Chromosome Polymorphism in the Ant, *Pheidole nodus*

Hirotami T. Imai[1] and Masao Kubota[2]

[1] National Institute of Genetics, Misima, Sizuoka-ken, 411 and
[2] Nakasone 13, Odawara, Japan

Abstract. A survey of chromosome polymorphism was made in populations of *Pheidole nodus* (Hymenoptera, Formicidae). A total of 1,666 males were collected from 11 localities in Japan. Four polymorphic karyotypes were observed: (1) n = 17 with 4 metacentrics (abbreviated as 4M), (2) n = 18(3M), (3) n = 19(2M) and (4) n = 20(1M). These differences are due to the Robertsonian type rearrangement. The karyotype 18(3M) is found in all the populations examined, but the others are more or less localized in their distribution. The 17(4M) appears mainly in Shikoku and the northern Kyūshū populations, 19(2M) along the Pacific coast of Honshū, Shikoku and Kyūshū, and 20(1M) in the eastern part of Honshū and Shikoku. This distribution pattern indicates that 18(3M) is the oldest, 19(2M) and 20(1M) are derived from 18(3M) by centric fission, and 17(4M) by centric fusion. The most probable mechanism of karyotype evolution in this species is considered to be the centric fission.

Introduction

It was previously reported that the myrmicine ant *Pheidole nodus* collected in Misima (Central Japan) showed a wide range of variation in chromosome number (n = 17–20) (Imai and Kubota, 1972). This type of chromosome polymorphism which was observed in some mammals (*e.g.* Matthey, 1963) is valuable for analysing population structure and karyotype evolution. In ants, Crozier (1969) has found it in an Australian ponerine ant, *Rhytidoponera metallica* but our study is the first case in Myrmicinae. The present paper deals with a population survey of the polymorphic karyotypes in *Pheidole nodus*, and discusses their possible evolutionary pathway based on their distribution pattern.

Materials and Methods

The ant *Pheidole nodus* F. Smith is a subtropical species occurring in the southern part of Japan (Yano, 1927). Their nests are mostly found in a naked wall of loam or in sandy soil lying between sea level and an altitude of 500 meters. A mature colony contains a few queens (1–64; Kuboki, personal communication), several thousand small workers and several tens of soldiers with large heads. The larvae of males and females (presumptive queens) develop in June and turn to pupae in early July.

Employing the improved squash method of Imai (1974), the chromosome preparations were made from pupal testes. Male material was preferred because karyotype analysis is easier in haploid than in diploid cells. The chromosome number of each individual was determined by counting at least ten good metaphase figures.

Observations

1. Geographic Distribution of Pheidole nodus in Japan

About a century ago, the first sample of *Pheidole nodus* was recorded by F. Smith (1874) from Hyōgo in Japan. Since then, this species has been found
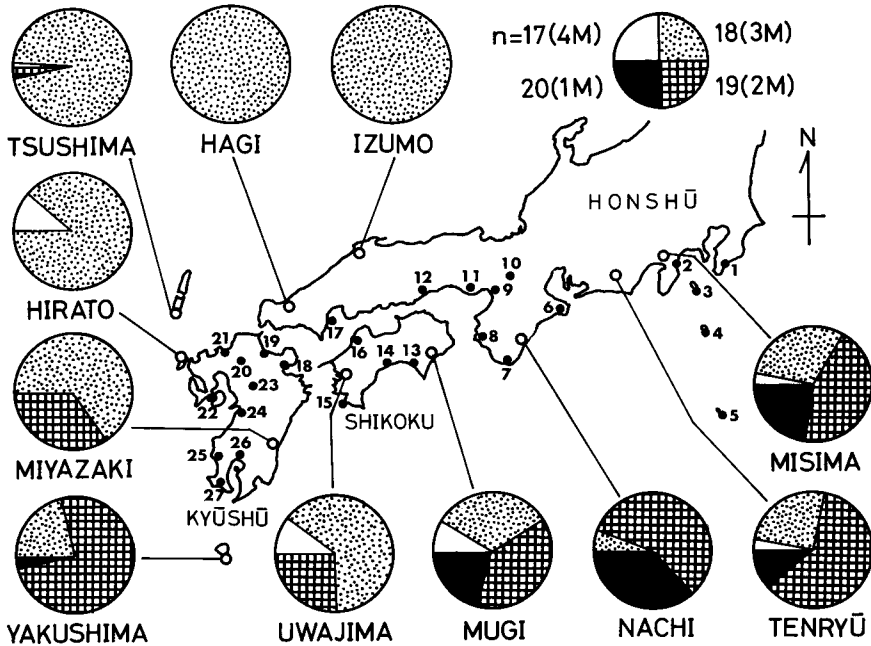
Fig. 1. Distribution of Pheidole nodus in Japan (•) and relative frequency of polymorphic karyotypes in various local populations (o). (1) Kamogawa (Yano, 1927). (2) Manazuru (Kondo; Bull. Nat. Hist. Hakone Jap. 1, 1961). (3) Ōshima (Teranishi; Zool. Mag. Jap. **41**, 1929). (4) Miyakejima. (5) Hachijōjima. (6) Shima. (7) Taiji. (8) Hinomisaki. (9) Ōsaka (Forel; Mitt. Schweiz. ent. Ges. **10**, 1900). (10) Kyōto (Morisita & Onoyama; Kyōto Pref. Gov. 1974). (11) Hyōgo (F. Smith, 1874). (12) Okayama (Wheeler; Bull. Amer. Mus. Nat. Hist. **22**, 1906). (13) Muroto (Okamoto, Gensei **3**, 1954). (14) Kōchi (Okamoto, 1954). (15) Kashiwajima (Okamoto, 1954). (16) Matsuyama. (17) Miyajima (Yano, 1927). (18) Morie (Teranishi, 1929). (19) Nakatsu. (20) Mt. Kosyo (Kuboki; Biol. Fukuokana **11**, 1971). (21) Fukuoka. (22) Nagasaki, (23) Ichinomiya (Wheeler; Boll. Lab. Zool. Gen. Agrar. Portici **21**, 1928). (24) Kumamoto (Wheeler, 1928). (25) Kushigino, (26) Kagoshima. (27) Makurazaki (Yano, 1927)

