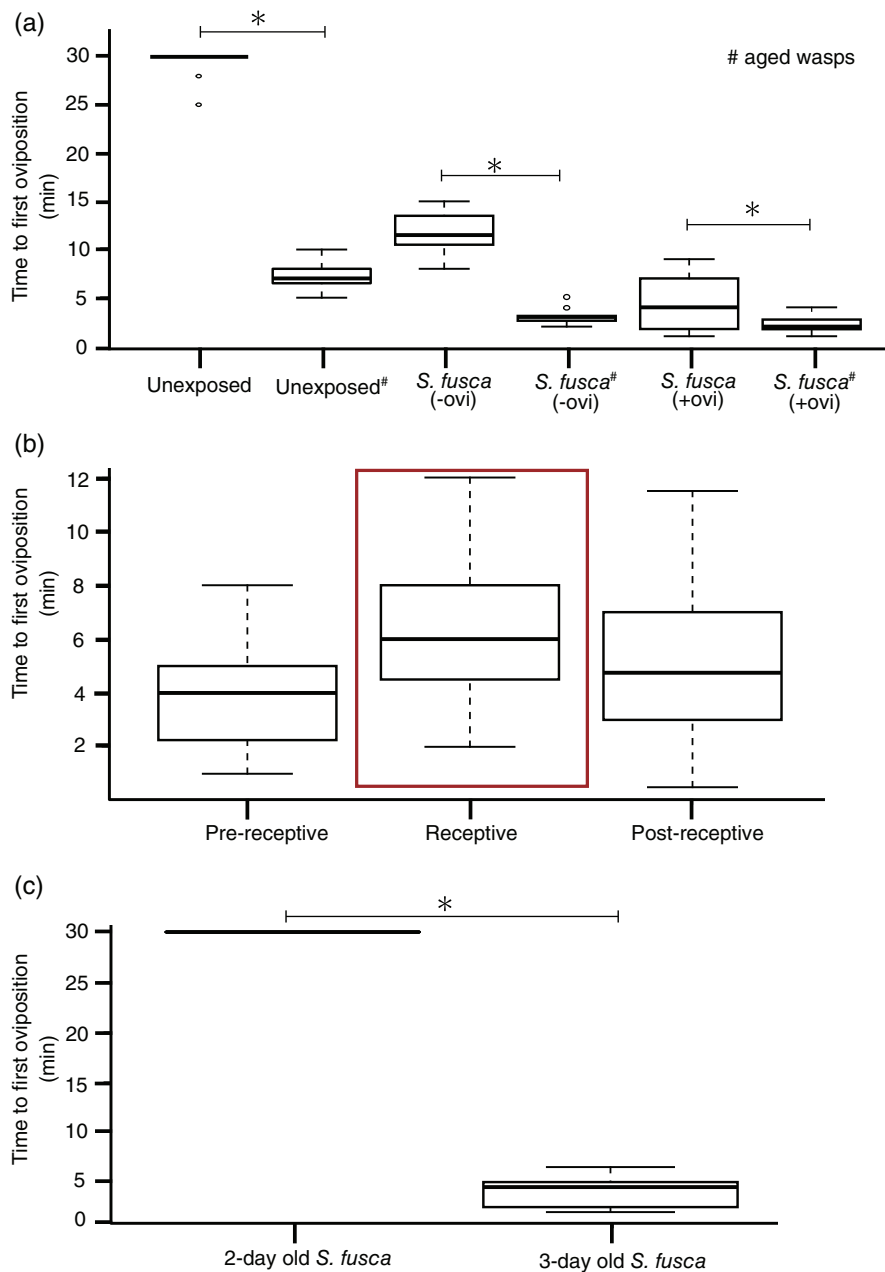
*Sycophaga testacea* (which oviposit specifically in the pre-receptive syconial phase) did not attempt oviposition on receptive or post-receptive phase syconia despite being aged for 4 days without any oviposition experience. None of the parasitoids showed any decrease in specificity due to ageing as they did not attempt oviposition in syconia with either inappropriate galler hosts or no galler hosts inside (Table 2). We could not carry out further experiments with parasitoids due to an extremely low survival rate of ageing parasitoids coupled with the infrequent simultaneous availability of aged parasitoids and syconia containing their galler hosts at the correct stage.

