

## SEX ALLOCATION IN THE ANT *COLOBOPSIS NIPPONICUS* (WHEELER).

### I. POPULATION SEX RATIO

EISUKE HASEGAWA

Department of Biology, Faculty of Science, Tokyo Metropolitan University,  
Minamiohsawa 1-1, Hachioji-si, Tokyo 192-03, Japan

**Abstract.**—The relative power of queens and workers at controlling sex allocation in the ant *Colobopsis nipponicus* is investigated in this study. Results show that *C. nipponicus* completely satisfies Hamilton's assumptions concerning colony social structure: monogyny, monoandry, and no worker reproduction. A genetic survey of the population structure rejects possibilities of local mate competition, local resource enhancement, and local resource competition, which all can bias population-allocation ratios from 0.5. Although these factors are absent, the observed sex-allocation ratio (male investment/total sexual investment;  $0.250 \pm 0.027$ ) is significantly biased toward females and is not different from the estimated optimal ratio for workers (0.252). Thus, it appears that workers are likely to win in conflicts over sex allocation with queens.

**Key words.**—Ants, queen-worker conflict, relatedness asymmetry, sex ratio, sociality.

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In a landmark paper, Hamilton (1964) argued that altruistic behavior of workers in eusocial Hymenoptera can be explained by genetic asymmetries of workers to their kin. Because of haplodiploid sex determination, a daughter of a singly mated female is more closely related to her sisters (by  $\frac{3}{4}$ ) than to her own offspring (by  $\frac{1}{2}$ ) or to her brothers (by  $\frac{1}{4}$ ). The inclusive fitness of workers can be increased when a worker rears her full sisters rather than her own daughters. Thus, kin selection may have favored altruistic behavior in which females tended to rear their sisters rather than their own offspring, leading to eusociality.

Trivers and Hare (1976) provided the first empirical support for Hamilton's kin-selection theory, which they amplified through their queen-worker conflict hypothesis. They argued that conflict arises between queens and workers over sex-allocation ratios because of asymmetries in relatedness among colony members. According to their argument, because queens are equally related to their daughters and sons (by  $\frac{1}{2}$ ), they should be favored to invest equal amounts of resources in each sex. By contrast, workers are three times more closely related to female reproductives than to males, and thus should be favored to allocate more resources to females, with an equilibrium ratio of investment of 3:1. Trivers and Hare put forth empirical data suggesting that sex allocation of monogynous ants was indeed biased toward females. This suggested that workers maximize their inclusive fitness by controlling sex-allocation ratios and that

kin selection may have played an important role in the evolution of eusociality in Hymenoptera. However, several alternative hypotheses have been proposed to explain the biased sex-allocation ratio in social Hymenoptera. These are based on local mate competition (LMC; Alexander and Sherman 1977), local resource competition (LRC; Clark 1978), and local resource enhancement (LRE; Schwarz 1987). Such interactions among related individuals affect the optimal sex allocation by changing the shapes of the fitness functions for males and females (Frank 1987; Pamilo 1991). LMC is defined as competition among related males for mating (Hamilton 1967). Limited dispersal of males from their natal nest, or inbreeding, or both, lead to LMC and result in a decreasing return to scale of the male fitness function (total return to a mother from investment in sons). As a result, the optimal sex allocation is biased toward females. In contrast, LRE (or LRC) affects the optimal sex allocation by changing the shape of the fitness function for females. If aggregation of related females within a small area results in improvements in, or competition for, resource availability, the shape of the female fitness function yields increasing, or decreasing, returns to scale and leads to female- or male-biased sex allocation. Additional empirical studies on the queen-worker conflict hypothesis and reanalyses of data have been conducted by many researchers (Pamilo and Rosengren 1983; Ward 1983; Herbers 1984, 1990; Nonacs 1986a,b; van der Have et al. 1988; Boomsma 1989). In their reanalysis of the em-

pirical data, Nonacs (1986a) and Boomsma (1989) emphasized that the general pattern of sex allocation in ants supports the Trivers-Hare hypothesis. There are, however, considerable variations in breeding parameters, such as mating frequency of the colony queen and worker reproduction, among monogynous ants. Moreover, these breeding parameters were not known in many of the studies analyzed in Nonacs (1986a) and Boomsma (1989). Mating frequency of the colony queen, worker reproduction, and genetic structure of the population are known to vary from species to species, even from population to population (Page 1986; Bourke 1988; Choe 1988; van der Have et al. 1988; Pamilo et al. 1992; Seppä 1992; Pamilo 1993). Few studies on sex allocation have clear data for all the above factors (Ward 1983; Ross and Matthews 1989). Thus, a thorough investigation of sex allocation for a species in one population is still important to evaluate the potential role of kin selection in the hymenopteran societies.