often by many authors from various localities in Japan (Fig. 1). So far as the data now available are concerned, the northernmost distribution is Kamogawa (Lat. 35°5′N; Yano, 1927) and Izumo (Lat. 35°30′N) of Honshū, and the southernmost sample is recorded from Yakushima (Lat. 30°20′N) of Kyūshū. The distribution pattern strongly suggests that *Ph. nodus* is one of the endemic species of Japan, occurring in the southern half of Honshū, Shikoku, Kyūshū and their adjoining islands.

2. Polymorphic Karyotypes of Pheidole nodus

The haploid number varies in this species from n = 17 to 20. Each karyotype is formulated as follows;

n = 4M + 0T + (11SM, ST & A + 2T) = 17 [17(4M)],
n = 3M + 2T + (11SM, ST & A + 2T) = 18 [18(3M)],
n = 2M + 4T + (11SM, ST & A + 2T) = 19 [19(2M)], and
n = 1M + 6T + (11SM, ST & A + 2T) = 20 [20(1M)],

where M, SM, ST, A, and T indicate meta-, submeta-, subtelo-, acro-, and telocentric chromosomes respectively (for chromosome figures, see Imai and Kubota, 1972). The 11SM, ST & A and 2T chromosomes enclosed in the parentheses were regularly found in all the karyotypes, while the numbers of the remaining metacentric and telocentric chromosomes varied between 4 and 1 and between 0 and 6, respectively. As the major fundamental number (NF) of those karyotypes is constant (NF = 31), the variation in chromosome number is of the so-called Robertsonian type.

Actually each of 18(3M) and 19(2M) karyotypes includes three different types due to meiotic segregation of heterozygote females for the polymorphic chromosomes. When we assign each of the arms involved in those Robertsonian polymorphisms a letter, A....H, and designate metacentrics by underlining the arms involved (AB): 18(3M) karyotype is subdivided into 18(A, B, CD, EF, GH), 18(AB, C, D, EF, GH) and 18(AB, CD, E, F, GH), and with the same manner 19(2M) is grouped into 19(A, B, C, D, EF, GH), 19(A, B, CD, E, F, GH) and 19(AB, C, D, E, F, GH). As the four metacentrics of *Ph. nodus* are, however, nearly identical in their size, and as banding analysis has not succeeded yet in ants, those six karyotypes mentioned above were not distinguished in the present experiments.

3. Geographic Distribution of Polymorphic Karyotypes

The chromosomes of *Ph. nodus* were examined in a total of 1,666 male individuals collected from 11 local populations: Misima, Tenryū, Nachi, Mugi, Uwajima, Miyazaki, Yakushima, Hirato, Tsushima, Hagi and Izumo (Fig. 1). The frequency of each polymorphic karyotype in those localities is summarized in Table 1.

