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Mating behaviour in the polygynous/polydomous wood ant *Formica aquilonia*

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The behaviour of reproductive ant females and males was examined in mating experiments in laboratory conditions. The aims were to identify mechanisms affecting sexual selection in ants in general, and to explore more specific issues applicable to the reproductive strategies present in ants like *F. aquilonia* characterized by a multinest/multiqueen (polydomous/polygynous) organisation of colonies. The mating process was described from video recordings. Multiple mating by both sexes was demonstrated and the observed mating frequency was in good agreement with results for the effective number of matings based on allozyme mother–offspring analyses. For females and males random mate choice was indicated. In choice experiments neither differences between the frequencies of intra- and interpopulation copulations occured, nor did the mating status of males and females affect the pattern of random mating. However, the number of preceding copulations affected the duration of different parts of the mating process in both males and females. Thus, female mating resistance tends to increase after several matings, whereas in males the duration of genital contact increases with the number of previous copulations. Also, male mating experience reduces the time used to get into genital contact, except when multiple copulations occur within a short time. The operational sex ratios in the experimental chamber did not affect the pattern of random mate choice. Spermathecal sperm counts correlated with the number of female matings and were comparable with both the estimated number of sperm for virgin males, and the spermathecal counts for old and young queens collected in the field. The findings are discussed in relation to general patterns of sexual selection in ants. Selection acting on male mating behaviour might drive both female and male multiple mating in *F. aquilonia*.

Introduction

A number of more or less unique factors affect sexual selection in ants. First, the extent to which investment in individual offspring reduces the queens' residual reproductive value, i.e. the amount of parental investment (Trivers 1972, Clutton-Brock 1991), is probably low. This is so, because investments in colony maintenance (workers) represent shareable resources (non-depreciable parental care, Altman *et al*. 1977) and thus the amount of parental investment does

not limit the number of sexual offspring produced (Wittenberger 1981). Neither is parental investment by males likely to be of any major importance since ant males generally die soon after mating. Thus, for ants the relative parental investment of the sexes is probably close to unity, and generates no unequivocal predictions for the operation of sexual selection, such as female choice (intersexual selection) or male–male competition (intrasexual selection). Second, differing inclusive fitness functions for queens and workers generate conflicts over the allocation of resources (Trivers & Hare 1976, Pamilo 1991, Bourke & Franks 1995, Crozier & Pamilo 1996). These conflicts are affected by the relatedness structure of the colony and are thus directly linked to the prevalent mating system. Third, the rule for ant females is that they have only one receptive period (e.g. Thornhill & Alcock 1983). In most ants a sperm supply for life is stored in the spermatheca of a female, probably during one single nuptial flight (but *see* also Rosengren *et al*. 1993: table 14.3). Fourth, ant males usually eclose without functional testes and the total number of sperm is stored in the seminal vesicles of sexually mature males (Hölldobler & Bartz 1985, but *see* also Heinze *et al*. 1993). Thus, the potential reproductive success of ant males is usually fixed and does not increase as a simple function of the number of matings. The last two phenomena probably both increase the role of intersexual selection in ants.

A great diversity of mating systems has been described in ants (e.g. Hölldobler & Wilson 1990). Both monandry and polyandry frequently occur (Page & Metcalf 1982). Male polygamy is common, even if males occasionally have enough sperm for just one mating (Keller & Passera 1992) or even less sperm than one female can store (Kerr 1961, Fjerdingstad & Boomsma 1998). Mating can take place in highly synchronised nuptial flights or during a less intensive flight season (Kannowski 1963). Furthermore, mating can take place in the air or on the ground, far from a nest nearby or even inside the nest (Fortelius *et al*. 1993, and references therein). In fact several different options may be used within one species (e.g. Rosengren *et al*. 1993).

Hölldobler and Bartz (1985) described two major syndromes of mating behaviour in ants. In one, "the female calling syndrome", females are more or less stationary and attract males by sex pheromones and mating takes place on the ground. Ants in this group typically have small mature colonies producing only a few sexuals each year. Big swarms of males from different colonies gathering on specific mating sites, characterise the other, "the male aggregation syndrome". These male swarms attract females from long distances by communal emission of a sex pheromone. Big mature colonies and a massive production of reproductives each year are characteristics of this second category.

