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POLYMORPHISM OF MALES IN *FORMICA EXSECTA* NYL. (HYM. : FORMICIDAE)

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SUMMARY

Males of *Formica exsecta* from two alpine valleys were found to belong to two significantly different size classes, called micraner and macraner. Nests contained either one or the other or both male types. Tot test whether both male types were haploid or one was diploid, chromosome numbers in brain cells from prepupae were counted and the relative DNA value of single nuclei from adult brains was determined. Most of the metaphase plates in brains from micraner as well as from macraner turned out to be haploid. The rest of the metaphases showed a 2n or 4n chromosome set. Workers had diploid brain cells together with some 4n cells. A difference between micraner and macraner was the percentage of cells with more than n chromosomes. All macraner had 90 % or more haploid cells in their brain while the percentage of haploid cells in micraner could be much lower, as low as 59 %. Only micraner showed chromosome numbers higher than 2n. DNA measurements gave principally the same result. Both male types exhibited the same low DNA value, lower than worker brain cells. In agreement with the chromosome countings, macraner had only one class of cells with a higher DNA value. In addition to the DNA values which are thought to represent the chromosome numbers n and 2n, lower values were found in macraner which are interpreted as degenerating nuclei. Both male types contained sperm.

The presented results show that in *F. exsecta* differences in male size are not induced by a haploid-diploid mechanism. All males were haploid. However, the frequency of endomitotic cycles, the doubling of the chromosome number without subsequent cell division, was lower in macraner than in micraner.

ZUSAMMENFASSUNG

Polymorphismus bei Maennchen von Formica exsecta **Nyl. (Hym. : Formicidae)**

Maennchen von *Formica exsecta* aus zwei Alpentaelern gehoeren zwei verschiedenen Groessenklassen an, Micraner und Macraner genannt. Es wurden Nester mit beiden Groessenklassen, aber auch solche mit nur je einer gefunden. Um zu untersuchen, ob

beide Maennchenklassen haploid oder eine diploid war, wurden die Chromosomezahlen in maennlichen Vorpuppen ermittelt und der relative DNA-Wert von einzelnen Gehirnzellen adulter Maennchen bestimmt.

Die ueberwiegende Anzahl aller Metaphaseplatten in Gehirnen von Maennchen beider Groessenklassen zeigte die haploide Chromosomenzahl. Der Rest der Metaphasen hatte 2n oder 4n Chromosomensaetze. Arbeiterinnen enthielten ueberwiegend diploide und daneben einige tetraploide Chromosomensaetze im Gehirn.

Ein Unterschied zwischen Micranern und Macranern war der Prozentsatz Zellen, die mehr als n Chromosomen hatten. In Macranern waren 90 oder mehr Prozent der Zellen haploid, waehrend in Micranern prozentual weniger haploide Zellen gefunden wurden. Dagegen enthielten nur Micraner Chromosomensaetze mit mehr als 2n. Die DNA-Messungen ergaben aehnliche Resultate. Beide Maennchentypen zeigten den gleichen niedrigen DNA-Wert, niedriger als der der Arbeiterinnen. Uebereinstimmend mit den Chromosomenzaehlungen hatten die Macraner nur eine Klasse hoeherer DNA-Werte. In Macranern wurde zusaetzlich eine Klasse DNA-Werte gefunden, die niedriger, war, als die, welche die n Chromosomenzahl repraesentiert. Diese Werte stammen vermutlich von degenerierenden Kernen.

Beide Maennchentypen enthalten Spermien.

Die vorliegenden Daten lassen den Schluss zu, dass bei *F. exsecta* die Maennchengroessenklassen nicht auf einen Haploidie-Diploidie-Mechanismus zurueckzufuehren sind. Einen Unterschied zwischen Micranern und Macranern ergab jedoch die Haeufigkeit endomitotischer Zyklen im Gehirn, also Verdoppellung der Chromosomenzahl ohne anschliessende Zellteilung. In den Gehirnen von Macranern wurden nur Zellen gefunden, die maximal einen endomitotischen Zyklus durchlaufen hatten. Micraner dagegen enthielten Mitosen mit einem vierfachen Choromosomensatz, entstanden durch zwei aufeinander folgende endomitotische Zyklen.

