

Michał WOYCIECHOWSKI

**Mating behaviour in the ant *Myrmica rubra*
(Hymenoptera, Formicidae) ***

[With 4 text-figs]

Zachowania kopulacyjne u mrówki *Myrmica rubra* (Hymenoptera, Formicidae)

Abstract. Experiments and observation of mating behaviour in *Myrmica rubra* during the nuptial flights in the Polish Carpathians showed that, in natural conditions, the majority of queens seem to be monandrous. Repeated matings of queens were explained by their inability to escape after the initial mating or by multiple male matings leading to the depletion of their semen. The duration of mating, depends on the male's previous matings, but also differs between different years. The queens' attractivity decreases in time after their last mating, but even after the last mating, multiple-mating queen is more attractive to males than a queen which mated only once. The results are discussed in terms of kin selection theory and worker reproduction.

I. INTRODUCTION

The formation of a large aerial aggregation of alate individuals during nuptial flights is a typical behaviour in *Myrmica rubra* LINNAEUS 1758 as well as in other species of that genus (NEUDECKER 1952, HUBBARD & NAGELL 1976, WOYCIECHOWSKI 1987). Alates forming such a swarm above a mountain top, trees or buildings often belong to many species (CHAPMAN 1954), each species occupying its own place, the same every year (WOYCIECHOWSKI 1990). In the species of the genus *Myrmica*, flight activities take place in the second half of summer and the beginning of autumn (WOYCIECHOWSKI 1990), depending on the weather conditions (BOOMSMA & LEUSINK 1981). During the day, the time of the swarming is typical for each species, although it partly coincides with that of other species (WOYCIECHOWSKI unpublished). The swarming distinctly synchronized in alates from many nests, surely minimizes the chances of, if not totally precludes, mating between familiars, and thus eliminates local mate competition. On the other hand, a group swarming brings about real dangers, e.g. death risk increasing with the distance to the swar-

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ming site or the attraction of predators by the concentration of a great number of vulnerable insects.

Data on the behaviour of particular individuals during mating in the genus *Myrmica* are scarce. The number of queen's matings is of special importance for such questions as the sex ratio in sexual castes (ALEXANDER & SHERMAN 1977) or workers' reproduction (WOYCIECHOWSKI & ŁOMNICKI 1989). As is implied by the theoretical model proposed by WOYCIECHOWSKI and ŁOMNICKI (1987), in nests headed by monandrous queens, i.e. those which mated only once, workers' reproduction is much more common than in nests with polyandrous queens. This general statement might concern particular nests of any species, or whole species and the strategies employed by them. The insufficient number of detailed observations on the number of queens' matings and on the origin of the males does not permit a verification of this hypothesis.

The production of male-determined eggs by the workers is a rule in *M. rubra* (BRIAN 1969, SMEETON 1981). Therefore one can expect, that queens of this species mate only once. To check whether this is the case was the main of this study. I also considered such behaviour of sexuals which would result in alternative reproductive strategies (FORTELIUS et al. 1987, KINOMURA & YAMAUCHI 1987).

II. STUDY AREA AND METHODS

I carried out my observations in the Polish Carpathians, in Male Pieniny range, on the northern slope of the Durbaszka Mt., or more precisely on a single rocky summit (850 m a.s.l.) some 20 m above the surrounding slope. This lone rock was the site of regular formation of multispecies aggregations of alate ants. *M. rubra* was one of the most frequent species. It is on this species that a series of experiments was carried out on 19th–21th September 1985, 3th–12th September 1986 and 21th–25th August 1987, between 8:00 and 16:00.

The experiments consisted in placing the captured alates immediately from the swarm, after an introductory visual determination, in a glass test-tube with a diameter of 2 cm. I then closed the test-tube with a cotton swab, leaving a free space of about 1 cm in height. Usually, a male placed in the immediate vicinity of a potential partner tried to mate as fast as possible. I termed this action „precopulation behaviour”. I recorded the number of such behaviours and their duration exact to 2.5 sec. I also measured the number and duration of the matings themselves. In the course of the experiment, I exchanged one of the partners while measurements were constantly taken. After the end of the observation, I preserved all individuals for precise identification. If two individuals of different species had been observed, that observation was not taken into consideration while the numbering of the subsequent partners was maintained. This situation, however, occurred only rarely.