No description of the mating activities of *F. aquilonia* is available, but several studies indicate a typical pattern within the genus *Formica*. Reproductives assemble at well-defined locations where females attract males by pheromone emission (Kannowski & Johnson 1969, Cherix *et al*. 1991, 1993, Walter *et al*. 1993). In addition, an optional pattern, labelled by Keller (1993) the polygyny syndrome, seems to be available for ants like *F. aquilonia*, characterised by a multi-queen and multi-nest (polygynous and polydomous) sociogenetic organisation (*sensu* Seppä 1994). In this case mating occurs in or near the nest with females joining the queen pool of either the native nest, or adjacent mature nests (Fortelius *et al*. 1993). Thus elements of both the female calling and the male aggregation syndromes seem to be included in the complex mating system prevalent among *Formica* ants.

In this study, I examined sexual selection in mating aggregations of *F. aquilonia* sexuals. I first describe the behaviours associated with the mating process and use this as a basis for more detailed analyses. Then I ask whether mate choice is random or whether discrimination indicating either intra- or intersexual selection can be detected. I also ask whether mate discrimination based on colony descent exists, what is the effect of mating experience on mate choice and mating behaviour, and what is the effect of the operational sex ratio on the mating behaviour? I also studied the transfer of sperm in laboratory conditions and estimated sperm counts for males and different categories of female sexuals.

Material and methods

The ant material

In this study I used alate (winged) females and males from two populations (Tr = Träskända and Å = Åker) of *Formica aquilonia* Yarrow. Both populations are located close to the city of Helsinki on the southern coast of Finland, and the distance between them is about 6 km. The experiments were carried out in 1991–1992. The Träskända population comprises about 150 nests on ca. 15 ha and the Åker population consists of about 70 nests on ca. 5 ha. The individuals from Åker originated from two nests (one for females and one for males) and were used only when interpopulation effects were tested. In all other experiments I used individuals collected from six different nests in Träskända (three for females and three for males). I collected alates during the nuptial flight period (end of May–beginning of June) from the surface of nests known to produce almost exclusively (ca. 90%) single-sex broods. Thus I assumed that neither males nor females had mated before the experiments. The fact that the females were virgin was confirmed by dissections of randomly selected individuals (method in Fortelius *et al*. 1993).

Experimental procedures

I made both direct observations (experiments 1–2) and video recordings (experiments 3–5) of the mating process. I kept all individuals, regardless of their origin or pretreatment, in identical laboratory conditions for about two hours before the experiments. All replicates of a given experiment were performed using simultaneously captured alates. For the different experimental setups fresh material was collected from the nests. As I could not control for possible differences between source nests or in alate age between the experiments, I did not test the results between different experimental setups. To allow quick identification I marked one of the individuals of the same sex in the three-specimen choice experiments (below), with paint on the thorax. I controlled for the effect of marking by systematically altering the category marked. The number of matings between marked and unmarked individuals did not differ over the experiments.

Video recordings were continued for 30 minutes and performed in a dome-shaped transparent plastic chamber (diameter 15 cm × 8 cm high). I controlled (optimized) the light, temperature and rH conditions to stimulate mating and enable a good quality video image (method based on Gösswald 1978, and own experience). A timer on the video image covered the timing of all experiments and specific observations during them.

Experiment 1

To test whether intra- and intersexual selection can be detected I observed the mating behaviour of males and females in the presence of several competitors. These experiments were performed outdoors during sunny weather in rectangular boxes with transparent covers (40 cm × 25 cm × 10 cm high). In each replicate I used five females and five males, marked with different colours on the thorax. All individuals were from the same population and in a given replicate the different sexes were from different nests while members of the same sex were from the same nest. I observed each replicate for 30 minutes and noted time and duration for each mating as well as the identity of the female and male involved.

Experiment 2

To test whether prior mating had an effect on male mating behaviour I successively (one at a time) presented a single male with five virgin females in a transparent plastic jar (diameter ca. 5 cm × ca. 10 cm high). For each mating, I noted both the duration and the mating number of the male. If the couple did not mate within ten minutes from the introduction of the female, she was replaced by another one. I terminated each replicate after 30 minutes, even if the male had not mated with the desired five females.

Experiment 3

To test for effects on mate choice by population descent I video recorded one virgin female from

each of the two populations together with one virgin male from one of the populations.

Experiment 4

To test whether mating status (virgin/non-virgin) affected mate choice in females and males I exposed nestmate sexuals, one of which had mated, with a virgin individual of the opposite sex from a different nest. When two females were placed with one male, one of the females was virgin and the other one had mated once, two to five hours before the recording started. When two males were placed with one virgin female, one of the males had mated with five different females, two to five hours before the experiment, while the other had not mated before.

