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Is the social parasite *Vespa dybowskii* using chemical transparency to get her eggs accepted?

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Abstract

Both avian and insect cuckoos must trick their hosts into accepting foreign eggs. In birds this is achieved through egg mimicry. Within the hornets the only known social parasite is the rare *Vespa dybowskii*. We investigated how the *V. dybowskii* queen induces tens or hundreds of host workers to accept her eggs and offspring. Since hydrocarbons function as recognition cues in social insects, we investigated these compounds from the surface of eggs and workers of *V. dybowskii*, both host species (*V. simillima* and *V. crabro*) and an additional four non-host species. We found that chemical mimicry of the hosts' colony odour and their eggs normally associated with wasps was not being employed by *V. dybowskii*. Chemical insignificance is also unlikely as the amounts of hydrocarbons extracted from parasite, host and non-host eggs were similar. Eggs of *V. dybowskii* may survive in part due to being chemically transparent, as methyl-branched compounds only represent a tiny proportion (<1%) of the parasites hydrocarbon profile but a large proportion (26–41%) in both host species. However, the functions of various hydrocarbon groups need to be investigated in the hornets before this new acceptance mechanism of parasite eggs and adults is understood.

Keywords: Cuckoo; Hornet; Mimicry; Cuticular hydrocarbons

1. Introduction

Tricking a host species into accepting eggs of a parasitic species has fascinated scientists for decades, as the interests of the cuckoo and host are diametrically opposed. In birds, the role of cuckoos in the evolution of the visual appearance of eggs and the egg recognition abilities of hosts are well understood (Brook and Davies, 1988; Davies, 2000). This co-evolution has led to the extraordinary levels of egg mimicry found in many bird cuckoos. Unlike avian cuckoos, which need to trick only two individuals (the parents), the social insect cuckoo queen needs to ''break into the colony fortress'' and then induce tens, hundreds or even thousands of host workers to rear her ''alien'' eggs. The exclusion of non-nest mates in social insects is based largely on chemical cues residing on the cuticle, with certain hydrocarbons known to play a crucial role in nest-mate recognition (Akino et al., 2004; Dani et al., 2005; Ozaki et al., 2005; Greene and Gordon, 2007; Martin et al., 2008a). Therefore, social parasites employ a variety of chemical methods to invade host colonies (Lenoir et al., 2001). The ability of a social parasitic queen to usurp a colony has been well studied in paper wasps (Cervo, 2006; Lorenzi, 2006), bumblebees (Zimma et al., 2003) and ants (Lenoir et al., 2001). However, the ability of social parasites to prevent their eggs from being destroyed by the host workers has only been investigated in the slave-making ant *Polyergus breviceps* (Johnson et al., 2005). The eggs of all social insects are visually indistinct but may have differing hydrocarbon profiles that will affect their ''chemical'' appearance as occurs in the adults (Martin et al., 2008b). It is has been shown that in most social insects, workers are able to discriminate between worker-laid and queen-laid eggs (Ratnieks et al., 2006) and possibly between eggs

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destined to become queens or workers (Lorenzi and Filippone, 2000). Therefore, just how social cuckoos are able to trick highly discrimative workers into accepting their eggs provides an intriguing evolutionary problem.

The hornets (*Vespa*) are an excellent model group since they are only 23 species, many occur sympatrically and their phylogenetic relationships have been investigated (Carpenter, 1987; Archer, 1991). They all have annual life cycles, a simple social structure and their biology is generally well known (Matsuura and Yamane, 1990). Within the Vespinae intraspecific queen usurpation is common (Archer, 1985; Martin, 1992). However, interspecific usurpation is rare with inquilinism been described in only five of the 60 species of Vespinae (Greene, 1991). Within the *Vespa* only *V. dybowskii* is a known social parasite or cuckoo. It is a rare species found only in the central mountains of Japan, North Burma, Eastern China and Ussuriland in Russia (Sakagami and Fukushima, 1957; Archer, 1992). Very little is known about the natural history of this species. It has been recorded founding nests independently (Sakagami and Fukushima, 1957) these are rarely encountered and their normal mode of colony foundation is by the queen invading well-established nests of *V. simillima* and occasionally *V. crabro*, killing the host queen and tricking the host workers into rearing her workers and sexuals. Both the host species have been shown to remove worker-laid eggs (Foster et al., 2002; Takahashi et al., 2003, 2007) so egg discrimination is known to take place and is probably chemically mediated. Furthermore, *V. dybowskii* forms a monophyletic group with *V. crabro* (Archer, 1992) so the Emery's rule may apply which states that social parasites tend to use hosts to which they are closely related (see Wilson, 1971), as this may allow them to mimic the hosts recognition systems more easily. However, *V. dybowskii* does not form a monophyletic group with its other, more commonly used, host *V. simillima*.