Among the four karyotypes, 18(3M) is found in all the eleven populations examined, but the other three are more or less localized in their distribution. As shown in Fig. 2, 17(4M) is mainly observed in Shikoku (Mugi and Uwajima) and the north of Kyūshū (Hirato), 19(2M) appears widely along the Pacific coast of Honshū (Misima, Tenryū and Nachi), Shikoku (Mugi and Uwajima) and Kyūshū (Miyazaki and Yakushima), and 20(1M) is restricted to the eastern part of Honshū (Misima and Tenryū) and Shikoku (Uwajima). Their distribution patterns and their geographical clines in frequency suggest that the center of distribution of 17(4M) would be in the north of Kyūshū or the west of Shikoku, and that of 19(2M) and 20(1M) somewhere at the Pacific coast of Honshū and Shikoku. If it is adequate to assume that the older karyotype occupies the larger geographical area, the relative ages of those karyotypes would be $17(4) \leq 20(1M) < 19(2M) < 18(3M)$, *i.e.*, 18(3M) is the oldest.

4. Karyotype Similarity and Geographic Distance

The results of the cytological examinations described in the preceding section reveal that the similarity between karyotypes of different localities is a function of the geographic distance between them. The similarity increases as the distance decreases (Figs. 1 and 2). The problem of genetical similarity in geographically structured populations has been mathematically studied by many authors since

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Table 1. Frequencies of polymorphic karyotypes of *Pheidole nodus* in various localities in Japan

Locality	No. of colonies examined	Karyotypes				Males, total no. studied
		17 (4M)	18 (3M)	19 (2M)	20 (1M)	
Misima	10	4 (2.6%)	51 (33.3%)	67 (43.8%)	31 (20.3%)	153
Tenryū	5	2 (2.4)	21 (25.0)	51 (60.7)	10 (11.9)	84
Nachi	10	0 (0.0)	7 (5.6)	72 (57.1)	47 (37.3)	126
Mugi	8	9 (8.5)	34 (32.1)	40 (37.7)	23 (21.7)	106
Uwajima	9	21 (9.9)	137 (64.6)	54 (25.5)	0 (0.0)	212
Miyazaki	9	0 (0.0)	72 (64.9)	39 (35.1)	0 (0.0)	111
Yakushima	6	0 (0.0)	29 (21.0)	104 (75.4)	5 (3.6)	138
Hirato	7	23 (11.4)	178 (88.6)	0 (0.0)	0 (0.0)	201
Tsushima	10	1 (0.5)	183 (97.3)	4 (2.1)	0 (0.0)	188
Hagi	8	0 (0.0)	133 (100.0)	0 (0.0)	0 (0.0)	133
Izumo	9	0 (0.0)	214 (100.0)	0 (0.0)	0 (0.0)	214
Total	91	60 (3.2)	1059 (57.5)	431 (30.7)	116 (8.6)	1666

Wright (1943). Following Malécot's (1948) concept, we calculated the probability that two randomly chosen genomes are identical in the present classification of karyotypes. Although the situation is not exactly the same as among allelic genes, we believe that the identity probability in the present case reflects to some extent the common origin. We are making an effort to obtain more detailed data to which the population genetics theory can be applied. When data equivalent to an allelic case become available, we can estimate various genetic and ecological parameters of great interest, such as the ages of the karyotypes. The actual calculation of the identity probability is done in the following way.

We first compute the frequency of each karyotype in each locality, as done in the preceding section. The frequencies are given in Table 1. For simplicity let P_{ij} be the frequency of karyotype i in location j. For example, if $i = 1$ corresponds to 17(4M) and $j = 1$ represents the Misima population, then $P_{11} = 0.026$. In a case of a single locality, the identity probability in location j is $\sum_i P_{ij}^2$. For example
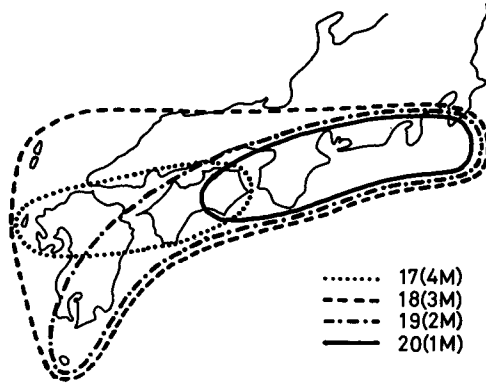
Fig. 2. Distribution patterns of polymorphic karyotypes