Experiment 5

To test whether the operational sex ratio influences mate choice I video recorded experiments with ten individuals of each sex from the same population with the following male to female ratio: 1/9, 3/7, 5/5, 7/3 and 9/1. All individuals of a given sex came from the same nest in a given replicate, but I used different source nests randomly across the entire experiments. I also used this setup to analyse short-term effects of previous matings, using the 1/9, 3/7, 7/3 and 9/1 replicates, as it was possible to track the rarer sex throughout the recordings.

Sperm counts

Old functional queens were collected by digging into a nest during egglaying (early April). Dealate young females were collected at the end of the the nuptial flight period (early June) from inside a nest. The age of the females was confirmed by ovarial development and absence of corpora lutea. In addition, females that had mated 1, 2, 3 and 5 times were obtained using the same procedure as described in experiment 2. Part of the males collected for the mating experiments were used for estimating the sperm count of virgin males.

The number of living spermatozoa was estimated using a modification of the technique used by Tschinkel (1987). Females and males were dissected, the spermatheca or seminal vesicles were ruptured in a small volume of Ringer's saline and the spermatozoa suspended. Between 0.5 and 2 ml (depending on the density of sperm) of the suspension was tranferred to a hemacytometer with 0.004 μl field volumes. From each suspension four samples were taken and for each sample the number of spermatozoa was counted in ten fields. Counts were used only if no statistically significant between-sample differences were detected.

Results

Mating behaviour

Based on behavioural observations on video recordings, I divided the mating process into three phases. The categorization is based on 34 replicates with initially virgin females and males present in an even sex ratio, and is in good correspondence with the copulatory behaviour described for *F. subintegra* and *F. montana* by Kannowski (1963).

Courting phase

This phase encompassed 32% of the time and was measured from the first physical contact between a male and a female to the moment they established genital contact. Usually males are the part actively seeking contact with females and they often use physical power to get into mating position, while females seem to resist mating.

Still phase

Immediately after the establishment of genital contact both sexes usually remain motionless for a while. This phase that encompassed 18% of the time I measured from the beginning of the genital contact to the first bigger movement of either sex. The female normally terminates the still phase by moving around with the male still bent over her back.

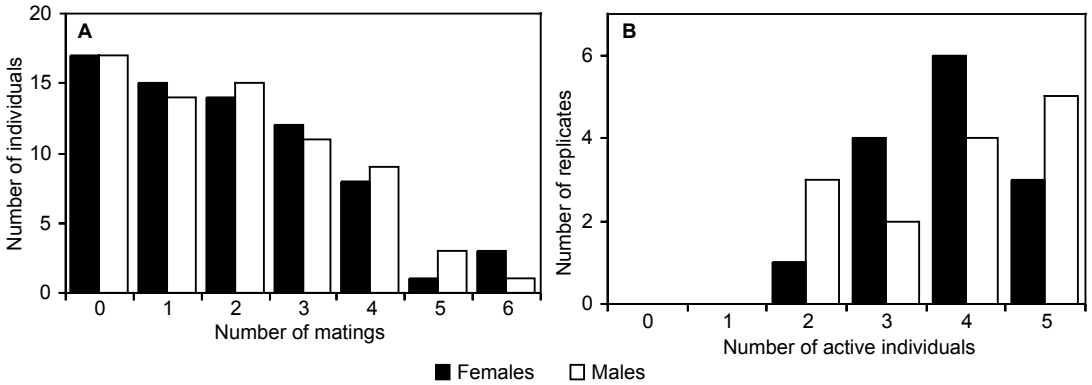

Fig. 1. — **A**: Mating frequency distribution of 70 females and males. Data were pooled over 14 replicates with 5 females and males in each. — **B**: The frequency distribution of replicates with none to all individuals mating at least once (active individuals). The distributions of the sexes did not differ significantly from each other (Kolmogorow-Smirnov test: $n = 14$, $p = 0.14$).

Terminal phase

This phase I measure from the end of the still phase to the end of genital contact, and it encompassed 50% of the time. The phase is characterised by the female actively trying to get rid of the male by fast movements and even biting.

