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Facultative social parasites mark host nests with branched hydrocarbons

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The chemical integration strategies of facultative social parasites of social insects have not received the scientific attention they deserve, even though there is considerable research being done on the strategies of obligate social parasites. We simulated intraspecific nest usurpations in the social paper wasp, *Polistes biglumis*, by dividing each nest into two parts and putting one half in the care of the original foundress and the other half in the care of a usurper. After 8 days, we removed and killed foundresses and usurpers, and later tested the responses of naïve, sister-offspring to them. In each half-colony, the offspring were more tolerant to the female that was last on the nest, regardless of whether she was the foundress or a usurper. This suggested that usurpers had the chemical means to be tolerated by the host offspring. Comparisons between the epicuticular hydrocarbon profiles of foundresses and usurpers showed that usurpers were neither chemically insignificant nor transparent, nor were they mimetic, as obligate parasites often are. Instead, usurpers had chemical profiles richer in methyl-branched hydrocarbons than those of the foundresses. Analyses of the hydrocarbon profiles of nest paper revealed that usurpers supplemented host nests with their own hydrocarbons, a sort of nest marking. As a result, the chemical profiles of the host nests became qualitatively more similar to those of the usurpers. These chemical strategies illustrate that branched hydrocarbons play a role as semiochemicals and that facultative parasites may not all be on the main pathway to obligate parasitism.

Parasites exploit any resources that free-living organisms offer: from molecular engines to whole cells, from whole organisms to the structures they make. In their struggle to exploit hosts, parasites provide some of the best evidence of adaptation by natural selection. Brood parasites are species that do not target organisms but rather the resources that free-living organisms build or produce: their nests, their parental care and/or their social structures (Wilson 1971; Rothstein 1990). Brood parasites exhibit traits that give them an advantage in fooling and exploiting their hosts and these traits are the results of reciprocal host–parasite interactions across evolutionary time (Brooke & Davies 1988). The recognition systems of birds and social insects protect nests from brood parasites. For example, the hosts of cuckoos discriminate between their own eggs and those of cuckoos visually, and so cuckoos lay visually mimetic eggs (Rothstein 1990; Rothstein & Robinson 1998). Furthermore, social insects distinguish nestmates from non-nestmates by means of chemicals, and social insect parasites (social parasites) trick their hosts about their own chemical identity (Bagnères & Lorenzi 2010). Doing so, social parasites enter host colonies, exploit host nests and use the host workforce for their own reproduction.

The information about chemical identity in social insects is conveyed by epicuticular hydrocarbon blends (Howard & Blomquist 2005; Blomquist & Bagnères 2010). Generally, individuals from different species have epicuticular hydrocarbon blends that differ in composition (Bagnères & Wicker-Thomas 2010). Within species, individuals from different colonies have hydrocarbon blends that differ in the relative proportions of their compounds (Bonavita-Cougourdan et al. 1987; Bruschini et al. 2010; Van Zweden & d'Ettorre 2010). Within colonies, nestmates recognize each other because their chemical profiles are very similar. Social parasites escape detection by hosts in at least three ways: they mimic the chemical profiles of their hosts (chemical mimicry, sensu Dettner & Liepert 1994); they have chemical profiles that are poor in hydrocarbons (chemical insignificance, Lenoir et al. 2001); and/or they lack some of the hydrocarbons of their hosts (chemical transparency, Martin et al. 2008a).

One illustrative example of chemical mimicry is that of the slavemaker *Polyergus* queens, which take on chemical profiles that

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match those of their *Formica* hosts (Habersetzer & Bonavita-Cougourdan 1993; D'Ettorre & Errard 1998; Johnson et al. 2001). Similarly, all three species of the obligate social parasite *Polistes* wasps perfectly mimic their congeneric host chemical profiles (Bagnères et al. 1996; Turillazzi et al. 2000; Lorenzi et al. 2004). The *Polyergus* parasite species and two of the *Polistes* species are also chemically insignificant when they invade host colonies (Lenoir et al. 2001; Lorenzi & Bagnères 2002, Lorenzi et al. 2004). These chemical strategies can only be explained as the result of host–parasite coevolution (Lorenzi 2006; Bagnères & Lorenzi 2010).

The original, basic function of insect hydrocarbons was probably physical: the limitation of desiccation. This function was probably coopted by a semeiotic function in a series of evolutionary steps that are mostly unknown (Le Conte & Hefetz 2008). Likewise, we know very little about the evolutionary steps that led social parasites to evolve chemical insignificance and chemical mimicry. In this respect, any research about the chemical mechanisms employed by nonspecialized, facultative, social parasites may contribute to identifying the steps towards the evolution of the chemical strategies that obligate parasites use to overcome host detection.

Intraspecific facultative parasitism is common among social insects (Hölldobler & Wilson 1990; Cervo 2006; Beekman & Oldroyd 2008) and intraspecific parasites have been pointed out as potential obligate preparasites or incipient obligate parasites (Taylor 1939; Savolainen & Vepsalainen 2003; Cervo 2006; Buschinger 2009). None the less, we have not come across any published research investigating the chemical ecology of intraspecific social parasites through the analysis of their hydrocarbon blends.

