

An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits?

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The processes allowing evolutionary transitions in resources used by parasitic wasps are largely unknown. Microhymenopteran communities associated with figs could provide a model system to investigate such transitions. We investigate here a species of genus *Idarnes*. The larvae generally develop as inquilines of the pollinating wasp larvae. However, in figs where the parasitic pressure is high, eggs are laid in developing seeds. These eggs turn into small males. This is the first report of seed consumption by a fig wasp. Using an alternative resource to produce small males could provide a pathway to select for increased ability to use this resource and hence provide an intermediate step for evolving the capacity to use new resources. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, 92, 9–17.

ADDITIONAL KEYWORDS: Agaonidae – *Ficus* – Hymenoptera – Moraceae – plant–insect interaction.

INTRODUCTION

Parasitica wasps contain a number of economically important seed eaters and parasitoids. Very little is known, however, on shifts in wasp feeding regimes. When consulting large taxonomic reviews, such as 'Australasian Chalcidoidea (Hymenoptera)' (Bouček, 1988), it is clear that there must have been quite a number of transitions in feeding regime from parasitoids to gallers or to seed eaters or reciprocally. Fine studies of particular groups are largely wanting (Ronquist, 1999), with the notable exception of Cynipidae. Phylogenies show that they were ancestrally parasitoids and that galling on plants evolved once. Within the galling clade, a monophyletic subgroup has become inquilines (Ronquist & Liljeblad, 2001).

The genus of *Ficus* may provide a good model system not only to illustrate the occurrence of shifts in wasp feeding regime over evolutionary times, but also

to document ongoing processes. *Ficus* offer an extreme example of an obligate mutualism between plant and insect with a community of associated wasps parasitic on the system (Weiblen, 2002). Each of the some 750 extant *Ficus* species is pollinated by one or several tiny species-specific agaonid wasps (Agaonidae; Chalcidoidea, Hymenoptera) (Cook & Rasplus, 2003; Molbo *et al.*, 2003). Female agaonid wasps: (1) enter the syconia (the urn-shaped *Ficus* inflorescences, with the flowers placed internally; hereafter referred to as the fig) through the ostiole (a pore, the unique connection between the inner and outer environments, more or less tightly closed by bracts); (2) oviposit into the ovaries of some of the female flowers; and (3) simultaneously pollinate (Jousselin *et al.*, 2001, 2003; Weiblen, 2002). Thus, sexual reproduction of the plant depends on the pollination services of agaonid fig wasps. On the other hand, each agaonid larva develops within a single galled fig ovule.

The sequence of fig development leads to a progressive shift in available resources. Gallers oviposit early

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in figs up to fig receptivity but space constraints probably prohibit later oviposition: the ovules swell rapidly after pollination so that there is no space left for new galls to develop. Indeed, the largest wasps are species ovipositing well before receptivity, enabling galls to become very large (Cook & Rasplus, 2003; Kjellberg *et al.*, 2005). It is reasonable to assume that wasps ovipositing after fig pollination should be inquilines or parasitoids because undeveloped ovules are withering.

Fig wasps are generally thought to use a single resource for their development, although it has been suggested that some inquilines could use galls initiated by different wasp species on the same fig host (Compton, Rasplus & Ware, 1994). Intense sexual selection leading to male fighting is largely documented in fig wasps and, in this context, selection for male size dimorphism is known to occur in some fig wasps (Bean & Cook, 2001; Pereira & Prado, 2005). In such species, wasps could be selected to lay male eggs in lower quality resources, producing sneaky small males in the process. Such a production of small males would broaden the range of resources available to the wasps and could constitute an initial transitory step towards adaptation to the use of a new resource. Indeed, in solitary parasitoids, it has been demonstrated that females should lay sons in lower quality hosts (small hosts) and that there is a positive correlation between host size and wasp size (King, 1987). In the present study, we document for the first time seed predation by fig wasps. Seeds appear to be used as a low quality resource to produce small males.

In figs sampled during April 2003, from a tree of *Ficus citrifolia* growing in the Universidade de São Paulo campus, Ribeirão Preto, São Paulo state (21°10'S; 47°48'W), we observed some males of *Idarnes* sp. (*carne* group) developing within structures that resembled fig seeds (hereafter referred to as bored structures) and that differed from the galls from which they usually emerge. These structures were eventually also observed in samples of *F. citrifolia* collected in trees located at two other places, Universidade Estadual de Londrina campus, Londrina, Paraná state (23°18'S; 51°09'W) and Universidade Estadual de Campinas campus, Campinas, São Paulo state (22°54'S; 47°03'W); but had been confused with seeds.

