Polygyny Increases Survival of Minor Workers and Mortality of Major Workers in Overwintering *Camponotus yamaokai* (Hymenoptera: Formicidae)

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Ann. Entomol. Soc. Am. 107(3): 702–707 (2014); DOI: http://dx.doi.org/10.1603/AN13063

ABSTRACT A higher proportion of polygynous ant species in northern areas suggests that cold climates select for increased queen number per colony. However, the types of social conditions within nests that allow polygynous species to inhabit cold climates are unclear. The Japanese twig-dwelling ant, *Camponotus yamaokai* Terayama & Satoh, 1990, which has on average two queens per colony, also inhabit colder areas than monogynous related species. *C. yamaokai* alates remain in natal colonies and always overwinter with workers. A previous study demonstrated that cohabitation by workers facilitates overwintering success of *C. yamaokai* queens. However, how queen number influences the survival of queens cohabitating with workers and the survival of other castes was not investigated. Here, we compared overwintering survival of queens and workers among experimental groups with different social structures. Wintering experiments revealed that queen survival did not differ between monogynous (one queen) and polygynous (two queens) groups but was correlated positively with worker number. Meanwhile both queen and worker number influenced worker survival, and these factors interacted with worker type (minor or major). Worker survival increased with increasing worker number, but the effect was more pronounced for major workers than for minor workers. With respect to queen number, minor workers survived longer in polygynous groups than in monogynous groups, whereas the opposite pattern was found in major workers. In other words, major worker mortality increased with increasing queen number. These results suggest that cohabitation with workers but not other queens facilitates queen survival, and that queen number increases survival in minor workers but increases mortality in major workers. Possible explanations for this latter pattern are discussed.

KEY WORDS social structure, polygyny, hibernation, over-wintering, myrmecology

Monogyny (single queen per colony) is considered to be a typical social system of ants. Phylogenetic studies indicate that monogyny is an ancestral trait (Hughes et al. 2008a,b). Meanwhile, polygyny (multiple queens per colony) has evolved frequently in every ant subfamily (Keller 1995, Heinze and Foitzik 2009). Intraspecific variation in queen number is also observed in some species (Brown and Keller 2000, Herbers and Johnson 2007, Meunier and Chapuisat 2009). A higher proportion of polygynous *Leptothorax* ants species in northern areas suggests that cold climates could be one factor selecting for increased queen number per colony (Heinze 1993, Heinze and Holldobler 1994). However, the types of social conditions within nests that allow polygynous species to inhabit cold climates are unclear (Heinze and Foitzik 2009).

This is the case in two common twig-dwelling ants in Japan—*Camponotus yamaokai* Terayama & Satoh, 1990 and *Camponotus nawai* Ito, 1914. They were described as the same species until 1990, owing to their similarities in ecology and morphology (Satoh 1990). However, interpopulational comparisons revealed that social structure and reproductive behavior were different among populations (Satoh 1989, Satoh et al. 1997). Terayama and Satoh (1990) described polygynous populations as a new species, *C. yamaokai*, and monogynous populations as *C. nawai*. *C. yamaokai* inhabits higher latitude zones in Japan than *C. nawai* (Terayama and Satoh 1990); when inhabiting the same latitude, *C. yamaokai* is observed at higher altitudes than are *C. nawai*. Their parapatric distribution suggests that the polygynous *C. yamaokai* is better adapted to colder climates than is the monogynous *C. nawai*. A laboratory experiment also revealed that *C. yamaokai* had higher cold tolerance than *C. nawai* (Shiroto et al. 2011). In this experiment, the coexistence of workers had a larger influence on queen survival than did cold tolerance of queens themselves. *C. nawai* foundresses always overwinter without...
workers after the nuptial flight, whereas *C. yamaokai* alates (newly emerged winged reproductives) remain in natal colonies and always overwinter with workers (Satoh 1989). Therefore, cohabitation by workers would allow *C. yamaokai* to achieve colony hibernation success (Shiroto et al. 2011).

