How Average Relatedness Affects the Frequency of Trophallaxis between Workers in an Experimental Colony of the Polygynous Ant, *Camponotus yamaokai*

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Abstract — To determine whether workers engage in trophallactic behavior preferentially toward highly related nestmates, we examined these behaviors between workers in colonies consisting of both kin and non-kin (mixed colony) and of only kin (pure colony). These behaviors were observed under sufficiently fed and in both starvation and normal feeding conditions. Workers in mixed colonies exhibited trophallactic behaviors equally toward kin and non-kin in both experimental conditions. The frequency of trophallaxis between workers in mixed colonies, however, tended to be lower than that in pure colonies, is especially under starvation conditions. The low frequency of trophallaxis among workers discussed in terms of the low degree of genetic relatedness among nestmates in mixed colonies.

Introduction

Eusocial insects, including ants, bees, wasps, and termites, live in groups that range from dozens to millions of individuals. They are socially altruistic. The existence of worker castes that exhibit altruistic behavior at the expense of their own fitness has always been puzzling. Hamilton (1964) proposed kin selection theory to explain how this altruistic feature of organisms can be favored if it increases the reproductive success of kin. Moreover, he suggested that the high genetic relatedness among females ($r=0.75$) resulting from haplodiploidy has contributed to the evolution of altruistic behavior in hymenopteran social insects. This hypothesis has been, however, somewhat controversial because of the repeated observations of polyandry and polygyny in eusocial insects (Moritz & Southwick, 1992; Bushinger, 1986; Bourke, 1988; Holldobler & Wilson, 1990). In colonies with low average relatedness, the workers' investment in their unrelated nestmates might not be beneficial to the donors. It might be beneficial, however, if it were directed preferentially toward highly related nestmates.

Several previous studies have suggested that honey bee workers might exhibit altruistic behavior that is preferential toward their kin (Visscher, 1986; Frumhoff, 1987; Page et al., 1989). It remains unclear, however, whether such suggestions, based on the results of experimental colonies with a low number of patriline or high phenotypic distinctiveness, is valid in nature. Therefore, it has also not yet been established whether honey bee workers can distinguish the extent of relatedness among their nestmates in a natural colony (Carlin, 1989, 1993; Moritz et al., 1996). Several previous studies also showed that workers' trophallaxis was not directed preferentially toward related nestmates in colonies (polygynous ants; Carlin et al., 1993, Snyder 1993; DeHeer & Ross 1997).

On the other hand, recent studies have suggested the possibility that workers can assess the mating frequency of queens in their colonies, which they then use to control the sex-ratio of the reproductive caste (Möller 1991, Sundström 1994). Furthermore, Evans (1995) also showed that workers could assess the re-
latedness asymmetry in their colonies, based on the diversity of genetic odor cues of their sexual larvae.

In the present study, we determine whether kinship is involved in the trophallaxis between nestmates. Trophallactic behavior in colonies with different genetic variations was observed under conditions in which the colonies were either supplied with sufficient food or were not fed. Furthermore, we compared the total frequency of the trophallaxis between workers in colonies with high genetic relationship to those in colonies with low genetic relationship under the both conditions, because the social interactions among all nestmates might change in response to genetic relatedness.

**Methods**

In a single nest of the polygynous ant *Camponotus yamaokai*, dozens of queens and hundreds of workers may live together. Almost all queens have matured eggs in their ovaries (Satoh, 1989, 1991). They seem to be closely related because the estimated relatedness based on DNA fingerprints is near 0.75 (Satoh et al. 1997).

In the present study, we observed trophallaxis behaviors in experimental colonies with varying genetic composition (“pure” or “mixed”) under different food conditions. We collected the nests of *C. yamaokai*, which inhabits the dead twigs of bamboo groves in Saitama and Chiba prefectures (central Japan) in October 1994 and January 1995, respectively. The distance between these populations is about 80 km. These nests were kept at 25°C and 16L: 8D. Young minor workers emerged in 5 original colonies from Saitama (SA-SE) and 3 original colonies from Chiba (CA-CC) between April and June. Within 24 h after emergence, some of them were selected from the original colonies to establish 2 types of experimental colonies. One contained 4 “pure colonies” (PA-PD), each consisting of 2 queens and 10 young minor workers from an original colony. The other type had 5 “mixed colonies” (MA-ME) consisting of 2 queens and 10 young workers obtained in equal numbers from both Saitama and Chiba original colonies, that situations presumably had low genetic relatedness. The brood in their original colonies were introduced into neither pure nor mixed colonies. Each ant was marked with a colored dot (Paint Markers, Mitsubishi, Japan) on the thorax and gaster, and transferred into plastic containers (dia.: 10 cm; ht.: 5 cm) with 2 artificial nests (plastic tubes: dia.: 7mm; length: 2cm). Thus, individual workers were allowed to select one of 2 tubes for their nest.

The ants in both pure and mixed colonies were sufficiently supplied with diluted honey and artificial pollen for honey bees (sufficiently fed conditions) for 7 days, and then their behavior was observed under the same conditions for 6 days (1 h per day).

We categorized their behavior as follows:

1. Trophallaxis: Mouth-to-mouth contacts lasting at least 5 s were regarded as trophallactic behavior. Dissection of the alimentary canal of the workers showed that colored honey-dew was transferred from donor to receiver when such mouth-to-mouth contact lasted at least 5 s.
2. Guarding: At least 1-min antennation against the edge of nest entrances.
3. Foraging: Feeding on food and/or water outside of the nest tube.
4. Self-grooming
5. Allo-grooming
6. Brood care: Antennating and tending of the eggs which each queen laid during the experimental periods.

As soon as observation under the sufficiently fed condition was finished, the ants were switched to starvation conditions in which only water was available for 7 days. Their behavior was then observed for 6 days (1 h per day).

**Results**

In both the pure and mixed colonies, all of the workers and queens stayed and cohabited in the same nest tube. In all mixed colonies under both conditions, workers did not perform trophallaxis toward related workers or queens from the same original colonies, but uniformly toward all workers and queens irrespective of their origins (Table 1).