Tsuji, K., Furukawa, T., Kinomura, K.,
Takamine, H., Yamauchi, K. 1991.

Reprint from

Insectes Sociaux

The caste system of the dolichoderine ant
Technomyrmex albipes (Hymenoptera: Formicidae): morpho-
logical descritpion of queens, workers and reproductively
active intercates

K. Tsuji, T. Furukawa, K. Kinomura, H. Takamine
and K. Yamauchi

The caste system of the dolichoderine ant *Technomyrmex albipes* (Hymenoptera: Formicidae): morphological description of queens, workers and reproductively active intercastes

K. Tsuji [1]*, T. Furukawa [2], K. Kinomura [3], H. Takamine [4] and K. Yamauchi [2]

[1] *Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Nagoya, 464-01 Japan*
[2] *Department of Biology, Faculty of Education, Gifu University, Gifu, 501-11 Japan*
[3] *Gifu-kita High School, Gifu, 502 Japan*
[4] *Okinawa Shogaku High School, Naha, 902 Japan*

Summary

Females of *Technomyrmex albipes* consist of winged queens, intercastes and workers. In established colonies, reproduction is performed by many intercastes (wingless females which have intermediate phenotypes between queen and worker characters). Dissection and morphological examination revealed that intercastes had a spermatheca, but workers did not. Intercastes can be divided into three classes: major intercastes with three ocelli, medium intercastes with one ocellus, and minor intercases without ocelli. Workers have no ocelli. The thoracic structure of intercastes gradually becomes more complex from minors to majors. The body size of intercastes gradually increases from minors to majors, and so does the number of overioles. The body size distributions of minor intercastes and workers overlap considerably, but the distributions of ovariole numbers overlap less. Winged queens had distinctly larger body sizes, more ovarioles and larger spermathecae than intercastes. Most intercastes were inseminated with developed ovaries and appeared to reproduce. The caste system and reproductive division of labour in *T. albipes* is compared to those of ant species in which permanently wingless females reproduce.

Introduction

In typical ant species, reproductive and non-reproductive castes differ phenotypically; the former have wings but the latter do not. In various ants, however, wingless individuals reproduce instead of winged queens. This phenomenon has become an important topic in ant biology (Crozier, 1982; Peeters and Crewe, 1984; Bolton 1986; Heinze and Buschinger, 1989). Several patterns of modifications in the morphology

---

* Present address: Entomological Laboratory, College of Agriculture, University of the Ryukyus, Nishihara-cho, Okinawa, 903-01 Japan.

of reproductive females and in the mode of reproductive division of labour have evolved in ants (reviewed by Peeters, 1991): 1) *ergatoid queens*, 2) *intercastes*, 3) *gamergates*, and 4) *parthenogenetic workers*. Ergatoid queens form a wingless reproductive caste which has evolved from winged queens. Their morphology is relatively fixed within a species, and their fecundity is similar to that of winged queens of other species, or even sometimes higher than winged queens (e.g. queens of *Eciton;* see discussion). Intercastes constitute a series of female forms exhibiting various combinations of queen and worker characters (i.e. intermediate phenotypes). These intercastes are generally not winged, although their alitrunk is distinguished by simplified flight sclerites. In many ants, intercastes occur by anomalies in the course of individual larval development, and they lack a spermatheca and have no reproductive function. However, in a few species they can mate and reproduce instead of the queens, e.g. in *Harpagoxenus sublaevis* and *Formicoxenus* spp. (Buschinger and Winter, 1975; Francoeur et al., 1985). Gamergates are mated reproductive workers that are found in some species of the subfamily Ponerinae, e.g. in the genera *Diacamma*, *Rhytidoponera* and *Ophthalmopone* (Wheeler and Chapman, 1922; Ward, 1983; Peeters and Crewe, 1984). They exhibit no distinction in external morphology to other conspecific unmated workers. Parthenogenetic workers produce diploid females without having been inseminated (thelytoky). This is known in the myrmicine ant *Pristomyrmex pungens* (Itow et al., 1984). Tsuji (1988, 1990) suggested that *P. pungens* may no longer be described as eusocial, because all the workers reproduce at one stage of their lifetime, and no workers are permanently sterile. To investigate the selective forces for the evolution of different types of queen replacement, it is not sufficient to simply compare the environmental difference between these species, because so far they are found within relatively restricted taxa, each of whom may have different phylogenetic constraints. Therefore, more empirical studies are needed on the replacement of winged queens in various taxa, especially outside the Ponerinae, Myrmicinae, Ecitoninae and Dorlinae.