#### Transition from non-acceptance to acceptance of unsuitable hosts for oviposition

The 2-day-old *S. fusca* wasps were not different from naive wasps in the time taken to attempt first oviposition on unexposed syconia as they did not attempt oviposition within 30 min. However, 3-day-old wasps readily accepted these unexposed syconia for oviposition (Kruskal–Wallis test,  $\chi^2_1 = 16.39$ ,  $n = 10$ ,  $P < 0.001$ ; Fig. 3c), as did 4- and 5-day old wasps (results not presented), suggesting that the transition to lower choosiness of oviposition hosts occurs between the days 2 and 3 of their lives.

## Discussion

Naive individuals of different species differed in their acceptance of hosts with different oviposition histories. Naive *Sycophaga fusca* preferred conspecific-exposed syconia over unexposed syconia. Naive parasitoids did not exhibit a difference in acceptance time of syconia containing alternative host galler species. The short-lived galler *S. fusca*, when aged and experiencing no diminution of egg load by preventing wasps from ovipositing (and thereby probably time-limited), deposited eggs in any offered host syconium. However, when allowed to oviposit during ageing, and thus having reduced its egg load, *S. fusca* wasps maintained their oviposition specificity, similar to naive, freshly eclosed wasps. Interestingly,



**Fig. 3.** Effect of ageing on host acceptance represented as box plots. Time of acceptance of: (a) receptive-phase unexposed syconia and syconia exposed to *Sycophaga fusca* with intact and excised ovipositors offered to naive versus 3-day-old (marked by #) *S. fusca* wasps (naive versus aged wasps on unexposed syconia,  $W = 256$ ,  $n = 16$ ,  $P < 0.0001$ ; naive versus aged wasps on syconia exposed to conspecific wasps with excised ovipositors,  $W = 256$ ,  $n = 16$ ,  $P < 0.0001$ ; naive versus aged wasps on syconia exposed to conspecific wasps with intact ovipositors,  $W = 136$ ,  $n = 16$ ,  $P < 0.0001$ ) (\*, significant difference at  $\alpha = 0.05$ ); (b) pre-receptive and post-receptive phase syconia with different oviposition histories offered to 3–5-day-old *S. fusca* compared with time taken to first oviposition attempt on receptive syconia by naive *S. fusca* wasps; (c) unexposed receptive-phase syconia offered to 2- and 3-day-old *S. fusca* wasps to determine the time of transition. Box plots show median (central line), first and third quartiles, and minimum and maximum values. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

another galler, *S. testacea*, did not accept an unsuitable host with ageing; the same result was obtained with the parasitoids. The transition from non-acceptance to acceptance of previously unacceptable syconia that were unexposed to oviposition occurred on the third day after eclosion for *S. fusca*, upon

completion of 25% of its life span. We therefore suggest that life-history traits such as life span or trophic position in species that use different oviposition substrates within the same plant host might affect oviposition response to stressful conditions.

**Table 1.** Effect of ageing on host specificity of individuals of the galler *Sycophaga fusca* with oviposition experience.

| Age of wasp (days) | Phase of syconia offered as substrate | Oviposition experience | Oviposition attempt |
|--------------------|---------------------------------------|------------------------|---------------------|
| 4 ( <i>n</i> = 12) | Unexposed pollen receptive            | Yes                    | > 30 min            |
| 4 ( <i>n</i> = 10) | Pre-receptive                         | Yes                    | No                  |
| 4 ( <i>n</i> = 15) | Post-receptive                        | Yes                    | No                  |

**Table 2.** Effect of ageing on host specificity of parasitoids without oviposition experience.

| Parasitoid species         | Age of wasp (in days) | Galler hosts inside the syconium | Host for the parasitoid | Oviposition attempt |
|----------------------------|-----------------------|----------------------------------|-------------------------|---------------------|
| <i>Apocrypta</i> sp. 2     | 10 ( <i>n</i> = 20)   | Pollinators                      | No                      | No                  |
| <i>Apocrypta</i> sp. 2     | 10 ( <i>n</i> = 22)   | No galls                         | No                      | No                  |
| <i>Apocrypta westwoodi</i> | 10 ( <i>n</i> = 11)   | <i>Sycophaga fusca</i>           | No                      | No                  |