Chemical mimicry or camouflage where the host achieves some degree of chemical congruency with the host is the best-known system used by parasites to persist within social insect colonies, e.g. (Lenoir et al., 1997; Akino et al., 1999). This is exemplified by parasitic queen *Polistes* wasps, which mimic very closely the odour of the colonies they usurp (Bagnères et al., 1996; Turillazzi et al., 2000; Lorenzi et al., 2004). Although additional parasite-specific hydrocarbons are used to change the usurped colony odour (Turillazzi et al., 2000). The mild convergence of chemical profiles of eggs of the slave-maker ant *Polyergus breviceps* and their *Formica* hosts appear an unlikely explanation for the adoption of parasite eggs (Johnson et al., 2005), suggesting other mechanisms may be operating. Other non-mimetic methods such as "chemical insignificance" where there is a lack of external chemical substances (Lenoir et al., 1999; Lambardi et al., 2007), or the use of deterrents (D'Ettorre et al., 2000; Ruano et al., 2005; Martin et al., 2007) exist but are less well studied.

The aim of this study was to investigate the method used by *V. dybowskii* to trick the host workers into accepting her eggs and preventing aggression between host and parasite workers. This was achieved by conducting a comparative GC–MS study investigating the surface chemistry of the eggs and workers of the cuckoo, its two host species and an additional four non-host species.

2. Methods

2.1. Sample collection

In Japan during August and September 2006 queen-laid eggs and adult workers were collected from seven different hornet species (*V. affinis*, *V. analis*, *V. crabro*, *V. ducalis*, *V. dyboskwii*, *V. mandarinia*, *V. simillima*). All colonies were collected at night and contained a single queen. With the exception of *V. affinis* that was collected from Ishigaki Island southern Japan, all other colonies were located in the same (Nagano-ken) mountainous region of central Japan. The *V. dyboskwii* queen-laid eggs came from four parasitized *V. simillima* colonies that were at various states of take-over. One colony contained approximately half host and half parasite workers, one colony had a few host workers remaining and two colonies were entirely composed of parasite workers. In all colonies the host *V. simillima* queen was missing and no host males were ever seen. The highly aggressive nature of *V. dyboskwii* combined with difficult access to nests meant that only 2–3 eggs could be collected from each colony. In addition to ten *V. dyboskwii* workers been collected, nine gynes (new queens) from two colonies were also caught. From all other hornet species, five queen-laid eggs and five adult workers from each colony were collected. From the seven hornet species a total of 88 eggs from 21 colonies were successfully analysed, along with 45 workers from 10 of the colonies and nine *V. dyboskwii* gynes (Table 1). Although only one colony was sampled for some non-host species, hydrocarbon profiles within a species are known to be remarkably consistent (Jallon and David, 1987; Howard, 1993; Symonds and Elgar, 2004; Martin et al., 2008b).

Each egg was carefully removed from its cell and placed into a vial into which 30 μl of hexane was added, this was evaporated, the vials sealed and kept below 5 °C. Due to the large size of hornets the hind-wings were removed from the adults and placed individually into vials into which 100 μl of hexane was added. This was evaporated, the wing discarded, the vial sealed and stored below 5 °C.

2.2. Chemical analysis

Just prior to analysis 30 μl of hexane containing C_{22}, an internal standard was added to the vials and samples were analysed on a HP 6890 GC (equipped with a HP-5MS column; length: 30 m; ID: 0.25 mm; film thickness: 0.25 μm) connected to a HP5973 MSD (quadrupole mass spectrometer with 70 eV electron impact ionization). Samples were injected in the splitless mode and the oven was programmed from 70 to 200 °C at 40 °C/min and then from 200 to 320 °C at 25 °C/min and held for 2 min at 320 °C.