Pamilo (1991) proposed a comprehensive model to estimate the optimal sex-allocation ratios separately for queens and for workers. Fitness was divided into return through males and return through females, thus, the model can incorporate the effects of LMC, LRC, and LRE separately. The linearity of the fitness function for each sex is affected by the existence of these variables. Degrees of such behavioral interactions among related individuals, in addition to relatedness asymmetries among colony members, can be evaluated from genotypic distributions in the breeding population. Thus, by using enzyme polymorphisms as genetic markers, the optimal sex allocations for queens and for workers can be determined in a population.

The main purpose of this study is to determine relative control of sex allocation by queens and workers and to evaluate importance of kin selection in the social system of *Colobopsis nipponicus* (Wheeler). I estimated all of the parameters required in Pamilo's model using various methods in the field. The ideal conditions assumed by Hamilton (1964)—monogyny, monoandry, and no worker reproduction (hereafter referred to as Hamiltonian conditions)—held. Mating structure and genetic relatedness among colony members were estimated using an allozyme polymorphism, combined with field observations of nuptial flights. Then, the observed sex-allocation ratio was compared with the expected ratios using Pamilo's model.

## MATERIALS AND METHODS

*Study Organisms.*—*Colobopsis nipponicus* is an arboreal ant occurring in warm regions of Japan. This ant is a completely dimorphic species, with workers having two physical subcastes (minor and major). Nests are made in hollows of dead twigs, which are preformed by coleopteran larvae, and only twigs attached to live trunks are used for nest sites. *Colobopsis nipponicus* has polydomous nest systems, which cause difficulties in determining colony boundaries (Snyder and Herbers 1991). However, behavioral interactions among nest members allow for clear identification of these boundaries (Hasegawa 1992). A genotype analysis of colony members also supports these boundaries, and *C. nipponicus* is invariably monogynous in mature colonies. The new generation of alates pupates from late June to early July, and nuptial flights occur after early August (Hasegawa 1992). Because of these features, all alates produced in a year can be collected in a given season. Furthermore, the nocturnal habits of this species allow collection of the entire nest by daytime sampling. All of these features make *C. nipponicus* one of the most favorable species for sex-allocation studies.

*Sampling and Colony Census.*—Sampling was conducted over three years (early July in 1990, late June in 1991 and 1992) in a coastal forest located in Amatsukominato, Chiba Prefecture, Japan. At this site, most nests of *C. nipponicus* were found in hollows of dead twigs attached to live trunks of the tree *Lythocarpus edulis*. In each year, 17, 17, and 8 trees were selected randomly within the study area of about 700 m<sup>2</sup> (10 × 70 m). All dead twigs attached to each tree were collected and examined for nests. Only twigs containing nesting *C. nipponicus* were returned to the laboratory and censused. All nest members were counted and identified to physical caste. Colony boundaries were determined by observing behavioral interactions between all possible nest pairs in each year (for the behavioral test, see Hasegawa 1992).

*Worker Reproduction.*—Ovariole development of workers was examined to estimate possible contributions to male production by workers. Five to 10 major and minor workers were selected randomly from both queenright and queenless nests from each of several mature colonies collected from May through October in 1991. All workers were dissected under a binocular microscope, and the length of the long axis

of the longest oocyte was measured to the nearest 0.025 mm using an ocular micrometer. The length of the 77 eggs laid in the collected colonies were also recorded.

To examine the effects of queen removal, a queenless colony consisting of 50 majors and 50 minors was established from members of a queenright nest and reared in the laboratory (23°C, 14 h L:10 h D, 60% R.H., abundant food supply). After isolation, 10 majors and 10 minors were dissected weekly to examine their ovarian development. Another 5 colonies, each of which consisted of 10 majors and 10 minors, were established from 5 mature colonies and were reared to confirm worker oviposition.