In *Polistes*, facultative social parasites (hereafter usurpers) invade host colonies during the founding phase, that is, the phase when the foundresses are the only adults in the colonies. Often, the targeted colonies are solitary foundations and hence a single foundress is the only adult defending her colony (Cervo & Dani 1996; Cervo 2006). Unlike most obligate parasites, usurpers attack, chase away or kill adult hosts. Then usurpers take over host colonies, comprising the host nests and the host immature brood, and 'wait' for the host brood to emerge. When the host brood emerge, usurpers dominate them and force them into rearing their own brood (Cervo & Dani 1996; Cervo 2006).

In the social wasp *Polistes biglumis* Linnaeus, the singly founded colonies are often the targets of conspecific usurpers (Lorenzi & Cervo 1995). We investigated whether usurpers are chemically insignificant, mimetic or transparent by simulating intraspecific nest usurpation in the laboratory. Although limited to one species, we also hope this study broadens our knowledge of female scent marking in animal conflicts in species other than paper wasps.

METHODS

Model Species

In the solitarily founding species *P. biglumis* (Lorenzi & Turillazzi 1986), up to 18% of the colonies are usurped by conspecific usurpers, possibly after they have lost their own nests (Lorenzi & Cervo 1995). Within a colony, the hydrocarbon profiles of adult wasps are similar to each other and to the profiles that cover the paper nest surfaces (Lorenzi et al. 1996). In contrast, hydrocarbon profiles differ between colonies (Lorenzi et al. 1997).

General Procedure

We split nests collected in the field into two parts: one half was reared by the original foundress (foundress half-colonies) and the other half was 'put up for usurpation' to an alien female (usurper; usurper half-colonies). Usurpers and 'host' colonies came from two populations separated by mountain barriers. Therefore we expected that usurpers would not be related to the colony they usurped.

We collected 24 colonies in Chalpe (Italian Western Alps: 44°56′N, 6°49′E) in mid-July 1997. At the same time, we also collected 24 *P. biglumis* colonies in Montgenèvre (French Hautes Alpes: 44°55′N, 6°43′E). The colonies were towards the end of the founding phase and single foundresses were on the nests. In the laboratory, we randomly chose 12 colonies from each collection site. We removed their foundresses and placed them separately in glass jars. We cut their paper combs in two with scissors so that the larger number of pupae was intact and the numbers of cells and old brood (large larvae and pupae) of the two parts were similar (foundress half-colonies: 17–28 cells; usurper half-colonies: 16–28 cells; Wilcoxon pairwise test: $Z = -0.420$, $P = 0.674$; foundress half-colonies: 5–8 brood; usurper half-colonies: 2–8 brood; $Z = -0.962$, $P = 0.336$).

Each half comb was separately fastened to the wall of a plastic box (18 × 12 cm and 11 cm high) and either its original foundress (foundress half-colony) or a usurper (usurper half-colony) was put into the box. If the colony came from one population, its usurper came from the other.

The half-colonies were kept under a 12:12 h light:dark photoperiod and supplied with honey, water and *Tenebrio molitor* larvae ad libitum. We kept foundresses and usurpers on their half-colonies for 8 days, then we removed and froze them in individual glass vials (−18 °C). We used them later in behavioural tests and chemical analyses (see below). Because foundress and usurper half-colonies had immature offspring, we waited for their emergence. The first offspring emerging from the foundress half-colonies were sisters to those emerging from the usurper half-colonies and both were daughters of their original foundresses (usurpers usually keep the first offspring of the foundress when they usurp host colonies, Lorenzi & Cervo 1992; Cervo & Lorenzi 1996). The offspring emerged after we removed foundresses or usurpers. Consequently, the offspring never met their foundresses or usurpers during adulthood and were immature in the 8-day period that foundresses and usurpers spent on the half-colonies. Therefore the offspring were naïve to foundresses and usurpers when we performed the behavioural tests.

Behavioural Tests

We tested whether the offspring discriminated between their foundress (i.e. their mother) and usurper (i.e. the usurper of their own half-colony or of their sisters' half-colony) by performing bioassays using the dead foundresses and usurpers. Bioassays using dead insects eliminate the confounding effects of behavioural or chemical actions by the insects introduced and are routinely used in recognition experiments in social insects (e.g. Bonavita-Cougourdan et al. 1987; Lorenzi et al. 1997; Ruther et al. 2002).

The tests were performed at least 1 day after the offspring emerged. At room temperature, we introduced the foundress and the usurper separately into each pair of half-colonies in random order and at least 1 h apart. We used forceps to keep foundresses and usurpers 1 cm from the nest surfaces. The tests lasted 1 min from the first unambiguous reactions by the offspring. The observer did not know whether the dead female was the foundress or the usurper in the test half-colony. We noted the occurrence of the following behaviours: biting, fleeing from the nest by flying, attempting to sting and grasping. In the behavioural tests, we recorded the responses by 1.13 ± 0.18 adult female offspring per foundress half-colony and by 1.33 ± 0.44 adult female offspring per usurper half-colony. The offspring tested did not differ significantly in age between half-colonies (on average: 3.18 ± 2.71 days old in foundress half-colonies; 6.38 ± 2.72 days old in usurper half-colonies; Wilcoxon pairwise test: $Z = -1.826$, $P = 0.068$).