Therefore, to verify that facultative seed predation was really involved, we performed a comparative anatomical study on these: (1) bored structures, (2) intact seeds; and (3) normal galls to assess whether wasps were developing within atypical galls or feeding on intact seeds. We also analysed quantitative data of wasp emergences and numbers of intact seeds and bored structures per fig to determine: (1) whether males that feed on seed are on average smaller in size (seeds as lower quality resources?) and (2) whether

seed predation is density dependent, occurring in figs presenting high densities of inquilines when better resources (pollinator galls) to exploit are scarce.

MATERIAL AND METHODS

STUDY SPECIES

In Brazil, *F. citrifolia* is pollinated by *Pegoscapus tonduzi* and associated with 14 nonpollinating chalcid wasp species (referred to as *Ficus eximia*; Pereira, Semir & Menezes, 2000). Among these nonpollinating species, genus *Idarnes* is the most represented, presenting three species with wingless males (two of the *carne* and one of the *flavicollis* group) and one species with winged males (*incerta* group). We have data showing that although the *Idarnes flavicollis* group and the *Idarnes incerta* group species are effectively phytophagous gallers, the *Idarnes carne* group species are inquilines or parasitoids (L. G. Elias & R. A. S. Pereira, unpubl. data). Species of the *flavicollis* and *incerta* groups colonize figs just before or simultaneously with pollinating foundresses and are able to develop in experimental pollinator-excluded figs. *Idarnes carne* group wasps colonize figs after pollination and were observed to oviposit in galls initiated by pollinating wasps (R. A. S. Pereira, unpubl. observ.). In the present study, we investigated seed predation by an *Idarnes* species belonging to the *carne* group, hereafter referred to as *Idarnes* sp. Voucher specimens have been preserved for future identification in a revision of the genus.

COMPARATIVE ANATOMICAL STUDY

We sampled a number of intact seeds, normal galls and bored structures from figs just after adult wasp emergence of a *F. citrifolia* tree growing in the Universidade de São Paulo campus, Ribeirão Preto, and preserved them in 70% alcohol for later examination by scanning electron microscopy and histological comparisons.

Additionally, to determine the stage of development of galls, pollinator larvae and seeds at the time of *Idarnes* sp. oviposition, we obtained histological serial sections in a sample of figs that *Idarnes* sp. females were ovipositing.

Material for scanning electron microscopy was dehydrated in ethanol–acetone series, critical-point dried in a Balzers CPD 030 apparatus, then mounted on metallic stubs with colloidal graphite and coated with gold in a Balzers SCD 050 sputter coater for 280 s. Material was observed and micrographs made with a Zeiss DSM 940 at 10 kV.

We made serial sections after gradual dehydration in alcoholic series, softening in a terpeniol solution during one week, and embedding in paraffin

(Johansen, 1940). Section thickness was 4–6 μm . We used 0.05% Toluidine Blue and Hematoxylin-Eosin to stain the cell walls and to detect phenolic compounds (O'Brien, Feder & McCully, 1964), and periodic acid Schiff (PAS) reagent to detect polysaccharides (Jenzen, 1962) in the nutritive tissues. Permanent slides were mounted in synthetic resin (Gerlach, 1969). Photomicrographs were taken using a Leica DML microscope.

QUANTITATIVE DATA COLLECTION AND ANALYSIS

We randomly collected 25 figs from a tree of *F. citrifolia* growing in the Universidade de São Paulo campus, Ribeirão Preto, prior to wasp emergence and employed usual techniques of fig wasp studies. They consisted of individualizing the figs in plastic flasks and allowing all the wasps to emerge before freezing for conservation and later counts. For each fig, we counted the numbers of wasps of each species, intact seeds, and bored structures. We measured fig diameter (estimate of fig size) and head width (estimate of wasp size) of each wingless male of *Idarnes* to the nearest 0.029 mm under a stereoscopic microscope. We also measured mesosoma length of females of *Idarnes* reared in figs with the highest number of bored structures (five figs with 19 or more bored structures each) and with the lowest number of bored structures (five figs with three or less bored structures each) to verify whether female sizes were associated with the occurrence of these bored structures. We used head width in males and mesosoma length in females because we have previously found on other fig wasps species that these measurements are: (1) among those that correlate best with the size of other organs and (2) are easy to measure on damaged wasps after their emergence or on wasps that have not yet emerged from their galls, thus reducing the risks of sample biases.