**Electrophoresis.**—In *C. nipponicus*, an esterase locus (*Est-II*) is polymorphic with two alleles, *F* and *S*. Subunit structure seemed to be trimeric, and FS heterozygotes express the products of both alleles. To estimate mating frequency of colony queens and relatedness among colony members, an electrophoretic analysis was conducted for samples from 1991 and 1992. From each of 73 nests, which were classified into 39 colonies in the 1991 sample, 6 males and 14 females were selected randomly and subjected to electrophoresis. Each individual was homogenized with 50  $\mu$ L of the electrode buffer (2.5 mM, Tris and 38.4 mM, Glycine, pH 8.3) in a micro test tube and centrifuged 5 min at 10,000 rpm at 4°C. Then, the supernatant was applied to a 7.5% polyacrylamide gel (gel buffer, upper layer: 0.06 M Tris-HCl, pH 6.7; lower layer: 0.38 M Tris-HCl, pH 8.9). In 1992, 10 female and 10 male larvae from each nest were examined in 20 mature colonies, and the colony queens from 14 founding colonies were electrophoresed.

**Occurrence of Nuptial Flights.**—Nuptial flights were investigated from August 6 to 21 1990. A trap, with a fluorescent light powered by dry-cell batteries, was set on a live *Lythocarpus edulis* trunk in the study area and was lit daily at 5:00 P.M. The males and females caught were counted the next morning. An additional 20 nests were collected on August 22, 1990, and colony composition was compared with the July sample.

**Sperm Counts of Males and Females.**—The numbers of spermatozoa in the spermatheca of females and those in the seminal vesicles of males were examined to clarify the relative number of copulations for both sexes. In 1990, 30 founding queens collected during field sampling and 105 males caught during nuptial flights were dissected under a binocular microscope; semen for each

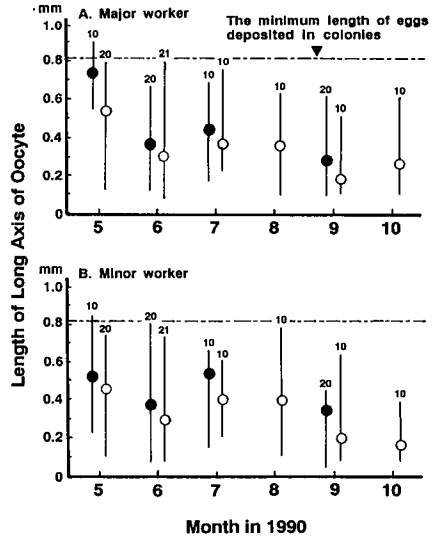


FIG. 1. Seasonal change in development of oocytes of majors (A) and of minors (B). The closed and open circles represent the mean length in queenright and queenless nests, respectively. The range is shown with the mean. The numbers on bars represent the number of individuals examined.

individual was diluted in 50  $\mu$ L of Borax Carmine solution, and sperm were individually counted using a hemocytometer.

## RESULTS

**Worker Reproduction.**—The seasonal change in development of workers' oocytes is shown in figure 1A,B. Although average length of oocytes decreased with the season, the difference between queenright and queenless nests was not significant for either subcaste [two-way ANOVA: for minors,  $F = 0.307$ ,  $df = (1, 113)$ ,  $P > 0.58$ ; for majors,  $F = 0.502$ ,  $df = (1, 113)$ ,  $P > 0.48$ ]. In a total of 272 oocytes examined, only 6 (2.21%) were longer than the minimum length of eggs actually deposited in colonies. Furthermore, all of the dissected workers possessed orange-colored oocytes with coarse yolk particles, an indication that these oocytes were probably not viable (see below). On the other hand, in the queen removal experiment workers laid many eggs after 5 wk following queen removal, and these eggs developed into males in the laboratory (data are not shown). These workers possessed well-developed, whitish oocytes with fine yolk, qualitatively different from the orange-colored oocytes mentioned above. The experiment also showed that 10% of workers began to develop whitish oocytes 3 wk after queen removal and

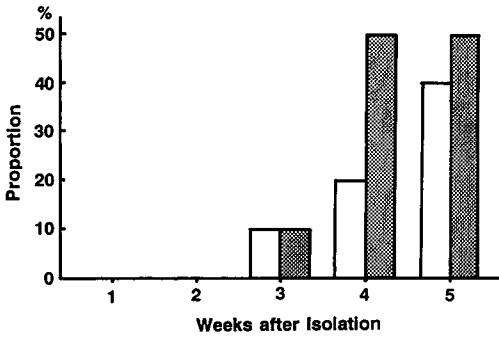


FIG. 2. The change in proportion of both subcastes that possessed viable oocytes in each week after queen removal. Opened and meshed bars represent minor and major workers, respectively.

