

Diversification in the use of resources by *Idarnes* species: bypassing functional constraints in the fig–fig wasp interaction

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Mutualisms such as the fig–fig wasp mutualism are generally exploited by parasites. We demonstrate that amongst nonpollinating fig wasps (NPFWs) parasitic on *Ficus citrifolia*, a species of *Idarnes* galls flowers and another species feeds on galls induced by other wasps killing their larvae. The galling wasp inserts its ovipositor through the fig wall into the fig cavity. The ovipositor then follows a sinuous path and is introduced through the stigma and style of the flower. The egg is deposited between the integument and nucellus, in the exact location where the pollinating mutualistic wasp would have laid its egg. Gall induction is a complex process. In contrast, the path followed by the ovipositor of the other species is straightforward: attacking a larva within a developed gall poses different constraints. Shifts in feeding regime have occurred repeatedly in NPFWs. Monitoring traits associated with such repeated evolutionary shifts may help understand underlying functional constraints. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 114–122.

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INTRODUCTION

Mutualism is a type of interaction that is beneficial for the species involved (Boucher, James & Keeler, 1982). However, most mutualistic systems, if not all, are susceptible to exploitation by species (parasites of mutualisms, often called cheaters) who take advantage of resources involved in the interaction without providing any benefit (Bronstein, 2001; Yu, 2001). In order to access resources involved in a mutualism, parasitic species may either mimic the way mutualists interact with each other or develop totally different ways of accessing them. For

instance, nonpollinating yucca moths oviposit after ovules have been fertilized, whereas the mutualistic pollinating moths oviposit during the period of receptivity: their ecology is radically different (Pellmyr, 2003). Conversely, in *Ficus sycomorus*, the wasp *Ceratosolen arabicus* is a mutualistic pollinator whereas *Ceratosolen galili* presents very similar behaviour including its period of oviposition, but does not carry pollen: their ecology is in this case highly similar (Galil & Eisikowitch, 1969). Different parasitic species may use different strategies to access the resources. Exploring their diversity may help us understand what the functional constraints are selecting for mutualistic behaviour and how they can be bypassed.

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Very little is known about the fine aspects of the parasites' actual strategies, thus limiting our understanding of such functional constraints. This is typically the case for the mutualism between fig trees and their pollinating agaonid wasps. Indeed, this ecological interaction is a well-known example of plant–insect mutualism (Weiblen, 2002; Herre, Jander & Machado, 2008), and a suite of specialized, species-specific, nonpollinating fig wasps (NPFWs) use resources involved in the fig–fig wasp mutualism, as they reproduce inside figs (Bronstein, 1992). The background biology of the fig–fig-pollinating wasp association can be summarized as follows: winged female pollinating wasps (1) enter the urn-shaped *Ficus* inflorescences (called figs or syconia) through an ostiole, (2) oviposit into the ovaries of female flowers, inducing a gall inside which their larvae will develop, (3) pollinate some of the female flowers, and, several weeks later (4) offspring female wasps emerge into the fig cavity and get loaded with pollen within the fig before exiting it in search of new receptive figs (Jousselin *et al.*, 2001).

NPFW species are generally specific to a plant host and present diversified larval biology (Cruaud *et al.*, 2011b). Most NPFWs oviposit into fig flowers, inserting their long ovipositors through the fig wall. The larvae develop within galled flowers and emerge as imagos into the fig cavity, more or less at the same time as adult pollinators. Some phytophagous gallers deposit their eggs early in fig development and gall fig wall tissues or flower primordia whereas most phytophagous gallers colonize figs at about the same time as the pollinating wasps and gall individual female flowers. Cleptoparasites are also phytophagous, but they oviposit in already induced galls and their larvae subsequently eliminate the galler larvae (Joseph, 1958). Finally, parasitoids feed directly on other fig wasp larvae (Tzeng *et al.*, 2008). Further, some wasp larvae are facultative seed eaters (Pereira, Teixeira & Kjellberg, 2007). Some nonpollinating phytophagous gallers enter the fig to oviposit at the same time as pollinating species (Galil, Dulberger & Rosen, 1970; Zhang *et al.*, 2009). These internal parasites belong to a series of different wasp lineages (Jousselin, Rasplus & Kjellberg, 2001), evidencing behavioural convergence in the way that resources are accessed. Nevertheless, the feeding regimes of NPFWs have been documented for very few species and are generally putative (e.g. Cruaud *et al.*, 2011b).