In experiment 1, when five females and five males were placed in the same mating chamber, a total of 134 matings across 14 replicates were observed. For both sexes the arithmetic mean number of matings was 1.91 based on the complete material, and 2.53 if the non-maters were excluded. The harmonic means for the pooled data set were 1.85 matings/female and 1.88 matings/male, and the range of the number of matings was between zero and six for both sexes. The mating frequency distributions of females and males were not significantly different (Kolmogorov-Smirnov test: $p = 1.0$) and both came close to a Poisson distribution (Fig. 1A). The maximum number of copulations observed in this study was nine for females and six for males.

The mean coefficient of dispersion, C.D. (Sokal & Rohlf 1980) for the number of matings, based on the fourteen replicates, was 1.47 for females and 1.44 for males. These means neither differed from each other nor from a theoretical mean of one, representing the C.D. of an ideal Poisson distribution (Kruskal-Wallis one-way ANOVA: $n = 14$, $H = 2.74$, $p = 0.26$). The mean number of specimens mating at least once was 3.8 for both sexes, demonstrating that in most replicates the majority of the sexuals took part in the matings (Fig. 1B). In addition, no differences in the time until mating (start of recording to mating) as a function of the number of previous copulations (1–6) were found; females and males mated on average within 12.37 ± 2.24 and 12.45 ± 1.96 minutes, respectively ($F_{5,134} = 0.71$, $p = 0.61$, and $F_{5,134} = 1.03$, $p = 0.40$, respectively). Thus no indications of non-random mating patterns emerged from the above analysis.

Neither males nor females showed any statistically significant preference for mating partners from the same or a different population with respect to any of the variables measured (experiment 3, Table 1). This result gives no support for a population effect in the polygyny syndrome scenario (above) with putative "stayer-females" either preferring or avoiding inter- or intrapopulation copulations.

Female mating status had no statistically significant effect on neither the number of matings per replicate, the duration of the genital contact nor the length of the courting phase (experiment 4, Table 2). However, the still phase was significantly shorter in non-virgin females (Table 2). Thus non-virgin females seem to opt for shorter copulations (start to move sooner), but males seemed able to keep the duration of genital contact more or less constant. Within the short time span of this experiment, the greater the

number of times a female had mated, the shorter the duration of the still phase was (experiment 5, Fig. 2B). This shows that there also is a quantitative component to the result reported above, that mated females start to move earlier than virgin females. Nevertheless, previous female matings did not increase the duration of the courting phase neither in experiments 4 nor 5. Thus females did not seem to use more time (and energy) to avoid additional matings (Table 2 and Fig. 2A).

The probability to obtain copulations did not alter with male mating status, but the duration of the courting phase was significantly shorter when males that have mated previously and allowed a period of recovery were involved (experiment 4, Table 2). Furthermore, males with previous mating experience tended to have longer periods of genital contact. This trend is clearly caused by a prolonged terminal phase involving no alteration of the still phase (Table 2). However, when males were not allowed a period of recovery but offered a continuous opportunity to mate, the courting phase remained constant (experiment 5, Fig. 2E). In experiment 2, males were

Table 1. Mating parameters for females from Åker (Å) and Träskända (Tr) when paired with males from Åker (mean ± S.E.).

	Female origin		*t*	df	*p*
	Åker	Träskända			
Frequency (matings/30 min)	2.43 ± 0.57	2.14 ± 0.96	0.26[1]	6	0.80
Time until mating (min)	12.76 ± 2.16	15.33 ± 2.71	0.75[2]	30	0.46
Duration of genital contact (sec)	55.02 ± 3.75	61.96 ± 3.70	1.31[2]	30	0.20
Duration of courting phase (sec)	25.29 ± 4.95	24.07 ± 7.16	0.146[2]	30	0.89
Duration of still phase (sec)	5.88 ± 2.14	5.60 ± 1.25	0.11[2]	25.4	0.91
Duration of terminal phase (sec)	49.14 ± 4.02	56.36 ± 3.45	1.34[2]	30	0.19

[1]paired *t*-test, [2]two-sample *t*-test.

Table 2. Comparision of matings for virgin and non-virgin females and males (mean ± S.E.). Significance indicated with boldface.

