

Annual life cycle and timing of male-egg production in the ant *Colobopsis nipponicus* (Wheeler)

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Summary

Life history parameters, including colony boundary, number of queens in a colony, seasonal change in colony-member composition and seasonal change in male-egg production were investigated for the arboreal ant *Colobopsis nipponicus* in the field. Colonies were shown to be typically monogynous and polydomous, with several different colonies inhabiting the same tree. The queen and workers overwinter only with larvae, which grow rapidly into new adults during early summer. Male-destined eggs were produced throughout the warm season with a conspicuous peak in summer that coincided with adult production. It is suggested that this might be related to seasonal changes in the food available to the queen.

Introduction

The accumulation of knowledge on annual cycles, such as the pattern of brood production, from a wide range of ant species is essential when generalizations about their ecology are being drawn. Community structures, interspecific interactions and specific life-history strategies of ants are known to be affected by the annual life-cycle of each species in the temperate region (Herbers, 1986, 1989; Yamaguchi, 1992). Yet, in spite of its potential importance, this has been studied for only a few species (Brian, 1957; Ito et al., 1988; Ichinose, 1986a; Mizutani and Imamura, 1980; Satoh, 1989). Even important reproductive information, such as the timing of male-egg production, has been reported for only a few species (Gösswald and Bier, 1955; Passera et al., 1988).

In this study, several important colony characteristics of an arboreal ant, *Colobopsis nipponicus*, were investigated in the field. Colony boundary and queen number were determined in addition to seasonal changes in colony composition and timing of male-egg production.

Materials and methods

Study organism and study site

Colobopsis nipponicus is a nocturnal, arboreal ant which nests in dead hollow twigs in evergreen broad-leaf forests in Japan. This species shows complete worker dimorphism; major workers are larger than minor ones and possess truncated heads. Foraging is performed solely by minor workers (Satoh, pers. comm.).

The study site was part of a coastal forest on a hill at Amatsukominato, Chiba, Japan. Most nests of *C. nipponicus* were found in hollows of dead twigs attached to live trunks of the tree *Lythocarpus edulis*. Nest density was high, the majority of dead twigs being occupied.

Colony sampling

Colony sampling was conducted monthly, from April 1990 to October 1991, in an area of about 500 m² (10 m × 50 m). At each sampling, several trees were selected randomly and all dead twigs attached to the trees were broken open. Twigs occupied by ants were brought back to the laboratory for further examination. Since *C. nipponicus* is nocturnal, daytime sampling allowed me to collect the entire nest. In the laboratory, the nest was censused for the numbers of adults of each caste, pupae, prepupae, larvae and eggs. Larvae were measured to the nearest 0.05 mm in body length using a binocular microscope and ocular micrometer, and were assigned to one of seven classes according to their length (see Tab. 1). Since *C. nipponicus* has two distinct worker subcastes, worker number is not the best measure of colony size, which is better expressed in terms of the total dry weight of workers. Dry weights of both worker subcastes were estimated from the regression equations of pupal dry weight of each subcaste on the number of major workers in the colony (Hasegawa, submitted).

Determination of the colony boundary

To avoid confusion between nest and colony, in this study, an occupied hollow twig was treated as a nest, and a group of nests, distinctly separated from the others by

Table 1. Arbitrary size classes of larvae for estimating total weight of brood

Size classes	Body length (mm)	Dry weight (mg) Mean ± S.D.	N
1	< 1.0	0.020 ± 0.006	30
2	1.0–1.6	0.071 ± 0.014	30
3	1.6–2.1	0.149 ± 0.025	30
4	2.1–2.7	0.271 ± 0.034	30
5	2.7–3.5	0.434 ± 0.066	30
6	3.5–4.5	1.354 ± 0.246	30
7	4.5 >	2.079 ± 0.131	24

Table 2. Criteria for assessing hostility and amicability between workers of different nests of *C. nipponicus*

Hostile interactions

- a. Intruder flees from recipients
- b. Recipients surround intruder
- c. Recipients pushes intruder with open mandibles
- d. Recipients sprays formic acid toward intruder
- e. Recipient and intruder lock together

