Host–parasitoid development and survival strategies in a non-pollinating fig wasp community

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A R T I C L E   I N F O

A B S T R A C T

In a tritrophic system, parasitoid development and galler host survival strategies have rarely been investigated simultaneously, an approach crucial for a complete understanding of the complexity of host–parasitoid interactions. Strategies in parasitoids to maximize host exploitation and in gallers to reduce predation risk can greatly affect the structure of tritrophic communities. In this study, the developmental strategies of galler hosts and their associated parasitoids in the tritrophic fig–fig wasp system are experimentally investigated for the first time. In this highly co-evolved system, wasp development is intrinsically tied with the phenology of the wasp brood sites that are restricted to the enclosed urn-shaped fig inflorescence called the syconium which can be regarded as a microcosm. Wasp exclusion experiments to determine host specificity, gall dissections and developmental assays were conducted with non-pollinating fig wasps in *Ficus racemosa*. Our results provide evidence for exceptions to the widely accepted koinobiont–idiobiont parasitoid dichotomy. This is also the first time fig wasps were raised *ex situ* from non-feeding stages onwards, a technique that enabled us to monitor their development from their pre-pupal to adult stages and record their development time more accurately. Based on variation in development time and host specificity, the possibility of a cryptic parasitoid species is raised. The frequency of different wasp species eclosing from the microcosms of individual syconia is explained using host–parasitoid associations and interactions under the modulating effect of host plant phenology.

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1. Introduction

In communities built on tritrophic interactions, gallers and parasitoids are engaged in strategies and counter-strategies. Species at higher trophic levels such as parasitoids can play an important role in maintenance of community structure by controlling the abundance of other species (Lawton and Strong, 1981; Hassell, 2000). In host–parasitoid interactions, the reproductive strategy of the parasitoid can further affect community dynamics (Kraaijeveld and Godfray, 1997; Ishii and Shimada, 2012). Based on development strategy, parasitoids are classified into koinobionts and idiobionts. A koinobiont allows its hosts to develop after parasitism, attacks the early, usually more abundant stages of its developing host (Askew and Shaw, 1986), and is predicted to have higher fecundity (Mayhew and Blackburn, 1999; Pennacchio and Strand, 2006). An idiobiont paralyses/kills its host immediately, attacks the later and static stages of host development (Askew and

Shaw, 1986) and is predicted to have lower fecundity (Mayhew and Blackburn, 1999; Pennacchio and Strand, 2006). Nearly all koinobionts are endoparasitoids whereas idiobionts attacking larval host stages are almost always ectoparasitoids (those attacking pupal stages may be endoparasitic) (Quicke, 1997). Ectoparasitoids generally do not face significant host immune defense although they often immunosuppress their hosts (Pennacchio and Strand, 2006) whereas endoparasitoids face a strong host immune defense response and have varied evasion mechanisms (Schmidt et al., 2001). From the host's perspective, risk of parasitism, i.e. predation, may increase with length of developmental stages as predicted by the slow growth–high mortality hypothesis (Clancy and Price, 1987) and therefore a host may accelerate through vulnerable stages to avoid predation. However, there is a trade-off between development rate and adult size. A host may develop rapidly but with reduced size or grow larger at the cost of longer development time (Harvey and Strand, 2002); a larger host may also serve as a high quality host in terms of nutrition available for the developing parasitoid (Mohamed et al., 2003) and may therefore carry the extra risk of being preferred for oviposition. The

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optimal developmental strategies of galler hosts and parasitoids to achieve maximum fitness with minimum predation risk and suitable offspring size will be dictated by a trade-off between such factors. A host−parasitoid association may be further complicated by a third species such as in tritrophic interactions where both host and parasitoids can be affected directly or indirectly by plant traits such as phenology and the possibility of plant sanctions (Pages et al., 2012; Borges, 2015a,b; Krishnan et al., 2015). A comprehensive study on the development and survival strategies of parasitoids and their hosts in a tritrophic system where a third interactant can act as a restricting factor can help us understand the dynamics of host−parasitoid interactions within a wider framework and in a natural context.

The fig−fig wasp system, with its species-specific pollinator and non-pollinators whose development is obligately linked with host plant development and therefore restricted in many ways, provides an excellent system to understand the interactions, reproductive strategies and complexity of population dynamics of the members of a tritrophic system (Borges, 2015a; Krishnan et al., 2015). The fig inflorescence or syconium can be considered a microcosm (Borges, 2015a) within which a multiplicity of ecological processes occur. The non-pollinating fig wasps (NPFWs) that oviposit from outside the syconium with their long ovipositors can be gall-inducing species, secondary gallers that modify galls made by primary gallers, inquilines or kleptoparasites of gall inducers, or parasitoids of other fig wasps (Joseph, 1959; Jousselin et al., 2008; Chen et al., 2013; Borges, 2015a). Although NPFWs attack figs at different stages of syconial development (Wang and Zheng, 2008; Ranganathan et al., 2010), they all need to complete their development at the same time in order to exit synchronously and opportunistically with the pollinators, since it is usually pollinator males that cooperate to cut exit holes through the wall of the otherwise sealed syconium allowing pollen-laden females and other fig wasps to escape (Cook and Rasplus, 2003; Herre et al., 2008; Suleman et al., 2012). Once pollinators have left the syconium, it ripens quickly, often within a few hours. Delayed departure from the syconium as a result of delayed eclosion would therefore expose wasps to consumption by frugivores or predation by ants (Bronstein, 1988). This requirement for inter-species congruence in eclosion time sets up an interesting arena for developmental conflict between gallers, parasitoids and host-plant with the earlier arriving gallers attempting to hasten syconium development time and the later-arriving parasitoids attempting to retard it in order to complete their development (Krishnan and Borges, 2014). Arrival of different galler species for oviposition at different stages of the fig development cycle also translates to a range of hosts available for parasitoids. Parasitoids capable of exploiting a wide range of host instars generally exhibit developmental plasticity (Harvey et al., 1994). The fig system provides an excellent system to understand this flexibility in parasitoid development since the development of their galler hosts is coupled with the host plant and more specifically with syconium development which in turn restricts the maximum development duration of parasitoids when parasitizing a later stage of the galler host. On the other hand, gallers are expected to have less intra-species variation in their development owing to their shorter oviposition windows in terms of days available for oviposition during syconium ontogeny (Ghara and Borges, 2010; Ranganathan et al., 2010). Although both gallers and parasitoids arrive for oviposition at specific ontogenetic stages of the syconium (Ranganathan et al., 2010) and gallers exploit specific fig inflorescence structures as oviposition sites (Ghara et al., 2011), the specificity of parasitoids for their galler hosts has not been experimentally investigated. Some predictions about host specificity have been made based on the frequency of wasp species developing within syconia (Wang and Zheng, 2008; Ghara et al.,