Yamauchi et al. (in press) found that in a dolichoderine ant *Technomyrmex albipes*, both winged and wingless males and reproductive females coexisted (see also Terron, 1972a, b). Winged females found new colonies independently after they are inseminated by winged males during the nuptial flight. Later, after the foundress queen dies, wingless females mate with wingless males within the nests, and enlarge their colony and multiply nests by fission (Yamauchi et al., in press). In this study, we describe the types of wingless reproductive females occurring in *T. albipes* by measurements of some morphological characters.

Materials and Methods

Field collection

This species forms huge polydomous colonies (Yamauchi et al., in press). Collection of a whole colony is extremely difficult. Instead, parts of colonies, i.e. nests, were sampled from the main island of Okinawa on May 25–27 and August 11, 1987. These samples were used for the following morphological measurements.

Measurement of morphology

In this work, hereafter, we use 'queen' and 'worker' to describe adult morphology only, not reproductive status (Peeters and Crozier 1988).

The following morphological characteristics were examined for both winged and wingless females: 1) maximum head width across compound eyes, 2) number of ocelli, 3) number of ovarioles in one ovary, 4) presence or absence of mature oocytes in ovaries, 5) presence or absence of spermatheca, 6) maximum width of spermatheca, and 7) presence or absence of sperm in spermatheca. Dissections were made using a dissecting microscope, and the width of the spermatheca was observed without a cover slip. The metric characters (1 and 6) were measured to the nearest 0.01 mm. The sampling method was varied for different characters, and is explained below.

Results

Qualitative classification

More than 300 wingless females and 500 winged females (queens) were haphazardly picked from a pooled sample from 11 nests which apparently belonged to different colonies (collected from different study sites at least 10 km distant from each other). They were dissected and their morphology examined. By this preliminary observation on the basis of their internal morphology, wingless females were divided into individuals with a spermatheca (we refer to these hereafter as *intercastes*) and those without spermatheca (hereafter *workers*). Intercastes were further subdivided into 3 classes: major, medium and minor intercastes, on the basis of their external morphology (Figs. 1–3). Major and medium intercastes have 3 ocelli and 1 ocellus respectively, while minor intercastes lack ocelli. Workers also lack ocelli. The thorax is largest and most queen-like in major intercastes, and becomes gradually smaller and simplified down the series to minor intercastes. Workers are indistinguishable from minor intercastes in external morphology, because they have similar body sizes.