INTRODUCTION

Male polymorphism is rather scarce among the ants. The few records include examples such as *Hypoponera eduardi* (FOREL) (LE MASNE, 1956), *H. punctatissina* (ROGER) (HAMILTON, 1979), *Cardiocondyla basesii* FOREL, *C. elegans* EMERY, *C. emeryi* FOREL, *C. wroughtonii* (FOREL) (BOLTON, 1982), *Formica naefi* KUTTER (KUTTER, 1957), *F. sanguinea* LATR., F. *pressilabris* NYL. (AGOSTI unpublished), and *F. exsecta* (PAMILO and ROSENGREN, 1984) and the *Rhytidoponera impressa* group (WARD, 1983). Whereas there are two distinct forms present in *H. eduardi, H. punctatissima* and *Cardiocondyla spp.* (winged and ergatoid males) a size dimorphism with smaller and larger males or micraner and micraner respectively exists in the other species. Such a species is the palearctic *F. exsecta,* often found in polydomous colonies. The male offspring is dominated by micraner. Several authors studying the taxonomy of *F. exsecta* during the last decades (e.g. DLUSSKY, 1967 ; DLUSSKY and PISARKI, 1971 ; KUTTER, 1977 ; COLLINGWOOD, 1979) recorded the rather variable interpolulation mean size of thoracal length but failed, lacking sufficient samples, to demonstrate the bimodal intrapopulation length distribution. This has

recently been shown by PAMILO and ROSENGREN (1984) and more extensively by FORTELIUS *et al.* (in press, 1987) for *populations* of Southern Finland and by AGOSTI (unpublished) for population in the Swiss Alps. A bimodal size distribution of males is also known for *Solenopsis invicta* BUREN, where the small males have a haploid chromosome set the large males are diploid (ROSS and FLETCHER, 1985), as shown by gel electrophoresis. By the same method PAMILO and ROSENGREN (1984) identified diploid males in *F. pressilabris* but the later authors did not report morphological characters of their specimens. We investigated *F. exsecta* which is close to *F. pressilabris* in two respects. We studied male size and carried out a cytological analysis to determine the ploidy of males, with the idea that large sized males could be diploid.

MATERIAL AND METHODS

Adult males were collected during August 1985 from populations which were known to have bimodal sized males. Two samples were taken from polydomous colonies with about 40 nests each, at 1,740 m and 1,820 m above sea level in the Val Trupchun, where the population was scattered along track up valley. Prepupae came from one nest in the Val Scarl (1,760 m) in August 1986. This polydomous colony found in a meadow consisted of about 80 nests. Both valleys, Val Trupchun and Val Scarl, are cross valleys to the Engadin in the canton Grison in Switzerland. Outbreeding is assumed between the colonies for mating flight occurs in the early morning after sunrise when the wind is blowing up valley.

Prepupae and workers were carried home and kept at roomtemperature until the prepupae were dissected. Voucher specimen were deposited in the collection of AGOSTI.

We found two size classes of prepupae corresponding to the two size classes of adult males. Some of the smaller prepupae did not contain gonads and were therefore classified as workers. For the remaining small prepupae and the large ones the male sex could be demonstrated by the presence of a male gonad.

For karyotyping and for counting the number of metaphases we used the following procedure for slide preparation :

Brains from prepupae were dissected out and kept for 2 h in Ringer solution to which 0,04 % colcemid was added. Then the tissue was fixed in 50 % acetic acid for about 2 min, transfered to acetic acid - orcein for 5 min. The brains were differenciated and squashed in 50 % acetic acid, the slides frozen in CO_2 to remove the cover slip. After dehydrating in alcohol the cells were mounted in Euparal.

The number of chromosomes higher than haploid were estimated but not counted in most of cells. The type of slide preparation can lead to breakage of the nucleus resulting in nucleus fragments which may contain one or more chromosomes. We decided to classify chromosome groups between 15 and 26 chromosomes as haploid cells. However, it is possible that such a fragment can orginate from a diploid or higher ploid cell. Most of the haploid cells show the exact haploid set of 26 chromosomes. Numbers from 27 to about 50 chromosomes were classified as 2n cells. Chromosone sets with more then 52 chromosomes were interpreted as polyploid cells.