	Gender	Treatment		*t*	df	*p*
		Virgin	Mated			
Frequency (matings/30 min)	female	2.00±0.87	1.56±0.53	0.64[1]	8	0.54
	male	0.77±0.31	0.77±0.22	0.00[1]	8	1.00
Time until mating (min)	female	15.74±1.66	14.44±2.79	0.42[2]	25	0.68
	male	5.16±1.55	7.67±2.27	0.91[2]	12	0.38
Duration of genital contact (sec)	female	63.00±5.15	72.13±9.72	0.83[2]	20.1	0.42
	male	62.74±4.76	84.00±21.84	0.95[2]	5.5	0.38
Duration of courting phase (sec)	female	24.22±5.07	20.58±5.82	0.46[2]	28	0.65
	male	79.71±17.80	30.50±3.16	2.72[2]	6.4	**0.03**
Duration of still phase (sec)	female	19.67±2.84	5.86±1.61	4.22[2]	26.2	**< 0.001**
	male	16.71±5.60	17.17±4.55	0.06[2]	11	0.95
Duration of terminal phase (sec)	female	43.33±6.13	66.27±10.26	2.01[2]	30	**0.05**
	male	46.03±5.94	66.83±19.93	1.00[2]	5.9	0.35

[1]paired *t*-test, [2]two-sample *t*-test.

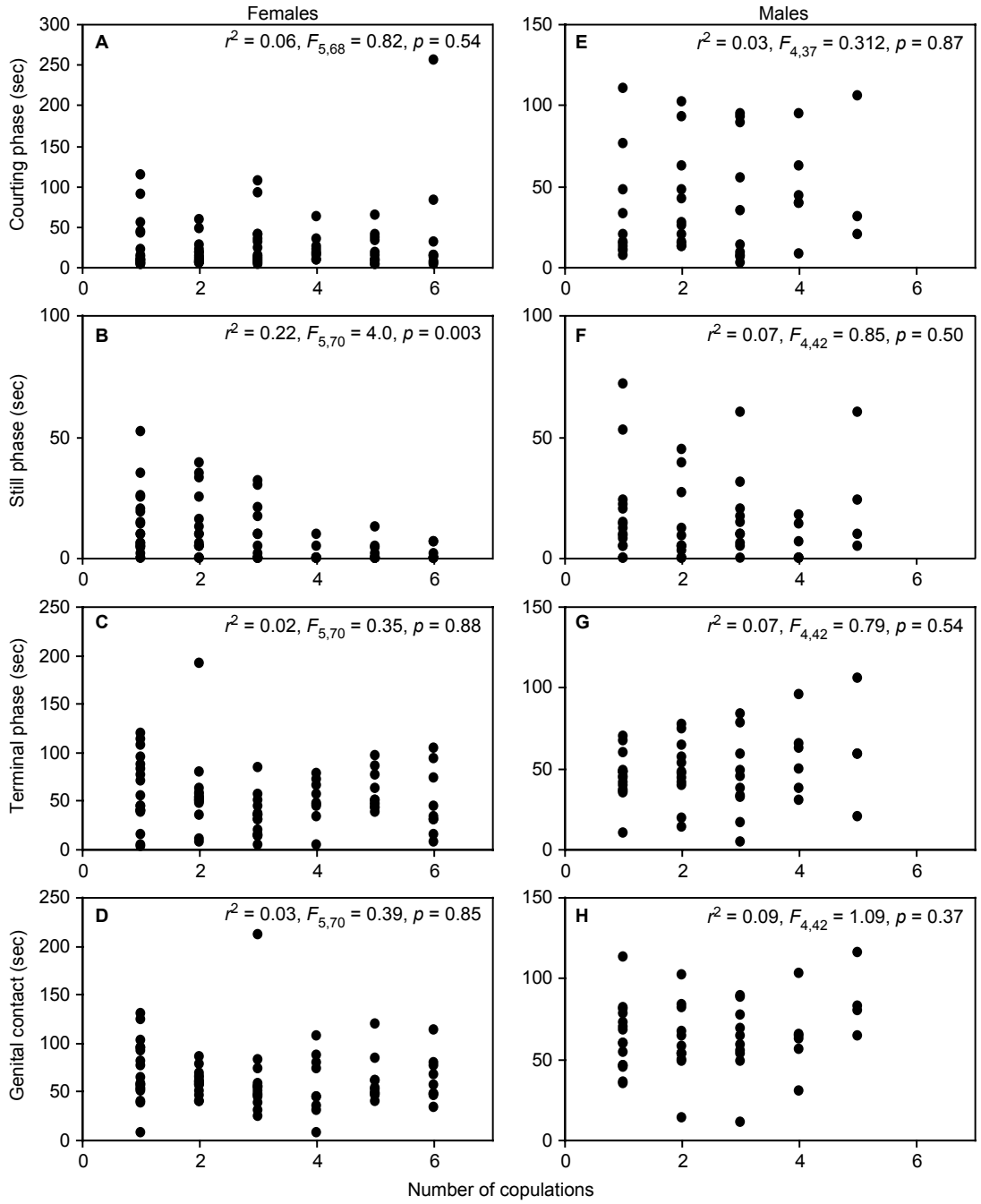

Fig. 2. The duration of the phases of the mating process as a function of preceding female and male matings. For females, data were pooled for copulations five and six as well as seven to nine. For males, data were pooled for copulations five and six.