Amicable interactions

- f. Intruder stays on brood pile
 - g. Allogrooming occurs between intruder and recipient
 - h. Recipient solicits intruder for regurgitation
 - i. Intruder regurgitates nutrient to recipients
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behavioural and genetic examinations, was referred as a colony. Many nests contained no dealate queen (see below), it was difficult to determine the colony boundary without behavioural and genetic analyses between members of nests (see Snyder and Herbers, 1991). A hostility test between two nest members, and an electrophoretic analysis of genotype of nest members was made. Hostility between workers was assessed by behavioural interactions for all possible pairs of the nests collected in each sampling. All members of a nest were placed in a small plastic box (65 × 50 × 25 mm) at least two days before tests. An alien major worker was introduced into the box, and interactions between the recipients and intruder were observed during at least three minutes. The criteria of hostility and amicability are presented in Table 2. When any hostile interactions (a to e in Table 2) were observed, the two nests were judged to belong to different nest groups. The occurrence of any amicable interactions corresponding f to i was taken to indicate that the nests belonged to the same nest group.

Genetic examination was made by polyacrylamide electrophoresis of larvae. In this population, one of the esterase loci (*Est-2*) was polymorphic with two alleles (F and S) which seemed to follow Mendelian inheritance (Hasegawa, unpublished). From each of 73 nests collected in June 1991, six male larvae were selected randomly and electrophoresed. The body shape of well-developed male larvae of *C. nipponicus* is more slender than female larvae; distinction was therefore easy. Since the queen produces all the males in a colony (Hasegawa, unpublished), the genotype of a nest queen could be determined from the genotype of the males.

Seasonal change in male egg production

Seasonal changes in the proportion of male-destined eggs were investigated by determining the karyotype of eggs present in the nests, using the method of Imai et al. (1977). Thirty eggs were selected at random from mature clonics, collected in each month during May–October 1991, and were karyotyped. Haploid eggs were regarded as males.

Results

Colony boundary

A total of 542 nests was collected in the study period. Two hundreds and forty-nine were queenright and 293 were queenless. These nests were classified into 286 colonies by the behavioural test. In June 1991, samples were also examined genetically: 103 nests collected from 17 trees were classified into 53 groups of nests, using worker-hostility tests. In every case, there was only one queenright nest per nest group. Within a nest group, the nest queen genotype, inferred from the male larvae present in each nest, was always consistent with the nest group being a genetically integrated unit, i.e. a colony. Figure 1 shows one example of results of analyses. As shown in Figure 1, multiple colonies inhabited a single tree. There was no case in which one colony nested among several trees.

Number of queens in a colony

In 286 colonies classified by the behavioural tests, 249 were queenright, of which 242 (97.2%) were monogynous and 7 (2.8%) contained two or three queens (polygynous). Dissections confirmed that all queens of polygynous colonies were inseminated and possessed well-developed oocytes. Polygynous colonies contained a few workers, and were significantly smaller than the monogynous colonies in their size (Mann-Whitney's U test, $z = 2.174$, $p = 0.03$), and in all but one case polygynous colonies contained minor workers only. In addition, all polygynous colonies were monodomous. Thus, it is suggested that the polygynous colonies were early stages in the colony life-cycle.

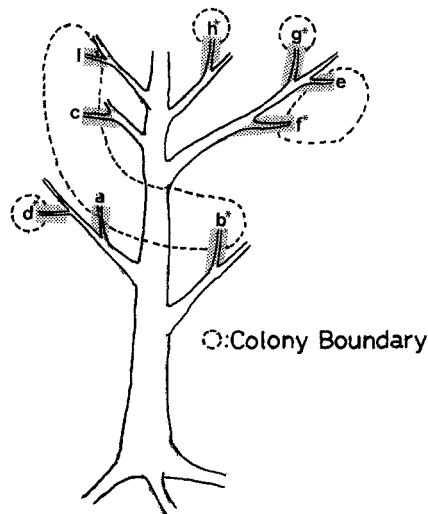


Figure 1. Colony structure of the ant *C. nipponicus*. A dotted circle represents a nest group (colony) defined by behavioural tests. Asterisk shows the nest where the queen was present