Table 2. Probability that two randomly chosen karyotypes from the specified location(s) are identical

	Misima	Tenryū	Nachi	Mugi	Uwajima	Miyazaki	Yakushima	Hirato	Tsushima	Hagi	Izumo
Misima	.345										
Tenryū	.374	.446									
Nachi	.344	.405	.468								
Mugi	.318	.337	.314	.299							
Uwajima	.329	.319	.182	.312	.492						
Miyazaki	.370	.375	.237	.341	.509	.544					
Yakushima	.407	.514	.456	.359	.328	.401	.614				
Hirato	.298	.224	.050	.294	.584	.575	.186	.798			
Tsushima	.333	.256	.066	.321	.634	.639	.220	.863	.947		
Hagi	.333	.250	.056	.321	.646	.649	.210	.886	.973	1.000	
Izumo	.333	.250	.056	.321	.646	.649	.210	.886	.973	1.000	1.000

in the Misima population, this probability is $0.438^2 + 0.333^2 + 0.203^2 + 0.026^2 = 0.345$ which appears as the first diagonal entry of Table 2. In a case of two different locations, say j and k, the probability is $\sum_i P_{ij}P_{ik}$. For example, between Uwajima and Miyazaki, $0.099 \times 0 + 0.646 \times 0.649 + 0.255 \times 0.351 + 0 \times 0 = 0.509$.

On the average the identity probability decreases as the geographical distance increases. This rather rapid decrease in the probability suggests that a strong local differentiation would exist in the ant population, and this in turn implies that the geographical flow of the ant genome is quite slow. In other words, the migration is very restricted in the ant species.

Discussion

1. Karyotype Evolution in Pheidole nodus

The cytological and population genetical analysis of the chromosome polymorphism in *Ph. nodus* suggest that the karyotypes 17(4M), 18(3M), 19(2M) and 20(1M) are evolved through at least the following five steps. Firstly, the ancestors of *Ph. nodus* had originally the monomorphic karyotype 18(A, B, CD, EF, GH), where the letters mean the arms of Robertsonian polymorphic chromosomes, *e.g.*, A and B are telocentric and CD is metacentric (details see observations in the present paper). Secondly, the first polymorphic karyotype 19(A, B, C, D, EF, GH) appeared perhaps in the eastern part of Honshū, and then spread along the Pacific coast of Honshū, Shikoku and Kyūshū. Thirdly, 20(A, B, C, D, E, F, GH) karyotype differentiated from 19(A, B, C, D, EF, GH) somewhere in the Pacific coast of Honshū. Since this karyotype arose more recently, the distribution of 20(1M) is restricted in the eastern part of Honshū and Shikoku. Fourthly, slightly after the appearance of 20(1M), the youngest karyotype 17(AB, CD, EF, GH) arose from 18(A, B, CD, EF, GH) in the northern part of Kyūshū or its neighbourhood. Finally, after these four polymorphic karyotypes were differentiated, some additional karyotypes such as 18(AB, C, D, EF, GH), 18(AB, CD, E, F, GH), 19(AB, C, D, E, F, GH) and 19(A, B, CD, E, F, GH) might have appeared secondarily in various local populations by the cross 17(AB, CD, EF, GH) × 20(A, B, C, D, E, F, GH), 17(AB, CD, EF, GH) × 19(A, B, C, D, EF, GH) or 18(A, B, CD, EF, GH) × 20(A, B, C, D, E, F, GH), though they could not be distinguished from each other for technical reason.

If the present interpretation is correct, we need to assume that at least one centric fusion 18(3M)→17(4M) and two centric fissions 18(3M)→19(2M) and 19(1M)→20(1M) must have occurred during the karyotype evolution of *Ph. nodus*.

There is another possible interpretation for their evolutionary pathway that the ancestral *Ph. nodus* had 17(AB, CD, EF, GH) karyotype and then three metacentrics AB, CD, EF dissociated independently by centric fission to form three polymorphic karyotypes, 18(A, B, CD, EF, GH), 18(AB, C, D, EF, GH) and 18(AB, CD, E, F, GH). If this assumption is correct, 19(2M) karyotypes can be generated in the meiosis of a heterozygote formed by mating 18(3M) × 18(3M), *e.g.*, 18(AB, CD, E, F, GH) × 18(A, B, CD, EF, GH), and with the same manner 20(1M) can be produced by the cross 18(3M) × 19(2M). This assumption seems, however, difficult to interpret the geographically clined distribution patterns demonstrated in Figs. 1 and 2. The same difficulty would appear when 20(1M) karyotype is assumed to be the oldest one.