Amongst NPFWs, Sycophaginae constitute a formidable tool for investigating convergent evolution of mechanisms of mutualism exploitation (van Noort & Compton, 1996). This monophyletic subfamily (Cruaud *et al.*, 2011a) includes species presenting supposedly distinct biologies – early and late gallers and cleptoparasites (Cruaud *et al.*, 2011b). Ancestral

Sycophaginae were probably gallers and at least two transitions to cleptoparasitism are thought to have occurred. Indeed, the genus *Idarnes* is divided into three species groups (Cruaud *et al.*, 2011a). Species of the *Idarnes incerta* and *Idarnes flavicollis* groups are believed to be gallers, and colonize figs early in development or simultaneously (respectively) with pollinating wasps, whereas species of the *Idarnes carme* group colonize figs after pollination and are probably cleptoparasites (Elias, Menezes Jr & Pereira, 2008). The same pattern is apparent within *Sycophaga* (*sensu* Cruaud *et al.*, 2011a, i.e. including former genus *Apocryptophagus*). Amongst the *Sycophaga* species colonizing *Ficus racemosa*, two species gall figs sequentially (*Sycophaga testacea* and *Sycophaga fusca*) and one (*Sycophaga agraensis*) is a cleptoparasite or a parasitoid of the pollinating agaonid wasp, ovipositing later in fig development (Wang & Zheng, 2008). The diversity of feeding regimes observed in both *Idarnes* and *Sycophaga* implies evolution of the capacity to use different resources throughout fig development (female flower primordia, female flowers, galled flowers, wasp larvae, seeds). The evolution of new exploitation strategies based on temporal shifts in resource access probably involves modification in oviposition mode (e.g. path followed by the ovipositor and precise location of egg deposition) and larval biology by character displacement (change in oviposition time) (Schluter, 2000; Sachet *et al.*, 2009). Such biological innovation may lead to subsequent radiation of NPFWs by host shift. The temporal and correlated feeding regime segregation seen in Sycophaginae also occurs in other mutualistic systems involving multiple closely related species. The yucca moths (Pellmyr, Leebens-Mack & Huth, 1996), the larch cone flies *Strobilomyia* (Sachet *et al.*, 2009), and cynipid wasps (Ronquist, 1999) suggest that diversification by character displacement followed by adaptive radiation is frequent in insects.

In order to confirm such a scenario, we demonstrate experimentally that a species of the *Idarnes flavicollis* group is a galler and that a species of the *Idarnes carme* group is a cleptoparasite. Further we present histological evidence for unsuspected adaptations in the path followed by the ovipositor and where eggs are laid.

MATERIAL AND METHODS

STUDY SPECIES AND AREA

We carried out field and laboratory observations of wasps associated with *Ficus citrifolia* P. Miller in Brazil in order to investigate this diversification in the use of resources. The study was carried out at the Universidade de São Paulo campus in Ribeirão Preto (21°10'S; 47°48'W). Six *F. citrifolia* trees were moni-

Table 1. Development of *Idarnes* (*Idarnes flavicollis* and *Idarnes carme* species groups) offspring in the absence and presence of the pollinating wasp species: the experimental design

Combination	Methodology
(1) unpollinated × <i>I. flavicollis</i>	Ten <i>I. flavicollis</i> females placed in each bagged branch, no pollination.
(2) unpollinated × <i>I. carme</i>	Ten <i>I. carme</i> females placed in each bagged branch, no pollination.
(3) pollination × <i>I. flavicollis</i>	Ten <i>I. flavicollis</i> females placed in each bagged branch 1 day after pollinator introduction.
(4) <i>I. flavicollis</i> × pollination	Ten <i>I. flavicollis</i> females placed in each bagged branch followed by pollinator introduction the following day.
(5) pollination × <i>I. carme</i>	Ten <i>I. carme</i> females placed in each bagged branch 15 days after pollination.*

*The timing of *I. carme* species oviposition was chosen following observations by Elias *et al.* (2008).

tored throughout their reproductive cycles during the years 2006–2007. *Ficus citrifolia* (subgenus *Urostigma*, section *Americana*) is a monoecious hemi-epiphytic tree, 3–6 m tall, which is abundant in disturbed habitats (R. A. S. Pereira, pers. observ.). In São Paulo state, it is pollinated by *Pegoscapus* sp. *Idarnes* is the most abundant genus of NPFW associated with *F. citrifolia*. We studied one species of the *I. carme* and one of the *I. flavicollis* species group, using them as model species.

WASP BIOLOGY

Experimental manipulations in the field were carried out to establish whether the investigated wasp species were gallers or cleptoparasites. In each monitored tree, 12 branches with very young figs were bagged with white fine-mesh fabric, precluding wasp access to the figs. Experimental manipulations consisted of exposing *I. flavicollis* and *I. carme* species group wasps to pollinated or unpollinated figs (Table 1) to test whether their offspring were able to develop in the absence of pollinators, i.e. whether they were gallers. All wasps used were collected at their emergence from figs from other *F. citrifolia* trees in the same area. Pollinated figs were obtained by introducing pollen loaded pollinating wasps into figs when they reached receptivity. The bag was removed and a single female pollinating wasp was placed with a fine brush near the fig ostiole to induce its entrance into the fig (five to ten figs per branch). Branches were bagged again to avoid uncontrolled access by any other wasps. We purposely introduced a single foundress per fig in order to keep figs unsaturated, ensuring that many flowers remained free for *Idarnes* oviposition.