Since the duration of behaviours observed diverged from the normal distribution, statistic comparisons employed the nonparametric KRUSKAL-WALLIS's test, yielding the test statistic value (H) and the probability (P) of rejecting the null hypothesis. Similarly, the chi-square test yielded the value of its statistics (χ^2) and the probability P , at the degree of freedom $df = 1$. Only the P value was given as the result of FISHER's exact test.

Experiment 1

I placed a queen with a male and then exchanged the male for a newly-captured one each 5 min. If mating took place before that time, I exchanged the male immediately after the mating. A series of nine males was still exchanged after the queen's last mating. I repeated this experiment many times in the three years; however, in 1987, subsequent males were exchanged for each queen only until her first mating.

Experiment 2

I placed six individually-marked males together with a queen, which I then exchanged for a new one each 5 min. Only if the queen mated did I exchange her immediately after the end of that mating. The exchange of the queens was continued until mating failed to occur with any of five consecutive queens. I repeated the experiment throughout 1986.

Experiment 3

I selected two groups of queens from experiment 2. The first consisted of those which mated with only one male which previously mated at least 5 times. The other included those which mated only once with a virgin male. The queens of both groups were deprived of the possibility for more matings for about 24 hrs. After that time, i.e. the next day, I subsequently exchanged not less than 10 males for each of those queens if she did not mate with any of them and not less than 10 males after queens' last mating (the males were exchanged according to the pattern in experiment 1).

This experiment, slightly modified, was repeated in the following year 1987. The queen had the possibility of only one mating (experiment 1) and it was put together, after some 24 hrs, with a male then exchanged five times according to the earlier principle.

III. RESULTS

Number and duration of the queen's matings

The examination of 39 queens in 1985 and 14 queens in 1986 (experiment 1) yielded the number of the queens' matings and their duration. On the average, the queens mated 1.94 times and, rejecting the 5 not mating queens 2.15 ti-

mes. The frequency distribution of queens with a given number of matings diverges from a random one (Fig. 1). The queens which mated only once were significantly more numerous than any other — those that did not mate at all and those that mated twice or more ($\chi^2 = 15.521$, $P < 0.001$).

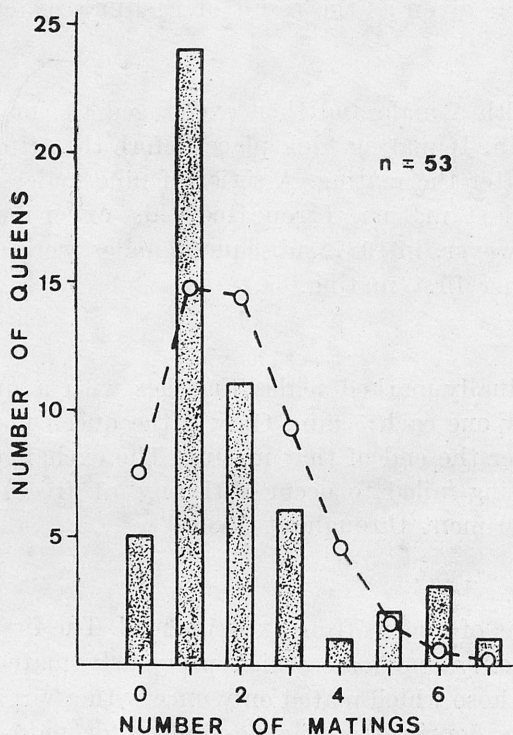


Fig. 1. Frequency distribution of the queens' matings and the expected Poisson distribution (dashed line)

The matings in 1985 (mean mating duration $\bar{x} = 68.9$ sec., number of observations $n = 80$) did not last significantly longer than in 1986 ($\bar{x} = 57.8$ sec., $n = 23$) (KRUSKAL-WALLIS'S test: $H = 1.903$, NS). As in the following year 1987, the queens were permitted only one mating; compared also was the duration of the first matings in the three years. In the first two years, the durations of the first queens' matings ($\bar{x} = 72.5$ sec., $n = 48$) were significantly longer than those in 1987 ($\bar{x} = 39.9$ sec., $n = 60$) ($H = 44.916$, $P < 0.001$). In 1985 and 1986, the duration of the queens' initial matings did not differ significantly from that of their consequent matings ($\bar{x} = 61.091$ sec., $n = 56$) ($H = 4.125$, NS).

