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Karyological Studies of Japanese Ants (*Hymenoptera, Formicidae*)

III. Karyotypes of Nine Species in *Ponerinae*, *Formicinae*, and *Myrmicinae*

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Abstract. The karyotypes of nine Japanese ants in three subfamilies (*Ponerinae*, *Formicinae*, and *Myrmicinae*) were successfully analysed by the improved squash technique. Three ponerine species had $2n=7$ and $n=4$ (*Ponera scabra*), $2n=22$ (*Brachyponera sinensis*), and $2n=28$ and $n=14$ (*Cryptopone sauteri*). Four formicine species had $2n=18$ and $n=9$ (*Camponotus* sp. and *C. tokioensis*), $2n=26$ and $n=13$ (*Camponotus japonicus*), and $2n=30$ and $n=15$ (*Lasius niger*). Two myrmicine species had $2n=18$ and $n=9$ (*Leptothorax congruus*), and $2n=37$, 38, 39, and $n=17$, 18, 19, 20 (*Pheidole nodus*). It was found that the variation of chromosome number observed in *P. nodus* was caused by Robertsonian type polymorphism.

Introduction

During the last two decades, cytological observations of ants have been remarkably developed; the details have recently been reviewed by Crozier (1970). Imai (1966) had applied the squash technique to ant chromosomes with the aim of analyzing their karyotype evolution. Since then, the chromosomes of 40 Japanese ants have been reported (Imai, 1966, 1969, 1970). The photographs obtained, however, were often not satisfactory for analyzing karyotypes, though they were sufficient for counting the chromosome numbers. Recently, Crozier (1968, 1970) developed an air-drying technique for the ant karyotype. We have also succeeded in obtaining very good photographs by the modified squash technique.

In order to accomplish the analysis of karyotype evolution in ants, it is of course necessary to accumulate much more data than is now available. Toward such accumulation, the present paper reports the karyotypes of nine Japanese ants observed by the improved technique during the summer of 1971.

Methods

The squash technique described by Imai (1966, 1969) was modified as follows: (1) Dissect out the desired organs (cerebral ganglia, ovaries, and testes) in a colchicine-hypotonic solution (0.01% colchicine in 0.45% sodium citrate solution) and leave them in the fresh hypotonic solution for 30 minutes at room temperature. (2) Transfer the organs on a slide slightly tipped to remove the hypotonic solution completely, and quickly tear the organs into pieces in a drop of 50% acetic-ethanol (2:3) fixative followed by squashing for one minute. (3) Put the squashed preparation on a block of dry ice for 2 or 3 minutes, tear off the cover glass using a razor, and then melt the ice by blowing air over it gently with a hair-dryer. (4) Place the slide in absolute glacial acetic acid for 30 seconds to reduce cytoplasmic staining, and dry it again by the hair-dryer. (5) Stain the preparation with a drop of 1% acetic-orcein for one minute and then cover it with a cover glass without sealing. (6) After examination of the preparation, strip the cover glass by dipping in running water and stock as a permanent preparation. This improved squash technique will be suitable for routine preparation of a large number of specimens.

Results and Discussion

Subfamily *Ponerinae*

Ponera scabra (Fig. 1, $2n=7$, $n=4$). Four (dipolid) female and two (haploid) male individuals were obtained from two colonies in Manazuru, Kanagawa prefect. The modal chromosome number was ascertained by observing 35 cells in the diploids and 41 cells in the haploids. The haploid karyotype obtained from testes of males consists of three submetacentrics and one small metacentric. On the other hand, the diploid karyotype obtained from cerebral ganglia of workers has one extremely large metacentric and six small metacentrics or submetacentrics, of which the first four chromosomes (Nos. 1–4) seem to correspond to those of the male haploid karyotype but chromosome No. 5 does not have a pairing partner, though the chromosomes Nos. 6 and 2, and Nos. 7 and 3 seem to be homologous pairs. The origin of this curious karyotype remains unknown. Anyway, $n=4$ is the lowest number in ants, elsewhere found only in the *Stenamma brevicorne* (Myrmicinae) observed by Hauschteck (1962).