In fig samples in which bored structures were found, we observed an over abundance of small wingless *Idarnes* males. We therefore used a series of linear models (LM) to determine the sizes of males that best explained the number of bored structures. We started with a multiple regression of number of bored structures (response variable) vs. number of males of each measured size (determined by the micrometric ruler accuracy: 0.171, 0.2, 0.229, 0.257, 0.286, 0.314, 0.343, 0.371, and 0.4 mm in head width) per fig. Then, using the simplifying assumption that all males from smaller sizes than the best correlated one in the previous analysis had also developed in seeds, we grouped these classes together and repeated the analysis to determine the simplest but equally good model to explain the number of bored structures. To verify whether using this male size threshold, we obtained a good approximation of the number of males that devel-

oped in seeds, we tested by paired *t*-test (Zar, 1996) whether the number of males up to this size differed from the number of bored structures. In addition, to sustain the idea that two types of resources of different quality were used, we tested whether the distribution of head width of all males differed from a unimodal distribution, using the DIP statistic (Hartigan & Hartigan, 1985) in the ODDJOB software, version 6.5 (Dallal, 1989).

We examined whether the utilization of seeds by *Idarnes* sp. occurred preferentially in figs with high densities of inquilines. To achieve this, and assuming that *Idarnes* sp. is an inquiline of *P. tonduzi* and estimating the number of males that had developed in bored structures, we created a variable that expressed the parasitic pressure per fig (the ratio inquiline *Idarnes*/galls available to parasitize). Where: (1) inquiline *Idarnes* = total number of *Idarnes* sp. less the number of *Idarnes* sp. males that had developed feeding on seeds and (2) galls available to parasitism = total number of *P. tonduzi* + number of inquiline *Idarnes*. We modelled the proportion of bored structures [number of bored structures/(number of bored structures + number of intact seeds) – response variable] as a function of the parasitic pressure, number of *I. flavicollis* group individuals and number of other fig wasp species per fig (explanatory variables). Fig diameter was included as a covariate but, because its effect was not significant, we excluded it from the final model. We used a general linear model (GLM) with binomial errors and the total number of seeds (intact + bored) as binomial denominator (Crawley, 1993). We performed all LM and GLM analyses using the statistical package S-Plus, version 6.1 (Insightful Corporation).

RESULTS

COMPARATIVE ANATOMICAL STUDY

At fig maturity, the morphological features of the bored structures and ovules presenting intact seeds were very similar, and strikingly different from the galls from which *Idarnes* sp. usually emerged (Fig. 1A, B, C). Bored structures were darker than intact seeds. We observed a single male of *Idarnes* sp. developing per bored structure (Fig. 1B). Seeds (Fig. 2A), bored structures (Fig. 2B) and galls (Fig. 2C) consisted of a lumen limited by layers of different tissues. These tissues were similar in all structures in terms of constituent cells, but differed in their arrangement around the lumen and number of cell layers.

At fig maturity, in the mature galls (Fig. 2C), the cells of the exocarp (outer epidermis) were hypertrophied, thick-walled and without phenols, different from seed exocarp cells which were rectangular, thin walled and with phenolic content. Below the outer epidermis were four to six layers of parenchymatic and