that the proportion of workers possessing whitish oocytes increased with time (fig. 2). These results indicate that worker reproduction did not occur in queenright colonies, even in queenless satellite nests, but colony orphaning results in male production by workers. To estimate the proportion of males produced by workers, brood composition and oocyte quality were examined for all colonies which produced alates. Workers possessing whitish oocytes were found in three colonies, all of which had no queen and contained only male brood; thus, these colonies seemed to be orphaned. As a result, males produced by orphaned workers numbered 38 of 1738 in 1990, 26 of 1184 in 1991, and 26 of 731 in 1992. Thus, 2.46% of the total male population was estimated to be produced by orphaned workers.

**Mating Number of Males and Colony Queen.** — Since the queen produced all males in a colony (see above), the genotype of the colony queen could be inferred from the genotype array of males

TABLE 1. Genotypes of males, females, the queen and her mate in colonies of *Colobopsis nipponicus*. The genotype of the colony queen and her mate is inferred from genotype arrays of males and females. *N*, number of colonies.

Colony queen	Genotype						<i>N</i>
	Females			Males		Queens' mate	
	SS	SF	FF	S	F		
SS	707			229		S	34
SS		126			46	F	4
SF	141	137		56	58	S	16
SF		17	25	14	10	F	3
FF		33			14	S	2
						Total	59

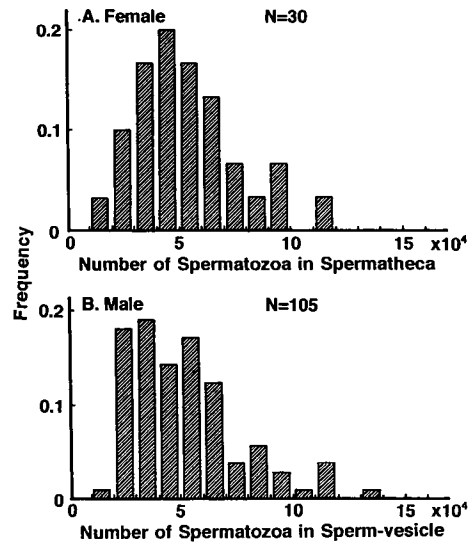


FIG. 3. Frequency distributions of spermatozoan counts in the female's spermatheca (A) and in the male's seminal vesicle (B).

in that colony. Thus, the mating frequency of the colony queen was estimated by comparing genotype arrays of female offspring to those of males. In 59 mature colonies, genotype arrays of female larvae were consistent with the assumption of monoandry of the queen (table 1). In addition, in colonies having two genotypes among females, the ratio of the two genotypes was not significantly different from 1:1 ( $\chi^2$  test, for all colonies,  $P > 0.05$ ). Therefore, the results show that the *F* and *S* alleles followed Mendelian inheritance and, more importantly, that the colony queen mated with a single male.

The frequency distribution of spermatozoan counts in semen from the spermatheca of queens and seminal vesicles of males are shown in figure 3. Since each distribution could be regarded as normal after a power transformation (*t*-test for skewness; for female,  $t = 0.427$ ,  $df = \infty$ ,  $P > 0.5$ ; for male,  $t = 0.454$ ,  $df = \infty$ ,  $P > 0.5$ ), their means and variances were compared. There were no significant differences between the two distributions in either variance [ $F = 1.178$ ,  $df = (29, 104)$ ,  $P > 0.25$ ] or mean ( $t = 0.473$ ,  $df = 133$ ,  $P > 0.5$ ). Since the two distributions were not statistically different from each other, relative copulation number of a male and a female must be equal. As demonstrated above, each colony queen mated with a single male. Thus, males appear to transport all sperm to the first-mated

TABLE 2. The average relatedness between different classes of individuals in *Colobopsis nipponicus*. *N* represents number of examined colonies or trees.

Pairs of individuals	Mean	SE	<i>N</i>
Females to females	0.745	0.051	59
Females to males	0.260	0.064	44
Queens inhabiting the same tree	0.045	0.100	16

female, so the average copulation number of males is also one.