Idarnes female wasps were also introduced into bags (ten to 15 wasps per branch) in different combinations (before and after pollination, and without pollinators, Table 1). Branches were kept bagged until just prior to wasp emergence from the fig. At that stage, figs were

collected, placed individually in 50 mL plastic flasks, and kept for approximately 48 h to allow emergence of wasps. Figs were cut open and emerged plus non-emerged wasps sorted to analyse (1) gall formation, (2) seed number, (3) empty flowers and bladder number, (4) offspring number, and (5) size of wasps (estimated from tibia, ovipositor, and mesosoma lengths). Around 60% of figs from unpollinated treatments aborted, against 20% from treatments involving pollinator introduction. We performed another experiment to assess whether *I. flavicollis* group wasps preferentially colonized pollinated or unpollinated figs (preference experiment). Three branches were bagged, but only half of the figs were pollinated in each of them. The following day, ten *I. flavicollis* group females were placed on each bagged branch, so wasps had the opportunity to choose between pollinated and unpollinated figs for oviposition.

OVIPOSITION MODE AND EGG DEPOSITION

We inspected naturally pollinated *F. citrifolia* trees to find *Idarnes* female attempting to oviposit into the figs and killed them with a few drops of chloroform. Figs were then dissected under a stereoscopic microscope to localize the position of the extremity of the ovipositor in the plant tissues. In addition, we checked whether figs had been pollinated or not (observing pollinating foundresses within the fig cavity).

We removed all flowers in which we found ovipositors to be inserted into the ovary, and fixed them for 24 h in FAA (formaldehyde – acetic acid – alcohol) 50; we then stored them in 70% alcohol (Johansen, 1940). We removed and prepared histological slides of the ovaries of the flowers. For that, ovaries were dehydrated in an ethanol–xilol series (about 2 h for each solution), softened in a terpenol solution for one week because the tissues are rich in lignin and cellulose, and individually embedded in paraffin and sectioned

Table 2. Number of wasp offspring (mean \pm SD) and other characteristics from experimental figs. Only infested figs were considered for calculation of each wasp species mean

Variables	Treatments*				
	<i>flavicollis</i>	<i>carme</i>	poll + <i>flavicollis</i>	<i>flavicollis</i> + poll	poll + <i>carme</i>
<i>Idarnes flavicollis</i> group	74.8 \pm 34.6	0	0	32.3 \pm 26.6	0
<i>Idarnes carme</i> group	0	0	0	0	8.5 \pm 7
<i>Pegoscapus</i> sp. (poll.)	0	0	127.5 \pm 36	67.3 \pm 63.5	150 \pm 55.2
Number of seeds	0	0	72 \pm 42	75 \pm 60	92 \pm 51
Number of empty flowers and bladders	207 \pm 97	NM	287 \pm 65	275 \pm 66	188 \pm 113
% fig abortion	59	100	12	32	11
Number of non-aborted figs	29 (29)	0† (0)	19 (0)	33 (13)	36 (9)
Fig diameter (cm)	1.2 \pm 0.1	NM	1.4 \pm 0.2	1.4 \pm 0.15	1.4 \pm 0.15
Number of trees	2	3	3	2	4

*See table 1 for details.

†Out of a total of 52 bagged figs.

Poll = pollinator (*Pegoscapus* sp.); NM = not measured; Number of non-aborted figs = number of figs that did not abort before wasp emergence stage; in brackets the number of figs with *Idarnes* offspring.

in a rotative microtome (6–8 μ m). Then, sections were placed on slides, deparaffinized in a xilol solution (1 min), rehydrated in an ethanol series (1 min each solution), stained with Safranin and Astra Blue, and mounted in synthetic resin (Gerlach, 1969). Slides were observed with a conventional light microscope. We processed 16 flowers oviposited by *I. flavicollis* group wasps and 18 by *I. carme* group wasps.

RESULTS

WASP BIOLOGY

Idarnes flavicollis group offspring developed regularly in figs in the absence of pollinators, whereas *I. carme* group offspring did not develop in any unpollinated fig (Table 2). Indeed, all experimental figs exposed only to *I. carme* wasps aborted and the female flowers were not swollen, indicating that *I. carme* wasps were not capable of inducing the transformation of flower ovaries into galls. When tested against pollinated figs, *I. flavicollis* group wasps developed only in the treatment in which they colonized figs before pollinators (treatment 4, Table 1). In the preference experiments all unpollinated figs ($N = 8$) presented gall induction, indicating that *I. flavicollis* group wasps had oviposited in them. By contrast, all pollinated figs ($N = 7$) produced only pollinator offspring. Moreover, *I. flavicollis* group wasps raised from unpollinated figs were significantly larger in size than those from figs with pollinating wasps [mesosoma length: pollinated (poll.) = 0.71 \pm 0.04 mm and unpollinated (unpoll.) = 0.74 \pm 0.05 mm, $t_{61} = -3.67$, $P < 0.001$; ovipositor length: poll. = 3.55 \pm 0.23 mm and unpoll. = 3.63 \pm 0.2 mm, $t_{61} = -2.18$, $P = 0.03$; tibia

length: poll. = 0.34 \pm 0.03 mm and unpoll. = 0.37 \pm 0.03 mm, $t_{61} = -4.42$, $P < 0.0001$. $N = 62$ wasps for both pollinated and unpollinated figs]. In spite of being capable of developing in the absence of pollinators, *I. flavicollis* group wasps were not able to naturally emerge from figs. Males of this species did not present the behaviour of making an aperture in the fig wall, indicating that *I. flavicollis* group wasps depend on the pollinator male service of opening the exit hole.