#### *Acceptability of hosts with different oviposition histories by naive NPFWs*

For a highly specific community of fig wasps, ours is the first study to show preference by wasps for syconia of the same developmental stage but varying in oviposition history. *Sycophaga fusca* preferred syconia exposed to oviposition by conspecifics over unexposed syconia. Preference for conspecific-exposed hosts for oviposition has been observed in many insects, as this increases mate availability, provides better resource utilisation, reduces risk of predator attack, and facilitates the overcoming of host plant defences (Denno & Benrey, 1997; Aukema & Raffa, 2004; Wertheim *et al.*, 2005; Desurmont & Weston, 2011). The preference of *S. fusca* for syconia previously exposed to oviposition by conspecifics can be attributed to the increased probability of finding mates for their offspring, as mating takes place only inside the syconium (Weiblen, 2002). Syconia that are completely unexposed to wasp visitation in the receptive phase (and therefore also likely to be pollinator-free) are usually aborted by the tree as they have not received pollen and hence will not produce seeds (Jandér *et al.*, 2012). This makes receptive-phase syconia unexposed to any wasps (control treatment) less suitable and therefore less preferred for oviposition.

In our study system, fig wasps have not only been observed to aggregate on syconia in the wild but also seemed to identify conspecific-exposed syconia in the experimental setup. Freshly eclosed naive *S. fusca* wasps preferred syconia bearing only footprints of the conspecifics over completely unexposed syconia. These results indicate that footprints by themselves can reveal information about syconium oviposition history as each wasp species has a unique chemical footprint signature (Yadav *et al.*, 2018). In addition to facilitating mate availability for offspring, joining conspecifics on the same host syconium for oviposition may also dilute the risk of predation by ants during oviposition (Wertheim *et al.*, 2005; Ranganathan & Borges, 2009; Bain *et al.*, 2014; Schatz *et al.*, 2014).

The parasitoid *Apocrypta* sp. 2 did not exhibit any preference between syconia containing either of its two galler hosts. While the two hosts may differ in their quality, with *S. testacea* probably being a higher-quality host than *S. fusca* owing to its larger size (Ghara & Borges, 2010; Yadav & Borges, 2017a),

they also differ in their abundance, with *S. fusca* being more abundant than *S. testacea* within host syconia (Ghara *et al.*, 2014). A combined effect of these two factors may result in the absence of preference for syconia containing either galler host.

#### *Effect of ageing and host deprivation on host acceptance for oviposition*

Although females exhibiting preference for oviposition sites may increase fitness through discrimination between suitable and unsuitable resources, being choosy may not be favourable when females are time-limited (Doak *et al.*, 2006). As mentioned earlier, egg limitation and time limitation can affect host acceptance differently. The short-lived wasp *S. fusca*, when aged without oviposition experience, showed a decline in time taken to accept a less preferred resource for oviposition; however, even under these conditions it maintained the relative ranking preference for resources of different quality/history supporting the hierarchy-threshold model.

When aged for the same duration with oviposition opportunity (which is expected to reduce egg load), the wasps did not hasten to accept an unsuitable resource. These results may suggest egg load as the determinant of hastening acceptance time. However, freshly eclosed pro-ovigenic *S. fusca* wasps carrying their maximum egg loads were reluctant to oviposit in less preferred resources (Fig. 2a).

These observations therefore suggest that ageing and egg load, when examined individually, may not have any effect on host acceptance; these findings are also supported by other studies (Prokopy *et al.*, 1993; Javois & Tammaru, 2006). The effect of ageing on resource acceptance observed in *S. fusca* appears to be the result of a possible interaction between biological age and egg load, as the wasps become time-limited with ageing. Additionally, females may also be able to assess host availability in the wild and accordingly modify their response even when change in egg load is negligible (Kagata & Ohgushi, 2002; van Alphen *et al.*, 2003); such factors may also have influenced our results.

Interestingly, *S. testacea*, another galler with life-history traits similar to *S. fusca*, did not attempt oviposition on inappropriate