continuously presented with a mating partner but the couple was isolated (no interference from other specimen). Under these conditions the duration of the genital contact increased significantly with the number of previous copulations (Fig. 3). In conclusion, not exhausted, non-

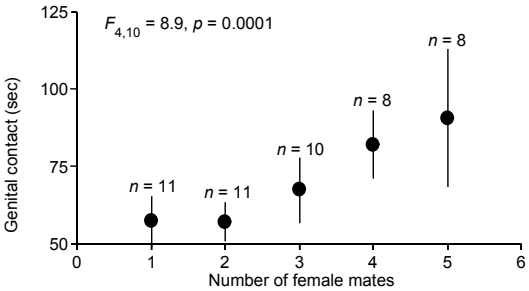

Fig. 3. Duration of genital contact (mean ± 95% confidence intervals) as a function of the number of preceding male copulations, with successive introduction of different females (experiment 2). n = number of replicates.

virgin males seem able to reduce the time needed for the courting phase and in general, non-virgin males, especially without interference from other specimens, stay in genital contact longer than do virgin males. For males, as for females, a quantitative effect of an increasing number of matings seems more important than the qualitative distinction, virgin *vs*. non-virgin.

When the operational sex ratio varied from one male per nine females to nine males per one female, the number of copulations per replicate followed a parabolic function of the sex ratio (Fig. 4A). This shows that the average mating frequency was highest when sex ratios were equal; when the proportion of males increased females mated more often and vice versa, although the effect for males is weaker (Fig. 4B). Interestingly the mean time to copulation for males increased when male bias increased (Table 3). This may indicate negative interactions between males. The duration of the entire mating process was not affected by the sex ratio although the still phase decreased with increasing male bias (Table 3). However, the number of copulations per female increased with male bias (Fig. 4B) and the duration of the still phase decreased with the number of times a female had mated (Fig. 2B). Thus the sex ratio *per se*, is not the likely explanation for the latter result.

Sperm counts

Nineteen females that were observed to mate once in the laboratory were dissected. Of these 6 had no detectable spermatozoa in the spermatheca. For the females that mated more than once in the laboratory no empty spermathecae were found. The number of spermatozoa increased almost linearly with the number of copulations for the range estimated here (1, 2, 3 and 5; Fig. 5). In total 59 old functional queens were dissected for sperm counts. Of these, 17 lacked detectable spermatozoa in the spermatheca, whereas all the 22 dissected young females mated in the wild had spermathcae containing living sperm. The mean estimated sperm count of the first season queens was 1 413 000, almost twice the corresponding estimate (893 100) for the 42 old queens with sperm in the spermatheca (Fig 5). The maximum estimate for a single first season queen was 2 513 000

Table 3. Duration of mating parameters under five different operational sex ratios.

Sex ratio male/ female	Time until mating (min)		Genital contact (Still + Terminal) (sec)		Courting phase (sec)		Still phase (sec)		Terminal phase (sec)	
	mean ± S.E.	n	mean ± S.E.	n	mean ± S.E.	n	mean ± S.E.	n	mean ± S.E.	n
1/9	9.42 ± 2.79	10	63.70 ± 5.31	10	25.67 ± 6.39	9	22.00 ± 5.68	10	41.70 ± 5.78	10
3/7	11.28 ± 1.64	37	64.24 ± 3.85	37	44.24 ± 6.15	35	13.81 ± 2.84	37	50.43 ± 3.55	37
5/5	10.80 ± 1.04	61	61.64 ± 2.12	61	27.90 ± 6.90	58	18.03 ± 2.43	59	43.81 ± 2.35	59
7/3	11.92 ± 1.12	56	64.77 ± 4.07	56	25.51 ± 3.36	55	9.34 ± 1.64	56	55.43 ± 4.15	56
9/1	13.52 ± 1.65	20	58.00 ± 6.12	20	33.53 ± 13.61	19	6.45 ± 1.98	20	51.55 ± 5.97	20
r_S	0.900		–0.300		–0.000		–0.900		0.800	
p	0.037		0.624		1.000		0.037		0.104	

r_S = Spearman rank correlation, n = number of replicates.