2015) and the similarity between gallers and parasitoids in cuticular hydrocarbon profiles (Ranganathan et al., 2015). Such questions are particularly challenging given the enclosed microcosm within which wasp development occurs. Furthermore, syconia that do not receive pollination, and in which an adequate number of developing pollinators and seeds are not present, may be aborted by the fig plant (Jousselin et al., 2003; Wang et al., 2010). Nevertheless, in our study system of *Ficus racemosa,* the role of life history traits, resource partitioning along space and time axes, and tools for resource partitioning between gallers and parasitoids for fig wasp community co-existence have been successfully investigated (Ghara and Borges, 2010; Ghara et al., 2011, 2015).

In the present study, in order to understand host−parasitoid interactions, we asked the following questions using experimental manipulations:

a) What are the galler host−parasitoid pairings in *Ficus racemosa*?
b) What are the ontogenetic, i.e. developmental stages of parasitoids and their hosts in this system?
c) Do non-pollinating galler hosts exhibit any strategies specific to increasing survival against their parasitoids?

We also examine whether the development and survival strategies of parasitoids and host gallers can explain the observed relative abundance of NPFWs that emerge from the microcosms of individual syconia, and whether general rules for assembly of fig wasp communities are deducible.

2. Methods and materials

2.1. Study system

Experiments were conducted on *Ficus racemosa* trees within the campus of the Indian Institute of Science, Bangalore, India (12°58′ N, 77°35′ E). The phenology of the monoecious *F. racemosa* can be divided into five stages (Ranganathan et al., 2010; adapted from Galil and Eisikowitch, 1968): A—pre-pollination phase; B—pollen receptive phase; C—interfloral phase; D—pollen donation and wasp dispersal phase; and E—seed dispersal phase (Fig. 1). There is one specific pollinator species, *Ceratosolen fusciceps*, whose progeny develops in some flowers at the expense of seeds. The community has six specific NPFWs (*Sycophaga stratheni, Sycophaga testacea, Sycophaga fusca, Sycophaga agraensis, Apocrypta* sp. 2, and *Apocrypta westwoodi*) that attack syconia at different stages of their development (Ranganathan et al., 2010) and differ in their oviposition windows, i.e. length of time during syconium development when oviposition occurs (Fig. 1).

The early arriving gallers (*S. stratheni* and *S. testacea*) develop in larger galls whereas later arriving gallers (*S. fusca* and *C. fusciceps*) develop in smaller galls (Ghara et al., 2015). These species show differential occupancy (different proportional abundances) within and between syconia with the pollinator being the most abundant species (Ghara et al., 2015). Of the galler hosts, *S. stratheni* is the largest (adult mass 0.85 ± 0.09 mg (wet weight)), *S. testacea* is of intermediate mass (0.35 ± 0.08 mg) with *S. fusca* (0.25 ± 0.08 mg) and *C. fusciceps* having the smallest mass (0.22 ± 0.06 mg); of the parasitoids, *S. agraensis* (0.24 ± 0.05 mg) and *Apocrypta* sp 2 (0.25 ± 0.04 mg) have equivalent masses while *Apocrypta westwoodi* is 4−5 times heavier (1.1 ± 0.17 mg) (n = 10 for all species; data from Ghara and Borges, 2010).

In the present study, of the seven fig wasp species in the community, only the abundant pollinator *C. fusciceps* and its putative parasitoid *S. agraensis* (Ghara et al., 2015) have not been investigated owing to the fragile nature of pollinator galls resulting in unsuccessful dissections. Exclusion experiments in which only
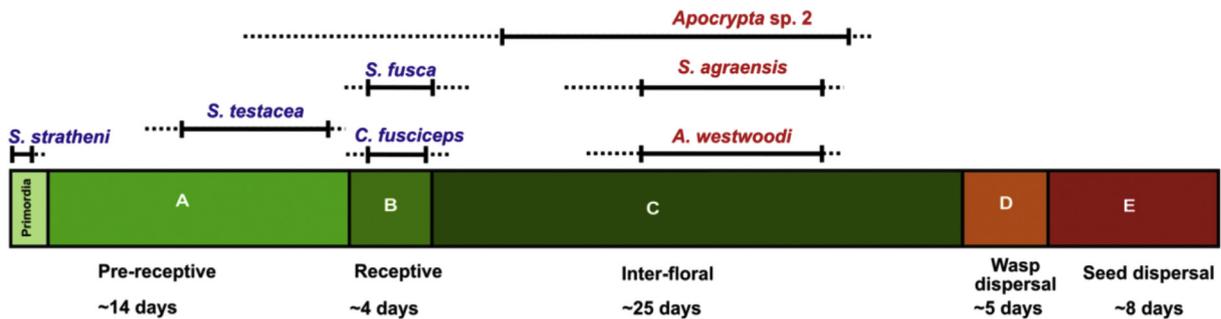
Fig. 1. Wasp arrival sequence for oviposition across syconium development phases (A–E) in Ficus racemosa (adapted from Ranganathan et al., 2010). Solid lines represent top 50% of wasp arrival while dotted lines represent the rest. Gallers are marked in blue and parasitoids are in red.