Meanwhile, an influence of queen number on colony hibernation is disputable in *C. yamaokai*. In acorn ants, *Temnothorax curvispinosus*, no difference in overwintering survival was detected between monogynous and polygynous colonies (Herbers and Johnson 2007). Our previous study also indicated that solitary queens of *C. yamaokai* survived as long as pairs of queens when they were maintained without workers under cold conditions (Shiroto et al. 2011). However, because *C. yamaokai* queens always overwinter with workers in field, we should investigate how queen number influences survivals in the presence of workers. Thus, the aim of the current study is to investigate whether the coexistence of workers alters the influence of queen number on queen survival. In addition, we also investigated how queen number influences worker survival.

**Materials and Methods**

**Ants.** The queen number per nest ranges from 0 to 46 in *C. yamaokai* (Satoh 1990). Nests of 11.6 and 18.4% are queenless and monogynous, respectively. Nests of 14.9 and 55.1% contain two queens and more than two queens, respectively. *C. yamaokai* has two worker castes (Satoh 1990). The size distribution of workers is bimodal. The head width of minor workers is smaller than 1.1 mm and that of major workers are larger than 1.2 mm. The workers of intermediate size are rare.

**Sample Collection.** Colonies of *C. yamaokai* were collected at Shimoda (34° 40′ N, 138° 50′ E) on 26–28 March 2009, and Fukusima (37° 46′ N, 140° 28′ E) on 5 and 6 April 2009. Ant colonies occupying dead branches and stems of dead bamboo were transferred into plastic bags. In the laboratory, ants were moved into plastic boxes (7 by 7 by 4 cm; Fig. 1A), in which they were maintained under a photoperiod of 10:14 (L:D) h and 15 ± 1°C until initiation of the wintering experiments on 13 April 2009. Red cellophane-coated plastic straws (5 mm in diameter and 4 cm in length) were placed in the plastic boxes as nesting sites (Fig. 1A). Fluon was painted on the sides of the boxes to prevent ants from escaping. We supplied an absorbent cotton containing yolk and honey and an absorbent cotton containing water as food. Absorbent cotton pads were changed every 4 d.

**Wintering Experiments.** To evaluate the influence of queen number and worker number on overwintering survival of individuals, we compared five treatment groups: 1) 2 queens, 10 major workers, and 70
Table 1. The compositions of castes for each treatment group

| Treatment groups | Queens | Major workers | Minor workers | n  |
|------------------|--------|---------------|---------------|----|
| 1                | 2      | 10            | 70            | 14 |
| 2                | 1      | 10            | 70            | 14 |
| 3                | 1      | 5             | 35            | 14 |
| 4                | 2      | 0             | 0             | 14 |
| 5                | 1      | 0             | 0             | 14 |

minor workers; 2) 1 queen, 10 major workers, and 70 minor workers; 3) 1 queen, 5 major workers, and 35 minor workers; 4) 2 queens without workers; and 5) 1 queen without workers (Table 1). For each treatment group, six and eight replicates were prepared from Shimoda and Fukushima populations, respectively. Field colonies had larvae and pupae, the number and developmental stages of which varied largely. Because it was difficult to assign immature stages homogeneously to treatment groups, we used only queens and workers to prepare colony fragments for the different treatment groups. To avoid pseudoreplication, we did not assign more than one colony fragment originating from the same colony to the same treatment group (Supp Table 1 [online only]).

Each fragment was moved into a plastic box (5.5 by 7.5 by 3 cm; Fig. 1B) with red celophane-coated plastic straws (5 mm in diameter and 4 cm in length). Fluon was painted on the sides of the plastic boxes. The plastic boxes were maintained under a photoperiod of 10:14 (L:D) h in an incubator (MIR-253, Sanyo Electric Co., Ltd, Osaka, Japan). Neither water nor food were provided to ants throughout the wintering experiment. The incubator temperature was set at 5.0 ± 1°C during the light period, at 0.4 ± 1°C during the first 10 h of the dark period, and at −3.3 ± 1°C during the remaining 4 h of the dark period. Temperatures of 5.0, 0.4, and −3.3°C are derived from the maximum, low, and minimum temperature in January, respectively, in Shizugawa (38° 14′ N, 141° 26′ E), which is the northern limit of C. yamaokai. Dry conditions also resemble the field environment of the Pacific side of Japan (Sekiguchi 1959).