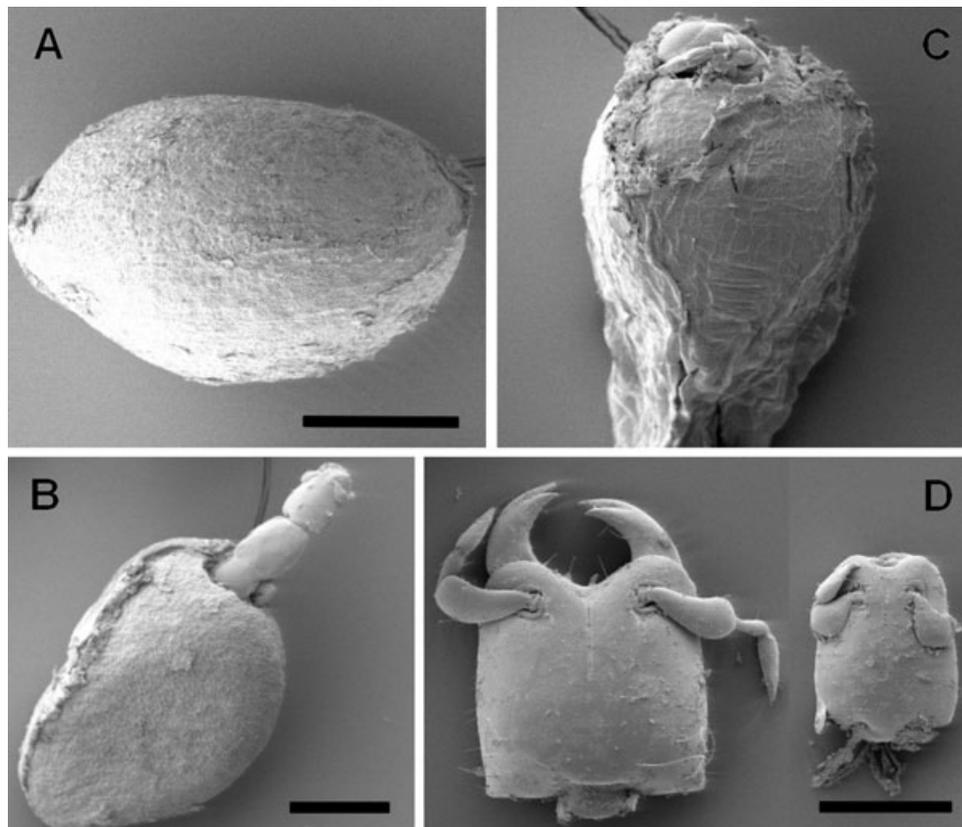


Figure 1. Scanning electromicrographs of intact seeds (A), bored structure with a small male of *Idarnes* sp. emerging (B), *Idarnes* sp. gall showing part of female head (C) and heads (same scale) of large and small males of *Idarnes* sp. (D) sampled in *Ficus citrifolia* figs. Scale bar: A, B, C = 0.5 mm; D = 0.25 mm.

phenolic cells and four to five layers of sclereids, which were similar to those found in the seeds. The prismatic crystals within this layer differed in their position compared with the seeds, occurring in the endocarp median layer. The gall inner layer had smaller and thinner-walled cells, also observed inside the lumen. The seed coat, consisting of phenolic cell layers and additional layers of parenchymatic cells, was not observed in the gall. An embryo with two fleshy cotyledons and endosperm cells occupied all of the seed cavity whereas some larval cells were found inside the gall lumen. Endosperm and embryo cells contained starch grains (Fig. 2A), evidenced by the PAS reagent. The anatomic structure of the bored structure was very similar to that of seeds (Fig. 2A, B). Remains of plant embryo tissues were found inside the lumen, indicating that a plant embryo had been present.

At the stage when females of *Idarnes* sp. were observed to oviposit, the developing seeds showed a globular embryo surrounded by endosperm cells at the beginning of their nuclear development, and few nucellar cells (Fig. 2D). The developing galls showed the lumen surrounded by several layers of hypertrophied parenchymatic cells with no starch grains

(Fig. 2E), produced simultaneously by a group of meristematic cells (Fig. 2F) that we observed near the vascular bundle. These parenchymatic cells (nutritive tissue) were apparently the resource available to the larva for its development.

SIZE OF MALES THAT DEVELOPED FEEDING ON SEEDS

Idarnes sp. males of the 0.257 mm in head size correlated best with the number of bored seeds, explaining 24% of the total variance (Table 1A). After pooling all males ≤ 0.257 mm, the remaining male sizes no longer correlated with the number of bored seeds (Table 1B). Therefore, we obtained the simplest model with two size variables (number of males ≤ 0.257 mm, explaining 39% of total variance of number of bored seeds, and males ≥ 0.286 mm nonsignificantly correlated; Table 1C). The mean \pm SD number of males up to 0.257 mm corresponded to the number of bored seeds (males = 10.1 ± 8.1 ; bored seeds: 11.1 ± 7.5 ; $t_{24} = -0.9$; $P = 0.387$).