*Relatedness between Individuals.*—Relatedness among colony members was estimated from genotype frequencies of 59 mature colonies collected in 1991 and 1992. I applied the identity-by-descent method (Queller and Goodnight 1989) to estimate relatedness among colony members. Table 2 shows the average relatedness of several classes of individuals. As described above, *Colobopsis nipponicus* completely fits the Hamiltonian conditions, which result in the estimated relatednesses closely matching Hamilton's (1964) predicted values. More importantly, the estimated relatedness between queens inhabiting the same tree was not significantly different from zero (one sample *t*-test;  $t = 0.450$ ,  $df = 15$ ,  $P > 0.5$ ). This means that queens inhabiting the same tree were not related to each other. Thus, the possibility of LRC (or LRE) can be rejected in this population because LRC (or LRE) requires aggregation of related females within a small area.

*Mating Structure of the Population.*—The occurrences of nuptial flights in 1990 are shown in figure 4. Apparently, multiple flights occurred during mid-August. The comparison of colony composition from July through August 1990 (table 3) showed that the proportion of alates among the colony members was the only significant difference. If each colony released alates in only a

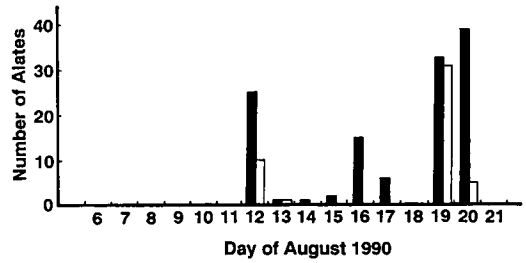


FIG. 4. Occurrences of nuptial flights and representation of two sexes in flights in *Colobopsis nipponicus* in the field in 1990. Closed and opened bars represent males and alate females, respectively.

single flight, the proportion of colonies having alates should have decreased in the August sample. Thus, each colony seemed to release alates gradually over several days, rather than contribute to only one mating flight.

Genotypes of the colony queen and those of her offspring are shown in table 1. The genotype frequencies of queens from 59 mature colonies did not deviate from Hardy-Weinberg equilibrium ( $\chi^2 = 0.04$ ,  $df = 1$ ,  $P > 0.9$ ). Under the assumption of random mating, mating-type frequency for these queens could be estimated from gene frequency in the population. The mating-type frequency for these queens was closely consistent with the frequency predicted under the random-mating assumption (fig. 5). All of these results indicate that *C. nipponicus* mated randomly in a large breeding population. Thus, LMC is unlikely in this population because LMC requires small breeding populations, or inbreeding, or both (Hamilton 1967).

*Colony and Population Sex-Allocation Ratio.*—To estimate colony and population sex-allocation ratios, dry weights of both sexes were determined for both the pupal stage and for adults participating in nuptial flights. Average dry weight

TABLE 3. Comparisons of colony composition of *Colobopsis nipponicus* from July through August. NS, not significant.

	Date of sampling		<i>P</i>
	July 19–22, 1990	August 20, 1990	
Number of colonies	34	7	
Proportion of colonies having alates	94.1%	85.7%	NS
The mean of total number of colony members ( $\pm$ SD)	542.5 $\pm$ 434.3	695.0 $\pm$ 496.0	NS
The mean proportion of alates in the colony population ( $\pm$ SD)	11.0 $\pm$ 7.4%	4.2 $\pm$ 6.3%	*

\*  $P < 0.03$ , Mann-Whitney *U*-test.

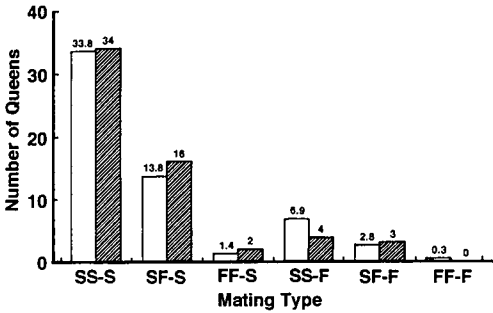


FIG. 5. Comparison between expected and observed numbers of mating types. Opened and meshed bars represent the expected and the observed number of each mating type, respectively. A  $\chi^2$  test showed no significant difference between them ( $\chi^2 = 1.626$ ,  $df = 2$ ,  $P > 0.3$ ). Calculation of  $\chi^2$  was based on four mating-type categories, SS-S, SF-S, SS-F, and others.