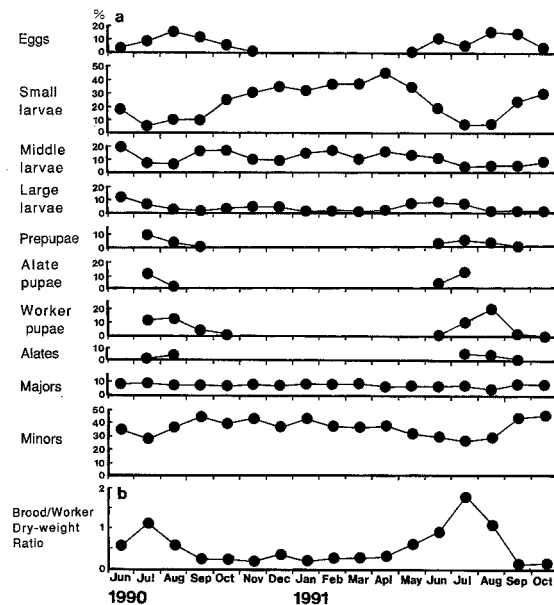


Figure 2. Seasonal changes in colony-member composition (a) and brood/worker dry-weight ratio (b) of *C. nipponicus*. The latter represents an index of amount of energy invested in brood. The ordinate in 2a represents the proportion of each class of individual to the total colony population

Seasonal change in colony composition

Figure 2a shows the seasonal change in the composition of colonies. Only adults and larvae were present during winter. Eggs were laid from May to October, and new adults appeared in summer. In Figure 2a, the larvae are divided into three principal size classes to clarify the growth pattern. The small class included the size classes 1 and 2 in Table 1, which are sizes of larvae soon after hatching from the eggs. The large class included the size classes 5, 6, and 7 in Table 1, destined to become minors (or males), majors and alate females, respectively. From Figure 2a it became clear that all eggs laid during the year developed into larvae by November. In addition, a portion of small larvae developed into medium- and large-sized larvae during the year, and overwintered. Pupation occurred in the next summer. Figure 2b shows seasonal changes in the ratio of the total dry weight of brood to the dry weight of the workers. The ratio changed seasonally with a conspicuous peak in July, indicating that larvae grew rapidly in early summer. On the basis of these results, the annual cycle of brood development in *C. nipponicus* is schematized in Figure 3. Although there may be a possibility that adult workers emerge from eggs laid early in the year, the majority of small larvae emerge from eggs laid in July–September, overwinter, and develop to pupa in the next year.

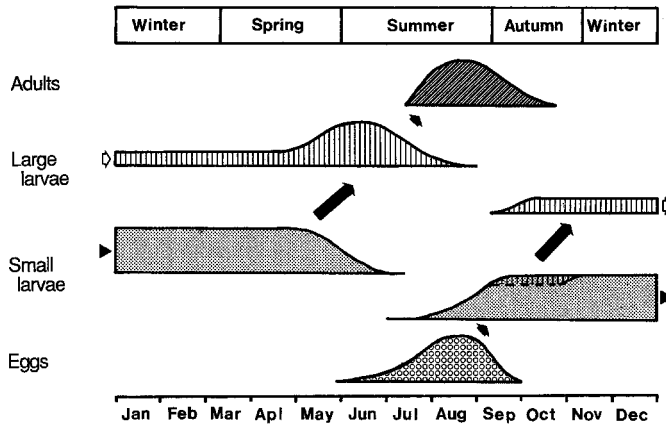


Figure 3. A schematic annual cycle of brood development in *C. nipponicus*

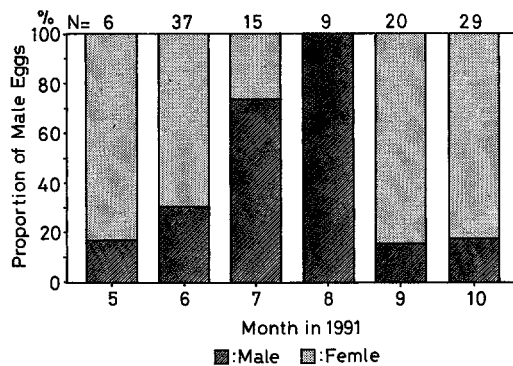


Figure 4. Proportions of male-destined eggs found in *C. nipponicus* colonies collected in the field during May–October 1991 (eggs possessing 25 chromosomes)