Brachyponera sinensis (Fig. 2, $2n=22$). Four diploid individuals were obtained from one colony in Manazuru. The diploid karyotype of this species, determined from 17 cells in the diploids, consists of five pairs of metacentrics, five pairs of submetacentrics, and one pair of acrocentrics or telocentrics. It was concluded that the *B. luteipes* reported by Imai (1969) is identical with the present species.

Cryptopone sauteri (Fig. 3, $2n=28$ and $n=14$). Twelve diploid individuals were obtained from two colonies and two haploid individuals from one colony in Manazuru. The modal chromosome number was decided by observing 28 cells in the diploids and 15 cells in the haploids. The haploid karyotype of this species consists of two submetacentrics or subtelocentrics and twelve acrocentrics or telocentrics. This result corrects the karyotype formula previously reported by Imai (1969).

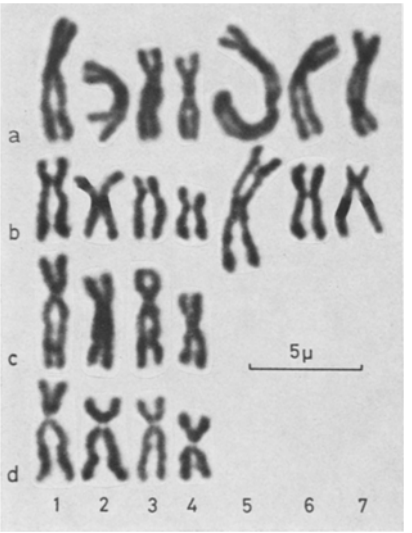

Fig. 1

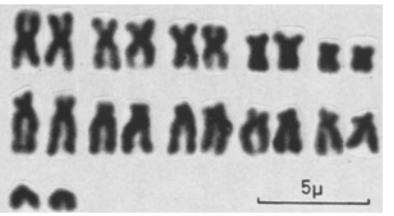

Fig. 2

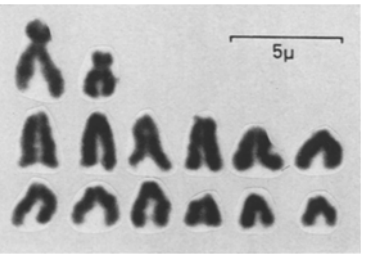

Fig. 3

Fig. 1 a–d. The karyotypes of *Ponera scabra*. a and b The diploid karyotypes, $2n=7$. c and d The haploid karyotypes, $n=4$

Fig. 2. The diploid karyotype of *Brachyponera sinensis*, $2n=22$

Fig. 3. The haploid karyotype of *Cryptopone sauteri*, $n=14$

Subfamily *Formicinae*

Camponotus (*Myrmamblys*) sp. 1 (Fig. 4a and b, $2n=18$, $n=9$). Seven diploid and two haploid individuals were obtained from two colonies in Misima, Shizuoka prefect. The modal chromosome number was decided by observing 20 cells in the diploids and 10 cells in the haploids. The diploid karyotype of this species consists of nine pairs of metacentrics or submetacentrics.

Camponotus (*Myrmamblys*) *tokioensis* (Fig. 4c, $2n=18$, $n=9$). Eight diploid and seven haploid individuals were obtained from two colonies in Manazuru and the chromosome counts are based on 25 cells in the diploids and 17 cells in the haploids. Karyologically, this species is identical with *C.* (*M.*) sp. 1 but the two species can be distinguished morphologically by the shape of their petioles. This species is the same species as *Camponotus* sp. (Imai, 1966) and *Camponotus caryae* group sp. (Imai, 1969).

Camponotus (*Camponotus*) *japonicus* (Fig. 5, $2n=26$, $n=13$). Four diploid and two haploid individuals were obtained from one colony in Misima. The chromosome number was determined from 25 cells in the dip-