OVIPOSITION MODE AND EGG DEPOSITION

Our results show that *I. carme* and *I. flavicollis* species groups have different strategies of colonizing figs and their larvae utilize distinct food resources. Wasps of *I. flavicollis* species group probe figs while they are receptive to pollinators [X (mean diameter) \pm SD: 0.98 \pm 0.9 cm, $N = 24$ figs]. Amongst the collected figs into which *I. flavicollis* wasps had inserted their ovipositors, two had not yet been entered by pollinating wasps, seven contained live pollinators and the other seven contained dead pollinators. However, in these seven figs, the ovules were not yet swollen showing that they had been pollinated at most one or two days before. *Idarnes carme* wasps probed significantly larger figs in which female flowers had already turned into galls and pollinator offspring was developing [X (mean diameter) \pm SD: 1.34 \pm 0.14 cm, $N = 28$ figs, $t_{43.6} = 12.04$, $P < 10^{-14}$]. *Idarnes flavicollis* group females presented an as-yet-undescribed mode of egg deposition for a NPFW (Fig. 1A, C). The ovipositor was inserted into flower ovaries through the flower stigma and style, following exactly the same path as the one followed by the ovipositors of the pollinating wasps

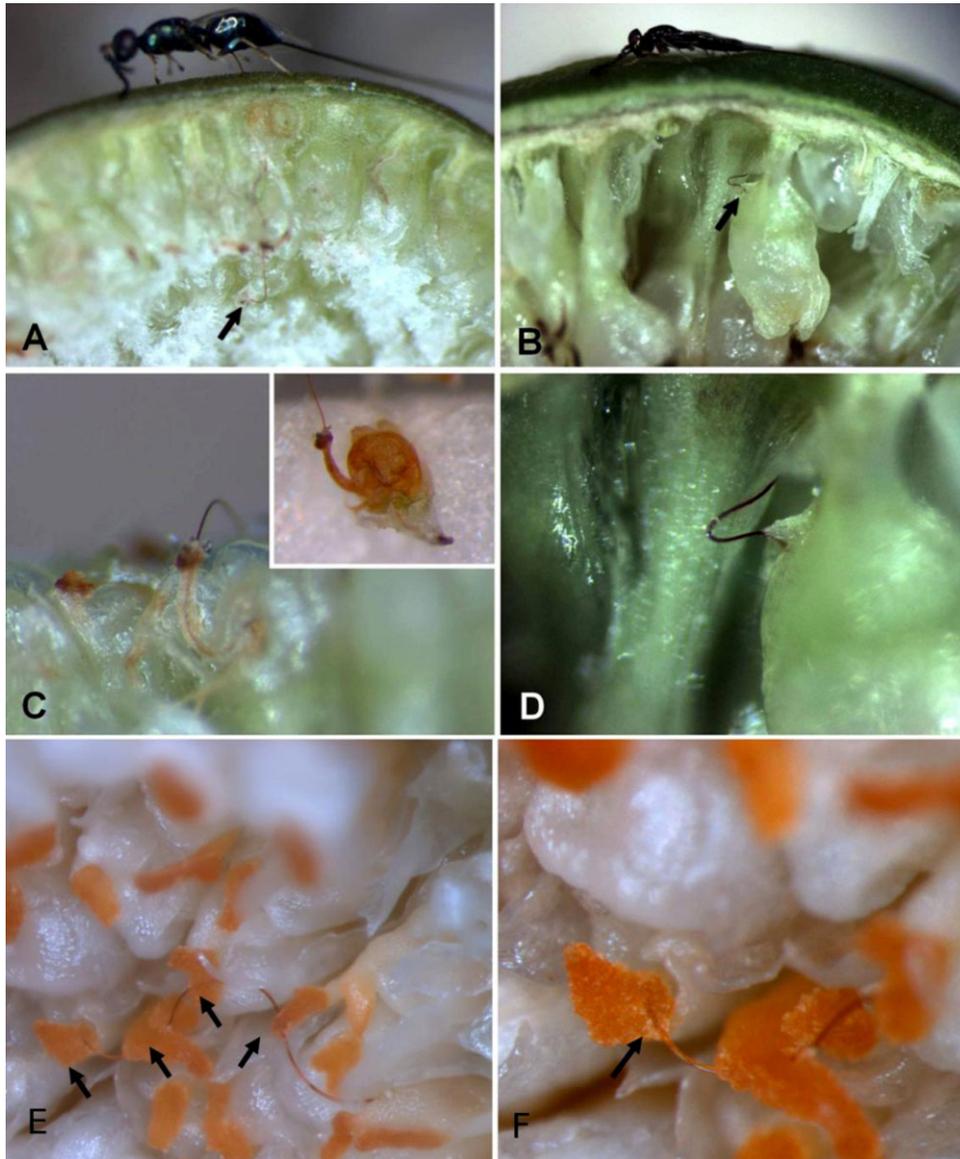


Figure 1. Oviposition by *Idarnes* species in *Ficus citrifolia* figs. A and C, oviposition by an *Idarnes flavicollis* female (receptive fig), note that the ovipositor is bent and inserted through the flower stigma (arrow in A). B and D, oviposition by an *Idarnes carme* female (fig with galls), note that the ovipositor is inserted through the gall wall (D and arrow in B). E, ovipositor of an *I. flavicollis* female inserted through several stigmas (arrows), for support. F, ovipositor inserted into a flower style through the stigma (arrow).