To measure the relative DNA content of nuclei the Feulgen method was applied. Brains of males and workers were dissected out and fixed in 50 % acetic acid for not more than ten minutes. The brains were placed in the fixative on a slide, covered with a siliconized coverslip, squashed and frozen on CO_2. After removing the coverslip the tissue

were hydrolysed for 12 min in 1N HCl at 60° C, cooled and stained in Schiff's reagent for 2 h. After that the slides were washed first in 10 % SO_2 watered three times for 5 min each and then in running tap water for another half hour. After dehydration in alcohol the slides were mounted in Euparal. For the DNA measurements we used a Zeiss Microskope-Photometer 01 applying the plug method. The relative DNA values are given in AU (arbitrary units) (RUTHMANN, 1966).

RESULTS

The size of the males

The distribution of the thoracal length of 61 males of the polydomous population from the Val Trupchun shows a bimodal distribution (*fig. 1*). The means of both the micraner and the macraner are significantly different ($X = 2{,}4$ mm resp. 2,9 mm, $p < 0{,}001$). In this population each nest exhibited only one male type. A different phenomenon existed in the population of the Val Scarl. Here both male types, micraner and macraner, occured together in the same nest. The mean values of the thoracal length were 2,5 mm for the micraner and 2,9 mm for the macraner, again a highly significant difference ($p < 0{,}001$). The Val Scarl type appears to be the common one as has been observed in other populations in Switzerland, for instance in the Val Sambuco (canton Tessin), Il Fuorn (canton Grison), and Bugnonet (canton Vaud).

The haploid chromosome number

Prepupae of two size categories of males, micraner and macraner, were analysed. Both exhibited 26 chomosomes in their brain cells as the modal chromosome number. This was decribed as the haploid chromosome number by HAUSCHTECK-JUNGEN and JUNGEN (1983). In *F. exsecta*, therefore, males are haploid, independent of individual size differences.

Workers were found to be diploid as expected, showing 52 chromosomes as the modal number.

The karyotype

In agreement with the results of HAUSCHTECK-JUNGEN and JUNGEN (1983) most of the chromosomes were medio- or submediocentric. For the smallest chromosomes it was not possible to determine the centromeric position.

Degree of ploidy in brain cells

In the brain of prepupae a certain variable number of mitoses can be found which permit the counting of the chromosome number and the determination of chromosome length and centromeric position. Most of the brain cells are already in the GO phase which means that they do no longer divide,