The number of ants surviving was recorded daily until all individuals were dead. We checked ant behavior daily to judge whether ants lived or died, when they walked around or moved antennae, legs, or both. When ants did not move, we removed those ants from the incubator and observed them for 3 min at room temperature. Ants that did not move within 3 min were removed as dead ants.

Statistical Analysis. The survival of ants was analyzed with the Cox proportional hazards regression model. Calculations were performed using the packages “coxme” (ver. 2.0) and “survival” (ver. 2.37-4) of statistical software R (ver. 3.0.0; R Development Team 2013). The first day of the wintering experiment to the last day observed alive was used as a dependent variable. Colonies from which queens were derived was set as a random factor. To analyze the survival of queens, we used the data of treatment groups 1) to 5). To analyze worker survival, we used the data of treatment groups 1) to 3). Model selection took place using a bidirectional stepwise procedure based on Akaike information criterion (AIC).

Results

The Cox model indicated significant influences of worker number on queen survival (Table 2). Queens survived longer with increasing workers within nests (z = −7.91, P < 0.001; Fig. 2). When workers did not exist within nests, half of queens died before 2 mo (median survival time [MST] = 58 d). When 40 and 80 workers coexisted, half of queens survived longer than 3 mo (MST = 97.5 and 115.5 d, respectively). Interpopulational difference in queen survival was significant but small (z = −1.96, P = 0.05; Table 2). Queens in a northern population (Fukushima, MST = 78 d) survived longer than queens in a southern population (Shimoda, 72.5 d; Fig. 2). Queen number did not influence queen survival (z = 0.53, P = 0.6; MLT50: 79.5 d in single queens vs 75 d in double queens; Fig. 2; Table 2).

The Cox model indicated that worker number also increased worker survival (z = −2.27, P = 0.023; Table 3; Figs. 3 and 4). However, a significant interaction between worker type and worker number indicated that survival of major workers increased with worker number more sharply than did survival of minor workers (z = −2.91, P = 0.004; Table 3; minor workers’ MST: 49 d with 40 workers vs 64 d with 80 workers; major workers’ MST: 75.5 d with 40 workers vs 75.5 d with 80 workers).

Queen number influenced worker survival (z = −2.45, P = 0.014; Table 3), and a significant interaction between worker type and queen number indicated that queen number influenced survival of minor and major workers differently (z = 3.39, P = 0.001; Table 3; Figs. 3 and 4). Minor workers survived longer with increasing queen number (MST: 57 d with 1 queen vs 66 d with 2 queens; Fig. 3). However, major workers survived for a shorter length of time with increasing queen number (MST: 80 d with 1 queen vs 70 d with 2 queens; Fig. 4).

Discussion

The current study revealed that queen number did not influence queen winter survival. In contrast, the cohabitation by workers increased queen survival re-
Regardless of queen number. This trend was the same for both populations. Consequently, queen number did not explain why \textit{C. yamaokai} colonies that have two queens on average (Satoh 1990) could inhabit colder area than the congeneric monogynous species, \textit{C. nawai}. Consistent with the present results, queen number did not influence overwintering success in the acorn ant (\textit{T. curvispinosus}; Herbers and Johnson 2007). Further studies over various taxa would reveal the role of queen number in overwintering colonies in social insects.