Quantitative comparison

Morphological measurements were made using other samples. Minor intercastes (n = 100) and workers (n = 100) were sampled from the same nest collected from Afuso, Onna. Winged queens, medium and major intercastes were not found in large numbers in one nest. Therefore, a pooled sample from the 11 nests was used, similar to the previous qualitative classification. The head width distributions were significantly different among the four categories (Kruskal-Wallis test, $X^2 = 276.9$, 4 d.f., P < 0.001). A posterior pairwise comparison between all categories was then performed by non-parametric Scheffé's test. The body size distributions of workers and minor intercastes overlap, and there was no statistically significant difference in their head width distribution (Scheffé's test, $X^2 = 0.91$, 4 d.f., P > 0.9). The difference in head width distribution between the major and medium intercastes classes was also non-significant ($X^2 = 4.97$, 4 d.f., P > 0.1). However, there were significant dif-
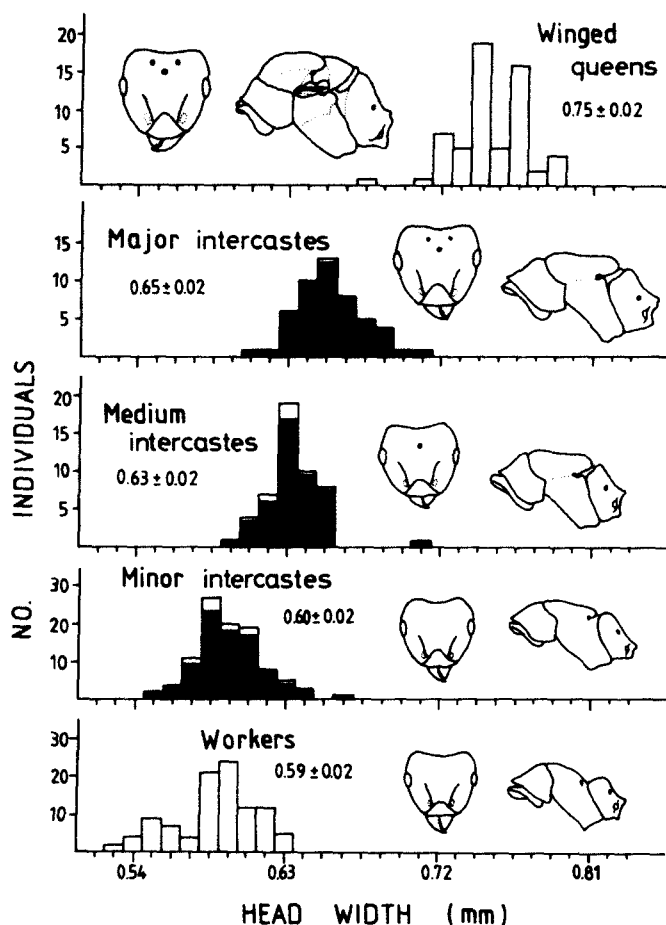
Figure 1. Frequency distributions of head width and drawings of thorax morphology. The mean and SD are indicated. Shaded portions of the histograms refer to inseminated individuals. Winged queens were collected from the nest before their nuptial flights, therefore all are uninseminated. There was significant difference in the distribution of this value among the five categories (Kruskal-Wallis test, $X^2 = 276.9$, 4d.f., $P < 0.001$). A posterior pairwise comparison was also made by Scheffé's method (see text)

ferences ($P < 0.05$) in all the other paired comparisons. The winged queens were the largest in body size among all female categories, and have a big, well-differentiated thorax with flight sclerites (Fig. 1).

Apart from the presence of spermatheca, the characteristic by which minor intercastes could be separated from workers, was the distinctly smaller numbers of ovarioles in workers (Fig. 2). There was a significant difference in average ovariole number between all categories of females (Scheffé's test, $P < 0.01$), except between major and medium intercastes ($X^2 = 4.15$, 4d.f., $P > 0.1$) (Fig. 2).

Spermathecal width was compared using a laboratory colony which originated from a nest collected from Afso in May 1987 (Fig. 3). Statistically significant differences in this value were detected (Kruskal-Wallis test, $X^2 = 58.5$, 3d.f.,
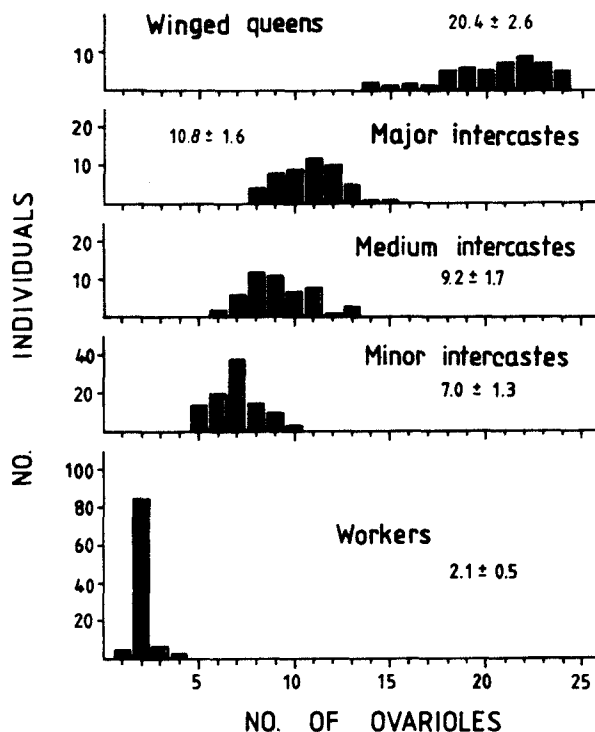
Figure 2. Frequency distributions of ovariole number per ovary. The mean and SD are indicated. There was significant difference in the distribution among the five categories (Kruskal-Wallis test, $X^2 = 333.5$, 4d.f., $P < 0.001$). A posterior pairwise comparison was also made by Scheffé's method (see text)

