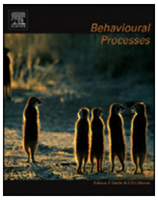
Behavioural Processes 91 (2012) 282–290

Contents lists available at SciVerse ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc



Queens defense by workers in the highly polygynous ant *Crematogaster pygmaea* (Hymenoptera: Myrmicinae)

Glauco Bezerra Martins Segundo[a], Jean-Christophe de Biseau[b], Yves Quinet[a,*]

[a] Laboratório de Entomologia, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Av. Paranjana, 1700, 60714-903 Fortaleza, CE, Brazil
[b] Evolutionary Biology and Ecology, CP 160/12, Université Libre de Bruxelles, Avenue F.D. Roosevelt 50, B-1050 Brussels, Belgium

ARTICLE INFO

Article history:
Received 26 April 2012
Received in revised form 10 August 2012
Accepted 21 September 2012

Keywords:
Crematogaster
Formicidae
Polygyny
Queen attractiveness

ABSTRACT

Some aspects of the biology of *Crematogaster pygmaea*, a highly polydomous and polygynous ant, are more commonly found in monogynous species. One such characteristic is the high attractiveness of its queens. In this study, this attractiveness was assessed under varying experimental conditions to investigate the factors responsible for its expression and variation, and to identify the nature of queen attractiveness. It was shown (1) that *C. pygmaea* queens are highly attractive to the workers that cluster on and around them (retinue), (2) that the attractiveness of *C. pygmaea* queens is context-dependent, i.e., it increases with increasing degree of potential danger to the queen, (3) that the attractiveness signal of *C. pygmaea* queens is chemically based, and (4) that this signal is persistent and apparently not colony-specific. The proposed hypothesis is that the *C. pygmaea* queens constantly release an attractiveness signal that is "read" by the workers, in a dependent way linked to the context, and that the main function of this attractiveness is to protect queens. This protection would have a high adaptive value in the context of the social structure and the reproductive strategies in *C. pygmaea*.

1. Introduction

Although pheromone-based communication has been extensively studied in social insects (Hölldobler and Wilson, 1990, 2009; Vander Meer et al., 1998), chemical interactions between queens and workers remain relatively poorly understood, particularly in ants (Kocher and Grozinger, 2011; Vander Meer and Alonso, 1998; Vargo, 1998).

Ant queens are generally attractive to their workers, the most spectacular examples being found in species with large monogynous colonies like New World army ants (e.g., *Eciton*, *Neivamyrmex*; Rettenmeyer et al., 1978), weaver ants (*Oecophylla longinoda* and *O. smaragdina*; Hölldobler and Wilson, 1983) or the African ponerine ant *Megaponera foetens* (Hölldobler et al., 1994). In those ants, the queens are permanently covered and/or surrounded by a court of workers (the retinue) with generally high and continuous worker turnover (Hölldobler and Wilson, 1983; Hölldobler et al., 1994; Rettenmeyer et al., 1978).

It has been demonstrated in several species that this attractiveness is mediated by pheromones, e.g., in *Pheidole pallidula* and *Lasius alienus* (Stumper, 1956), *Neivamyrmex* spp. (Watkins and Cole, 1966), *Camponotus pennsylvannicus* (Fowler and Roberts, 1982), *Solenopsis invicta* (Glancey, 1980; Glancey et al., 1984; Jouvenaz et al., 1974; Vander Meer et al., 1980; Vargo and Hulsey, 2000), *Myrmica rubra* (Coglitore and Cammaerts, 1981), *Linepithema humile* (Cariou and Passera, 1990), *Myrmecia gulosa* (Dietemann et al., 2005).

The functions of this attractiveness have never been extensively investigated, but tending, contact communication, and defense have been most frequently suggested. In *O. longinoda* and *O. smaragdina*, the queen is frequently licked and fed by the retinue (Hölldobler and Wilson, 1983). On the other hand, in *M. foetens* and *M. gulosa*, the queen is neither fed nor groomed by the retinue, suggesting that queen attractiveness is not related to queen-tending in these species (Dietemann, 2002; Hölldobler et al., 1994).

