

Research article

Spermatogenesis of diploid males in the formicine ant, *Lasius sakagamii*

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Summary. The diploid chromosome number in the female formicine ant, *Lasius sakagamii*, is 30, while the haploid number in normal males is 15. Six of the 30 studied colonies (20%), however, contained many diploid males. Although the body size of the diploid males was, on average, larger than that of the haploid males, both types of males showed normal external and internal morphologies. Furthermore, there was no reduction in chromosome numbers in either diploid or haploid males during spermatogenesis. The spermatid nuclei of diploid males, however, were significantly larger than those of haploid males, and the amount of DNA in the diploid males, as measured by flow cytometry, was twice as much as that in the haploid males, clearly indicating that diploid males produce diploid sperm.

Key words: Diploid males, diploid sperm, *Lasius sakagamii*, polygyny, spermatogenesis.

Introduction

In Hymenoptera with haplo-diploid complementary sex determination, diploid individuals develop into males if their sex-determining alleles are homozygous (Crozier and Pamilo, 1996). To date, diploid males have been reported in 17 ant species (Crozier and Pamilo, 1996). Although these males are generally thought to be sterile (Pamilo et al., 1994), they seem to be fertile in a few species (Crozier and Pamilo, 1996; Krieger et al., 1999). Few detailed studies have been made in ants, however, with regard to spermatogenesis. In the present study, we describe the characteristics of spermatogenesis in some detail and the external morphology of diploid males in the polygynous formicine ant, *Lasius sakagamii*.

Material and methods

We collected male pupae and adults from 30 colonies inhabiting sandy herbaceous or bare ground along the Nagara River, Gifu, Japan, from June to July in the period 1996–1999.

Chromosomal preparations were made using the testis of pink-eye white pupae and an improved air-drying technique (Imai et al., 1988). In addition to karyotype analysis, we also measured the nuclear size of the spermatids in these chromosomal preparations (100 nuclei each of 5 individuals).

For histological studies, white as well as black pupae and adult males were fixed in Bouin solution, embedded in Spurr's low-viscosity medium, and sectioned at ca. 10 µm with glass. The sagittal sections were stained on a hot plate with Mallory's solution (1% methylene blue/1% azure II in 1% sodium borate).

After determining the ploidy in one part of the testes of 4 haploid and diploid males, we determined the relative amount of DNA in the remaining part using flow cytometry. Cell nuclei were first separated from the cells and stained with test reagents: DNA reagent kit for animal cell analysis: mono separate solution of nuclei and color staining solution containing fluorescent coloring DAPI (4',6-diamino-2-phenylindole). The nuclei were then analyzed in the flow cytometer (PA type: Partec GmbH/Munster, Germany).

After the testes were removed for chromosome preparation, the pupae and adults were kept in a 100% alcohol solution for morphological study. Measurements of the maximum head width across the eyes, the maximum thorax width just in front of the forewings, and the maximum eye length were made using a binocular dissecting microscope.

Results

1. Spermatogenesis of haploid and diploid males

Maturation division

Figure 1 shows the chromosomes of haploid and diploid males at metaphase. The haploid and diploid numbers were 15 and 30, respectively. The karyotype consisted of 14 (28) acrocentric and one (2) metacentric chromosomes. Figure 2 presents the chromosomes at anaphase. The chromosomal

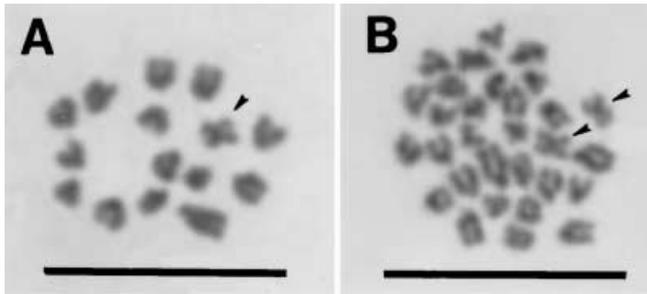


Figure 1. Chromosomes of *Lasius sakagami* males. **A** haploid male; **B** diploid male. Arrow: metacentric chromosomes. Scale: 10 μ m

number was 30 in the haploid males and 60 in the diploid males at the beginning of anaphase. Later in anaphase, this number was divided into half. Thus, no reduction of chromosomal numbers occurs throughout maturation division in either type of male.