(Grandi, 1929; Galil & Eisikowitch, 1969). Ovipositors inserted into the style were recorded from both unpollinated and pollinated figs. As *Idarnes* oviposit from outside the fig, ovipositors had to be bent about 120° so that they could be inserted into the flower via the stigma (Fig. 1A, C, F). Histological sections revealed that *I. flavicollis* eggs were laid between the inner integument and the nucellus of female flower ovaries (Fig. 2A), in exactly the same location as are fig pollinator eggs (Grandi, 1966; Verkerke, 1989; S. J. Gonzalez, unpubl. data for *F. citrifolia*). To reach the

stigma of the flower into which the egg was laid, the ovipositor was introduced into the fig cavity and inserted through successive stigmas so that the ovipositor was held in place. In this species, as in other species of subgenus *Urostigma* section *Americana*, the flower stigmas form a platform but are separated from each other as the papillae are not fused: a synstigma *sensu stricto* is not formed. Hence the ovipositor has to be curved to bridge gaps between successive stigmas (Fig. 1E). *Idarnes carme* wasps insert their ovipositors straight through the gall wall and ovipositor bending

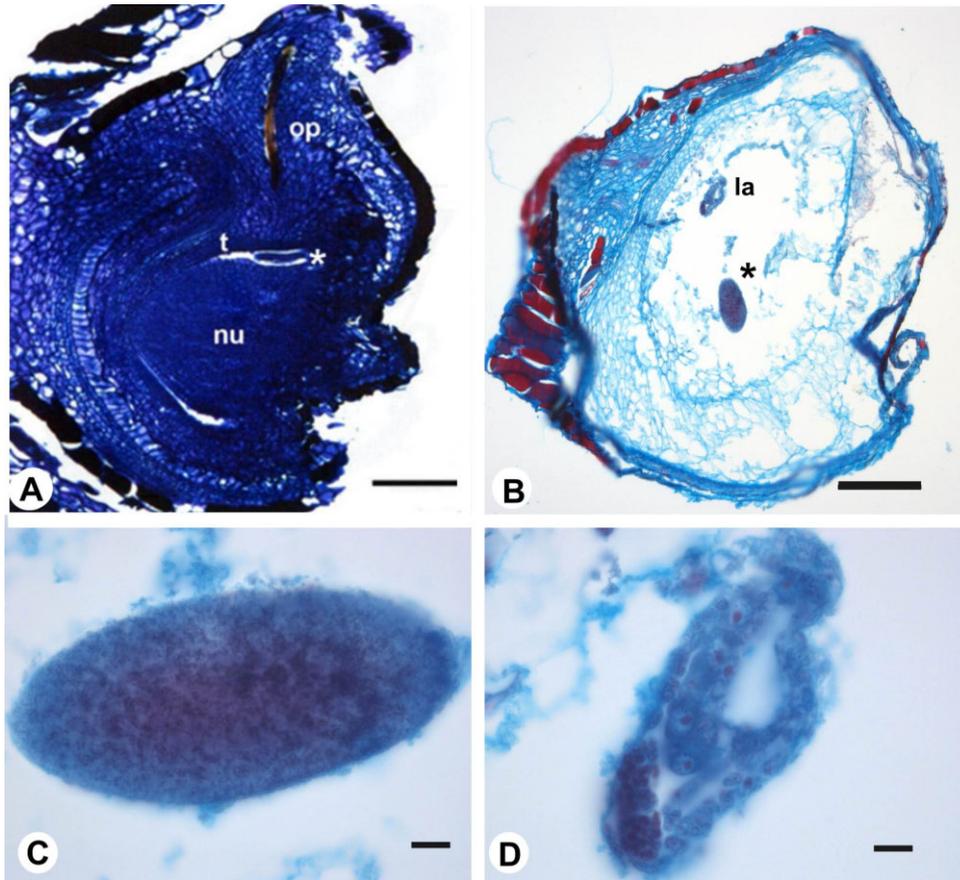


Figure 2. Microphotographs of flowers containing *Idarnes* eggs. A, unpollinated flower oviposited by an *Idarnes flavicollis* species group female, showing part of the ovipositor (op) and the egg (beside *) deposited between the nucellus (nu) and the inner integument (t). B, ovary of pollinated flower, oviposited by *Pegoscapus* sp. (la, larva) and by *Idarnes carme* sp. 1 (egg beside *). C, detail – *I. carme* species group egg. D, detail – *Pegoscapus* sp. larva. Scale bars: A, B = 100 μm ; C, D = 6 μm .

was not observed (Fig. 1B, D). Their eggs are laid inside the embryo sac cavity, where a pollinator larva is also located (Fig. 2B, C, D). These wasps are cleptoparasites or parasitoids of other wasps' larvae.