The presence of a retinue around the queen has also been interpreted as a defensive behavior in species in which the queen moves between nests or migrates regularly, like in New World army ants (Ecitonini) (Rettenmeyer et al., 1978) or in *M. foetens* (Hölldobler et al., 1994). In *O. longinoda*, the queen that migrates to a new leaf nest "is totally covered by a retinue of major workers, many of which having the gaster raised in a defensive posture" (Hölldobler and Wilson, 1983). This phenomenon was first described in the New World army ants (Ecitonini). During the statary phase in army ants colonies, the queen is surrounded by a group of small workers in the bivouac, but during the nomadic phase, a retinue is formed of numerous workers moving with the queen through the emigration columns (Rettenmeyer et al., 1978). In *O. longinoda*, the queen that migrates to a new leaf nest "is totally covered by a retinue of

---

* Corresponding author at: Rua Noruega, 244, 60710-780 Fortaleza, CE, Brazil. Tel.: +55 3101 97 49.
E-mail address: yvesq@terra.com.br (Y. Quinet).

0376-6357/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.beproc.2012.09.009

major workers, many of which having the gaster raised in a defensive posture" (Hölldobler and Wilson, 1983). A nomadic lifestyle and the need for the queen to be protected during emigrations is also the hypothesized function of high queen attractiveness in *M. foetens* (Hölldobler et al., 1994).

More fundamentally, queen attractiveness is now regarded as an element of the chemically mediated system that allows colony cohesion and regulation of reproduction, via primer and releaser pheromones produced by the queens (Kocher and Grozinger, 2011; Le Conte and Hefetz, 2008; Vargo, 1998). Cuticular hydrocarbons acting as contact pheromones have been shown to be involved in the discrimination of fertile and non-fertile individuals in more than 20 ant species from 16 genera and several subfamilies, and are most likely used by workers as fecundity signals (reviewed in Liebig, 2010; Monnin, 2006; Peeters and Liebig, 2009). In some cases, it was demonstrated that those putative fecundity signals allow the workers to direct their attention to the most fertile queens in polygynous species such as *Leptothorax* sp. (Ortius and Heinze, 1999) or *Formica fusca* (Hannonen et al., 2002). In *S. invicta*, the chemical compounds responsible for queen attractiveness are produced in the poison gland (Glancey et al., 1984) and most likely also in part in the postpharyngeal glands (Vargo and Hulsey, 2000). In polygynous colonies of *S. invicta*, the higher attractiveness of the dominant queen and the consequent monopolization of workers' attention result in a higher rate of trophallaxis with workers and higher ovipositional rate (Chen and Vinson, 2000).

Extreme queen attractiveness to workers has been reported only in monogynous species with large colonies (Hölldobler and Wilson, 1983; Hölldobler et al., 1994; Rettenmeyer et al., 1978). Even in polygynous species like *L. humile*, artificial reduction to monogyny leads to a higher queen attractiveness (Keller, 1988).

Crematogaster pygmaea is a highly polygynous ant (Quinet et al., 2009) whose queens show strong attractiveness to workers (Hamidi, 2010). Other features of its biology are more typically found in monogynous species, like strong queen/workers dimorphism (Fig. 1), occurrence of nuptial flights, ability of young queens to found colonies in an independent and claustral way (at least under laboratory conditions), capacity of workers to lay fertile eggs when no queen is present, and presence of colony boundaries (multicoloniality; Hamidi, 2010; Hamidi et al., 2012; Quinet et al., 2009). Understanding the function of the high queen attractiveness in *C. pygmaea* is therefore of particular interest.

In this work, laboratory bioassays and field experiments were used to measure the attractiveness of *C. pygmaea* queens, to precise the conditions under which this attractiveness occurs, and to investigate the signals responsible for it.