Note that *Sycophaga* is the correct name for *Apocryptophagus* with which it has been synonymised. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specific wasps were allowed to oviposit within fig syconia were conducted to investigate the host specificity of parasitoids. The results were followed up with gall dissections. Dissections were also performed to investigate whether parasitoids are endo-or ectoparasitoids and koinobionts or idiobionts and to study the developmental stages of NPFWs.

2.2. Galler host–parasitoid pairs: exclusion experiments

Fig syconial bunches (10–15 syconia per bunch) were bagged (covered in mesh bags) in the flower primordia stage to prevent unwanted wasp species from entering or ovipositing into syconia. Based on the observed oviposition windows (Fig. 1), only certain galler host–parasitoid combinations were deemed possible. Twenty wasps of each non-pollinating galler species and pollinator were released into each bagged syconial bunch (*S. testacea* in A-phase, *C. fusciceps* in B-phase and *S. fusca* in B-phase) followed by 20 of each parasitoid species (*Apocrypta* sp 2 in B and C-phase and *Apocrypta westwoodi* in C-phase) to obtain the following galler–parasitoid combinations: *S. testacea* – *Apocrypta* sp 2, *S. fusca* – *Apocrypta* sp 2, *S. testacea* – *Apocrypta westwoodi*, *S. fusca* – *Apocrypta westwoodi*, *C. fusciceps* – *Apocrypta* sp 2, and *C. fusciceps* – *Apocrypta westwoodi*. Syconia were allowed to complete their development through C-phase, were collected in D-phase and the eclosing wasps were allowed to emerge from the syconia in the lab. The identity of the wasp species emerging out of syconia was noted. The experiment was repeated 3–5 times for each putative host–parasitoid combination (Table 1). Since *S. stratheni* is a rare galler wasp, it could not be included in this study.

2.3. Ontogenetic stages: gall dissection and ex-gall development

Gall dissections were carried out in physiological saline under a stereomicroscope (Zeiss Stemi, 2000-C). Images were taken using a Carl Zeiss AxioCam MRc camera and a Leica M205c microscope. After the introduction of wasps in the monitored bunches, 3–4 syconia were removed every 24 h and gall dissections were carried out to follow the developmental stages. Developmental stages of *S. testacea*, *Apocrypta* sp 2 and *Apocrypta westwoodi* were observed and noted. Developmental stages of *S. fusca* could not be completely determined due to the fragile nature of their galls resulting in unsuccessful dissections. Wasps in their pre-pupal stage were transferred from galls into a petri dish containing tissue moistened in HL-3 buffer, sealed with parafilm and maintained in an incubator at 25° C where they completed their development and emerged as active adults. We refer to this period outside the gall as ex-gall development.

3. Results

3.1. Galler host–parasitoid pairs: wasp exclusion experiments

The results of wasp exclusion experiments to determine host specificity of the parasitoids are provided in Table 1. *Sycophaga testacea* was a common host for the parasitoids *Apocrypta* sp 2 and *Apocrypta westwoodi* since both these parasitoids developed successfully in syconia in all fig bunches previously exposed to oviposition by *S. testacea*. *Sycophaga fusca*, on the other hand, was parasitized only by *Apocrypta* sp 2 since *Apocrypta westwoodi* failed to develop in any syconia with *S. fusca* oviposition. None of these parasitoids developed in syconia with only developing pollinators.

Table 1

Wasp exclusion experiments to determine the galler host–parasitoid pairs in *Ficus racemosa*. Galler hosts and parasitoids were released into the bagged syconial bunches at their respective arrival times during fig development. Wasp species emerging out of syconia at wasp dispersal phase were noted.

Fig wasp pair	Galler species released	Parasitoid species released	Number of experimental replicates (fig syconial bunches)[a]	Wasp species that completed their development
1	S. testacea	Apocrypta sp 2	3	S. testacea, Apocrypta sp 2
2	S. fusca	Apocrypta sp 2	3	S. fusca, Apocrypta sp 2
3	C. fusciceps	Apocrypta sp 2	5	C. fusciceps only
4	S. testacea	A. westwoodi	3	S. testacea, A. westwoodi
5	S. fusca	A. westwoodi	3	S. fusca only
6	C. fusciceps	A. westwoodi	3	C. fusciceps only

[a] Syconia in each bunch ranged from 10 to 15 in number. Only a fraction (5–6 syconia per bunch) of these syconia completed development owing to the absence of the pollinator.

It was difficult to carry out exclusion experiments with *S. stratheni* owing to its rarity but dissections of its galls provided evidence of it being a host for both parasitoids (n = 23; parasitoid larvae were observed to be in first or second instar stage inside the galler larva). *Apocrypta* sp 2, therefore, appears to be a generalist predating on three non-pollinating gallers (*S. stratheni*, *S. testacea*, and *S. fusca*), while *A. westwoodi* is more specialised, parasitizing only the early large gallers *S. stratheni* and *S. testacea*.