Nuclear size of spermatids

The spermatids of the diploid males appear to normally metamorphose into sperm. Dissection and histological preparation revealed that the testes of 3 black pupae and 4 adults that were assumed to be diploid males because of their greater body size, were filled with numerous sperm. The nuclear size of the diploid sperm was apparently larger than that of haploid sperm (Figs. 3, 4): The mean maximum diameter of

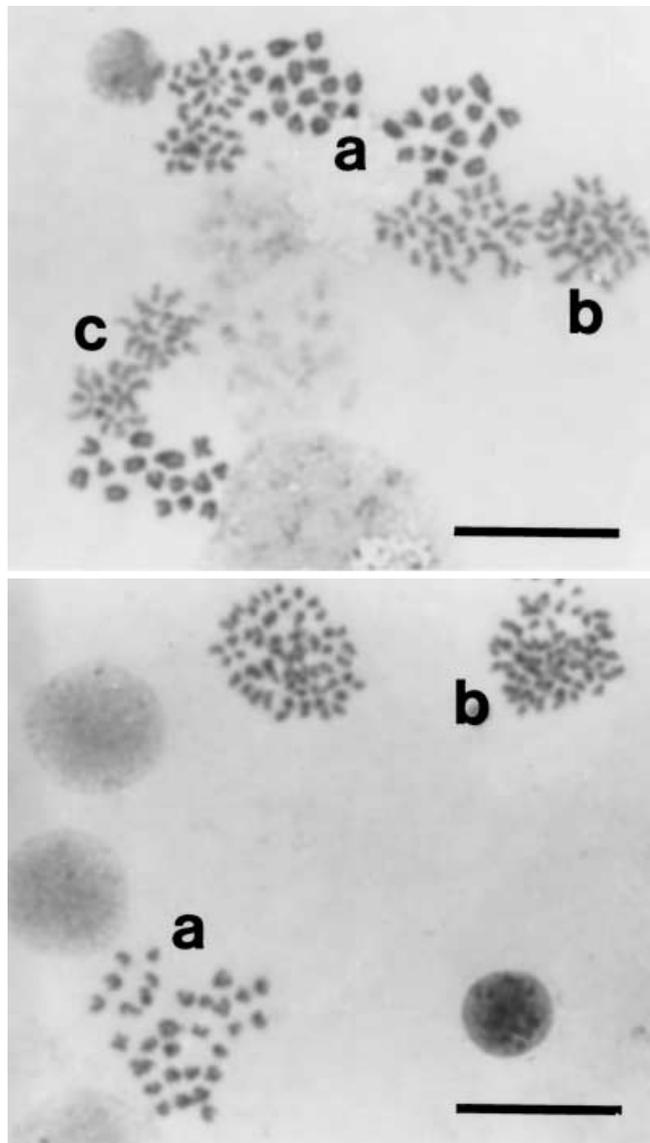


Figure 2. Chromosomes at various phases of maturation division in haploid and diploid males. Above: haploid male; bottom: diploid male. a: metaphase; b: beginning stage of anaphase; c: final stage of anaphase. Scale: 10 μ m