2. Materials and methods

2.1. Subjects and housing conditions

C. pygmaea is a highly polygynous and polydomous ground-dwelling species that occurs in open and sandy areas in the littoral zone of the state of Ceará (Northeastern Brazil), and also in the "caatinga" zone, a seasonal xerophilous thorn woodland/shrubland that covers most of the state of Ceará (Quinet et al., 2009). Its extensive colonies are formed of tens of small nests interconnected by a network of surface trails that also lead the foragers to herbaceous plants where they search for nectar or honeydew, their main food source (Quinet et al., 2009). Each nest is a simple straight vertical gallery up to 30 cm in length with 1–4 horizontal chambers containing up to 15 queens, as well as brood and workers (Quinet et al., 2009). The queen/workers ratio, which approaches 1/100 (Quinet et al., 2009), is similar to that found in tramp species like *L. humile*, *Monomorium pharaonis* or *Wasmannia auropunctata* (Passera, 1994).

Nearly one hundred nests of *C. pygmaea* were excavated in 2010 and 2011, in 7 colonies: 3 located on the campus of the State University of Ceará (3°47′S 38°33′W), in Fortaleza (littoral zone), 3 in Eusébio (littoral zone; 3°51′S 38°25′W), 17 km from Fortaleza, and 1 in the "Serra das Almas" Reserve ("caatinga" zone; 5°08′S 40°51′W), 300 km from Fortaleza. From those nests, 47 laboratory colonies, with a mean of 6 queens each, hundreds of workers and brood were produced. All of the queens and workers in laboratory colonies originated from the same field colony and from nests as close as possible to each other. Each laboratory colony was kept in a plastic box (30 cm × 20 cm and 7 cm high) with sides coated with Fluon®, at a constant temperature of 30 ± 2 °C with a 12:12 L:D photoperiod, and with glass test tubes as nesting sites. The ants were fed ad libitum on sugar water and dead *Tenebrio molitor* larvae.

Nearly all experiments were conducted with *C. pygmaea* workers and queens derived from colonies kept in the laboratory for 2–8 months before the experiments were made.

Workers of 3 ground-dwelling ant species (*Solenopsis* sp.1, *Solenopsis* sp.2 and *Ectatomma suzanae*), all living in the same areas as *C. pygmaea*, were used as alien ants in some experiments. One to three colonies (or pieces of colonies) of each species were collected on the campus of the State University of Ceará and kept in the same laboratory conditions as for *C. pygmaea*. *Solenopsis* sp.1 is a monomorphic species whose workers size (±3.1 mm in length) is slightly larger than that of *C. pygmaea* workers (±2.6 mm in length). *Solenopsis* sp.2 is a polymorphic species with large variation in size. Only the smallest (roughly the same size as *C. pygmaea* workers with a ±2.8 mm length) and the largest workers (almost two times larger than the *C. pygmaea* workers, with a ±4.6 mm length) were used in the experiments. *E. suzanae* is a monomorphic species whose workers are much larger (±9.2 mm in length) than *C. pygmaea* workers.

2.2. Experimental devices and procedures

2.2.1. Attractiveness of C. pygmaea queens

The attractiveness of *C. pygmaea* queens was first observed and quantified in the field, in 2 experimental contexts: immediately after nest excavation and after queens were placed on a colony trail (the experiments are detailed in Section 2.3.1).

In more controlled laboratory experiments, the attractiveness of *C. pygmaea* queens was assayed with a procedure similar to that used by Keller and Passera (1989) to test the attractiveness of *L. humile* queens. The tested *C. pygmaea* queen was confined in a wire-mesh ring (2 cm in diameter, 5 mm high) placed in the center of a Petri dish (8.5 cm in diameter) with Fluon®-coated sides. The bottom of the dish was covered with a white paper disc that was changed before each new experiment or set of experimental treatments. The wire-mesh ring allowed the *C. pygmaea* workers, but not the queen, to pass through. A piece of glass covering the wire-mesh ring prevented the queen from escaping.

Before an experiment (or a set of experimental treatments) was initiated, 50 *C. pygmaea* workers were placed in the Petri dish and allowed 5 min to acclimatize before the tested object (queen or dummy) was introduced inside the wire-mesh ring. Ten seconds after the experiment (or the treatment) began (t_0), and then every 3 min for 30 min, 2 types of measurements were made: the number of workers on the queen (or dummy) and the number of workers around, and in physical contact with it (by convention, the number of workers around the queen was called the retinue to distinguish it from the number of workers on the queen). The attractiveness of the queen (or dummy) in a specific experiment was recorded as