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Finding hidden females in a crowd: Mate recognition in fig wasps

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ABSTRACT

Multi-species mating aggregations are crowded environments within which mate recognition must occur. Mating aggregations of fig wasps can consist of thousands of individuals of many species that attain sexual maturity simultaneously and mate in the same microenvironment, i.e, in syntopy, within the close confines of an enclosed globular inflorescence called a syconium – a system that has many signalling constraints such as darkness and crowding. All wasps develop within individual galled flowers. Since mating mostly occurs when females are still confined within their galls, male wasps have the additional burden of detecting conspecific females that are “hidden” behind barriers consisting of gall walls. In *Ficus racemosa*, we investigated signals used by pollinating fig wasp males to differentiate conspecific females from females of other syntopic fig wasp species. Male *Ceratosolen fusciceps* could detect conspecific females using cues from galls containing females, empty galls, as well as cues from gall volatiles and gall surface hydrocarbons.

In many figs, syconia are pollinated by single foundress wasps, leading to high levels of wasp inbreeding due to sibmating. In *F. racemosa*, as most syconia contain many foundresses, we expected male pollinators to prefer non-sib females to female siblings to reduce inbreeding. We used galls containing females from non-natal figs as a proxy for non-sibs and those from natal figs as a proxy for sibling females. We found that males preferred galls of female pollinators from natal figs. However, males were undecided when given a choice between galls containing non-pollinator females from natal syconia and pollinator females from non-natal syconia, suggesting olfactory imprinting by the natal syconial environment.

1. Introduction

Mate recognition is often the first and most important step in reproduction. For those organisms that mate in large aggregations, mate recognition must occur in crowded conditions. Such aggregations can either consist of conspecifics as in garter snakes (Shine and Mason, 2001) and bark beetles (Byers and Wood, 1980) or of multiple species as in mycophagous *Drosophila* (Jaenike et al., 1992), fiddler crabs (Detto et al., 2006), *Anopheles* mosquitoes (Diabaté et al., 2006) and fig wasps (Janzen, 1979; Weiblen, 2002; Herre et al., 2008; Ghara and Borges, 2010). Mate recognition signals function not only as barriers to hybridisation in mixed-species aggregations, but are also important for reproductive isolation in sympatric species. The evolution of mate recognition signals can often be important in the radiation and maintenance of species isolation in species that are sympatric and syntopic sharing not only geographic ranges, but also habitats within these ranges (Symonds and Elgar, 2004; Mullen et al., 2007; Smadja and Butlin, 2009; Nanda and Singh, 2011). Species specificity of premating signals in sympatric species is higher than those between allopatric species (Butlin, 1987; Coyne and Orr, 1989; Noor, 1999). Such signal specificity is also high in sympatric species under syntopic conditions (Shine et al., 2002).

In crowded and dark spaces, chemical (Partan and Marler, 2005) and/or vibratory signals (Hebets and Papaj, 2005) could conceivably be more important than visual signals in mate

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recognition. In crowded situations where several species are simultaneously sexually mature and are ready to mate within the same micro-environment, i.e. in syntopy, the ability of males to accurately find a conspecific female becomes crucial. Such systems afford valuable insights into mechanisms by which mate recognition could occur under signalling constraints. Since sex pheromones could function over longer or shorter distances depending on their volatility, it is expected that heavier relatively non-volatile cuticular hydrocarbons could serve as contact sex pheromones (Singer, 1998; Ginzel, 2010), while compounds with low volatility could serve as mate recognition signals over short distances without contact having to occur (Yoshida, 1978; Simser and Coppel, 1980). In species in which females are hidden by physical barriers, males have to find such hidden females using signals that can either penetrate or coat such barriers such as the retreat silk of ant-mimicking spiders (Borges et al., 2007) and the cocoons of hymenopteran parasitoids (Howard, 1993), or employ proxies for female signals as in male gall wasps which use altered host plant volatiles to find females hidden within plant stems (Tooker et al., 2002; Tooker and Hanks, 2004), or male *Heliconius* butterflies that use host plant volatiles and the presence of immature larvae to find females developing within pupal cases which they proceed to guard until female eclosion (Estrada and Gilbert, 2010; Estrada et al., 2010).