The head size distribution of the 609 measured males was not unimodal (DIP statistic = 0.122; $P < 10^{-3}$). In figs presenting almost no consumed seeds

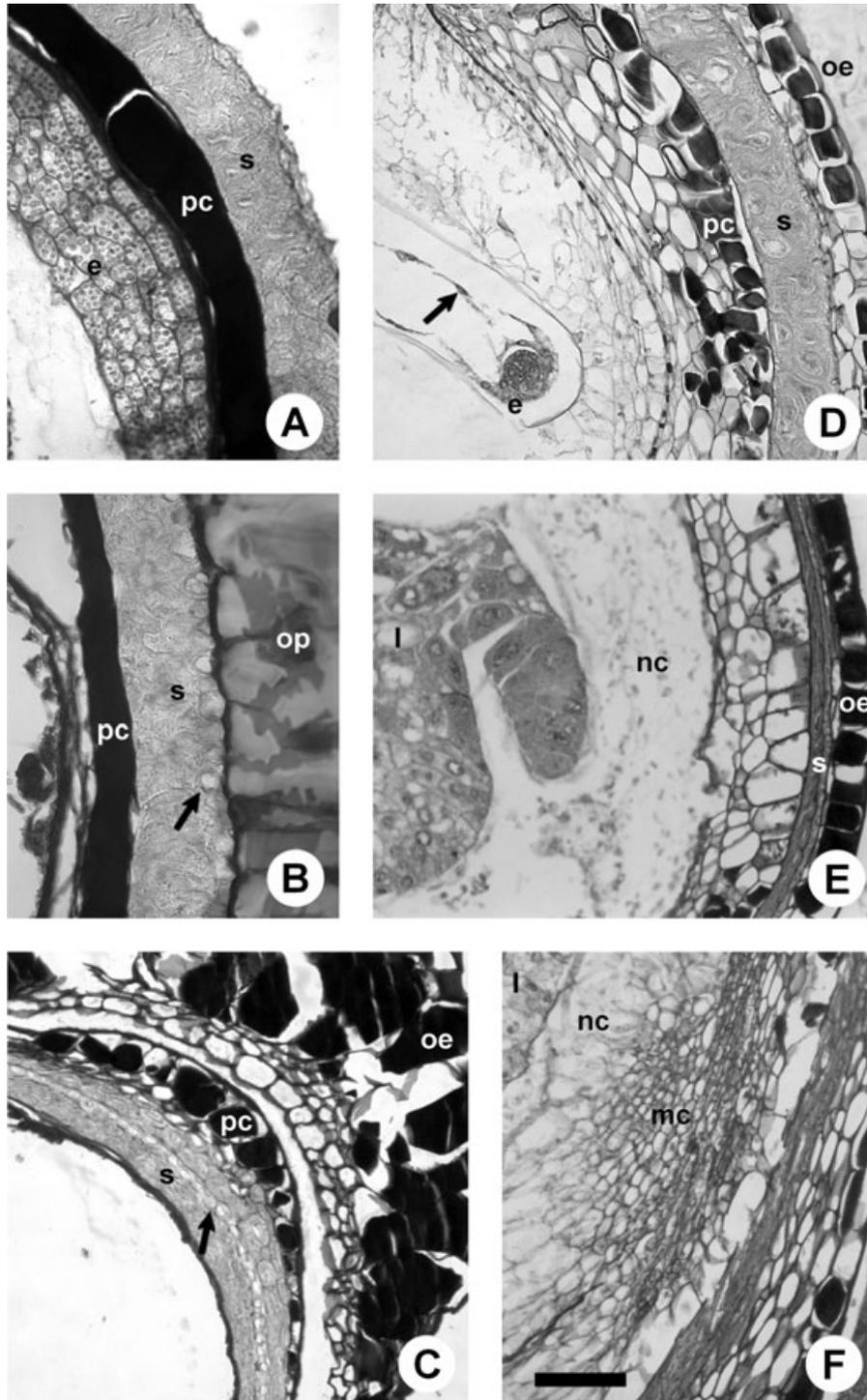


Figure 2. Longitudinal sections of *Ficus citrifolia* seed, bored structure and gall. A, B, C, at fig maturity; D, E, F, at the stage of *Idarnes* sp. oviposition. A, intact seed. Note the starch grains in embryonic cells (e). Outer epidermis was crushed. B, bored structure. Note the crystalliferous sclereids (arrow) in the outer layer. C, gall. Note the crystalliferous sclereids in the median layer (arrow). D, developing seed at the stage when *Idarnes* sp. oviposit. Note the globular embryo (e) and the first divisions of endospermic nuclei (arrow). E, developing gall at the stage when *Idarnes* sp. oviposit. Note the nutritive cells (nc) surrounding the larvae (l). F, detail of the meristematic cell group (mc) that appears to produce nutritive cells (nc). e, embryonic cells; l, larvae; mc, meristematic cells; nc, nutritive cells; oe, outer epidermis; pc, phenolic cells; s, sclereids. Scale bar: A, B, C = 120 μm ; D, E = 80 μm ; F = 86.5 μm .