$P < 0.001$). As the sample size was equal ($n = 20$), a posterior pairwise comparison was made by Tukey's method rather than Scheffé's. There was a significant difference in spermathecal width between winged queen (after mating in the laboratory) and mated intercastes ($P < 0.01$, Tukey's test). The distributions of spermatheca width largely overlapped among intercastes (Fig. 3), though the differences in all paired comparisons were statistically significant (Tukey's test, $P < 0.01$), except that between medium and major intercastes ($P > 0.05$).

In short, there are relatively distinct differences (with little overlap) between winged queens and intercastes in all the quantitative characters examined (head width, number of ovarioles and spermathecal width). On the other hand, between workers and intercastes there is a large overlap in the distribution of head width, and only little overlap in the number of ovarioles. No intercastes with small vestigal wings, which were sometimes found in *Leptothorax nylanderi* (Plateaux, 1970), were observed in *T. albipes*.

In all the field samples, the proportion of inseminated individuals among intercastes was extremely high, and was on average more than 95% (Fig. 1, for details see Yamauchi et al. (in press)). Most inseminated intercastes had developed ovaries, often with mature oocytes, that were characterized by a relatively hard corion and
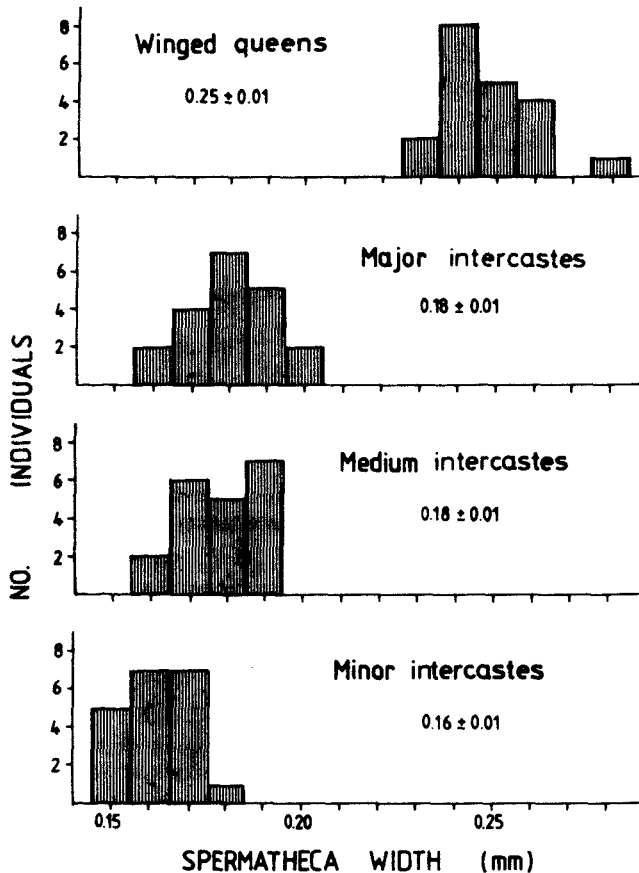
Figure 3. Size-frequency histograms of spermathecal width. The mean and SD are indicated. There was significant difference in the distribution among the four categories (Kruskal-Wallis test, $X^2 = 58.5$, 3 d.f., $P < 0.001$). A posterior pairwise comparison was also made by Tukey's method (see text)