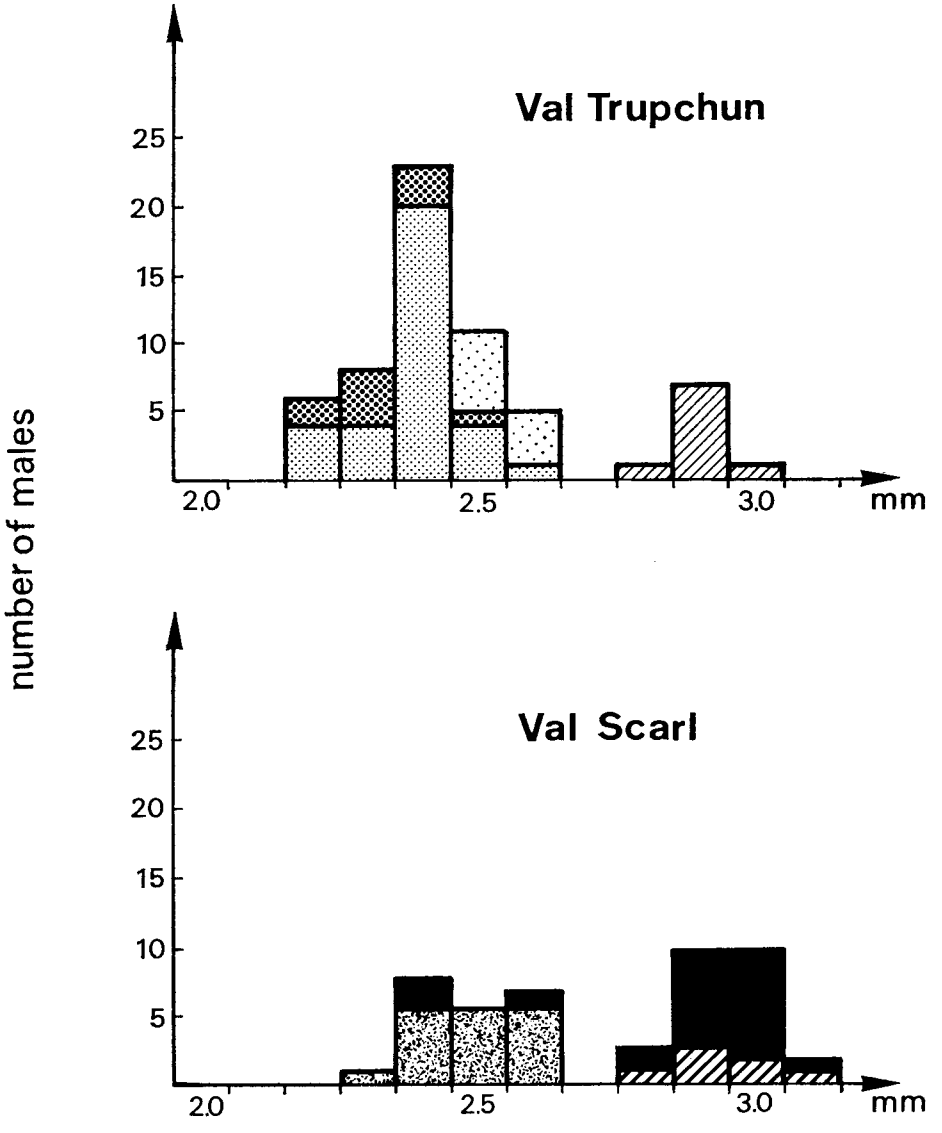


Fig. 1. — Distribution of the thoracal length of males from the Val Trupchun (62 males measured) and the Val Scarl (47 males measured). The different labels indicate diferent nests. The thoracal length is representative for the all over body size.

Abb. 1. — Verteilung der Thoraxlaengen von Maennchen aus dem Val Trupchun (62 Maennchen gemessen) und dem Val Scarl (47 Maennchen gemessen). Die verschiedenen Schraffuren bezeichnen verschiedenen Nester. Die Toraxlaenge wird als repraesentativ fuer die Koerpergroesse angenommen.

and DNA replication is finished. Therefore DNA measurements of single nuclei represent the final degree of ploidy.

Table 1 shows the percentage of haploid, diploid and chromosome numbers higher than diploid in brain cells of male prepupae.

In all males haploid nuclei were the most frequent ones, but in all twelve males analysed diploid nuclei also occurred. Additionally cells with a higher chromosome number than 2n were found in four males.

According to the chromosome number observed in metaphase plates, males can be grouped into two different classes : those which showed more than 90 % haploid mitoses and those in which only 59-76 % of mitoses were haploid and 24-41 % mitoses had a diploid or higher ploid chromosome number. Some cells were estimated to have an oktoploid chromosome set. In

Table I. — Frequencies of mitoses with different chromosome numbers in the brain of *F. exsecta*. Male and worker prepupae except the worker at' originated from the same nest. The male prepupae Nr. 1 - 7 had been smaller than the male prepupae Nr. 8 - 12. Therefore we classified Nr. 1 - 7 as micraner and 8 - 12 as macraner.

Tabelle I. — Frequenzen von Mitosen mit verschiedenen Chromosomenzahlen aus dem Gehirn von *F. exsecta*. Sowohl die Vorpuppen von Maennchen wie auch die von Arbeiterinenn mit Ausnahme der Arbeiterin at' stammen aus dem selben Nest. Die maennlichen Vorpuppen Nr. 1 - 7 waren kleiner als die Vorpuppen Nr. 8 - 12. Nr. 1 - 7 wurden daher als Micraner, Nr. 8 - 12 als Macraner betrachtet.