3.2. Ontogenetic stages: gall dissections and ex-gall development

The different developmental stages of NPFWs obtained from dissections and lab rearing are shown in Fig. 2 and Fig. 3 (for detailed description see supplementary material S1). Gall dissections supported the results on host specificity of the parasitoids from the exclusion experiments. The first and second instar larva of *Apocrypta* sp 2 were observed developing inside the gall but outside the body of the larva (of each host species), feeding on cell-free gall fluid (Fig. 3C). The host larva was alive at this stage but motionless and presumed dead by the last larval stage when no remnants were found (n = 34). The parasitoid *Apocrypta* sp 2 seems to exhibit the dual behavior of being phytophagous in the early stages and entomophagous in later stages when it kills the host. The manner in which it allows the galler 'host' to develop further after attacking the gall might be considered analogous to koinobiosis; however, since the host insect itself is not attacked at this stage, it is not a koinobiont parasitoid in the strict sense. It is, therefore, categorised as a phytoentomophagous ectoparasite. The egg and first instar larva of *A. westwoodi*, on the other hand, was found inside the corpse of a pre-pupal stage host and this parasitoid parasitises the late fourth instar and early pre-pupal stages of its host (Fig. 3F); *A. westwoodi* is, therefore, a completely entomophagous idiobiont endoparasitoid.

Superparasitism was observed in a few galls (n = 10) in which more than one first instar larva of *Apocrypta* sp 2 were found; also there was often a combination of a single first/second instar endoparasitic idiobiont larva of *A. westwoodi* and one first instar larva of *Apocrypta* sp 2 inside the same *S. testacea* or *S. stratheni* gall in C-phase (n = 4). It is not known whether both individuals ever successfully develop fully.

We were able to rear both males and female wasps of gallers and parasitoids from the early pupal stage onwards in the lab ex-gall with no external nutrient media. In the early-arriving gallers (*S. stratheni* and *S. testacea*) the transition from fourth instar to early pupal stage took only ~12 h (n = 28) as compared to parasitoids that took 5−6 times longer through this transition (n = 15). We also succeeded in raising *Apocrypta* sp 2 males in the lab from the fourth instar larval stage itself.

3.3. Development time and host−parasitoid interactions

From the wasp exclusion experiments and dissections, we find that the smaller parasitoid *Apocrypta* sp 2, during its arrival in A−B phases, attacks the early instar larvae of *S. stratheni* and *S. testacea* and takes ~45 days to complete its development (Fig. 4). However, after syconium pollination, it parasitises the early instar larvae of *S. fusca* and takes ~25 days only to complete its development thus exhibiting flexibility in its pre-adult life span (Fig. 4) as well as host diversity. The larger parasitoid *Apocrypta westwoodi*, on the other hand, lays its eggs into the last larval stages of *S. stratheni* and *S. testacea* during its arrival in C-phase and completes its development in only ~20 days (Fig. 4). The early arriving gallers *S. stratheni* and *S. testacea* have the longest development time of ~60 and ~50 days respectively with maximum time spent in all the larval stages combined. The later arriving parasitoids have shorter larval stages and complete their development faster than the early arriving gallers (Fig. 4).

4. Discussion

In the five examined fig wasp species within the seven fig wasp community of *Ficus racemosa*, *Apocrypta westwoodi* is an endoparasitic idiobiont parasitizing only hosts with large galls whereas *Apocrypta* sp 2 is an ectoparasitic, phytoentomophagous parasite
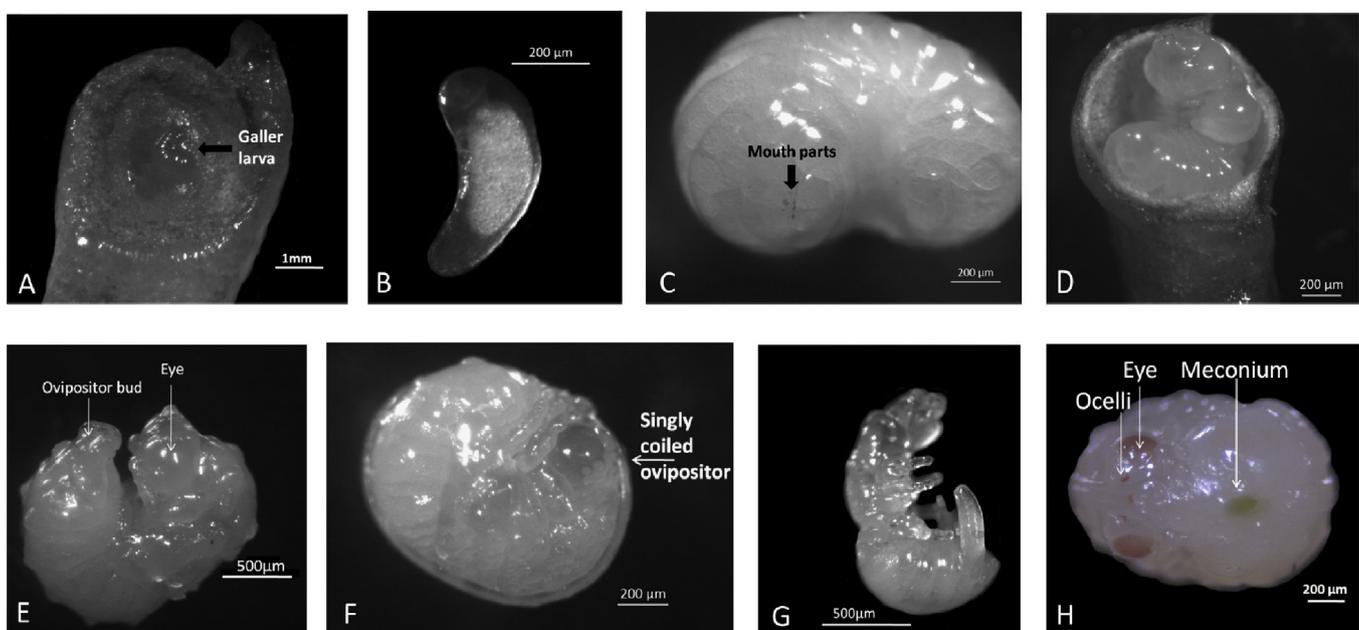