of adult males ( $0.335 \pm 0.032$  mg, mean  $\pm$  SD,  $N = 173$ ) was slightly, but significantly, lighter than that of male pupae ( $0.456 \pm 0.048$  mg,  $N = 100$ ;  $t$ -test,  $t = 25.489$ ,  $P < 0.001$ ), whereas the average dry weight of adult females ( $2.721 \pm 0.287$  mg,  $N = 63$ ) was about 1.4 times as much as pupal weight ( $1.963 \pm 0.157$  mg,  $N = 170$ ), and the difference was highly significant ( $t$ -test;  $t = 25.483$ ,  $P < 0.0001$ ). Among these values, pupal dry weight of males and adult dry weight of females were used to estimate colony and population sex-allocation ratios, because dry weight is a good estimator of "production cost" (Danforth 1990; but also see Boomsma 1989). Furthermore, as dry weight of alate pupae is constant regardless of colony size in *C. nipponicus* (Hasegawa MS), substitution of average values should give an accurate estimate of the allocation ratio of each colony. Figure 6 shows the contribution to total breeding dry-weight mass by colonies belonging to different classes of sex-allocation ratios. Distributions of allocation ratios were unimodal in each year and have similar peaks, so the data were combined for the three years (fig. 6D) to obtain the population-wide sex-allocation ratio in this population. The population sex-allocation ratio (male investment/total sex investment) was  $0.250 \pm 0.027$  (mean  $\pm$  SD; for discussion of data weighting, see Boomsma 1988).

**Optimal Sex-Allocation Ratio.**—The results could be used to estimate the optimal sex-allocation ratios for both workers and queens by using Pamilo's (1991) model. The absence of LMC, LRC, and LRE results in linearity of fit-

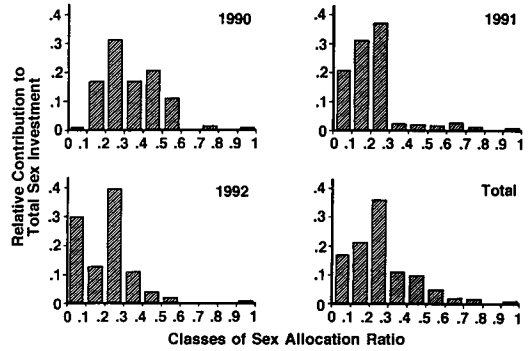


FIG. 6. The relative contributions to total breeding dry-weight mass by colonies that belonged to different sex-allocation classes. Sex-allocation ratio represents proportion of male investment in total sex investment.

ness returns to investment for both sexes. The colony queens are equally related to daughters and sons, whereas the female nestmates are three times more closely related to one another than to nestmate males. In addition, the proportion of males produced by orphaned workers was estimated to be 2.46% of the total male population. Thus, all of the parameters required in Pamilo's model were estimated in this study. The estimated optimal sex-allocation ratio for workers is 0.252 and for queens is 0.503. The observed population ratio ( $0.250 \pm 0.027$ ) is significantly different from the queen's, but not from the workers', optimum (one-sample  $t$ -test: for queen's optimum,  $t = 9.37$ ,  $P < 0.001$ ; for workers' optimum,  $t = 0.07$ ,  $P > 0.8$ ).

## DISCUSSION

This study shows that in *Colobopsis nipponicus*, the colony queen mates with a single male and that workers do not produce males under queenright conditions, even in queenless satellite nests. In addition, mature colonies of *C. nipponicus* are invariably monogynous (Hasegawa 1992). Thus, all the Hamiltonian conditions for colony structure are realized in this population. This situation maximizes the relatedness asymmetries of queens and workers to alates (Trivers and Hare 1976). Relatedness estimations suggest that there is actual asymmetry among colony members (table 2). Thus, there must be a conflict between the two parties over sex-allocation ratios as predicted by Trivers and Hare.

The observed sex-allocation ratio is significantly biased toward females. Alexander and Sherman (1977) stated that LMC, not queen-

worker conflict, is the cause of biased sex allocation in ants. In this population, however, LMC was unlikely. LMC is defined as competition for mating among related males (Hamilton 1967). However, the mating mode of *C. nipponicus* males, which randomly mate only once in large breeding populations, would seem to result in almost no competition among related males. In addition, if there is any amount of LMC, a theoretical model predicts that a colony would invest a constant amount of resources to males and the rest to females (Yamaguchi 1985; Frank 1987). Consequently, the total sex investment would be expected to correlate with the investment in females, but not males. There is, however, a strong positive correlation in this population between total sex investment and investment in males (Hasegawa MS). LRE, another factor by which sex allocation can become biased toward females, was also rejected in this study. LRE requires aggregation of related females within a small area. However, neighboring queens nesting on the same tree were not related. Thus, neither LMC nor LRE can explain the female-biased sex-allocation ratio observed in this population.

