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Evolution of a soldier caste specialized to lay unfertilized eggs in the ant genus *Crematogaster* (subgenus *Orthocrema*)

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ABSTRACT

Among social Hymenoptera, only some ant genera have more than one morphological kind of non-reproductive adults. Individuals that are bigger than ordinary workers can function for defence and/or food storage. In *Crematogaster* (*Orthocrema*) *smithi* from Arizona, a third caste exists in addition to winged queens and workers; it is intermediate in size, weight and morphology, and individuals lay many unfertilized eggs that are mostly eaten by larvae (Heinze et al., 1995, 1999). We studied another three species belonging to the subgenus *Orthocrema*: *Crematogaster pygmaea* from Brazil, *Crematogaster biroi* and *Crematogaster schimmeri* from Taiwan. Using scanning electron microscopy and ovarian dissections, we show that 'intermediates' are a patchwork of queen-like and worker-like traits, just as in *C. smithi*; importantly the combinations differ across species. 'Intermediates' are numerically few in the colonies, and in *C. pygmaea* they are produced seasonally. Using histology we confirmed the lack of a spermatheca, thus they are not ergatoid queens. Based on the similarity of their mosaic phenotypes with those in other ant lineages, we suggest that *Orthocrema* 'intermediates' are a soldier caste with a specialized trophic function. This soldier caste has been reported in other *Orthocrema* species from Madagascar, Guinea and Costa Rica, suggesting that it is widespread in this subgenus.

1. Introduction

Division of labour is especially efficient in ant societies because of a marked morphological specialisation among female nestmates. In addition to winged queens (adapted for colony foundation and elevated fertility) and wingless workers (adapted for maintenance activities on the ground), additional morphological castes occur sporadically across different lineages. Ergatoid (permanently wingless) queens reproduce in many species that have shifted away from independent colony foundation (Peeters, 2012). Soldiers bigger than ordinary workers exist in unrelated genera and have a variety of functions that include colony defence, food storage or seed-milling (Molet et al., 2012). Since both ergatoid queen and soldier castes evolved repeatedly, their morphological characteristics are highly heterogeneous.

Crematogaster is the third largest genus of ants (478 current species; subfamily Myrmicinae), and is a common and conspicuous component of tropical faunas on all continents. Workers are monomorphic or exhibit continuous size polymorphism (Longino, 2003). Wingless individuals with a big gaster were reported long ago in *Crematogaster minutissima* (Holliday, 1903) and *Crematogaster biroi* (Bingham, 1903 p. 138). Heinze et al. (1995, 1999) showed in *Crematogaster smithi* that a 'third caste' ('neither queens, nor workers') is intermediate in size, weight and morphology between workers and winged queens. These intermediates do not perform foraging, maintenance, or defensive duties. They are never inseminated, but they lay viable eggs that are mostly eaten by larvae. Similar wingless intermediates are known in other species of the subgenus *Orthocrema* (Bernard, 1952; Longino, 2003; Hamidi, 2010; Blaimer, 2012a), but the use of a variety of terms ("large workers", "ergatoids", "intercastes", "ergatogynes", "intermediate workers") has prevented a comparative perspective.

The subgenus *Orthocrema* (108 species) is strongly divergent and sister to all other *Crematogaster* (Blaimer, 2012b). We compared

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the morphology of 'intermediates' in *Crematogaster pygmaea* from Brazil, and *C. biroi* and *Crematogaster schimmeri* from Taiwan. We show that 'intermediates' are mosaics of queen-like and worker-like morphological traits. Importantly, they all lack a spermatheca, hence they cannot reproduce sexually and are not ergatoid queens. Based on comparisons across ant lineages, we argue that these 'intermediates' are a soldier caste with an exclusively trophic function.

2. Methods

2.1. Colony sampling

All three species nest in the ground. Colonies of *C. pygmaea* are highly polygynous and polydomous, with dozens of nests (simple 20 cm vertical tunnels connecting 1–4 horizontal chambers) connected by trails (Quinet et al., 2009). A total of 136 nests were excavated in Fortaleza and Eusébio (State of Ceará, Brazil) in December 2010–February 2011 (end of dry season), and mid-April 2011 (rainy season) (Table S1). The habitat was open areas in the savanna-like "tabuleiro", adjacent to the littoral zone. Ten nests were also excavated in February 2011, in Serra das Almas Reserve, 300 km south of Fortaleza, in the "caatinga" zone, a seasonal xerophilous thorn shrubland that prevails on the semi-arid lowlands and covers most of Ceará State. Workers were not counted but Quinet et al. (2009) found a queen/worker ratio of 1:100.

Twenty-five queenright nests of *C. biroi* were collected in Taiwan, in Hushan (Yunlin county), Lienhuachih and Huisin (Nantou county), and Chuchih (Taipei county), during 2010–2011 (Table S1). Nests occur in rotten wood, in soil, and under stone in broad-leaved forests. In these same habitats (Hushan, Chichi (Nantou county) and Luyeh (Taitung county)), we also collected six queenright nests of *C. schimmeri*.