Fig. 2. Developmental stages of galler *Sycophaga testacea.* (A) first instar larva within a gall, (B) second instar larva, (C) third instar larva, (D) fourth instar larva within a gall, (E) primary pupa (female), (F) intermediate female pupa, (G) intermediate male pupa, (H) intermediate pupa (female) with meconium.
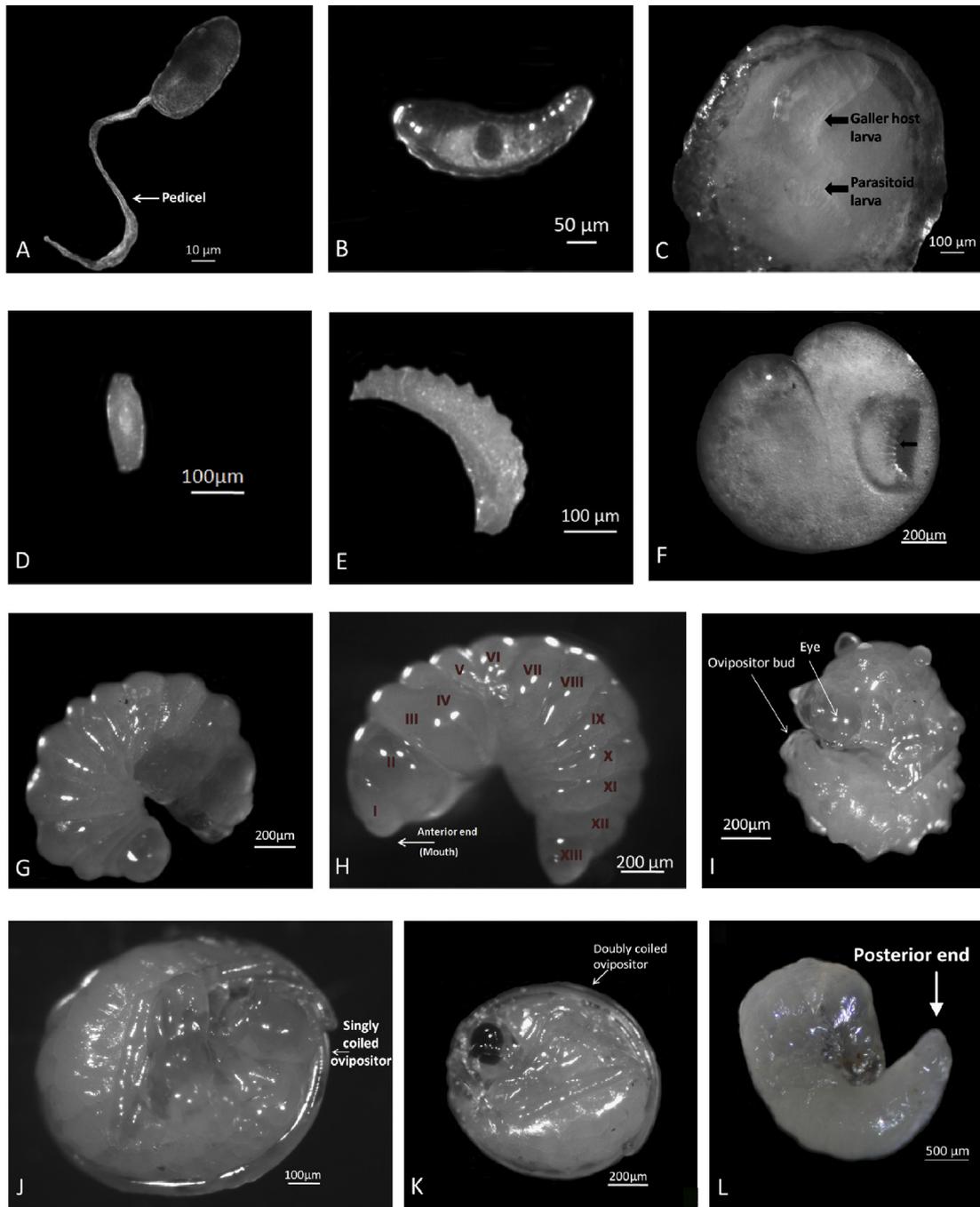
Fig. 3. Developmental stages of parasitoids. Initial stages are different for *Apocrypta* sp.2 (A–C) and *Apocrypta westwoodi* (D–F) and late stages are common (G–L). (A) *Apocrypta* sp 2 egg dissected out from a parasitized *S. testacea* gall, (B) first instar larva, (C) second instar larva, (D) *A. westwoodi* egg dissected out from a parasitized *S. testacea* larva, (E) first instar larva, (F) second instar larva, (G) third instar larva, (H) fourth instar larva showing thirteen distinct segments, (I) primary pupa (female), (J) intermediate female pupa (*Apocrypta* sp 2), (K) intermediate female pupa (*A. westwoodi*), (L) intermediate male pupa. Details about these stages are available in the Supplementary Information.

preying on all three non-pollinating gallers and exhibiting large variation in its total development time with different hosts. However, none of these parasitoids parasitize the abundantly available pollinator *C. fusciceps*. We succeeded in raising fig wasps *ex situ* from the early pupal stage onwards and observed that the transition of the early-arriving gallers from the fourth instar to early pupal stage was rapid and could be a possible strategy to escape predation by *A. westwoodi* by accelerating through the vulnerable pre-pupal stages.