I have been checked whether all matings of the queens have the same impact on their consequent mating behaviour. For that, I selected 8 queens which mated only once the day before, with a male earlier mating many times, as well as 8 other queens which also mated only once the day before, but with

a virgin male. It was made possible for all those queens to continue mating (experiment 3 of 1986). In the first of the groups described, 7 queens resumed mating while only one did not. In the second group, the proportions were reversed: 2 and 6 queens respectively. This difference is significant (FISHER's exact test: $P < 0.05$). None of the queens undertaking another mating mated more than twice.

Precopulation behaviour

The results described in this section concern the behaviour of the males placed in the presence of queens mating at least once in the course of all observations (experiments 1 and 3).

Often a queen did not mate immediately after encountering a male (experiment 1). The duration of this precopulation behaviour, aimed at provoking mating before the queen's first mating, did not differ significantly in the three consecutive years (1985: $\bar{x} = 31.3$ sec., $n = 35$; 1986: $\bar{x} = 30.4$ sec., $n = 13$; 1987: $\bar{x} = 28.0$ sec., $n = 60$; $H = 2.422$, NS) and was 29.3 sec. on the average. Each consecutive queen's mating occurred after ever longer attempts of the male; sometimes only the second or third sexually-active male was permitted to mate. The measurements of the duration of precopulation behaviour in all males between subsequent queens' matings showed significant differences (before first mating: $\bar{x} = 47.7$ sec., $n = 48$; before second: $\bar{x} = 120.7$ sec., $n = 23$; before third: $\bar{x} = 144.3$ sec., $n = 14$; before fourth and further: $\bar{x} = 254.4$ sec., $n = 17$; $H = 29.419$, $P < 0.01$).

Not only the duration, but also the number of precopulation behaviours to which a male was forced in order to obtain a partner differed depending on whether this was before the first or before the repeated mating of a queen (experiments 1 of 1985 and 1986). The first mating of a queen occurred on the average after 1.1 such attempts by the male, while a second mating, if it took place at all, followed on the average 2.1 such attacks. This difference is confirmed by the fact that 42 queens took part in mating after the first attack of a sexually-active male, while only 6 queens were attacked by males more times. Among the queens which mated more than once, and taking into account only their second mating, 13 queens took part in it after the first attack by the male and 11 queens had to be attacked more than once. These differences are significant (FISHER's exact test: $P < 0.05$).

Not all queens were similarly attractive to males, for not always did the males exhibit sexual activity towards the encountered queens. I have checked if the attractiveness of the queens, measured in the frequency of the males active towards them, depends on the earlier matings of those queens (experiments 1 in 1985 and 1986). First, I investigated whether queens are attractive to the same extent from the first to the last mating. Thus only the time from the beginning of the experiment to the last mating of the queen was considered. During that time, I have not observed that, until the first

mating of the queen, the numbers of sexually-active (50) and inactive (0) male would distinctly differ from the corresponding numbers (80 and 5) of males placed with the queens after their first mating (FISHER's exact test: $P < 0.05$).

The frequency of sexually-active males was also considered when they were placed with the queens after their last mating. Here, however, two groups of males were examined. The first included the males placed with the queens which earlier mated only once. The other group consisted of the males placed with the queens which earlier mated at least twice. I have established that there were 139 sexually-active and 79 inactive males in the first group. In the second, the frequency of inactive males distinctly dropped as 72 were active and only 19 inactive. Those differences are significant ($\chi^2 = 6.994$, $0.01 < 0.001$). The fact that even after a queen's last mating the males exhibit a greater interest in those which earlier mated more times, and the decrease with time of the attractiveness of the queens after their last mating was confirmed by conducting a covariance analysis (Fig. 2).

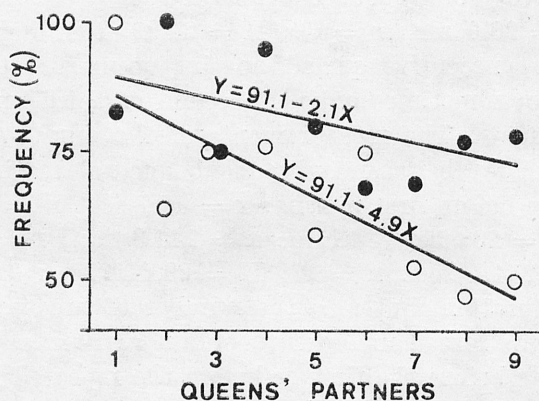


Fig. 2. Frequency of sexually active males (%) among the consecutive partners of the queens after their last mating — males in presence of queens which earlier mated only once (open circles) or more then once (solid circles). Analysis of covariance to comparison of regression lines: significance of the regression coefficient: $F = 13.116$, $P < 0.01$; significance of the difference in the slopes $F = 2.231$, NS; elevation: $F \rightarrow 7.752$, $0.05 < P < 0.01$; intercept, NEWMAN-KEULS test: $q = 4.096$, $0.05 < P < 0.01$)