2.2. Histology

The posterior half of the gasters of 5 'intermediates' of *C. biroi* and *C. pygmaea* were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Tissues were postfixed in 2% osmium tetroxide in the same buffer, dehydrated in a graded acetone series and embedded in Araldite. Serial semithin sections with a thickness of 1 μm were made with a Leica EM UC6 ultramicrotome and viewed with an Olympus BX-51 microscope to check for the eventual presence of a spermatheca.

2.3. Other morphological observations

Specimens of the three castes of *C. pygmaea* (5 queens, 5 workers, 2 'intermediates'), *C. biroi* (3 queens, 5 workers, 3 'intermediates'), *C. schimmeri* (2 queens, 5 workers, 5 'intermediates') were measured (total length from middle of clypeus to tip of gaster; width of first gaster segment) and weighed. Specimens of the three species were coated with a thin gold layer by sputtering, and examined with a JEOL JSM-6360 scanning electron microscope. Gasters were dissected in all three castes of the three species, to describe ovaries, yellow bodies, and check for eventual presence of a spermatheca.

3. Results

3.1. Occurrence of 'intermediates' in colonies

Seventy-one nests belonging to one *C. pygmaea* colony excavated in April (rainy season) yielded 14 'intermediates', present in eight of the 25 more populous nests (with two or more queens). In contrast, 75 nests (from 4 colonies) excavated a few months earlier (end of dry season) yielded only four 'intermediates' (Table S1).

C. biroi colonies ($n = 25$) were monogynous with 12–479 workers and 1–5 'intermediates' (26 in total). Colonies of *C. schimmeri* ($n = 6$) were also monogynous with 314–626 workers and 14–23 'intermediates' (103 in total) (Table S1).

3.2. External morphology of 'intermediates'

Winged queens and workers of all species are highly divergent in total size, and the 'intermediates' fall in between (Fig. 1). Both linear measures and weights were intermediate (Table 1). Size differences in head and thorax are clearly illustrated in Figs. 2 and 3.

In *C. pygmaea*, 'intermediates' have less protruding eyes than winged queens, with a number of ommatidia (130 ± 6) midway between workers (54 ± 5) and queens (267 ± 6; Table 1). Similarly in *C. biroi* and *C. schimmeri*, ommatidia numbers of 'intermediates' are in-between queens and workers (Table 1). Compared to queens, the ocelli in 'intermediates' are reduced although very distinct (lens and small patch of photoreceptors) in *C. pygmaea* and *C. biroi*, while they are not seen in *C. schimmeri* (Fig. 2, Table 1).

Ant queens have a flight thorax in which the second segment (mesonotum) is greatly enlarged because it functions to attach the considerable wing muscles. The first segment (pronotum) is strongly reduced, unlike in workers where winglessness causes it to be more prominent (Keller et al., submitted for publication). *Orthocrema* queens have indeed a large raised mesonotum that completely hides the pronotum in dorsal view (Fig. 3). In workers, although pronotum and mesonotum are fused, the former is obviously bigger. In 'intermediates', despite winglessness, the mesonotum is bigger than in workers but the pronotum is visible dorsally (Fig. 3). Moreover, 'intermediates' exhibit striking interspecific differences in thorax structure. In *C. biroi*, a suture (i.e. line or groove indicating the junction of two formerly articulated sclerites) cannot be seen between pronotum and mesonotum. In *C. schimmeri*, the pro-mesonotal suture is distinct and importantly, the mesonotum is less reduced in size (Fig. 3). Too few specimens were examined by SEM to assess intraspecific variability in 'intermediates'.

3.3. Internal morphology of 'intermediates'

Dissections of 'intermediates' revealed striking differences in numbers of ovarioles: in-between queens and workers in *C. pygmaea* and *C. biroi*, but same as queens in *C. schimmeri* (Table 1). Yolky oocytes were present (Fig. 4A–C) and eggs were laid before dissections in *C. biroi*. Dark yellow bodies were conspicuous at the base of ovarioles in 'intermediates', indicating that many eggs had been laid earlier. Unlike in queens, a spermatheca was not found in 'intermediates', and this was confirmed by both transverse and longitudinal histological sections (Fig. 4D–E).

4. Discussion

4.1. Orthocrema *'intermediates' are a mosaic of queen-like and worker-like traits*

Our morphological data in *C. pygmaea*, *C. biroi* and *C. schimmeri* show that all have a third caste combining morphological traits from winged queens and workers. Individuals with intermediate size are very conspicuous (Fig. 1) given the striking size differences between queens and workers in the subgenus *Orthocrema*. The gaster of 'intermediates' is particularly big, and this reflects a higher number of ovarioles compared to workers (Table 1). Most individuals dissected had either active ovaries (i.e. many developing