Table 1. Series of linear model analyses used to explain the number of bored seeds in function of the number of males of different sizes

Factors: head size (mm)	Standardized coefficient	<i>t</i>	Percent of variance*	<i>P</i>
(A) R^2 adj. = 0.635; $F_{9,15} = 5.637$; $P = 0.002$				
0.171	-0.023	-0.173	0.05	0.865
0.200	0.234	1.406	3.01	0.180
0.229	0.430	2.636	10.57	0.019
0.257	0.923	3.967	23.94	0.001
0.286	0.505	2.289	7.97	0.037
0.314	-0.907	-2.697	11.07	0.017
0.343	0.413	2.262	7.78	0.039
0.371	-0.043	-0.242	0.09	0.812
0.400	0.305	1.619	3.99	0.126
(B) R^2 adj. = 0.552; $F_{6,18} = 5.933$; $P = 0.001$				
Small (≤ 0.257)	0.845	4.705	41.29	$< 10^{-3}$
0.286	0.223	1.083	2.19	0.293
0.314	-0.296	-1.153	2.48	0.264
0.343	0.218	1.179	2.59	0.254
0.371	0.090	0.490	0.45	0.630
0.400	0.069	0.391	0.28	0.701
(C) R^2 adj. = 0.567; $F_{2,22} = 16.7$; $P < 10^{-4}$				
Small (≤ 0.257)	0.684	4.655	39.10	$< 10^{-3}$
Large (≥ 0.286)	0.184	1.256	2.85	0.222

A, initial model using all observed sizes; B, males ≤ 0.257 mm were lumped together, assuming that all had developed as borer; C, nonsignificant classes were lumped together as larger males (≥ 0.286 mm in head width).

N, sample size (25 figs).

*Change in R^2 caused by the removal of the factor from the model.

(three or less bored seeds), only 28% of the males were smaller than 0.257 mm in head width, vs. 49% in figs with 19–27 consumed seeds (Fig. 3A, B), whereas the distribution of mesosoma length of females reared these two groups of figs were very similar (Fig. 3C, D). Hence, the shape of the distributions of head sizes and observational data (Fig. 1B, D) suggests that, among the insects produced, only males developed on seeds and that these males were on average smaller than males reared in galls.

RESOURCE AVAILABILITY VS. SEED UTILIZATION

Table 2 summarizes the main characteristics of the study sample. This sample showed high nonpollinating wasp infestation (mainly *Idarnes* sp.) and low abundance of *P. tonduzi* compared with approximately 200 pollinating wasps commonly observed in figs with low nonpollinating wasp infestations (R. A. S. Pereira, unpubl. observ.).

The proportion of bored seeds correlated positively with inquiline parasitic pressure ($P < 10^{-3}$; Table 3). The proportion of bored seeds was negatively correlated with the number of *I. flavicollis* group individuals developing within a fig and did not correlate with numbers of other wasps (Table 3).

DISCUSSION

The present study is the first report of any fig wasp feeding on seeds and it is also the first report of fig wasps feeding on two different types of resources. Indeed, the anatomic structure we observed and the differences between wasp galls and seeds are very similar to those described by Verkerke (1986) in a highly detailed histological analysis. The bored structures presented tissue structures very similar to those of intact seeds, but anatomically different from gall tissues. This leads us to conclude that the bored structures were effectively young seeds on which larvae of the studied *Idarnes* species had fed. The quantitative data set shows that this species develops both feeding on seeds and as an inquiline on galled flowers. The inquiline status is in agreement with the species of the *Idarnes carme* group being observed to arrive several days after pollinators, when the female flowers that were pollinated or transformed into galls had started enlarging. At that stage, no space is left for new gall development and this confirms that these wasps when developing in galled flowers cannot be primary gallers. Moreover, the negative impact that *Idarnes* exerts over pollinator brood (Pereira, 1998) confirms that these species are pollinator inquilines.