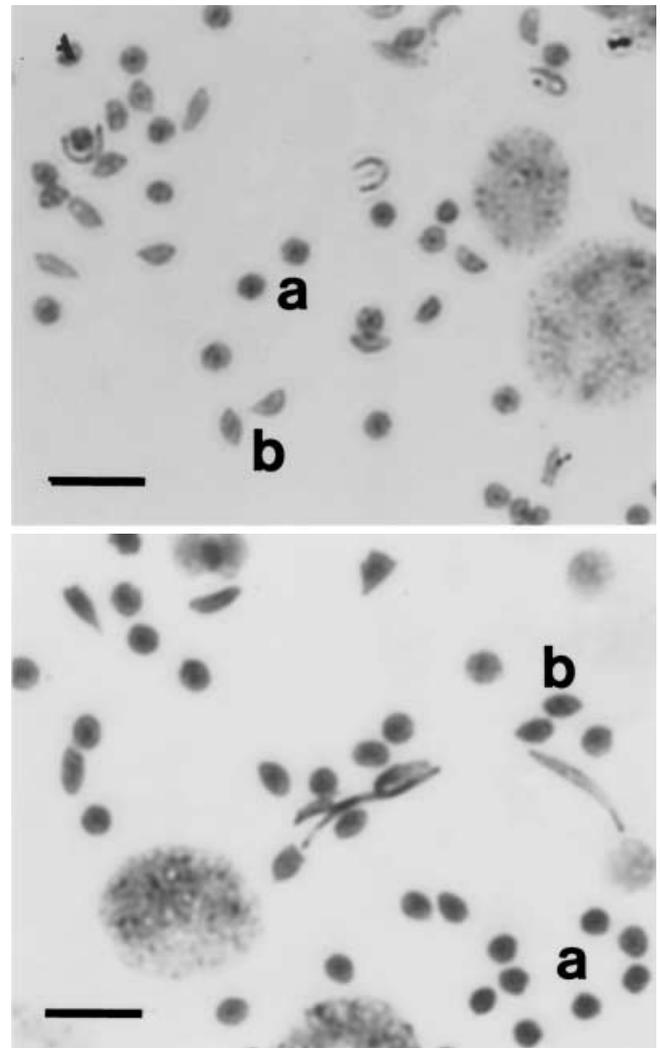


Figure 3. Spermatids of haploid and diploid males. Above: haploid male; bottom: diploid male. a: nucleus of spermatid; b: spermatid at the beginning of metamorphosis. Scale: 10 μ m

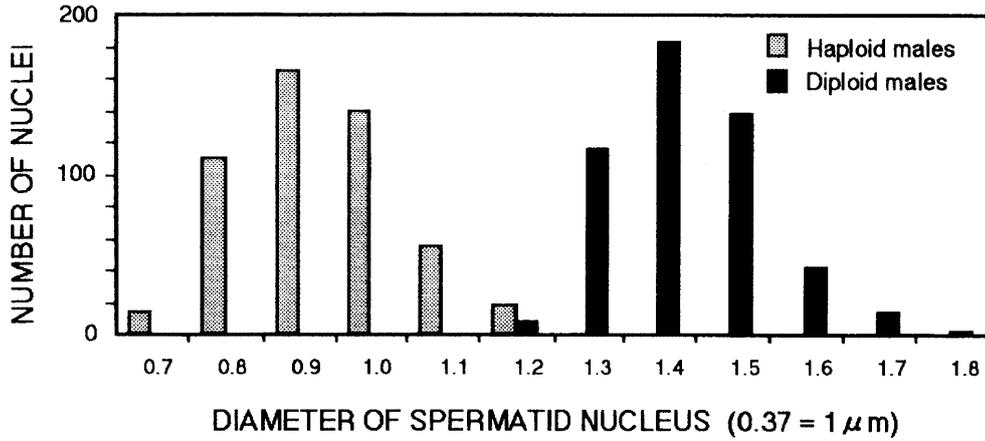


Figure 4. Frequency distribution of spermatid nucleus size in haploid and diploid males

the nuclei was 2.5 ± 0.3 (SD) μm in haploid males ($n = 500$) and 3.7 ± 0.3 (SD) μm in diploid males ($n = 500$).

DNA quantities

The results of flow cytometry are shown in Fig. 5. Fluorescence intensity was measured for ca. 1500 and ca. 2000 cells from 4 haploids and 4 diploids, respectively. Both diploid and haploid males showed two peaks of cell frequency with

regard to DNA quantity. The cells of haploid males that contained 50 FL DNA were mostly spermatids and those with 100 FL DNA mostly spermatocytes. In contrast, the cells of diploid males containing 100 FL DNA were mostly spermatids, while those with 200 FL DNA were mostly spermatocytes. The amount of DNA in the spermatids and spermatocytes of diploid males was almost twice as much as that of haploid males. Therefore, we concluded that all of the spermatids of the diploid males were diploid.

2. External morphology

Although male size varied between the colonies, the average size of the diploid male pupae was greater than that of the haploid pupae in each colony (Figs. 6, 7). The frequency distribution of adult size corresponded with that of the whole pupae with regard to haploid and diploid males. Therefore, the largest adult males can be regarded as diploid. However, we could not find any abnormal external and internal morphologies in these males.

3. Frequency of diploid males in the field

Diploid males were found in 6 of the 30 colonies studied (20%). These diploid male-producing colonies were located