The difference between the observed allocation ratio and estimated ratios was significant for the queens', but not the workers', optimum. This fact suggests that the workers win in conflicts with the queens and succeed in controlling the sex allocation toward their favorable value. The observed ratio (0.250) is exactly the predicted value at which workers maximize their inclusive fitness. In *C. nipponicus*, worker control in sex-allocation ratios would be a kin-selected trait because workers do not lay eggs. Thus, the results suggest that relatedness asymmetry may affect social interactions within societies through kin selection. Studies have shown that relatedness of female nestmates in polygynous wasps and ants is low (Pearson 1983; Ross and Carpenter 1991; Kaufmann et al. 1992). Accordingly, several alternative hypotheses have been proposed to explain the origin of eusociality in polygynous societies, such as the polygynous-family hypothesis (West-Eberhard 1978) and the communal-aggregation hypothesis (Itô 1993). These alternative hypotheses emphasize the importance of ecological factors, such as high survival rates of polygynous colonies, rather than the role of kin selection in the course of eusocial evolution. However, Ross and Matthews (1989) demonstrated that a Hamiltonian condition is likely to occur in a

primitively eusocial wasp, *Microstigmus comes*, and that sex allocation of *M. comes* is biased toward females. They argued that because sex-allocation ratio in *M. comes* is biased toward females, kin selection likely played an important role in the origin of eusociality in this species. Even in polygynous swarm-founding wasps, cyclical oligogyny and high relatedness among swarm members have been demonstrated (Strassmann et al. 1991, 1992). High relatedness among workers would favor their helping behavior as a kin-selected trait. Although high relatedness among female nestmates and female-biased sex-allocation ratio tell us little about the origin of eusociality (Seger 1991), kin selection still seems to be a primary factor which affects social interaction within eusocial societies of Hymenoptera.

Why workers do not lay eggs may be another outcome of queen-worker conflict. If workers voluntarily keep from ovipositing, they would lay eggs soon after queen removal. However, worker oviposition starts one month after queen removal; thus, the queen may suppress worker reproduction in some way. Two different hypotheses have been proposed for regulation of worker reproduction by queens: the pheromonal-control hypothesis (Fletcher and Ross 1985), and the sexual-deception hypothesis (Nonacs and Carlin 1990; Nonacs 1993). Pheromonal control is a physiological suppression of workers' ovariole development by secretions from queens. The sexual-deception hypothesis proposes that queens control male parentage by hiding the sex of their offspring until it is too late for the workers to replace queen's males with their own eggs. Although the results reported here do not elucidate the mechanism in question, further research into these mechanisms would bring new insights into the nature of queen-worker conflict in *C. nipponicus*.

Finally, the unimodal distribution-of-allocation ratio in *C. nipponicus* is consistent with the prediction of Boomsma and Grafen (1990), that the optimal allocation ratio of each Hamiltonian ant colony converges to a single value, whereas factors which disturb Hamiltonian conditions, such as polygyny, polyandry, and worker reproduction, lead to variable sex-allocation ratios among colonies. Unimodal sex-allocation ratios are very rare in ants, and indeed have been reported in only one case, *Tetramorium caespitum* (Brian 1979; Nonacs 1986a). The European *T. caespitum* is obligately monogynous, thus the

Hamiltonian conditions are likely to occur in that species. In *C. nipponicus*, although the distribution of sex-allocation ratio is unimodal, there is substantial variation among colonies (fig. 6). Sex-ratio variability in ants can be explained by three different hypotheses: the constant-male hypothesis (Frank 1987); the relative-relatedness-asymmetry hypothesis (Boomsma and Grafen 1990); and the resource-availability hypothesis (Nonacs 1986a). Some authors have emphasized resource availability for colonies (Nonacs 1986b; Backus and Herbers 1992); however, there is little empirical data on this factor (Backus and Herbers 1992). Factors affecting variability in sex-allocation ratios must be analyzed separately from the factors that affect the population ratio. This issue is beyond the scope of this study and will be reported in detail elsewhere (Hasegawa MS).

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Corresponding Editor: R. Holt