Meanwhile, queen number influenced overwintering worker survival significantly, but trends were different between minor and major workers. Minor workers survived longer in polygynous groups than in monogynous groups. In contrast, major workers survived for a shorter length of time in polygynous groups than in monogynous groups, although they survived longer with increasing worker number. This suggests that other workers help major worker survival, but that multiple queens may decrease their survival. One possible reason for this result is that the labor load of major workers increases with queen number. Tsuji et al. (2011) reported in \textit{Diacamma sp.} that nonreproductive workers survive for shorter periods in colonies with reproductive workers compared with those without, because reproductive workers increase the labor loads of nonreproductive workers. In \textit{C. yamaokai}, minor workers are observed usually to engage in outside labor, whereas major workers tend to stay a long time inside nests and around queens. Therefore, if major workers care for queens predominately, queen number per major workers would increase their energetic costs. However, the division of labor between minor and major workers has not been investigated quantitatively in \textit{C. yamaokai}. In addition, because the activity of ants is low under overwintering conditions, the labor load of major workers would not be expected to increase much with queen number.

Alternatively, major workers may play the role of honeypots, as reported in a congeneric ant, \textit{Camponotus nipponicus} (Wheeler) (Hasegawa 1993). It is known that trophallaxis from workers to queens can

![Fig. 2. Cumulative proportion of \textit{C. yamaokai} queens surviving over time under different social conditions. Top and bottom graphs show Fukushima (northern) and Shimoda (southern) populations, respectively. Left and right graphs show monogynous (1 queen) and polygynous (2 queens) groups, respectively. Dotted lines, broken lines, and solid lines show groups without workers, with 40 workers, and with 80 workers, respectively.](https://academic.oup.com/aesa/article-abstract/107/3/702/56915)

| Table 3. Results of the Cox proportional hazards regression model for worker survival |
|-----------------------------|--------------|---------|--------|
| Factors                    | Coefficients |  z      |  P     |
| Population                 | -0.31        | -2.62   | 0.009  |
| Queen number               | -0.16        | -2.45   | 0.014  |
| Worker number              | -0.01        | -2.27   | 0.023  |
| Worker type                | -0.24        | -0.56   | 0.300  |
| Worker type \times Queen number | 0.44       | 3.39    | 0.001  |
| Worker type \times Worker number | -0.01    | -2.91   | 0.004  |

Interactions not listed in this table were excluded from the stepwise procedure. The reference point of this model is the mean survival of minor workers in the Shimoda population.
improve the nutrient and moisture conditions of queens (Kaspari and Vargo 1995). We observed trophallaxis between queens and major workers under breeding conditions. However, frequency of trophallaxis under overwintering conditions was not quantified, and the direction of food transfer has not been investigated.

Alternatively, crowding by workers could decrease metabolic rates of queens by avoiding moisture loss during dry winters, as reported in the diapausing tropical beetle, *Stenotarsus rotundus* (Tanaka et al. 1988). However, this factor does not explain why queen number per worker was correlated negatively with major worker survival. Because the nest of *C. yamaokai* is long and narrow (≈5 mm in diameter), workers could reduce moisture loss from a nest by crowding at the nest entrance. Therefore, a slight difference of queen number (1 vs 2) would not influence the workers’ efforts to keep moisture conditions high within the nest.

In addition, Kaspari and Vargo (1995) suggest that workers surrounding a queen could act as a temperature buffer. *C. yamaokai* workers also could reduce heat loss from a nest by crowding at the nest entrance. However, there is no information on major workers’ ability to produce heat in *C. yamaokai*. If many major workers were necessary to prevent a queen from freezing, queen number per worker would influence the energetic costs and survival of major workers. Further studies are needed to investigate how number of major workers influences body temperature of queens.

Thus, the proximate factors underlying different influences of queen number on minor and major workers is still unclear. To elucidate these factors, we should investigate the division of labor between worker subcastes and the physiological conditions of *C. yamaokai* under overwintering conditions.

**Acknowledgments**

Our work has been supported by Grant-in-aid for Young Scientist (Start-up) (#19870002) and Inamori foundation grant. We thank Ann M. Fraser for generously helping with editing.

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Received 23 April 2013; accepted 28 February 2014.