I have also observed that the queens mating at least once became, after some time, less attractive to males and gave up further mating. Of the 60 queens which mated only once, I randomly selected 13 the following day and placed consecutive males in their presence (experiment 3 of 1987). While there were 61 sexually active and only 1 inactive male on the first day, i.e. since the last mating, the same numbers the day after that mating were respectively 41 and 24 ($\chi^2 = 25.025$, $P < 0.001$). This result was confirmed by the significantly lower interest of the males in the queens the day after their first mating. It should also be stated that of the 13 mentioned queens only one took part in only one next mating on the day after their first mating. If, on the other hand,

the queens were sexually attacked by males immediately after their first mating, 24 of those queens mated only once while the other 24 took part in at least one additional mating (experiment 1, Fig. 1). This difference is significant (FISHER's exact test: $P < 0.05$).

Number and duration of the males' mating

An average male could mate 3.0 times within not more than 70 min and the maximum number of observed matings of a male within that time was 10 (experiment 2; Fig. 3). The duration of a male's mating, lasting from 10 to 1330 sec., increased significantly in the consecutive mating ($H = 39.673$, $P < 0.001$; Fig. 4).

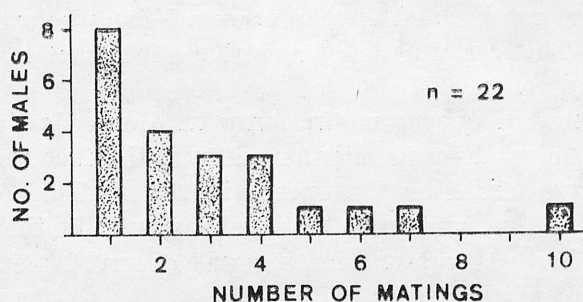


Fig. 3. Frequency distribution of the males' matings

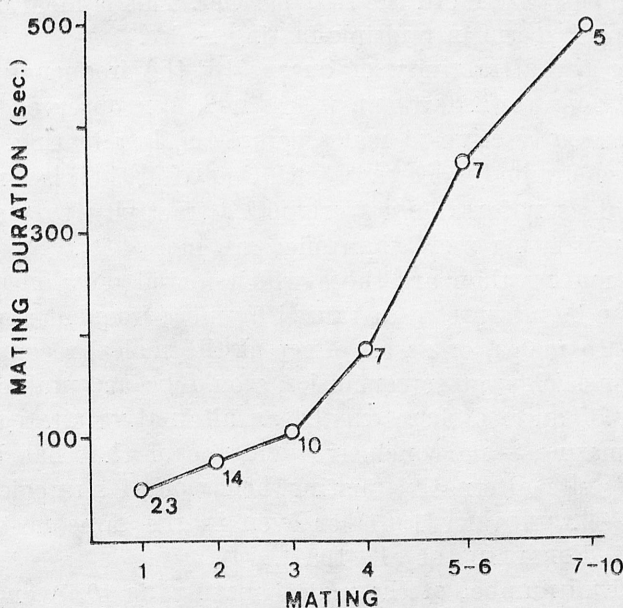


Fig. 4. Mean duration of the consecutive matings of the males. (On the diagram, numbers of matings are given)

IV. CONCLUSIONS

1. The queens of *M. rubra* do not select their partners in mating. As a rule, a queen mates with the first sexually-active male encountered although there are, on the average, several hundred of males per each queen in a cloud of swarming ants (WOYCIECHOWSKI 1990).

2. The first mating of a queen usually follows the first attack by a male. However, in order to persuade the queen for another mating, the males have to double the number much of such attacks. In natural conditions, a queen has then the possibility to avoid further matings.

3. As a rule, the queens unattacked immediately after the first mating do not take part in consecutive matings.

4. The time in which a male actively attempts mating is the shortest before a queen's first mating and increases several times before her consecutive matings (Fig. 3). This confirms the thesis that the queen can avoid repeated matings in natural conditions.

5. The frequency of queens mating only once exceeds that which could be expected from a random distribution, despite the fact that the more frequent matings were forced on the queens in the artificial conditions of the experiment. The mean number of matings observed in the experiment, 2.1, in those queens whose at least one mating was observed seems to be higher than in natural conditions.