DISCUSSION

Our results provide the first non-ambiguous evidence for the presence of both gallers and cleptoparasites within *Idarnes*, a monophyletic genus (Cruaud *et al.*, 2011a). Indeed, although a series of observations suggested that *I. flavicollis* were gallers (Pereira *et al.*, 2007; Elias *et al.*, 2008), no direct evidence demonstrating this assertion had yet been published. Further, only 59% of figs only exposed to *I. flavicollis* aborted compared to over 90% aborted figs when pollinators were introduced without pollen (for Panama, Jander & Herre, 2010, for São Paulo state, R. A. S. Pereira, unpubl. data). This shows that *I. flavicollis* is more efficient at inhibiting fig abortion

than pollinators without pollen. Nevertheless, *I. flavicollis* has not been selected to limit abortion of unpollinated figs because its offspring do not cut exit holes from figs and hence cannot emerge from pollinator-free figs. We suggest that figs containing a sufficient number of flowers developing into galls or seeds will not abort (S. J. Gonzalez, unpubl. data). The pollinator of *F. citrifolia* systematically carries pollen (Jander & Herre, 2010) and may have developed the strategy of systematically using pollen to initiate endosperm development instead of galling flowers. Interestingly, *I. flavicollis* does not oviposit successfully in figs colonized 24 h before by the pollinator whereas the pollinator readily colonizes figs in which *I. flavicollis* oviposited 24 h before. The reasons for this asymmetry would be worth exploring. Similarly, although previous data on *I. carme* strongly suggested that these wasps were cleptoparasites (Elias *et al.*, 2008), this is the first time that the precise location of egg deposition has been observed,

confirming the hypothesis. A similar situation is found in another genus within subfamily Sycophaginae. Indeed, data strongly suggest that, on *F. racemosa*, *S. testacea* and *S. fusca* are galls and that *S. agragensis* is a cleptoparasite (or a parasitoid) (Wei *et al.*, 2005; Sun, Wang & Hu, 2008). These three wasp species belong to a different monophyletic lineage within Sycophaginae (Cruaud *et al.*, 2011a). The precise location of egg deposition is strikingly different between the two *Idarnes* species. *Idarnes carme* oviposits in the swollen embryo sac cavity whereas *I. flavicollis* lays its eggs in precisely the location where the pollinator would have laid its egg. Precise location of egg deposition is known for *Sycophaga sycomori*, another Sycophaginae species, which galls flowers at the time of fig receptivity. Surprisingly, *S. sycomori*, a wasp that enters figs as the pollinators do, does not introduce its ovipositor through the styles as does *I. flavicollis* but along them and deposits its egg in a quite different position, within the embryo sac (Galil *et al.*, 1970). This suggests that the behaviour of galling flowers at fig receptivity may have two evolutionary origins in the Sycophaginae. Nevertheless, both strategies mimic aspects of pollinating wasp behaviour. Indeed the pollinating wasp egg is deposited between the inner integument and the nucellus, just where *I. flavicollis* eggs are deposited, but upon hatching, the pollinator larva migrates rapidly into the embryo sac, to the location where the *S. sycomori* egg is deposited (Verkerke, 1987, 1989; S. J. Gonzalez, unpubl. data). These observations suggest that galling a receptive flower is a highly constrained process and may hence require mimicking the behaviour of the pollinator. The narrow temporal window for fig colonization by these galls and the extremely precise oviposition mechanism seen in these wasps confirm that inducing a gall is a rather complex achievement (Shorthouse, Wool & Raman, 2005) and must be under strong selective pressure. Comparing the two galling Sycophaginae also evidences how species may be locked into particular strategies because of their history. It would have been simpler in functional terms for *S. sycomori* to oviposit through the style as the pollinators do and for *I. flavicollis* to oviposit without having to pass through the style. This is not the case and *I. flavicollis* has evolved a highly sophisticated way of inserting its ovipositor into styles following a complex path. Verkerke (1987) suggested that the morphology of female flowers (disposition of the vascular bundle, fusion of ovary inner and outer integuments) was such as to guide the ovipositor of the pollinator so that the egg was deposited in a very precise location. We have seen here that *I. flavicollis* has evolved the striking feature of ovipositing through the flower stigma and style, even though