Mate recognition by male wasps in the nursery pollination mutualism between fig and fig wasps has to function under the constraints of crowding, darkness, syntopy and simultaneous sexual maturation of multiple fig wasp species as well as the possible requirement for proxies for the location of hidden females. In this mutualism, individual fig wasps develop in galled flowers within an enclosed, globular, thick-walled inflorescence called the fig syconium, while many of the uniovulate flowers also develop seeds after being pollinated by mutualistic fig wasps. Wingless male fig wasps emerge first from their galls, and either release females from their galls before mating or insert genitalia into female-containing galls to mate with virgin females (Weiblen, 2002; Cook and Rasplus, 2003; Cook and Segar, 2010). Depending on the fig species and syconium size, syconia may contain hundreds to thousands of flowers (Janzen, 1979; Verkerke, 1989; Kjellberg et al., 2001; Cook and Rasplus, 2003), with hundreds of developing wasps and seeds. Besides the mutualistic fig wasp species (belonging to Agaonidae), most species of figs are parasitised by other galler, inquiline or parasitoid fig wasp species (belonging to other subfamilies in the Chalcidoidea), all of which also develop within individual galls within the enclosed syconium. Between 1 and 30 species of syntopic fig wasps could occupy the syconia of a single fig species (Cook and Rasplus, 2003; Cook and Segar, 2010). In some fig species, two species of congeneric pollinating wasps may also develop within the same syconium (Michaloud et al., 1996). Therefore, if males are able to detect the pre-mating chemical signals of different fig wasp species, they can be expected to experience a chemical equivalent of the cocktail party problem encountered in acoustic communication in a noisy environment (Bee and Micheyl, 2008), i.e., the need to perceive specific mate recognition signals in a noisy chemical environment. Furthermore, since in some fig species such as *Ficus racemosa*, females cannot free themselves from their galls and rely upon males to do so (A. Krishnan, pers. observ.), males need to either use a species-specific chemical or vibratory signal that is emitted by females themselves and that can pass through the gall wall, or rely on proxy cues that coat the gall wall and indicate the presence of conspecific females within galls. Furthermore, since male wasps (especially the pollinator males) have a short lifespan of 24–48 h (Kjellberg et al., 1988; Ghara and Borges, 2010) within which they have to mate as well as cut an opening in the syconium wall to release pollinator females, male pollinator wasps should also be under intense selection pressure to identify galls containing conspecific females quickly and accurately.

Most often, fig wasp males mate with females found within their own syconium since males usually die within their natal syconium (Galil and Eisikowitch, 1968; Herre et al., 2008; Ghara and Borges, 2010). However, in some species, fig wasp males may leave their natal syconium and mate with females from other syconia (Greeff, 2002; Greeff et al., 2003, 2009). This may be to avoid inbreeding (Greeff et al., 2009), although it is believed that fig wasps with their haplodiploid sex determination system can tolerate high levels of inbreeding (McKey, 1989); often only a single or few foundress female wasps may lay eggs within individual syconia (Kathuria et al., 1999; Zavodna et al., 2007). Consequently, sib matings are likely to be quite frequent (Zavodna et al., 2007). However, whether male fig wasps prefer female signals from their natal syconia or from non-natal syconia is not clear, though studies on the pollinator *Pegoscapus assuetus* seem to indicate that males prefer females from natal syconia (Frank, 1985). The cues employed by *P. assuetus* males to differentiate between females from natal and non-natal syconia were not investigated.

With this background, we have used *F. racemosa* to understand how male pollinator fig wasps solve the problem of finding hidden females. We determined if male *Ceratosolen fusciceps* can distinguish between galls of conspecific females from those of non-pollinator females (which can co-occur with pollinators within the same syconia) on the basis of (1) whole galls (with the gall occupant within the gall); (2) empty galls (gall occupant removed to remove potential vibratory signals); (3) volatile signals from empty galls and (4) surface hydrocarbon signals of empty galls. We also conducted choice assays to test if males prefer galls containing females from non-natal fig syconia (which are definitely non-sibs) to females from natal fig syconia (which have higher probabilities of being sibs).

2. Materials and methods

2.1. Species biology and study site

The syconia of *F. racemosa* are pollinated by the mutualistic agaonid wasp *Ceratosolen fusciceps* Mayr and are also host to six other species of non-pollinating fig wasps in the subfamilies Sycophaginae and Sycoryctinae respectively (gallers – *Apocryptophagus stratheni* Joseph, *Apocryptophagus testacea* Mayr, *Apocryptophagus fusca* Girault and the parasitoids – *Apocryptophagus agraensis* Joseph, *Apocrypta westwoodi* Grandi and *Apocrypta* sp. 2) (Ghara and Borges, 2010) that develop within them. Although wasp dispersal phase syconia containing all 7 species of wasps are rare, syconia containing 3–4 wasp species are quite common (Ghara, 2012). Pre-dispersal stage syconia are generally 13–35 mm in size and can contain 2000–7000 flowers. Each syconium can contain from 0 to 1200 developed wasps, of which 10–40 percent are males (M. Ghara, A. Krishnan and R.M. Borges, unpublished data). Males of all 7 species of wasps reproducing in *F. racemosa* are wingless, usually die within their natal syconia, and have never been observed to exhibit aggressive or fighting behavior. All species of wasps are sexually mature at the same time; females of all species leave the syconium concurrently.

We collected pre-dispersal stage syconia (in which male wasps had exited their galls, but female wasps were still within their galls) from in and around the Indian Institute of Science campus in Bangalore, India (12°58'N, 77°35'E). We cut open syconia to collect males of the pollinator wasp (*C. fusciceps*) and galls containing females of pollinator and the non-pollinating wasps