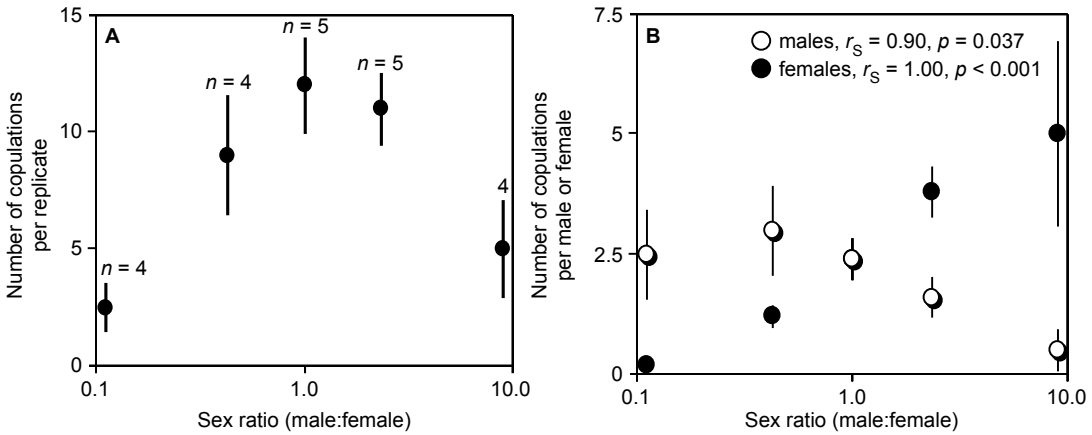

Fig. 4. Number of copulations in 30 minutes (mean ± S.E.) as a function of the operational sex ratio. The male/female ratios were: 1/9, 3/7, 5/5, 7/3 and 9/1. — **A**: number of copulations per replicate, — **B**: number of copulations per female and male, n = number of replicates, r_S = Spearman rank correlation.

as compared with 5 156 000 for a female induced to mate five times in the laboratory. The mean estimate for virgin males with degenerated testes was 5 568 000 (Fig. 5), and the maximum estimate for a single male was 12 000 000.

Discussion

Here I have shown that both sexes of *F. aquilonia* mate multiply. The mean mating frequency (around two times) observed here for females, corresponds well with the estimated effective mating frequency based on allozyme mother-offspring analyses in the same species (Pamilo 1993). Also, the estimates of spermathecal contents of wild-captured first season females in this study, roughly corresponds to the sperm transferred during two matings (Fig. 5). The number of copulations observed across the experiments varied between one and nine, which is wider than the range (1–6) observed by Pamilo (1993). However, as he points out, the genetic estimate is likely to be an underestimate since males with identical genotypes at the studied loci are not separable from each other. Thus most copulations in *F. aquilonia* apparently result in sperm transfer. This conclusion is further supported by the dissections, which showed a linear increase in sperm counts as a function of the mating frequency. However, about one third (6/19)

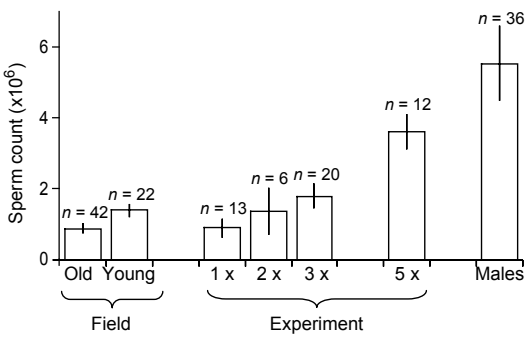

Fig. 5. Sperm counts in different categories of sexuals (mean ± 95% confidence intervals). n = number of replicates.

of the observed copulations did not result in sperm transfer. In *F. aquilonia* some degree of insemination competition (*sensu* Page 1986) has been indicated by the allozyme analysis. Pamilo (1993) found that as a rule one male fathers well over 50% of the offspring. Although this result suggests a first-male advantage, offspring analyses from queens with identified successive mating types are required to confirm it (cf. Sundström & Boomsma 2000). Interestingly, the morphology of the spermatheca may be related to the pattern of sperm displacement. Spheroid shape, as is the case here and the rule for ants, usually correlates with a first male advantage while tubular shape correlates with a last male advantage (Walker 1980, and references therein).