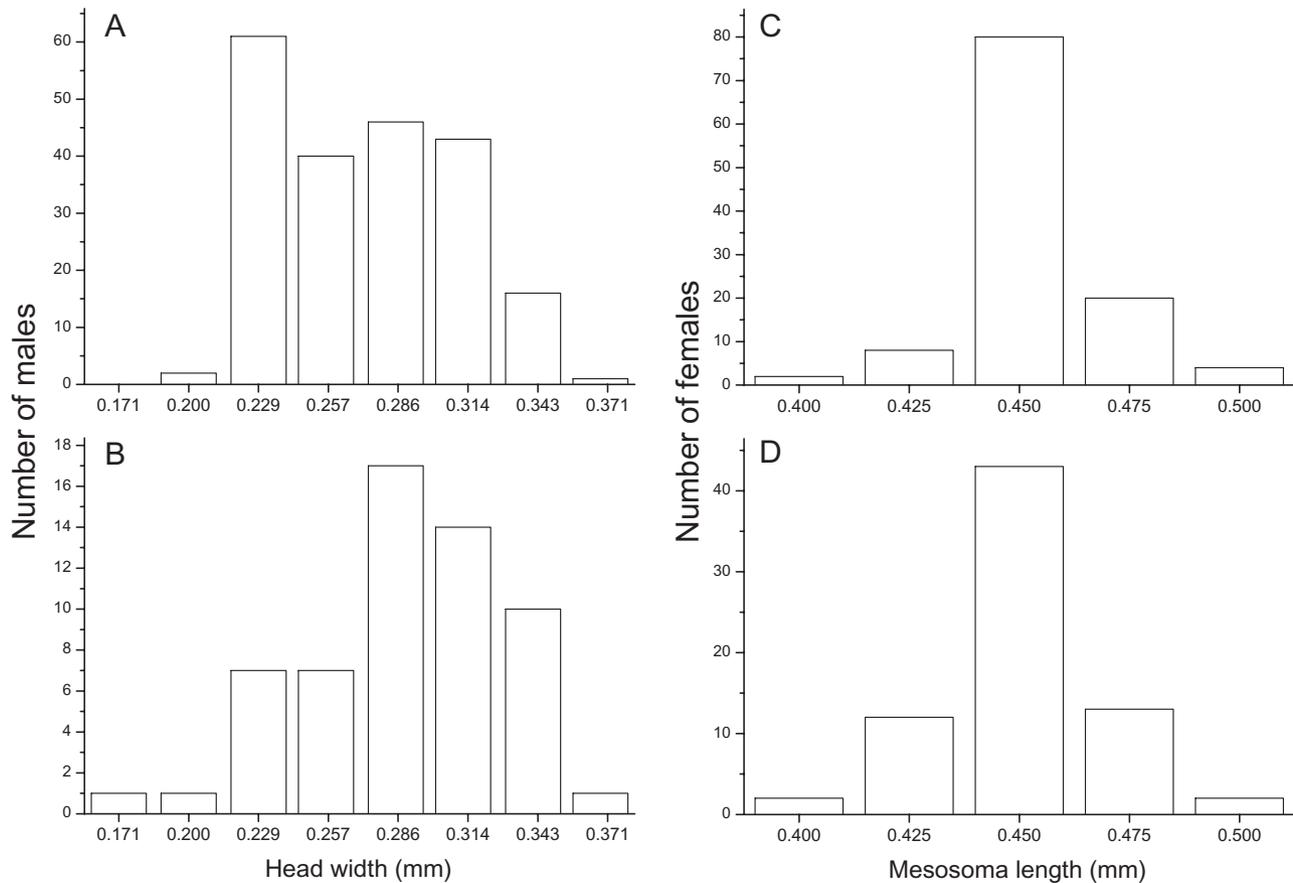


Figure 3. Distributions of head width of males (A, B) and mesosoma length of females (C, D) of *Idarnes* sp. reared in figs with the highest number of bored seeds (110 bored seeds in five figs; sample sizes: A = 209 males; C = 114 females) and with the lowest number of bored seeds (seven bored seeds in five figs; sample sizes: B = 58 males; D = 72 females).

Table 2. Characteristics of the figs sampled in a crop of *Ficus citrifolia* in Ribeirão Preto

Variables	Mean per fig	Standard deviation	Minimum – maximum
Intact seeds	96.8	37.9	38–172
Bored seeds	11.1	7.5	0–27
Small males of <i>Idarnes</i> (c)*	10.1	8.1	0–31
<i>Pegoscapus tonduzi</i>	31.5	30.7	0–138
<i>Idarnes</i> (c)	44.8	23.5	15–108
<i>Idarnes</i> (f)	4.8	11.6	0–56
Other species	21.1	13.8	0–57
Parasitic pressure†	0.58	0.17	0.21–1

(c), *carne* group; (f), *flavicollis* group.