4.1. Host specificity of parasitoids

Apocrypta westwoodi has 4–5 times the body mass of the other parasitoids in the system (Ghara and Borges, 2010), and subsequently attacks only the two gallers of the largest size (*S. stratheni* and *S. testacea*) in the community (Ghara and Borges, 2010). The lack of coherence observed between the weight of the parasitoid *Apocrypta westwoodi* and its galler host *S. testacea* (data from Ghara and Borges, 2010) may be the result of sampling. It is possible that *A. westwoodi* sampled for weights in that study were those that
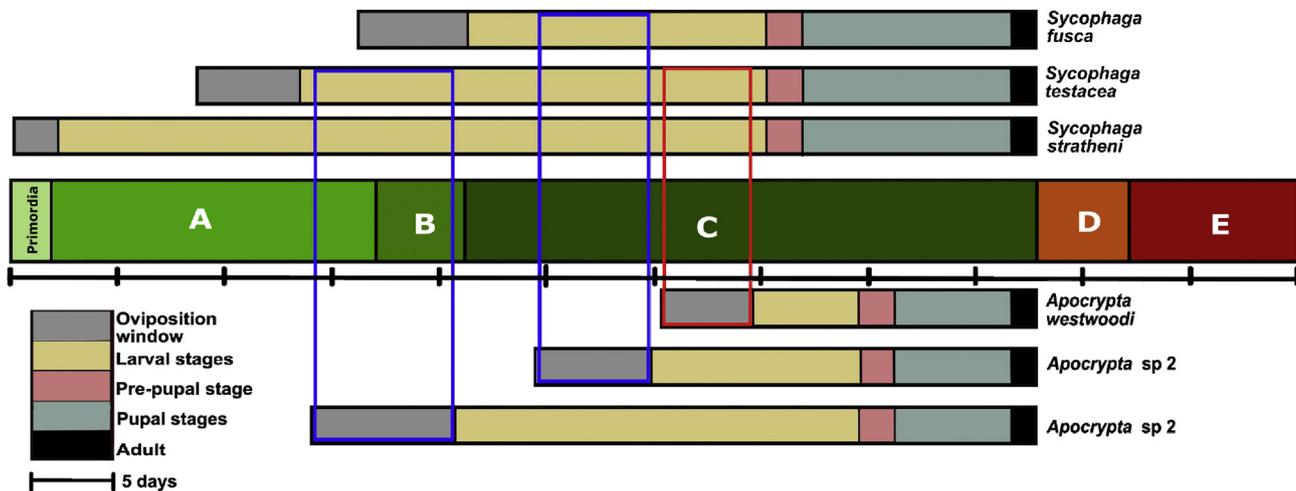
Fig. 4. Developmental stages of the non-pollinating fig wasps along the fig syconium development timeline. Blue and red rectangles represent the galler host specificity of the two parasitoids *Apocrypta* sp 2 and *A. westwoodi* respectively. *Apocrypta westwoodi* parasitises *S. stratheni* and *S. testacea* while *Apocrypta* sp. 2 predates upon all three non-pollinating gallers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

developed inside the galls of the much larger but rarer host *S. stratheni*.

Large galls contain larger hosts that are assumed to be of higher quality than small hosts due to higher resource availability for parasitoid development (Charnov and Skinner, 1985; Mohamed et al., 2003). None of the *Apocrypta* parasitoids attack the pollinator wasp (also see Godfray, 1988, for similar observation in *Ficus hispidiodes*: syn: *F. hispida*); however, the galler *S. fusca* that oviposits concurrently with the pollinator is common prey for the much smaller *Apocrypta* sp 2. The nutritional availability is *S. fusca* galls is probably suitable for the smaller *Apocrypta* sp 2. While *C. fusciceps* galls have the same developmental duration, size and morphology as those of *S. fusca* (Fig. S1B), and is also the most abundantly available potential host, that *C. fusciceps* is not a host to *Apocrypta* may be due to an inability to evade host defences, difference in gall fluid composition (that needs to be characterized), difference in tissue origin of the gall and thereby larval resources (e.g. nucellus versus endosperm), or to a lack of recognition of *C. fusciceps* as potential hosts (preliminary results).

Apocrypta sp 2 exhibits large variation in its total development time. This variation could be result of developmental plasticity owing to its wide prey base (Harvey et al., 1994). However, variation in development time could also be indicative of cryptic species in this parasitoid as observed in *Drosophila serrata* (Schiffer et al., 2004). The coexistence of cryptic species of pollinating fig wasps has been demonstrated in several *Ficus* species (Molbo et al., 2003) and it is possible that there is a cryptic species in our system as well with one species of *Apocrypta* sp 2 attacking *S. fusca* and the other attacking the other two *Sycophaga* gallers. However, molecular analysis is needed to investigate this further. Presence of cryptic species might also lead researchers to underestimate the species richness of communities in fig wasp assemblages that have been considered undersaturated (Hawkins and Compton, 1992).