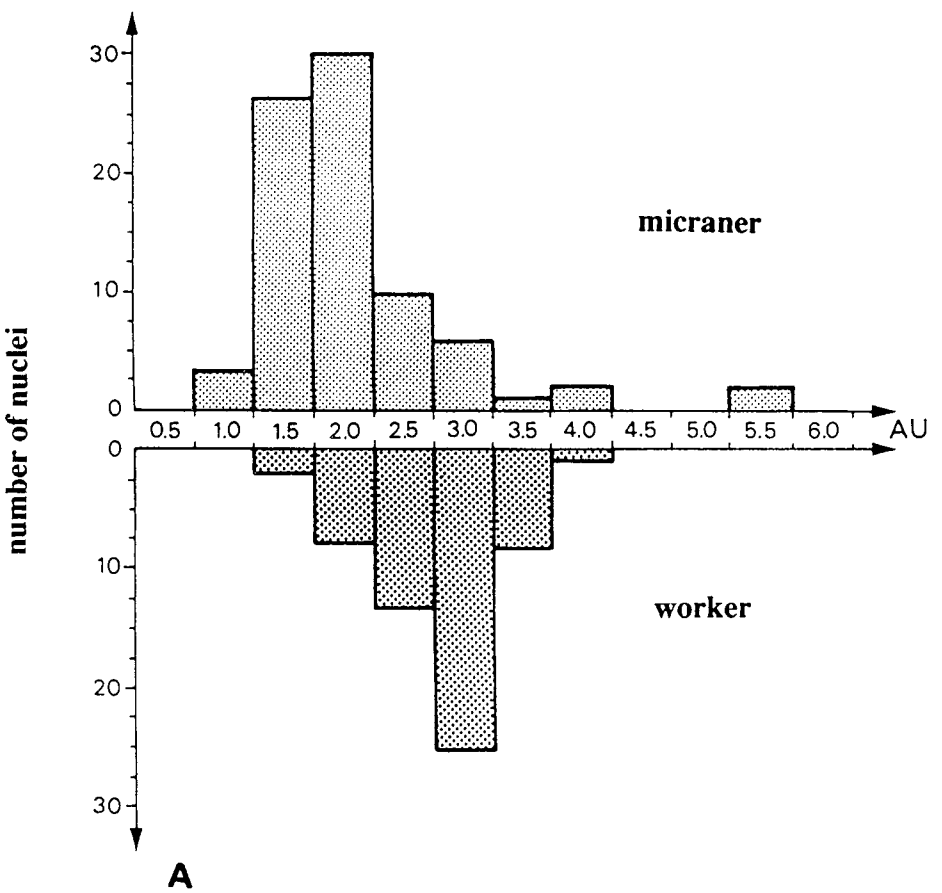
	% Metaphase			
Micraner number	Haploid (n)	Diploid (2n)	Polyploid (4n) or > 4n)	Number of nuclei analysed
1	59	35	6	17
2	70	24	5	99
3	64	18	18	141
4	76	24	0	42
5	62	35	3	66
6	91	9	0	92
7	95	5	0	99
Macraner number				
8	94	6	0	31
9	95	5	0	59
10	90	10	0	30
11	95	5	0	42
12	93	7	0	15
Worker number				
13	0	100	0	15
14	0	93	7	15
at'	0	96	4	373

males with 90 % or more haploid mitoses no nuclei with more than 2n could be found.

Micraner were found in both groups, in the group of males which had mostly haploid mitoses in the brain as well as in the group of males in which many diploid and higher ploid mitoses occurred. All macraner showed mostly haploid mitoses, never more than 7 % diploid mitoses and no higher ploid mitoses in the brain. Thus some micraner had a high, others a low amount of diploid mitoses while the macraner uniformly had a low amount of diploid mitoses in their brain. The percentage of haploid mitoses in the brain does not discriminate between the micraner and macraner.

In workers only diploid and tetraploid mitoses were found.