2. Fusion and Fission in Animal Karyotype Evolution

The available data for animal karyotypes suggest that the so-called Robertsonian type rearrangement has played an important role in karyotype evolution (see White, 1973). Although the term Robertsonian rearrangement refers to centric fusion, centric fission (= misdivision) and centric dissociation, it usually implies centric fusion alone.

As an alternative to this fusion hypothesis, it has been proposed by some animal cytologists that karyotype alteration can also occur by centric fission (*e.g.*

John and Hewitt, 1966, 1968, Todd, 1970). This hypothesis is severely criticized by White (1973), but the data suggesting centric fission have been accumulating (*e.g.* Webster *et al.*, 1972; Takagi and Sasaki, 1974).

Structural chromosome changes have usually been interpreted on the following four assumptions: (1) Two-break rearrangements, (2) a unitary structure of the centromere, (3) an interstitial location of the centromere, and (4) the sealing off of a broken end by a telomere (Muller, 1940; White, 1973). These assumptions allow for karyotype evolution by centric fusion but not by centric fission. On the other hand, John and Hewitt (1966, 1968), studying the karyotype evolution of grasshoppers, have proposed alternative possibilities: (1) Either one or two-break rearrangements involved, (2) a double structure for at least some centromeres, (3) a terminal as well as an interstitial location of the centromere, and (4) the sealing off a broken end without the use of an existing telomere. These possibilities, if correct, allow the karyotype evolution by centric fission as well as centric fusion.

There is enough evidence supporting the latter possibilities. The double structure of the centromere has been demonstrated in plants (Lima-de-Faria, 1956) and also in animals (John and Hewitt, 1966). In orthopterans, the existence of telocentric chromosomes has been shown by John and Hewitt, (1966, 1968) and Southern (1969). As to the telomere, Muller and Herskowitz (1954) could not always rule out exceptions. In fact, a number of deficient chromosomes viable inspite of the loss of their original telomeres by terminal deficiency have been found in maize (McClintock, 1941) and in *Drosophila* (Lindsley and Grell, 1967).

White (1973) has emphasized the instability of telocentrics, adopting Rhoades' (1940) conclusion that telocentrics tend to form iso-chromosomes and are eliminated by natural selection. However, there is notable evidence that stable telocentrics as well as unstable ones do exist in many plant species (*e.g.*, Strid, 1968). In animals, John and Hewitt (1968) noted telocentrics which were produced by centric fission and survived through at least five mitotic generations. Recently, more stable telocentrics are reported in grasshoppers (Southern, 1969), in the Chinese Hamster (Kato *et al.*, 1973), in a vole (Fredga and Bergström, 1970) and in a shrew (Olert, 1973). The telocentrics of *Pheidole nodus* studied in the present paper are probably highly stable ones. Mark (1957) concluded that stable telocentrics are surely present but rare in nature because of genetical imbalance in meiosis. In this connection, Darlington and LaCour (1950) have pointed out long ago that misdivision of the centromere must be expected in varied degrees in most species but that only very few of the new telocentrices will be stabilized, and become a source of increasing the basic chromosome number.

To overcome the difficulty due to the instability of telocentrics and the *de novo* formation of the centromere, White (1973) suggested centric dissociation as a mechanism increasing chromosome number, which is a kind of translocation occurring between a large bi-armed chromosomes and a small donor chromosome. This hypothesis, however, introduces another difficulty that supernumerary chromosomes (or B-chromosomes) as the donor of the centromere are too rare in animals to be the responsible mechanism in increasing the chromosome number, except some insects such as the Orthoptera (Hewitt, 1973) and the Coleoptera

(White, 1973). Since there are no supernumerary chromosomes in *Pheidole nodus*, the dissociation hypothesis is not adopted in this paper.

The evidence mentioned above, if correct, strongly suggests that centric fissions seem to occur in nature and do play a significant role in karyotype evolution. Our purpose is, of course, not to deny the importance of centric fusion in karyotype evolution, but to emphasize also the importance of centric fission as well. Indeed, both fusion and fission are observed in the present material, *Pheidole nodus*. It is, however, unknown how frequently centric fissions are fixed in animal karyotype evolution. A quantitative approach of karyotype evolution attempted by Imai (1975) will provide relevant information to this problem.

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