4.2. Developmental strategies of parasitoids

The developmental strategies of parasitoids have been extensively studied (Harvey and Strand, 2002; Harvey, 2005; Pennacchio and Strand, 2006; Jervis et al., 2008; Harvey and Malcicka, 2016) and dichotomized, based on suites of traits that they possess, into endoparasitic koinobionts and ectoparasitic idiobionts (Askew and Shaw, 1986) with some exceptions reported in ichneumonids

(Gauld, 1988; Pennacchio and Strand, 2006). Here, we find more evidence for exceptions to this dichotomy. In the fig–fig wasp system of *F. racemosa*, the ectoparasitic *Apocrypta* sp 2 keeps its host alive by laying its egg away from the host; the larva feeds on gall tissue (fluid) in its initial stages and is therefore phytophagous early in its life and an idiobiont in later stages; this species can be correctly termed phytoentomophagous. A mixed entomophagous-phytophagous feeding habit has also been observed in eurytomid and torymid wasps although these are entomophagous in the early stages and feed on plant tissue once the host has been consumed (Hawkins and Goeden, 1984; Leggo and Shorthouse, 2006). Feeding on plant tissue first while the host is alive, as observed for *Apocrypta* sp 2, may serve to continue stimulating gall development and associated sequestration of resources into the gall.

The endoparasitoid *A. westwoodi* kills its host immediately and feeds on the dead larva and can, therefore, be classified as a true idiobiont. In the Ichneumonidae and Braconidae, idiobiont endoparasitism is postulated to have arisen from idiobiont ectoparasitism in order to attack more exposed hosts whereas koinobiont ectoparasitism is considered to be an evolutionary transition stage between idiobionts and endoparasitic koinobionts (Gauld, 1988; Pennacchio and Strand, 2006; Quicke, 2015). Furthermore, idiobionts are generally ectoparasitoids or endoparasitoids of pupae while koinobionts are usually endoparasitoids (Quicke, 1997; Pennacchio and Strand, 2006). Given that in the fig system, each host is concealed inside a gall in a syconium, it is possible that the strategies of parasitoids of our fig–fig wasp system could be driven by the strength of their defense mechanisms against host immune response. *Apocrypta* sp 2, in its earlier stages, may not be equipped to face host immune response and, therefore, develops away from the host but inside the gall until it reaches the second/third instar when it can attack the host. *Apocrypta westwoodi* might also be killing its host immediately to avoid the host immune response. *Apocrypta* sp 2 is therefore an ectoparasitic phytoentomophagous parasite, and not a koinobiont in the true sense while *A. westwoodi* is an endoparasitic idiobiont. In the light of exceptions to the conventional dichotomy (Gauld, 1988; Quicke, 2015) and failure of predictions based on it (Mayhew and Blackburn, 1999; Harvey, 2005; Boivin and Ellers, 2016), we suggest that the developmental strategies of parasitoids should be studied along the continuum of a combination of life-history traits instead of under such strict dichotomies.

In terms of development time, the late-arriving parasitoids complete their development much faster than the early-arriving gallers (Fig. 4) in order to synchronize their exit from the syconium with the pollinators during the wasp dispersal phase. Although the development rate of these late-arriving parasitoids is faster such that they complete their development in ~20 days compared to ~50−60 days in gallers, yet in order to successfully complete development, these late-arriving parasitoids delay the development time of syconia by 2−3 days (Krishnan and Borges, 2014) in order to exit the syconium concurrently with pollinators and other wasp inhabitants.

4.3. Developmental strategies of gallers

Coupling host survival strategies with parasitoid developmental strategies can advance our understanding of the dynamics of a host−parasitoid association with better precision (Hassell, 2000). In *F. racemosa,* the early-arriving gallers *S. stratheni* and *S. testacea*, which produce the largest galls, have the longest total development time of all wasps developing within the syconium. Slow development also translates to increased time spent in each developmental stage and therefore increased vulnerability to parasitoids (Clancy and Price, 1987), and this may be why the early gallers hasten the development time of the fig syconium (Krishnan and Borges, 2014). We also observed that the transition of the early-arriving gallers from fourth instar to early pupal stage was very fast (~12 h) and could be a possible strategy to escape predation by accelerating through the fourth instar pre-pupal stage that is vulnerable to attack by *A. westwoodi*. This might result in scarcity of hosts for parasitoids, and consequently the occurrence of superparasitism as in several hymenopteran parasitoids (Gu et al., 2003; Dorn and Beckage, 2007) and as observed in a few instances during this study. Survival of the extra larvae may be affected by factors such as physical attack, encapsulation or toxic chemical secretions (Vinson and Hegazi, 1998) but these remain to be investigated.

It is interesting that only the last larval stage undergoes developmental acceleration. This may be due to restriction on total development time so as to synchronize the exit from the syconium with pollinators during the wasp dispersal phase and also the requirement for longer feeding stages (early larval stages) to achieve the larger size of the adults. Males of each species complete their development ~1−2 days before females but since it is difficult to differentiate between sexes before the pupal stage (with the methodology used in this study), it is not possible to confirm whether the total difference is due to accelerated development by males only (Gu et al., 2014) and/or to delay in eclosion time of females. That males should eclose earlier than females is understandable as males compete to inseminate as many virgin females as possible while females are still in their galls inside the syconium to increase their reproductive opportunity (Doyon and Boivin, 2006).

Our study provides insight into the comparative developmental biology of non-pollinating fig wasps for the first time. Only few studies have documented gall induction and early developmental stages for pollinating fig wasps and a non-pollinating galler wasp (Jansen-González et al., 2012, 2014) and the gallers studied in our system have similar developmental stages and fluid feeding habit described for a pollinating fig wasp (Jia et al., 2014). However, we find the NPFWs lack the shriveled ovary integument covering their larva inside the galls as described for the pollinator *C. solmsi marchali* (Jia et al., 2014) since pollinators gall fully developed flowers that are ready to be pollinated. The habit of freely floating and feeding in the fluid filling the gall (observed to be cell-free/non-cellularised in *F. racemosa* gallers) appears to be true for all galler species in the fig system. The gall continues to contain fluid till the

third instar larval stage. The quantity of gall fluid within the galls of different species (gall size as proxy for fluid inside the gall) and the variation in time spent in larval (feeding) stages (Fig. 4) possibly accounts for the sizes of the adult gallers. However, the size difference between species could also be due to the nutritional composition of fluid inside the galls which needs characterization.