opaque coloured yolk. Workers had no mature oocytes, although they had trophic oocytes, which characteristically have a soft membrane, transparent yolk and are smaller than mature fertile oocytes. On average, approximately 40% of the population of wingless individuals within nest are intercastes, of which the majority are minor intercastes, the remainder being workers. Wingless males comprised only a small portion (0.14% $\pm$ 0.4 s.d.) of the adult population in the nest. The proportion of each morphological categories will be shown in detail in another publication (Yamauchi et al., in press).

Discussion

In the majority of ants, winged queens (which dealate after the nuptial flight) mate and reproduce, but a number of species are now known in which permanently

wingless individuals are the functional reproductives. This brings complications to the nomenclature, because such forms must be distinguished from workers. Buschinger (1987) and Peeters and Crozier (1988) have advocated the need to use a combination of morphology and function to describe the biological characteristics of adult females. Peeters (1991) recognized two distinct adult forms, which appear to be morphologically intermediate between workers and winged queens. Both of these forms were confusingly called 'ergatogynes'. One category consists of ergatoid queens that are wingless members of a morphologically specialized reproductive caste. Ergatoid queens have evolved from winged queens. Their fecundity is almost equal to (or higher than) that of winged queens of related species, and they exhibit little morphological variation within species. The other category comprises the intercastes, which represent various graded stages in a series connecting workers and winged queens, and which occur together with the queen in the same species.

We used the term intercastes for the wingless females with a spermatheca in *T. albipes*, since they fit the following two characteristics of intercastes. First, they showed continuous variation in both internal and external morphology. Second, they occurred together with conspecific winged queens (ergatoid queens do not occur with winged queens within a species, except in *Hypoponera eduardi*, in which, however, two types of queens do not occur in the same colony (Le Masne, 1956). However, the situation in *T. albipes* is unusual, since intercastes occur in various ants but have no reproductive function. In a few leptothoracine spcies where reproductive intercastes are known, colonies are functionally monogynous with a single reproductive intercaste in each colony (Buschinger and Winter 1975, 1978; Francoeur et al., 1985; Heinze and Buschinger, 1987). In contrast, in *T. albipes*, almost all intercastes were inseminated and seemed reproductive, and their number per colony was extremely high.

Intercastes of *T. albipes* are different from the gamergates found in some Ponerinae, because the former can be morphologically distinguished from the worker caste by the presence of a spermatheca. The failure of virgin intercastes and workers to reproduce (Yamauchi et al., in press) also suggest they do not perform thelytoky, as has been reported in workers of *Cataglyphis cursor* (Cagniant, 1982) and *Pristomyrmex pungens* (Itow et al., 1984).

Terron (1972 a, b) reported that an African *Technomyrmex* species had major and minor workers. The former had ocelli and a functional spermatheca, and produced reproductive eggs. He suggested that this species might be *T. albipes*. However, the present study has shown that in Japanese *T. albipes* there is an intercaste without ocelli, and this was not reported in Terron's study. Minor intercastes were the most abundant of all categories of intercastes in the Japanese *T. albipes*. Further taxonomic studies are required to clarify whether Terron's and our species are the same.

Yamauchi et al. (in press) suggested that wingless reproductives in *T. albipes* evolved to support secondary polygyny (Hölldobler and Wilson, 1977). By mating within their natal nest, wingless males and females succeed the single dealate queen which founded the colony independently. Colonies are polydomous, but have a clear colony boundary, owing to discrimination of their colony members (Tsuji, in prep.). Therefore, wingless sexuals are probably inbred. They can enlarge the colony and perform nest multiplication by fission; once a colony is established, it may last almost eternally.

