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[SHORT COMMUNICATION]

The Intra-colony Inbreeding of *Leptothorax makora* (Hymenoptera: Formicidae)

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Numerous attempts have been made by scholars to show various patterns in the sociality of social insects in the wild (*e. g.*, Lin and Michener, 1972; Holldobler and Wilson, 1977, 1990; Keller, 1991, 1993; Bourke and Frank, 1995; Heinze and Tsuji, 1995; Crozier and Pamilo, 1996). In the last few decades, a lot of evolutionary ecologists have paid attention to 'polygyny' (Wilson, 1974; Holldobler and Wilson, 1977; Rosengren *et al.*, 1993) (in this paper, we defined 'polygynous' colony as a colony with more than one queen), because it is a point of contradiction in Hamilton's (1964a, b) kin selection theory. Recently, some scholars thought that the hazard of solitary colony founding during dispersal and after colonization are likely to be key factors in selecting for polygyny (Keller, 1991; Tinaut and Heinze, 1992; Herbers, 1993; Heinze, 1993; Bourke and Heinze, 1994; Keller and Reeve, 1994).

If the hazard of solitary colony founding during dispersal is high in polygynous ant species, how do new foundress queens and males mate without dispersal? In theory, the possibility of inbreeding within a colony may be one of the mechanisms of mating without dispersal in ants, because of the haplo-diploid sex determination in which a mated female can lay both fertilized (female) eggs and unfertilized (male) eggs. In practice, however, only a few attempts have so far been made at whether ants, especially polygynous ant species, have the ability of inbreeding within a colony.

There are many species of polygyny in ant genus *Leptothorax* (Hymenoptera: Formicidae, Myrmicinae). At least 13 *Leptothorax* species are distributed in Japan (Terayama *et al.*, 1992). *Leptothorax makora*, that was classified into *Leptothorax* sp. 9., is one of the species in which the percentage of polygyny is high, especially in June, that is, just after mating and dispersal season (Murase *et al.*, 2000). These results suggest that the adoption of new foundress queens in established colonies is more dominant in *Leptothorax makora* than in other closely related *Leptothorax* species in which solitary colony founding by foundress queens is more common (Hamaguchi and Kinomura, 1996; Murase *et al.*, 2000).

The purpose of this study is to test the ability of intra-colony inbreeding in *Leptothorax makora* by rearing in the laboratory. We observed dispersal and mating behavior, and examined inbreeding within a colony.

The nest size of *Leptothorax makora* is about 30 workers. Their reproductive season is thought to consist of one brood per year early in the spring. The body length of workers is about 2–2. 5 mm (Terayama *et al.*, 1992). We collected all ant colonies from the slopes of secondary deciduous forests near the Nagara River, Gifu City (35° 27'N, 136° 47'E, 30–100 m at sea level), Gifu Prefecture, in the central part of Japan. *Leptothorax makora* utilizes fallen twigs, the caliber

of which range from 1.5–3.0 mm available for nest sites, on the ground (Murase *et al.*, 2000). We sampled 30 ant colonies in the field in April 2003 (pre-dispersal season). We spent six days (20 h) on collecting twigs. We randomly picked up and dissected fallen tree twigs on the forest floor, and checked them to see whether an ant nest was founded or not. We brought the ant colonies to a laboratory on the same day. We reared them in plastic cases individually until late August. For observation of inside nests, we put two glass tubes (with a diameter of 6 mm and 5 cm long in length) in each plastic case as their nest sites. We observed the dispersal and mating behavior of new foundress queens and males everyday from end of May to end of July. We observed the mating behavior between the new foundress queens and the males from 4 o'clock to 8 o'clock (early in the morning) and from 12 o'clock to 16'clock (daytime). In August, we dissected the new foundress queens and examined their seminal receptacles under a light microscope.

The numbers of colonies that were observed to have both offspring (*i. e.*, new foundress queens and males) were eight. The total number of new foundress queens in the eight colonies was 24. The new queens came back to their mother's nest (the glass tube initially supplied) again after walking around the nest. They did not use another glass tube as the new nest site. The hazard of solitary colony founding of *Leptothorax makora* during dispersal may be the highest among three sibling species that inhabit the closed microhabitats. In one species, *Leptothorax congruus*, there is one queen per colony, while in the other species, *Leptothorax spinosior*, the number of queens per colony differs among populations (Hamaguchi and Kinomura, 1996). The inter-specific difference of environmental factors, especially some factors affect the hazard of solitary colony founding such as twig density, among these three ant species may influence number of queens per colony.

The mating behavior between new foundress queens and males was observed, in all cases, in the early morning. Sperm was found in the seminal receptacles of all new foundress queens (24 individuals) (Fig. 1). Twenty foundress queens in the nests where no males were present were chosen, dissected and examined. Mature eggs were observed in the ovarioles, while sperm was not found in the seminal receptacles of these queens. The results showed that *Leptothorax makora* has the ability of inbreeding between new foundress queens and the nest mate males. Intra-colony

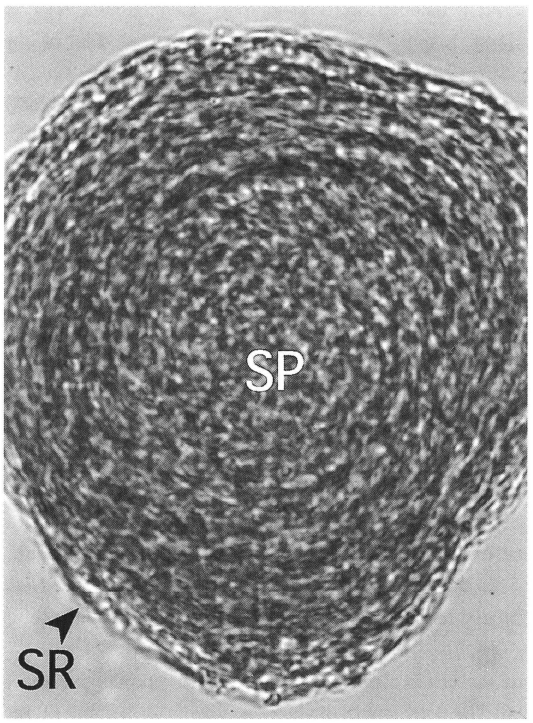

Fig. 1 Sperm within a seminal receptacle of a new foundress queen of *Leptothorax makora*. The diameter of the seminal receptacle is 30 μm. SP: sperm, SR: seminal receptacle.

inbreeding may contribute to the high percentage of polygyny in *Leptothorax makora*.

In the future, we need to observe occurrence of intra-colony inbreeding in the other two sibling species, and estimate the hazard of solitary colony founding in each ant species.

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