The frequency of cell divisions between males varies dramatically. The highest number of divisions recorded in a worker brain of *F. exsecta* was 373. The worker came from a nest in Pfynwald (canton Valais, Switzerland) in



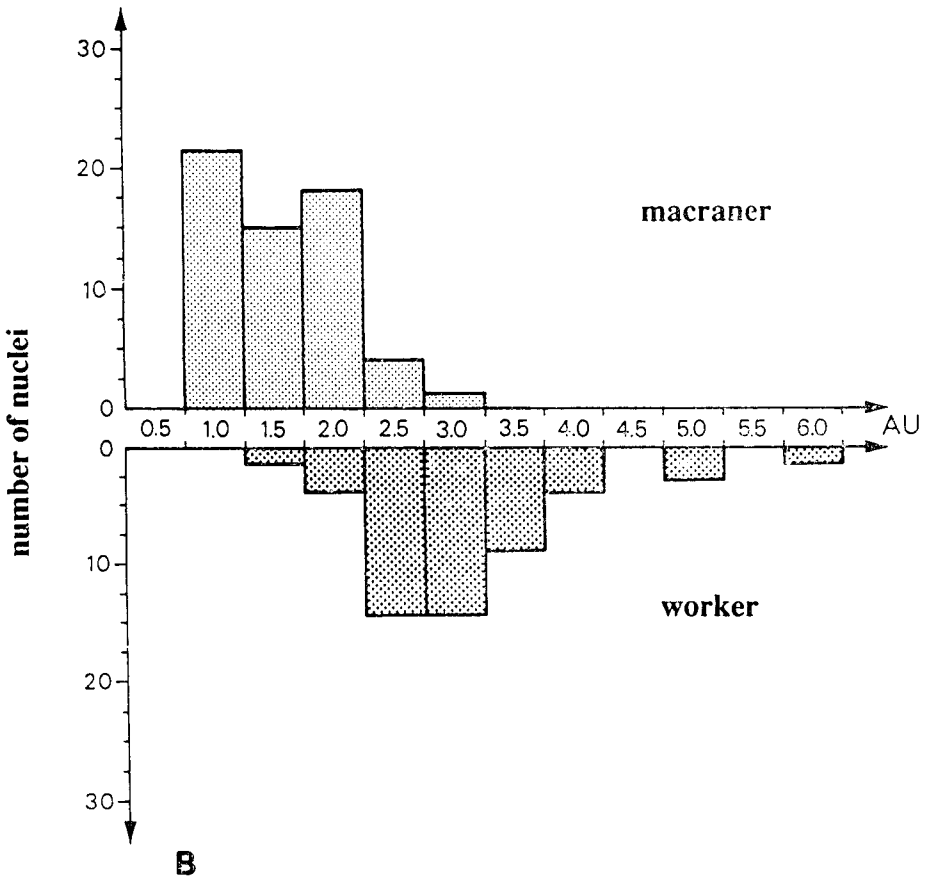


Fig. 2 a and 2 b. — The relative DNA amount in AU (arbitrary units) of brain cells of *F. exsecta*. Males (upper colomns) and workers (lower columns) were analysed. The ants in *figure 2a* came from one nest, those in *fig. 2b* from another. All males from the former nest had been smaller (micraner) than those from the latter (macraner).

Abb. 2 a und 2 b. — Der relative DNA-Wert von Gehirnzellen von *F. exsecta* in willkuerlich gwaehlten Einheiten. Es wurden Maennchen (obere Saeulen) und Arbeiterinnen (untere Saeulen) analysiert. Die Ameisen von *figure 2a* stammen aus einem, die von *figure 2b* aus einem anderen Nest. Die Maennchen des ersteren waren kleiner (Micraner) als die des letzteren (Macraner).

1970. In the ants collected in 1986 the maximum number was 141, while most of the numbers of mitoses per brain were lower than 100. Brains with less than 10 mitoses are not included here.

The relative DNA content of single brain cells was measured with the Feulgen method. Males as well as workers were analysed. The material came

from two nests, one with only micraner, the other with only macraner. The results are shown in *Fig. 2.* The most frequent DNA content of micraner, was to be found in the class of 1,5 - 2,0 AU while the corresponding one for workers from the same nest was found in a higher class, namely between 2,5 - 3,0 AU. This result is in agreement to the modal chromosome numbers : n for males, 2n for workers.

In both males and workers nuclei were found which had a DNA content corresponding to higher chromosome numbers, 2n in males and 4n in workers. The highest DNA value was measured in males, and the chromosome numbers higher than 4n were also only found in males.