A similar life cycle may have evolved in the ponerine species *Rhytidoponera metallica*. In most *Rhytidoponera* species, the queens have disappeared, and instead mated workers reproduce (Haskins and Whelden, 1965; Ward, 1981). In *R. metallica*, winged queens continue to be produced, albeit erratically (mated queens have never been found in field colonies). Ward (1986) succeeded in getting a mated queen to found a new colony in the laboratory. After her death, the observed workers calling in order to attract males. C. P. Haskins (personal communication) succeeded in maintaining a colony of *R. metallica* for more than 26 years in the laboratory, because workers inbred with males. These laboratory data suggest that the ancestral condition in *R. metallica* may have been both long-range dispersal via solitary winged queen and gamergate reproduction leading to polygyny and colony budding (Ward, 1989). In *T. albipes*, both the winged and wingless reproductives are viable components of the reproductive cycles. Inter- and intra-species variation in the proportion of dispersal to non-dispersal individuals may be explained by the genetic model of dispersal polymorphism (Hamilton and May, 1977).

Despite the phylogenetic distance and the difference in relative importance of winged and wingless reproductives between *R. metallica* and *T. albipes*, the function of wingless reproductive females seems similar: to succeed the queen as supplementary reproductives. Why did sexual reproduction by workers evolve in *R. metallica*, while reproduction by intercastes evolved in *Technomyrmex albipes*? This may be explained by phylogenetic and morphological constraints. In *Rhytidoponera* spp., workers have a functional spermatheca, as do workers of many ponerine species. In higher ants, such as the subfamily Dolichoderinae, workers do not have a spermatheca, and therefore cannot function as supplementary reproductives to produce female offspring. In *T. albipes*, however, the production of intercastes with a spermatheca provides an additional option: a large number of them can mate and reproduce in each colony. There is another example in the formicine ant *Cataglyphis cursor*, which may suggest the importance of the ability of workers to reproduce sexually as a constraint for the evolution of sexual reproduction by workers. Workers of *C. cursor* do not possess a spermatheca, like those of other formicine species. Sexually active intercastes have not evolved in this species. Instead, workers of *C. cursor* reproduce parthenogenetically, yielding a new queen and workers, when a colony is orphaned (Lenoir & Cagniant, 1986).

Acknowledgements

References

Bolton, B., 1986. Apterous females and shift of dispersal strategy in the *Monomorium salomonis* – group (Hymenoptera: Formicidae). *J. Nat. Hist. 20*:267–272.

Buschinger, A., 1987. Polymorphism and reproductive division of labor in advanced ants. In: *Chemistry and Biology of Social Insects* (J. Eder, H. Rembold, Eds.), Verlag J. Peperny, Munich, pp. 257–258.

Buschinger, A. and U. Winter, 1975. Der Polymorphismus der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.). *Ins. Soc. 22*:333–362.

Cagniant, H., 1979. La parthénogénèse thélytoque et arrhénotoque chez la Fourmi *Cataglyphis cursor* Fonsc. (H. F.). Cycle biologique en élevage des colonies avec reine et des colonies sans reine. *Ins. Soc. 26*:51–60.

Crozier, R. H., 1982. On insects and insects: twists and turns in our understanding of evolution of sociality. In: *The Biology of Social Insects* (J. M. Breed, C. D. Michener and H. E. Evans, Eds.), Westview Press, Boulder, Colorado, pp. 4–10.

Francoeur, A., R. Loiselle and A. Buschinger, 1985. Biosystematique de la tribu leptothoracini (Formicidae, Hymenoptera) 1. Le genre *Formicoxenus* dans la Région holarctique. *Naturaliste Can. (Rev. Ecol Syst.) 112*:343–403.

Hamilton, W. D. and R. M. May, 1977. Dispersal in stable habitats. *Nature 296*:578–581.

Haskins, C. P. and R. M. Whelden, 1965. "Queenlessness", worker sibship and colony vs population structure in the Formicid genus *Rhytidoponera*. *Psyche 72*:87–112.