they probe the fig from outside, and lay their eggs exactly in the same place as the pollinator (S. J. Gonzalez, unpubl. data for the pollinator of *F. citrifolia*, Condit, 1932; Johri & Konar, 1956; Grandi, 1966 for other fig-pollinating wasps). This supports the suggestion by Verkerke (1987) that these morphological features have evolved to guide the ovipositor and are hence the product of the mutualism between *Ficus* and fig-pollinating wasps. The path followed by the *I. flavicollis* ovipositor illustrates the complex problem of how the length of an ovipositor can be bent so that its tip can explore the fig and the egg can be laid in a suitable location. The ovipositor is formed by three pieces of chitin clipped together and gliding with respect to each other, which is controlled by muscles within the gaster of the wasp (Snodgrass, 1993). As such, the ovipositor cannot follow complex paths if it is not anchored in tissues. This is seen in the path of the *I. flavicollis* ovipositor within the fig cavity: it is anchored in the flower stigmas. The bending path between stigmas suggests that the tip of the ovipositor is easily bent, a feature that also probably facilitates the proper positioning of the ovipositor to penetrate into styles. Little is known in NPFWs about how ovipositors are inserted and their actual paths within; available observations suggest large diversity amongst wasp species. Observations on two species of cleptoparasitic *Philotrypesis* that oviposit soon after receptivity show that to reach an ovary the ovipositor is inserted into the flower through its pedicel (Y. Dubois, unpubl. data; Compton & Nefdt, 1988). In another cleptoparasite or parasitoid associated with *Ficus burtt-davyi*, *Sycoryctes* sp., the path of the ovipositor may go through an ovary to enter another one or go through the basal bracts (Compton & Nefdt, 1988). *Sycoryctes* sp. always oviposits late in fig development, on average much later than the *Philotrypesis* associated with figs of the same *Ficus* species. We may surmise that once flowers are well developed, the path followed by the ovipositor is much less constrained: flowers are more densely packed together and flower abortion risks have become small. Finally, *Watshamiella* spp. guard ovipositing females of other NPFW genera, and insert their ovipositor into exactly the same location as the wasp they guarded, suggesting that the ovipositor will follow exactly the same path as that of the previous wasps, allowing easy parasitism (Compton *et al.*, 2009). Indeed, the fig-associated wasps system is a hugely versatile model (Herre *et al.*, 2008). Documenting the actual feeding regime of NPFWs will enable us to define better the costs that they impose on the mutualism between figs and fig-pollinating wasps, as the correlative approach easily leads to misleading inferences (West *et al.*, 1996).