The situation in the nest with macraners is less clear. In males a series of cells were found which had a lower DNA value than most of the cells from micraner. The highest values, and there were only few, correspond to the DNA value of diploid cells. This underlines the result that in macraner only haploid and diploid cells were found. The distribution of DNA values from workers were similar in both nests, the nest with only micraner and the one with only macraner. In workers from the latter nest some higher ploid cells were measured.

In some macraner, nuclei were found which had an extremely low DNA value. This type of nuclei was not present in micraner. The following observation may explain these low DNA values. Highly heteropycnotic nuclei were frequent in most of the brains of micraner, macraner, and workers. Degenerating nuclei were often found. It is important to note the observation of defective celle divisions in several brains. Chromatin pieces were found which were not included in the daughter nuclei. This suggests a loss of chromosomal material from the nuclei and could explain the lower than normal DNA amount observed in the daughter nuclei.

Sperm production

The gonadal duct of micraner and macraner were found to contain sperm.

DISCUSSION

In the FOREL-collection of the Museum d'Histoire naturelle at Geneve, four pins are labelled as "TYPUS" of *F. exsectopressilabris* FOREL which originate from different localities in southern central Europe, one from the Engadin. FOREL (1874) stressed in his description of the male intermediate size, a position inbetween the larger *F. exsecta* and the smaller *F. pressilabris.* Accepting *F. exsecta* as being one species (KUTTER, 1957, 1977) we need an explanation for the existence of the two male types present in the Engadin.

The question whether the size polymorphism of males of *F. exsecta* is induced by a haploidy-diplody polymorphism could be solved in the present

investigation. All males were haploid. The situation found by Ross and FLETCHER (1985) in *S. invicta* is not true for *F. exsecta*. Large males in *S. invicta* had been diploid and showed aspermy, while in both male types of *F. exsecta* sperm were found. In contrast to *F. pressilabris* we did not find a single diploid male in *F. exsecta*. The causal explanation for the size polymorphism remains to be demonstrated.

Additionally we found a high frequency of endomitotic cell cycles in the male brains which suggests new questions. In workers as well as in males a high chromosome number often found was 4n. Therefore it may be possible that in workers and males the function of one cell type is dependent on the tetraploid chromosome set. In males one more cell cycle is needed than in workers, to reach the 4n set. No interpretation can be offered to explain the lack of 4n cells in large males and in some of the small ones. A possible explanation would be that in those males the 4n cells are replaced by a higher number of haploid cells than small males have. Eventually transcriptional capacity is determined by the total DNA amount of a cell cluster, independent of the number of cells in the cluster as shown in *Drosophila*. Nurse cells of *Drosophila* eggs carrying the mutant dic (dicephalic) (eggs with dic show nurse cells at both egg poles) being higher ploid if the number of cells per pole cluster is lower than normal. As in *F. exsecta* the onset of a new replication cycle is not known in *Drosophila* (BOHRMANN *et al.*, 1986).

Another unusual observation was the differences between male prepupae concerning the number of mitoses per brain. We suspect that these differences indicate differences in the stage at the time of dissection rather than individual differences between males. It is not known how the onset of the cell cycles in brain cells is regulated.

A possible explanation of the different male types in the colony cycle described by FORTELIUS *et al.* (1987 in press) may be that one type develops from eggs layed by the queen the other type comes from eggs oviposited by workers. Worker oviposition is widespread among ants (WILSON 1971) : in the genus *Formica* known in *F. exsecta* (PAMILO and ROSENGREN, 1983), in *F. polyctena* Foerster (EHRHARDT, 1962) and in *F. rufibarbis* Fabr. (HOHORST, 1972), supporting the prediction by means of the genetical theory of the social behavior that workers should favor their own sons over their brothers (WILSON, 1971). After PASSERA (1984) egg laying of queens and workers may by synchronous in the same nest. According to FORTELIUS *et al.* (1984) micraner and macraner may represent two different reproductive strategies in the colony cycle : outbreeding and inbreeding respectively. In another well studied hymenopteran species, the anthophorid bee *Centris pallida* FOX (ALCOCK, 1979) large males patrol on the ground to copulate with the freshly emerged females. Small males hoover and mate with flying virgin females. Thus two different sized male types render a higher mating rate of the females.