host syconia despite being aged without oviposition experience. Also, *S. fusca* and *S. testacea* have different oviposition substrates (pre-receptive versus receptive syconia); therefore initial gall development probably occurs in different physiological environments for these species. Nevertheless, we expected *S. testacea* with ageing to experience a time limitation similar to or even greater than *S. fusca* due to its shorter life span (~6 vs ~8 days; Ghara & Borges, 2010); however, the observed absence of any attempt to oviposit in receptive-phase syconia by *S. testacea* may be the consequence of a hardwired behaviour resulting from morphological constraints to oviposition due to ovipositor length. The community of fig wasps in this system, which oviposit in a temporal sequence along the development of the syconium (Fig. 1), also exhibit a corresponding increase in the length of their ovipositors in order to drill through the growing syconium wall to access internal oviposition sites (Ghara *et al.*, 2011, 2014). *Sycophaga testacea* oviposits into a relatively smaller pre-receptive phase syconium (Fig. 1) and has a small ovipositor that cannot access the florets in larger syconia characteristic of later developmental stages. *Sycophaga testacea* does not encounter much variation in syconium size during the pre-receptive stage, making all syconia at that stage accessible to its ovipositor; it might therefore be evolutionarily costly to have a longer ovipositor. Such a morphological constraint can limit possible behavioural plasticity similar to the neural constraints that have been erected as factors in affecting behaviour and limiting insect host range (Dusenbery, 1992; Bernays & Weislo, 1994; Hopper *et al.*, 2013). The costs and trade-offs associated with plasticity can be decisive in shaping the dynamic nature of host acceptance (Relyea, 2001).

The longer-lived parasitoids did not show any decrease in specificity although they were aged without any oviposition experience for about 50% of their life span. This is suggestive of a premium on high specificity at all ages if the potential cost of ovipositing in a wrong host is zero reproductive success. Parasitoids may also be overcoming the effect of the possible age–egg load interaction discussed for *S. fusca* through oosorption, which can reduce egg load while increasing survival and longevity under stressful conditions (Rosenheim *et al.*, 2000; Jervis *et al.*, 2001); they could, therefore, avoid the negative effects of time limitation at the cost of reduced realised fecundity in order to maintain specificity. However, the phenomenon of oosorption needs to be confirmed in parasitoids of the fig–fig wasp system.

#### *Transition from non-acceptance to acceptance of unsuitable hosts for oviposition*

The time taken to accept a host syconium for oviposition by the short-lived *S. fusca* began to decline as early as 2 days after eclosion. This can be better understood in an ecological context when the age of wasps is converted to proportion of life span. A span of ~2 days for *S. fusca* is equal to about 25% of its life span (life span of *S. fusca* ~ 6–8 days; Ghara & Borges, 2010) and acceptance of a less preferred host could clearly have certain costs and unknown benefits. Rejection of a less preferred host in the absence of a suitable host would also mean flying in search of a preferred host on another fig tree as fig

trees mostly exhibit within-tree synchrony bearing syconia of the same stage (Janzen, 1979; Bronstein, 1989). As flight is energetically expensive in these wasps (Venkateswaran *et al.*, 2017), such choosy wasps may experience a further reduction in life span before a suitable host is encountered, precluding the laying of their full egg complement. In addition, *S. fusca* is generally more abundant (Ghara *et al.*, 2014), resulting in greater competition for oviposition sites. The time spent to assess host quality may, therefore, be more costly in this species, favouring less choosy individuals. Accepting a less preferred host and yet maintaining a preference ranking, should a suitable host be encountered, may be beneficial to these short-lived wasps.

Under particular stresses such as ageing, the oviposition decisions of species may be affected by life-history traits and trophic levels. For example, pro-ovigenic individuals may change oviposition preference due to a decrease in their life expectancy if they have an intact egg load; however, the same stress may not have a similar effect on synovigenic species, as lack of resources may also restrict egg development, resulting in a lower egg load (Rosenheim, 1999). Synovigenic species can therefore afford to continue to be choosier for a higher proportion of their life span and this may explain the behaviour observed for parasitoids in our system. In the fig–fig wasp system, temporal windows for oviposition differ for galls and parasitoids, with pro-ovigenic galls having much shorter windows compared with synovigenic parasitoids (Ghara & Borges, 2010; Ranganathan *et al.*, 2010). Pro-ovigeny may be adaptive when resources are temporally or spatially limited, whereas synovigeny may be favoured when chances for future reproduction are higher. Thus, selection for flexibility in host acceptance is likely to be different for pro- and synovigenic species. We show that non-pollinating fig galls differ in their acceptance of syconia with different oviposition histories. The observed effects can be summarised by two sets of possible explanations: (a) differences in species biology and limits to behavioural plasticity as observed for species within the same genus with similar life-history traits (galls *S. testacea* and *S. fusca*); and (b) differences in some life-history traits such as life span and initial egg load along with trophic level (galler versus parasitoids) as observed across genera (*Sycophaga* galls versus *Apocrypta* parasitoids). Choosiness or lack thereof could also be a function of an interaction between egg load and individual age; however, an empirical quantification of egg load across treatments in these wasps would be needed to test this hypothesis further.

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