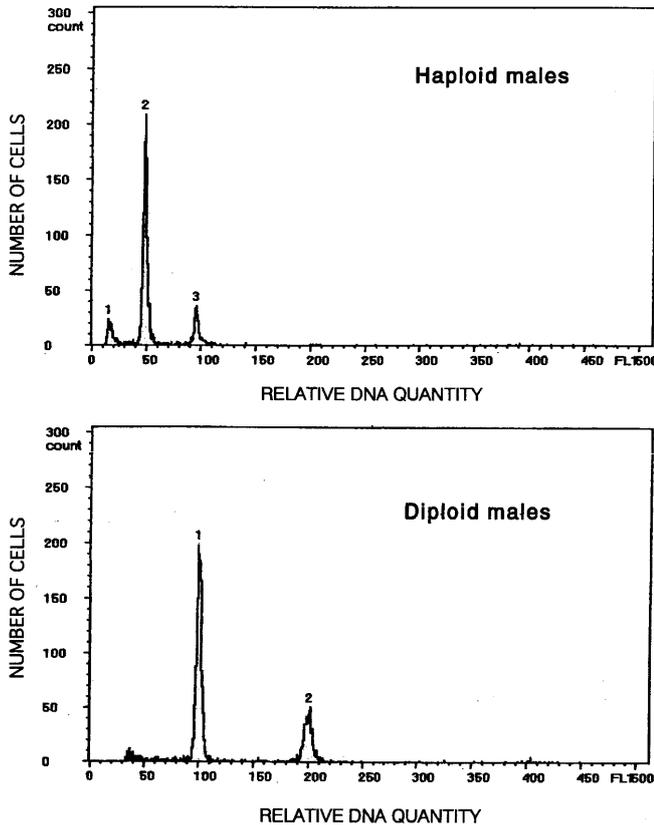


Figure 5. DNA quantities of germinal cells in haploid and diploid males. Peak 1 of haploid males is noise

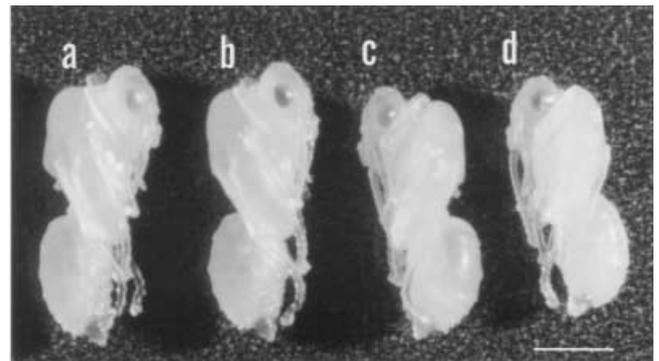


Figure 6. Haploid and diploid male pupae. a and b: diploid male; c and d: haploid male. Scale: 1 mm