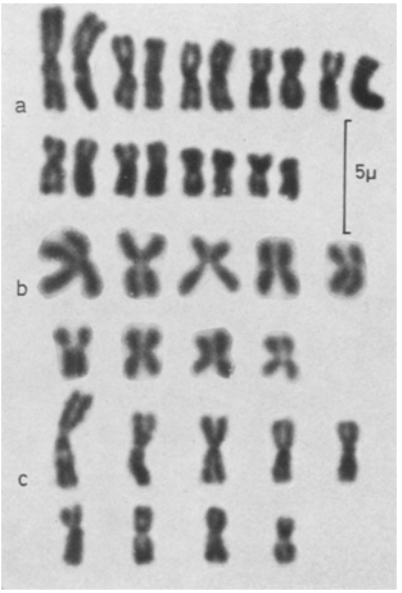

Fig. 4

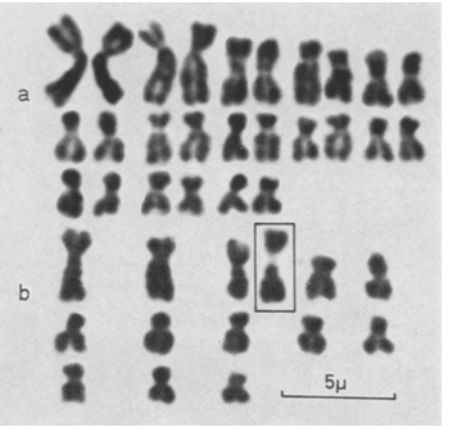

Fig. 5

Fig. 4a–c. The karyotypes of two species of *Camponotus* (*Myrmamblys*). a and b The diploid and the haploid karyotypes of *C.* (*M.*) sp. 1, $2n=18$ and $n=9$. c The haploid karyotype of *C.* (*M.*) *tokioensis*, $n=9$

Fig. 5a and b. The karyotypes of *Camponotus* (*Camponotus*) *japonicus*. a The diploid karyotype, $2n=26$. b The haploid karyotype, $n=13$. The framed third chromosome has a remarkable secondary constriction

loids and 20 cells in the haploids. All chromosomes are metacentrics or submetacentrics with a continuous range of size, among which the third chromosome shows a remarkable secondary constriction at the proximal part of the short arm (Fig. 5, framed). This species is the same species as *Camponotus* sp. collected at Hodogaya, Kanagawa prefect (Imai, 1969). In *C. japonicus*, individuals having $n=14$ were collected at Sugadaira, Nagano prefect (Imai, 1966, 1969). The present result suggests, therefore, that our *C. japonicus* includes two species of races that are different karyologically, or else that the species is polymorphic.

Lasius niger (Fig. 6, $2n=30$ and $n=15$). Six diploid individuals were obtained from one colony in Misima, one diploid and three haploid individuals from another colony in Manazuru. The modal chromosome number was decided by observing 35 cells in the diploids and 20 cells in the haploids. The diploid karyotype of this species consists of 14 pairs of acrocentrics or telocentrics, and one pair of medium size metacentrics.

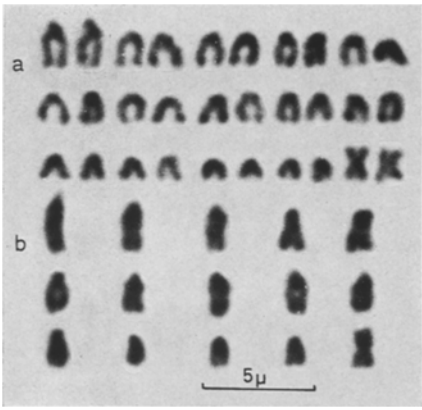

Fig. 6

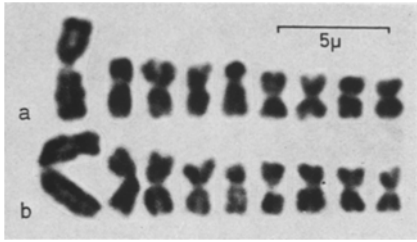

Fig. 7

Fig. 6a and b. The karyotypes of *Lasius niger*. a The diploid karyotype, $2n = 30$. b The haploid karyotype, $n = 15$

Fig. 7. The haploid karyotypes of *Leptothorax congruus*, $n = 9$

Subfamily *Myrmicinae*

Leptothorax congruus (Fig. 7, $2n = 18$ and $n = 9$). Four diploid and two haploid individuals were obtained from one colony in Misima, four haploid individuals from the other colony in Manazuru. The modal chromosome number was decided by observing five cells in the diploids and 55 cells in the haploids. The haploid karyotype of this species consists of one large metacentric and eight small metacentrics or submetacentrics.