*Number of males ≤ 0.257 mm in head width.

†Proportion of pollinator galls in fact occupied by *Idarnes* sp. (see Materials and methods).

Sample size = 25 figs.

We only observed males developing in seeds and not females. Furthermore, although the presence of bored seeds was associated with more small males being produced, there was no relationship between presence of bored structures and females size. This suggests that

either founding females only laid male eggs in seeds or that female larvae did not manage to complete their development in seeds. The reduced size of males developing on seeds is probably due to resource limitation in terms of quantity or quality. Oviposition occurs at a

Table 3. Results of the general linear model analysis used to explain the proportion of bored seeds in function of 'parasitic pressure' (see Materials and Methods) and occurrence of other nonpollinating fig wasps

Factors	Slope	d.f.	χ^2_*	<i>P</i>
Parasitic pressure	1.376	1	14.032	$< 10^{-3}$
<i>Idarnes (flavicollis)</i>	-0.029	1	10.778	0.001
Other species	0.001	1	0.019	0.891

N, sample size (25 figs); d.f., degrees of freedom.

*Change in deviance caused by the removal of the factor from the full model.

Model statistics: $R_L^2 = 0.278$, $\chi^2_3 = 26.5$, $P < 10^{-5}$.

stage when the embryo and endosperm of *F. citrifolia* seeds are relatively early in their development, and therefore their cells most probably do not contain large amount of reserves, whereas gall development has already been initiated by the original galler when *Idarnes* sp. oviposits. Furthermore, the galls' inner tissue layers present specialized cells for larval nutrition, rich in proteins and lipids (Verkerke, 1987, 1988).

Our data show that intact seeds are used as an alternative food resource mainly when few galls are left available for oviposition within the fig. This behaviour may be favoured by the hymenopterans' ability to control offspring sex (Cook, 1993). The production of males in lower quality resources, as reported in several parasitoid species (King, 1987), can be facilitated by reduced fitness loss associated with alternative mating strategies. Indeed, at least in some species of nonpollinating fig wasps, small males do not defend mates in the cavity but rather mate among the flowers (Bean & Cook, 2001; Pereira & Prado, 2005). Moreover, the occurrence of small males in inquiline species (e.g. *Philotrypesis* and *Apocrypta*) appears to be associated with alternative mating strategies between nondispersing males, whereas in galler species, reduced males appear to be associated with dispersal polymorphism (e.g. *Ottetisella*, Pienaar & Greeff, 2003).

Bored seeds were also observed in other highly infested *F. citrifolia* crops in Brazil, suggesting that this alternative resource utilization may occur frequently within the species. Our results provide insights for understanding the proximate causes that regulate male intraspecific variation. Jousselin, van Noort & Greeff (2004) showed that *Philotrypesis* male morphology is evolutionarily labile and suggested that the ability of *Philotrypesis* species to produce both small and big fighting morphs is 'the result of changes in regulatory factors rather than mutations in critical pathways as these would be more difficult to reinstall'. Therefore, seed predation deserves to be investigated

in other inquiline fig wasps groups to examine the evolutionary pattern of this behaviour.

For the fig-fig wasp mutualism, *Idarnes* species (as well as other nonpollinating species; Kerdelhué & Rasplus, 1996; Kerdelhué, Rossi & Rasplus, 2000) represent a cost to the plant's male function (pollen dispersal) because their numbers are negatively correlated with pollinating wasp brood sizes (West & Herre, 1994; West *et al.*, 1996; Pereira, 1998). Alternative seed predation adds a direct cost to the plant's female function, which makes this inquiline a more virulent parasite of the fig-agaonid wasp mutualism. Interestingly, the evolution of the cost to the mutualism in this *Idarnes* goes in the opposite direction to the one occurring in some others lineages in which parasitic fig wasps have become mutualists (Jousselin, Rasplus & Kjellberg, 2001): natural history particularities may account for diversified outcomes of the ecological interactions in the fig-fig wasp system.

Such plasticity in parasitica wasp larva feeding regime as documented in the present study has hardly been reported for other species. Nevertheless, there is strong evidence that feeding regime may change profoundly over time, as documented in Cinipidae or in *Megastigmus* (Rouault *et al.*, 2004). Facultative use of lower quality resources to produce males may be an important initial step in a shift in the feeding regime because it allows progressive adaptation to the new resource.

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