Heinze, J. and A. Buschinger, 1987. Queen polymorphism in a non-parasitic *Leptothorax* species (Hymenoptera, Formicidae). *Ins. Soc. 34*:28–43.

Heinze, J. and A. Buschinger, 1989. Queen polymorphism in *Leptothorax spec. A:* its genetic and ecological background (Hymenoptera: Formicidae). *Ins. Soc. 36*:139–155.

Hölldobler, B. and E. O. Wilson, 1977. The number of queens: an important trait in ant evolution. *Naturwissenschaften 64*:8–15.

Itow, T., K. Kobayashi, M. Kubota, K. Ogata, H. T. Imai and R. H. Crozier, 1984. The reproductive cycle of the queenless ant *Pristomyrmex pungens*. *Ins. Soc. 31*:87–102.

Le Masne, G., 1956. La signification des reproducteurs aptères chez la fourmi *Ponera eduardi* Forel. *Ins. Soc. 3*:239–259.

Lenoir, A. and H. Cagniant, 1986. Role of worker thelytoky in colonies of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae). *Entomol. Gen. 11*:153–157.

Peeters, C., 1991. Ergatoid queens and intercastes in ants: two distinct adult forms which look morphologically intermediate between workers and winged queens. *Ins. Soc. 38*:1–15.

Peeters, C. and R. Crewe, 1984. Insemination controls the reproductive division of labour in a ponerine ant. *Naturwissenschaften 71*:50–51.

Peeters, C. and R. H. Crozier, 1988. Caste and reproduction in ants: not all mated egg-layers are "queens". *Psyche 95*:283–288.

Plateaux, L., 1970. Sur le polymorphisme social de la fourmi *Leptothorax nylanderi* (Förster), I: Morphologie et biologie comparées des castes. *Annls. Sci. nat Zool. 12e série 12*:378–384.

Terron, G., 1972a. La ponte des ouvrières fécondés chez une fourmi camerounaise du genre *Technomyrmex* Mayr: mise en évidence d'une descendance ouvrière. *C. R. Acad. Sci. Paris ser. D 274*:1516–1517.

Terron, G., 1972b. Observations sur les mâles ergatoides et les mâles chez une fourmi du genre *Technomyrmex* Mayr (Hym., Formicidae, Dolichoderinae). *Ann. Fac. Sci Cameroun 10*:107–120.

Tsuji, K., 1988. Obligate parthenogenesis and reproductive division of labor in the Japanese queenless ant *Pristomyrmex pungens*. Comparison of intranidal and extranidal workers. *Behav. Ecol. Sociobiol. 23*:247–255.

Tsuji, K., 1990. Reproductive division of labour related to age in the Japanese queenless ant *Pristomyrmex pungens*. *Anim. Behav. 39*:843–849.

Ward, P. S., 1981. Ecology and life history of the *Rhytodoponera impressa* group II. Colony origin, seasonal cycles, and reproduction. *Psyche 88*:109–126.

Ward, P. S., 1983. Genetic relatedness and colony organization in a species complex of ponerine ants. I. phenotypic and genotypic composition of colonies. *Behav. Ecol. Sociobiol. 12*:285–299.

Ward, P. S., 1986. Functional queens in the Australian greenhead ant, *Rhytidoponera metallica* (Hymenoptera: Formicidae). *Psyche 93*:1–12.

Ward, P. S., 1989. Genetic and social changes associated with ant speciation. In: *The Genetics of Social Evolution* (M. D. Breed and R. E. Page, Eds), Westview Press, Boulder, pp. 123–148.

Wheeler, W. M. and J. W. Chapman, 1922. The mating of *Diacamma*. *Psyche 29*:203–211.

Yamauchi, K., T. Furukawa, K. Kinomura, H. Takamine and K. Tsuji (in press). Secondary polygyny by inbred wingless sexuals in the dolichoderine ant *Technomyrmex albipes*. *Behav. Ecol. Sociobiol.*