The male mating system

Based on results in this study, male mating tactics are best interpreted in terms of a scramble competition, where males avoid aggressive interactions altogether and instead try to secure matings as fast as possible (Thornhill & Alcock 1983). Such a tactic can be predicted in short-lasting mating assemblages, especially if one can expect a first-male advantage.

Further support for this idea comes from the analysis of male mating experience. Males use less time for their first copulations (stay in genital contact for a shorter period) than for later ones (Fig. 3). The proximate reason for this result may be male exhaustion and/or slower rate of sperm transfer. On the ultimate level it may indicate that males are selected to minimise the time spent per mating. That physical exhaustion may limit the number of times a male can mate within a short time range is supported by the fact that the courting phase was shorter for non-virgin than for virgin males if the former are allowed to rest before remating (Table 2). Also, one male with nine females did not achieve more copulations than each of five males with five females (Fig. 4B).

The male mating potential under the prevalent conditions could be estimated to be about three copulations in 30 minutes (Fig. 4B). Also, when male bias increases the number of copulations per male decreases. This result together with the positive correlation between male bias and time until mating (Table 3), may be interpreted as a sign of male–male interaction. However, the number of copulations per female increases with increasing male bias (Fig. 4B), and female mating resistance increases with the number of copulations she has endured (Fig. 2B). Thus, males may also obtain fewer copulations because of female behaviour.

The female mating system

In social Hymenoptera, several hypotheses explaining the evolution of polyandry on the basis of colony-level selection have been put forward (Crozier & Page 1985, Hamilton 1987, Sherman *et al*. 1988, Crozier & Pamilo 1996, Crozier & Fjerdingstad 2001). These hypotheses, however, have been examined for a number of *Formica* species and particularly in highly polygynous species like *F. aquilonia*, colony-level selection may not be of major importance for the evolution of polyandry (Pamilo 1993, Pamilo *et al*. 1993, Boomsma & Ratnieks 1996).

On the individual level, non-selective polyandry has been interpreted in terms of females avoiding costs of resistance to mating (Parker 1970, Walker 1980, Thornhill & Alcock 1983). The patrolling behaviour of *Formica* males (Kannowski & Johnson 1969, Cherix *et al*. 1993) may create a cost to female mating resistance, as males may detect females that are about to leave the mating locations. The fact that the still phase decreased in duration when females had mated more than three times suggest that females indeed actively resist mating, at least when denied the possibility to leave. However, none of the other phases were affected by female mating frequency, which indicates that the effort to resist multiple mating is not very pronounced.

The comparatively high degree of polyandry observed in *F. aquilonia* may best be interpreted in terms of a male-induced polyandry. Cole (1983) suggested that the correlation between the occurrence of female polyandry and large colony size in ants, strongly argues for polyandry as an adaptation to the maintenance of long-lived large colonies because queen(s) heading a large colony needs to store large numbers of sperm, more than one male can supply. Although, e.g. Crozier and Page (1985) find this hypothesis "highly implausible", mainly because of the expected strong selection for bigger male ejaculates, it is noteworthy that sperm counts in the genus *Atta* have demonstrated the existence of a female sperm storing capacity several times the sperm count of individual males (Kerr 1961, Fjerdingstad & Boomsma 1998).

In *F. aquilonia*, however, one male could easily fill the spermatheca of a female (Fig. 5), but the maximum number of spermatozoa observed to be transferred during one copulation in this study was about one third of the mean estimate for the male sperm count (1 831 000/5 568 000). In fact the number of sperm stored increased linearly from one to five copulations, with no sign of decline. This sug-

gests that one of the sexes limits the number of sperm transferred to the spermatheca during copulation, or that females expel sperm immediately after copulation. Dissections of mated males showed that males use only a small part of their total sperm count during one copulation, excluding female sperm expelling. These empirical observations suggest that males may face a trade-off between the number of sperm spent on each mate and the total number of mates. This is so, firstly because the total number of sperm a male can transfer during its life is fixed, and increasing the number of mates does not increase the potential reproductive success, as long as all sperm is used. On the other hand, the low probability of a given female ever becoming a nest queen may shift the optimum towards a higher number of mates than needed just for a complete use of sperm. A first-male advantage (above) introduces time as a constraint, and might still bias the trade-off towards a higher number of less complete inseminations. Thus females might find themselves forced to a particular form of the sperm-replenishment polyandry (Thornhill & Alcock 1983).

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