To our knowledge, this is the first attempt to raise fig wasps outside the gall from the pre-pupal stage onwards under laboratory conditions. This innovative technique will make it possible to explore many questions that are limited by our inability to handle developing wasps outside syconia. We hope to extend this ex-gall technique to even earlier stages of development.

4.4. Can developmental strategies explain the composition of wasps emerging from a syconial microcosm?

Species co-existence and structuring in fig wasp communities have been studied for several fig−fig wasp systems (Wang and Zheng, 2008; Ghara and Borges, 2010; Ghara et al., 2015; Segar et al., 2013, 2014). Species richness, abundance and systematics have been investigated by phylogenetic studies at local and global levels (Hawkins and Compton, 1992; Cruaud et al., 2011; Segar et al., 2013). Across fig communities, there are differences in species richness but the ecological structure comprising of five guilds; i.e. pollinators, large gallers, small gallers, parasitoids of small gallers and parasitoids of large gallers; and their proportional abundance, was found to be similar across continents (Segar et al., 2013). At the syconium level, occupants change along syconium ontogeny and trophic interactions can greatly affect the diversity and frequency of wasps emerging from each syconium (Segar et al., 2013; Ghara et al., 2015; Krishnan and Borges, 2014; Krishnan et al., 2015). Our investigations into the parasitic network and developmental strategies of members of the wasp community provide insights into mechanisms governing community assemblage that can explain the cross-continental similarity in patterns of fig wasp assemblage within syconia.

The early larval stages of the two early arriving large galler species, *S. stratheni* and *S. testacea,* are attacked by *Apocrypta* sp 2 and the last larval stage is predated upon by *A. westwoodi*. Both these gallers face the additional risk of losing all their offspring if pollinators do not later enter this microcosm which could result in this syconium being aborted (Jandér and Herre, 2010). The high predation and abortion risk, coupled with a short life span of 1−2 days (Ghara and Borges, 2010), possibly explains the observed rarity of the largest and earliest-arriving galler species *S. stratheni* within individual syconia (Ghara et al., 2015). *Sycophaga testacea* (another early-arriving large galler with the same parasitoids and similar abortion risks) has, however, a relatively higher abundance than *S. stratheni* within individual syconia (Ghara et al., 2015) and this can be explained by lower predation risks from parasitoids. *Sycophaga testacea* has a longer oviposition window (Fig. 1) and therefore larvae of only early-arriving individuals, that reach the fourth instar by C-phase, are vulnerable to attack by *A. westwoodi* (Fig. 4). On the other hand, larvae of late arriving *S. testacea*, that are still in early larval stages during C-phase, face predation only by *Apocrypta* sp 2 (Fig. 4). This predation is further diluted by the fact that *Apocrypta* sp 2 also exploits the abundantly available *S. fusca* as a host in C-phase. As in other examples of facilitation within the community ecology of fig wasps within fig microcosms (Krishnan et al., 2015), *S. fusca* can indirectly facilitate *S. testacea* by sharing predators (and vice versa) resulting in an observed higher abundance of *S. testacea*. The small galler *S. fusca* arriving in the pollen-receptive phase has the highest abundance among non-pollinating gallers (Ghara et al., 2015). Its arrival time overlaps with that of the pollinator (Ranganathan et al., 2010) and therefore the probability

of ovipositing in a syconium with risk of abortion due to lack of pollination is minimal (Ghara et al., 2015). This species has only a generalist parasitoid as predator and with the diluted predation risk can, therefore, afford to lay more eggs per syconium.

In parasitoids, ovigeny index has been linked with parasitoid development mode (Jervis et al., 2001). Koinobionts, compared with idiobionts, produce smaller eggs, have a higher realized fecundity, and a higher maximum rate of oviposition during their short adult life spans (Mayhew and Blackburn, 1999). *Apocrypta* sp 2 is a small sized generalist unconventional 'koinobiont' (phytoentomophagous) capable of preying on all non-pollinating gallers whereas the large-sized *A. westwoodi* is a specialist idiobiont as it predates only on the last larval stage of galler species that produce large-sized galls. Generalist behavior, high abundance of one of its hosts, high fecundity, dual feeding behavior and specificity for relatively longer larval stages seem to be major factors behind the high abundance of *Apocrypta* sp 2 within individual syconia. The late-arriving *A. westwoodi*, owing to its specificity for the short vulnerable stages of its rarer hosts and its low lifetime fecundity, is found in lower numbers within individual syconia.

In addition to galler–parasitoid associations, plant traits such as syconium volume and within-tree asynchrony may also affect resource abundance for both gallers and parasitoids (Krishnan et al., 2015) and affect the population abundance of the whole community.

5. Conclusions

Our study attempts to understand the developmental and survival strategies of galler hosts and their parasitoids in the microcosm of *F. racemosa*. Our contributions towards raising fig wasps outside syconia and our findings on host specificity of parasitoids in relation to life-history traits open up new aspects of exploration in such tritrophic systems. These findings along with the possibility of cryptic species in the fig–fig wasp brood-site pollination mutualism should help us understand not only the dynamics of entire fig wasp communities but also the survival of the mutualism in such a complex and multiply parasitized system.

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Appendix A. Supplementary data

Supplemental Information includes description of developmental stages of non-pollinating gallers and parasitoids. Supplementary figure S1 shows the adults of all the seven species of wasps and galls induced by different gallers.

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2017.04.001

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