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REFERENCES

- Boucher DH, James S, Keeler KH. 1982.** The ecology of mutualism. *Annual Review of Ecology and Systematics* **13**: 315–347.
- Bronstein JL. 1992.** Seed predators as mutualists: ecology and evolution of the fig pollinator interaction. In: Bernays E, ed. *Insect-plant interactions*. Boca Raton, FL: CRC Press, 1–43.
- Bronstein JL. 2001.** The exploitation of mutualisms. *Ecology Letters* **4**: 277–287.
- Compton SG, Nefdt R. 1988.** Extra-long ovipositors in Chalcid wasps: some examples and observations. *Antenna* **8**: 102–105.
- Compton SG, van Noort S, McLeish M, Deeble M, Stone V. 2009.** Sneaky African fig wasps that oviposit through holes drilled by other species. *African Natural History* **5**: 9–15.
- Condit IJ. 1932.** The structure and development of flowers in *Ficus carica* L. *Hildargia* **6**: 443–481.
- Cruaud A, Jabbour-Zahab R, Genson G, Couloux A, Peng YQ, Yang DR, Ubaidillah R, Pereira RAS, Kjellberg F, van Noort S, Kerdelhué C, Rasplus JY. 2011a.** Out-of-Australia and back again: the worldwide historical biogeography of non-pollinating fig wasps (Hymenoptera: Sycophaginae). *Journal of Biogeography* **38**: 209–225.
- Cruaud A, Jabbour-Zahab R, Genson G, Kjellberg F, Kobmoo N, van Noort S, Yang DR, Peng YQ, Ubaidillah R, Hanson PE, Santos-Mattos O, Farache FHA, Pereira RAS, Kerdelhué C, Rasplus JY. 2011b.** Phylogeny and evolution of life-history strategies in the Sycophaginae non-pollinating fig wasps. *BMC Evolutionary Biology* **11**: 178–192.
- Elias LG, Menezes AO Jr, Pereira RAS. 2008.** Colonization sequence of non-pollinating fig wasps associated with *Ficus citrifolia* in Brazil. *Symbiosis* **45**: 107–111.
- Galil J, Dulberger R, Rosen D. 1970.** The effects of *Sycophaga sycomori* L. on the structure and development of the synconia in *Ficus sycomorus* L. *New Phytologist* **69**: 103–111.
- Galil J, Eisikowitch D. 1969.** Further studies on the pollination ecology of *Ficus sycomorus* L. (Hymenoptera, Chalcidoidea, Agaonidae). *Tijdschrift voor Entomologie* **112**: 1–13.
- Gerlach D. 1969.** *Botanische mikrotechnik*. Stuttgart: Georg Thieme Verlag.
- Grandi G. 1929.** *Studio morfologico e biologico della Blastophaga psenes*. Bologna: Societa Tipografica Gia Compositori.
- Grandi G. 1966.** Simbiosi mutualistiche intercorrenti fra i *Ficus* e i Calcidoidei Agaonidi. In: Grandi G, ed. *Instituzioni di Entomologia Generale*. Bologna: Calderini, 327–333.
- Herre EA, Jander KC, Machado CA. 2008.** Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annual Review of Ecology, Evolution and Systematics* **39**: 439–458.
- Jander KC, Herre EA. 2010.** Host sanctions and pollinator cheating in the fig tree-fig wasp mutualism. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **277**: 1481–1488.
- Johansen DA. 1940.** *Plant microtechnique*. New York: McGraw-Hill Book Company Inc.
- Johri BM, Konar RN. 1956.** The floral morphology and embryology of *Ficus religiosa* Linn. *Phytomorphology* **6**: 97–111.
- Joseph K. 1958.** Recherches sur les chalcidiens, *Blastophaga psenes* (L.) et *Philotrypesis caricae* (L.), du figuier (*Ficus carica* L.). *Annales des Sciences Naturelles, Zoologie* **11^e serie**: 197–260.
- Jousselin E, Hossaert-McKey M, Vernet D, Kjellberg F. 2001.** Egg deposition patterns of fig pollinating wasps: implications for studies on the stability of the mutualism. *Ecological Entomology* **26**: 602–608.
- Jousselin E, Rasplus JY, Kjellberg F. 2001.** Shift to mutualism in parasitic lineages of the fig/fig wasp interaction. *Oikos* **94**: 287–294.
- van Noort S, Compton SG. 1996.** Convergent evolution of agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. *Journal of Biogeography* **23**: 415–424.
- Pellmyr O. 2003.** Yuccas, yucca moths and coevolution: a review. *Annals of the Missouri Botanical Garden* **90**: 30–35.
- Pellmyr O, Leebens-Mack J, Huth CJ. 1996.** Non-mutualistic yucca moths and their evolutionary consequences. *Nature* **380**: 155–156.
- Pereira RAS, Teixeira SP, Kjellberg F. 2007.** An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits? *Biological Journal of the Linnean Society* **92**: 9–17.
- Ronquist F. 1999.** Phylogeny, classification and evolution of the Cynipoidea. *Zoologica Scripta* **28**: 139–164.
- Sachet JM, Poncet B, Roques A, Després L. 2009.** Adaptive radiation through phenological shift: the importance of the temporal niche in species diversification. *Ecological Entomology* **34**: 81–89.
- Schluter D. 2000.** *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Shorthouse JD, Wool D, Raman A. 2005.** Gall-inducing insects – nature’s most sophisticated herbivores. *Basic and Applied Ecology* **6**: 407–411.
- Snodgrass RE. 1993.** *Principles of insect morphology*. London: Cornell University Press.
- Sun B, Wang R, Hu Z. 2008.** Diet segregation of fig wasps and the stability of fig-fig wasp mutualism. *Biodiversity Science* **16**: 525–532.
- Tzeng HY, Tseng LJ, Ou CH, Lu KC, Lu FY, Chou LS.**

- 2008.** Confirmation of the parasitoid feeding habit in *Sycoscapter*, and their impact on pollinator abundance in *Ficus formosana*. *Symbiosis* **45**: 129–134.
- Verkerke W. 1987.** Syconial anatomy of *Ficus asperifolia* (Moraceae), a gynodioecious tropical fig. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C* **90**: 461–492.
- Verkerke W. 1989.** Structure and function of the fig. *Experientia* **45**: 612–622.
- Wang RW, Zheng Q. 2008.** Structure of a fig wasp community: temporal segregation of oviposition and larval diets. *Symbiosis* **45**: 113–116.
- Wei ZD, Peng YQ, Xu L, Yang DR. 2005.** Impact of *Oecophylla smaragina* on the percentage number of offspring of pollinator and nonpollinating wasps on *Ficus racemosa*. *Zoological Research* **26**: 386–390.
- Weiblen GD. 2002.** How to be a fig wasp. *Annual Review of Entomology* **47**: 299–330.
- West SA, Herre EA, Windsor DM, Green PRS. 1996.** The ecology and evolution of the New World non-pollinating fig wasp communities. *Journal of Biogeography* **23**: 447–458.
- Yu DW. 2001.** Parasites of mutualisms. *Biological Journal of the Linnean Society* **72**: 529–546.
- Zhang FP, Peng YQ, Compton SG, Zhao Y, Yang DR. 2009.** Host pollination mode and mutualist pollinator presence: net effect of internally ovipositing parasite in the fig–wasp mutualism. *Die Naturwissenschaften* **96**: 543–549.