Pheidole nodus (Figs. 8 and 9, $2n = 37$, 38, 39, $n = 17$, 18, 19, 20). Three diploid and 153 haploid individuals were obtained from ten colonies in Misima. The modal chromosome number of each individual was determined from ten cells. Imai (1969) reported the chromosome number of this species as $2n = 38$ and $n = 19$, from a sample collected at Taiji, Wakayama prefect. The present observation indicates, however, that *P. nodus* in Misima district shows a wide range of variation in the chromosome number. All haploid karyotypes of this species observed had fundamentally 11 submetacentrics or subtelocentrics and two acrocentrics or telocentrics. On the other hand, the karyotypes having $n = 20$, 19, 18, and 17 include different numbers of metacentrics and telocentrics, viz., one metacentric and six telocentrics (Fig. 8a), two metacentrics and four telocentrics (Fig. 8b), three metacentrics and two telocentrics (Fig. 8c), and four metacentrics and no telocentrics (Fig. 8d), respectively. These series of variation in chromosome number appear to indi-

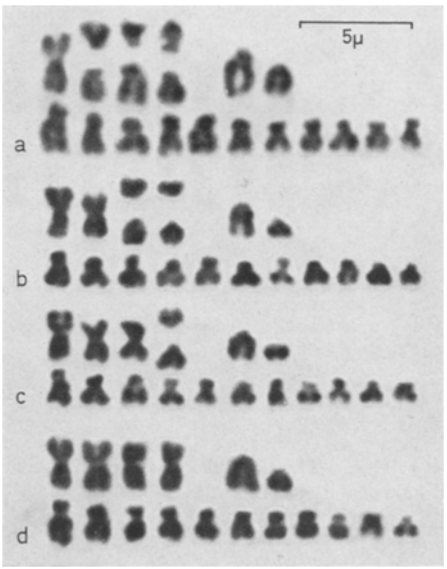

Fig. 8a–d. The haploid karyotypes of *Pheidole nodus*. a The karyotype having one metacentric, $n = 20$. b The karyotype having two metacentrics, $n = 19$. c The karyotype having three metacentrics, $n = 18$. d The karyotype having four metacentrics, $n = 17$

Table 1. Intra- and inter-colonial variation of the haploid males of *Pheidole nodus* having different chromosome numbers

Colony No.	Frequency of the males having each Chromosome number and metacentrics (M)				Males, total no. observed
	17 (4M)	18 (3M)	19 (2M)	20(1M)	
1	—	1	8	1	10
2	—	2	3	—	5
3	—	5	4	1	10
4	—	9	23	13	45
5	—	2	3	4	9
6	—	—	7	6	13
7	4	4	3	—	11
8	—	16	10	4	30
9	—	9	4	—	13
10	—	3	2	2	7
Total	4	51	67	31	153

cate Robertsonian relationship. This type of polymorphism was observed in an Australian ponerine ant, *Rhytidoponera metallica* (Crozier, 1969); ours is the first case in the *Myrmicinae*. It was found that the frequency of each karyotype varied remarkably within a colony as well as among colonies (Table 1). The intracolonial variation of the karyotypes suggests the presence of queens (reproductive females) having different diploid

Fig. 9a–c. The diploid karyotypes of *Pheidole nodus*. a The karyotype having three metacentrics, $2n = 39$. b The karyotype having four metacentrics, $2n = 38$. c The karyotype having five metacentrics, $2n = 37$

karyotypes. In fact, the diploid karyotypes of three females collected from the same colony observed at pupal stage were $2n = 39(3M)$, $38(4M)$, and $37(5M)$, where M is the number of metacentrics. These karyotypes were interpreted as follows: $39(3M) = 19(2M) + 20(1M)$, (Fig. 9a); $38(4M) = 19(2M) + 19(2M)$, (Fig. 9b); and $37(5M) = 18(3M) + 19(2M)$, (Fig. 9c). If this interpretation is correct, the chromosome polymorphism observed in males should also be found in females. If this is the case, the presence of seven different diploids can be expected, *i.e.*, $40(2M)$, $39(3M)$, $38(4M)$, $37(5M)$, $36(6M)$, $35(7M)$, and $34(8M)$, though only three of these karyotypes, $39(3M)$, $38(4M)$, and $37(5M)$ were observed in this sample. As far as the present observations are concerned, no gross morphological differences were found among those karyotypically different individuals. The details will be published in a separate paper.

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