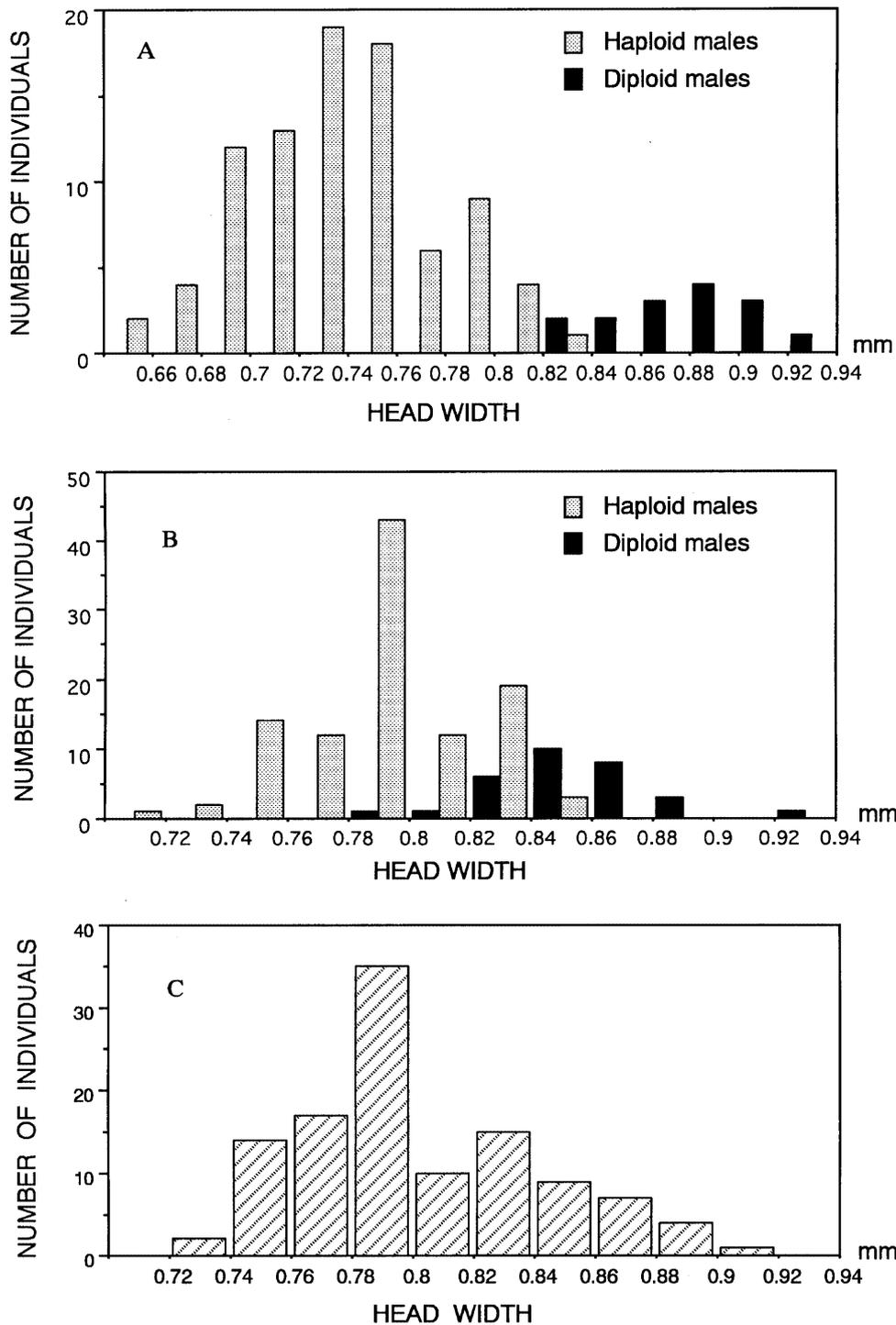


Figure 7. Frequency distribution of head width in haploid and diploid male pupae and male adults. A: pupae of Colony 96-1; B: pupae of Colony 98-3; C: adults of Colony 98-3

at sedimentary areas just adjacent to the river; they were observed to sometimes sink beneath the flood water and be partially destroyed. In these diploid male-producing colonies, the ratio of diploid to total number of males per colony varied from 13% to 90%, with a mean of 34% (n = 6).

We also observed that diploid males appeared only at the time of normal sexual production, as reported in *Formica* spp. (Pamilo et al., 1994), suggesting that younger stages of these males were eliminated at other times of year.

Discussion

So far, diploid males are known for 17 species of ants (Crozier and Pamilo, 1996). Most of these species are either parasitic or polygynous. In *Lasius sakagami*, which is a polygynous and polydomous species (Yamauchi et al., 1981), we found a frequent occurrence of diploid males. The body size of the diploid males was generally larger than that of the haploid males. This is also seen in *Solenopsis invicta* (Ross

and Fletcher, 1985). One of the causes of this size difference could be the difference in the size of the cell nucleus because of different amounts of DNA.

Based on all of the present data of chromosomal analysis, measurements of spermatid nucleus size, and DNA quantities, it is certain that these diploid males produce diploid sperm. Diploid sperm are also found in *Bracon* (Ichneumonoidea), *Nasonia* (Chalcidoidea), and *Bombus* (Apidae), but not previously in ants. The occurrence of triploid or tetraploid individuals in some ant species, however, probably reflects the occurrence of diploid males (diploid sperm) (Crozier and Pamilo, 1996; Krieger et al., 1999). In fact, a triploid worker was found in a colony of the myrmicine ant, *Crematogaster* sp. (Imai et al., 1977) and surprisingly high levels of triploidy (12%) were observed in non-reproductive females in an introduced polygynous *Solenopsis invicta* population in the USA (Krieger et al., 1999). A tetraploid worker was found in the formicine ant, *Camponotus* sp. (Imai et al., 1977) and completely triploid males could be produced in an inbred strain of *Harpagoxenus sublaevis* (Fischer, 1967; cited from Loiselle et al., 1990).

Diploid *Solenopsis invicta* males were listed by Ross and Fletchers (1985) to take part in normal summer mating flights. Although we never observed the copulation of diploid males with females in *L. sakagamii*, these ants did show precopulation behavior: antennation, mounting of females, and searching female genitalia in artificial nests. Moreover, in a few workers of this species, three bands were observed at an esterase allozyme region with 4 alleles (Yamauchi and Hashikura, unpubl. data), suggesting they were triploid and therefore daughters of diploid males.

It is conceivable from the above-mentioned facts that diploid males might be fertile and their triploid daughters might also be partially fertile. This has been seen in some other ant species e.g., *Bracon* (Whiting, 1939) and *Nasonia* (Whiting, 1960; Macy and Whiting, 1969). Since diploid males may have an important influence on sex ratios, population dynamics, social evolution, etc., further detailed studies

are needed on the frequency and the fertility of these males, especially these of polygynous species.

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