6. One of the causes of the queens' polyandry (Fig. 1) might be the males' multiple mating. If a male mates more times, its later partners decide to mate again more frequently than did the first partner. This is most probably caused by the depletion of sperm in multiple-mating males.

7. Measuring the attractivity of queens by the frequency of males' pre-copulation behaviour towards them, it has not been observed that the queen would become less attractive to males before the last mating. The males' interest in the queen gradually decreases with time after their last mating (Fig. 2) and the queen does not regain its original attractivity to males.

8. The queens mating more than once are more attractive to males even after their last mating than are those which mated only once (Fig. 2). This results might also be interpreted as caused by more frequent matings of queens more attractive to males, or as an effect of the males' recognition of multiple-mating queens. This latter conclusion would be acceptable if, in at least a small number of queens, there existed an alternative strategy consisting in their multiple mating — no conclusive evidence of that has been found. In some species of ants alternative mating behavioural strategies occur within one population (MARIKOVSKY 1961) or even in the same nest (KINOMURA & YAMAUCHI 1987, FORTELIUS at all. 1987).

9. The potential number of the males' matings is 10, even within a short time (Fig. 3). A male is able to mate about 3 times on the average.

10. The duration of mating depends on many factors such as previous ma-

tings of the male, but it also varies each year. The prolongation by several times of the following matings of the male (Fig. 4) confirms the earlier thesis on the depletion of its sperm. The duration of the mating is not influenced by the previous sexual behaviour of the queen.

Results from 2 to 5 suggest that, in natural conditions, a single mating is the most frequent strategy of *M. rubra* queens. This agrees with the hypothesis presented in the Introduction, which connects queen mating frequency and worker reproduction in eusocial *Hymenoptera* (WOYCIECHOWSKI & ŁOMNICKI 1987). The single mating of the queens and the multiple mating of the males in *M. rubra* is also suggested as an explanation of the more than 1:500 male-biased sex ratio in a cloud of swarming ants (WOYCIECHOWSKI 1990). This does not preclude the possibility that even in natural conditions some of queens mate more than once.

All of the above conclusions have been forwarded with the awareness of the fact that some of both the queens and of the males captured in the experiment have already mated before. It has been decided, however, that such situations were few and influenced neither the results nor the interpretation presented. It can also be assumed that, estimating the mean number of the queens' matings, the conditions of the experiment, not totally natural, compensated if not exceeded the effect of pre-experiment matings.

Institute of Systematics and Evolution of Animals
Polish Academy of Sciences
ul. Sławkowska 17, 31-016 Kraków, Poland

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STRESZCZENIE

Prowadząc eksperymenty i obserwując zachowanie kast seksualnych u *Myrmica rubra*, w czasie rójki w Polskich Karpatach, uzyskano wyniki uzasadniające twierdzenie, że w naturalnych warunkach większość królowych jest monandrycznych, czyli kopuluje tylko z jednym samcem (rys. 1). Ponieważ już wcześniej stwierdzono, że u tego gatunku mrówek samce są najczęściej synami robotnic, powyższe rezultaty są zgodne z hipotezą uzasadniającą reprodukcję robotnic monandrią królowych gniazd eusocialnych *Hymenoptera*. Za przyczyny powtórnych kopulacji części królowych uznano uniemożliwienie im ucieczki bezpośrednio po pierwszej kopulacji oraz wielokrotne kopulacje samców, a tym samym niewystarczające ilości ich spermy dla dalszych partnerek. Stwierdzono także, że królowa nie dobiera sobie partnera, kopulując z reguły z pierwszym aktywnym seksualnie samcem. Królowa jest dla samców najbardziej atrakcyjna do czasu kiedy skłonna jest kopulować, po ostatniej kopulacji atrakcyjność królowej stopniowo maleje z czasem. Mimo to nawet po ostatniej kopulacji królowa, która kopulowała wielokrotnie, jest dla samców bardziej atrakcyjna niż królowa, która kopulowała tylko raz (ryc. 2). Samce w krótkim czasie są zdolne kopulować nawet do 10 razy, średnio mogą jednak kopulować 3-krotnie (ryc. 3). Czas kopulacji trwający od 10 do 1330 sekund jest zależny od wcześniejszych kopulacji samca (ryc. 4), ale też zmienia się w poszczególnych latach.