Seasonal change in male-egg production

In *C. nipponicus*, the chromosome number of diploid eggs is $2n = 50$, and that of haploid eggs $n = 25$. Analysis showed that the proportion of male-destined eggs changed during the year (Figure 4). Although some male-destined eggs were laid during the period May–October, when *C. nipponicus* is active, haploid egg production predominated in July and August. Twenty of 24 (83.3%) eggs examined were male-destined in July and August, whereas in the other periods, only 20 of 92 (21.7%) were haploid.

Discussion

The study has shown that *C. nipponicus* is a monogynous and polydomous species. This type of colony structure is known in several ant species (Kloft and Hölldobler,

1964; Ichinose, 1986 b; Snyder and Herbers, 1991; Walker, 1984), but the function of polydomous nests is almost unknown. Nest sites for arboreal ants are considered to be a limited resource (Herbers 1986, 1989; Yamaguchi, 1992). Since multiple colonies of *C. nipponicus* inhabit the same tree (Fig. 1) and compete for nest sites with each other (Hasegawa, submitted), polydomy may be a method of expanding the nest space.

The existence of small incipient, polygynous colonies is interesting. Colony founding by multiple queens (pleometrosis) is generally followed by elimination of all but one queen after emergence of the first workers (Bartz and Hölldobler, 1982; Hölldobler and Taylor, 1983; Rissing and Pollock, 1986). Therefore, since large colonies of *C. nipponicus* invariably contained a single queen, elimination of supernumerary queens must occur in this species.

C. nipponicus produce eggs and small larvae in one year, which overwinter and continue to grow in the summer of the next year. A portion of larvae must develop into new queens in early summer, because most larvae do not reach queen-destined size (size class 7 in Table 1) by April. Therefore, resource availability during this period would be critical for sexual production in *C. nipponicus*. The relationship between resource availability and sex allocation is an important unsolved problem in ant biology (Nonacs 1986), which awaits further empirical studies. It is also noteworthy that *C. nipponicus* overwinters without adult sexuals, because many *Camponotus/Colobopsis* species overwinter with sexuals (Ito et al. 1988; Satoh, 1989).

Timing of male-egg production is poorly known in ants. Only a few studies have focused on this aspect (Gösswald and Bier, 1955; Passera et al., 1988). Passera et al. (1988) showed that while male larvae of *Iridomyrmex humilis* were found in colonies throughout the year, adult males appeared only periodically. They argued that some of the male larvae are fed to other colony members. In *C. nipponicus*, 40 of 116 eggs examined were male-destined (34.5%). This proportion was much higher than that of adult males to whole brood in the reproductive season. For example, 17.7% of whole broods in July 1990 and June 1991 was male. It seems probable that some of the male-destined eggs, especially those produced early, are fed to other colony members.

It is interesting to speculate why most male-destined eggs are laid in July and August, even though some are produced throughout the warm season. During this period, the new generation of alates emerges from pupae (Fig. 2a) and wait to participate in nuptial flights. During this waiting, the weight of alate females increases by a factor of 1.4 (Hasegawa unpublished, see also Boomsma and Isaaks 1985), while new workers continue to eclose. Consequently, the high amounts of energy invested in brood during this period (Fig. 2b) might result in a poor nutrition for the queen. It seems very probable that the high production of haploid eggs is stimulated by such social factors, rather than environmental cues such as the photoperiod. Further studies of the relationships between the nutritional condition and the sex of eggs laid by *C. nipponicus* might resolve this.

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