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References

ALCOCK J., 1979. — The evolution of intraspecific diversity in reproductive strategies in some bees and wasps. In : M.S. Blum and N.A. Blum (eds.), *Sexual selection and reproductive competition in insects.* Academic Press, New York, 381-402.

BOHRMANN J., KIEFER G., SANDER K., 1986. — Inverse correlation between mean nuclear DNA content and cell number in nurse cell cluster of *Drosophila. Chromosoma, 94,* 36-44.

BOLTON B., 1982. — Afrotropical species of the myrmicine ant genera Cardiocondyla, Leptothorax, Melissotarsus, Messor and Cataulacus. *Bull. Br. Mus. nat.* Hist. (Ent), *45* (4), 307-370.

COLLINGWOOD C.A., 1979. — The Formicidae (Hymenoptera) of Fennoscandia and Denmark. *Fauna Ent. Scand., 8,* 1-174.

DLUSSKY G.M., 1967. — Ants of the genus Formica. *Izdatelstvo* " Nauka ", Moscow, 237 pp. (in Russian).

DLUSSKY G.M., PISARSKI B., 1971. — Rewizija polskich gatunkow mrowek (Hymenoptera : Formicidae) z rodzaju, Formica L. *Fragm. Faun.* (Warsaw), *16,* 145-224 (in Polish).

EHRHARDT H.J., 1962. — Ablage übergrosser Eier durch Arbeiterinnen von *Formica polyctena* Foerster (Ins., Hym.) in Gegenwart von Königinnen. *Naturw., 49* (22), 524-525.

FOREL A., 1874. — Les fourmis de la Suisse. - *Neue Denkschr. allg. schweiz. Ges. Naturw.,* Zürich, *26,* 452 pp.

FORTELIUS W., PAMILO P., ROSENGREN R., SUNDSTROEM L., in press. — Male size dimorphism and alternative tactics in *Formica exsecta* ants (Hymenoptera, Formicidae). *Ann. Zool. Fennici, 24* (in press).

HAMILTON W.D., 1979. — Wingless and fighting males in fig. wasps other insects. In: M.S. Blum and N.A. Blum (eds.), *Sexual selection and reproductive competition in insects.* Academic Press, New York, 167-220.

HAUSCHTECK-JUNGEN E., JUNGEN H., 1976. — Ant chromosomes I. - The genus Formica. *Ins. Soc., 23* (4), 513-524.

HOHORST B., 1972. — Jahreszeitliche Veraenderungen der Ovariolen bei Arbeiterinnen von *Formica rufibarbis* Fabr. (Hym, Form. *Ins. Soc., 19,* 403-404.

KUTTER H., 1957. — Zur Kenntnis schweizerischer Coptoformicaarten (Hym. Form.). *Mitt. Schweiz. Ent. Ges., 30* (1), 1-24.

KUTTER H., 1977. — Hymenoptera Formicidae. *Insecta Helvetica, 6,* Zuerich, 298 p.

LE MASNE G., 1956. — La signification des reproducteurs aptères chez la fourmi *Ponera eduardi* Forel. *Ins. Soc., 3,* 239-259.

PAMILO P., ROSENGREN R., 1983. — Sex ratio strategies in Formica ants. *Oikos, 40,* 24-35.

PAMILO P., ROSENGREN R., 1984. — Evolution of nesting strategies of ants : Genetic evidence from different population types of Formica ants. *Biol. J. Linn. Soc., 21,* 331-348.

PASSERA L., 1984. — L'organisation sociale des fourmis. *Bios,* Univ. Paul Sabatier, Privat, Toulouse, 360 pp.

ROSS K.G., FLETCHER D.J.C., 1985. — Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera : Formicidae), and its evolutionary significance. *Evolution, 39* (4), 888-903.

RUTHMANN A., 1966. — Methoden der Zellforschung. *Kosmos, Franckh' sche Verlagshandlung,* Stuttgart, 301 pp.

WARD P.S., 1983. — Genetic relatedness and colony organization in a species complex of ponerine ants. *Behav. Ecol. Sociobiol., 12,* 285-299.

WILSON E.O., 1971. — The insects societies. *Belknap Press, Cambridge,* MA, 548 pp.