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Diploid males and colony-level selection in *Formica* ants

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It is suggested that the evolution of polyandry by social hymenopteran queens is caused by colony-level selection, either because polyandry affects the distribution of non-functional diploid males in colonies (the load hypothesis) or because it increases the genetic diversity of the worker force (the diversity hypothesis).

Diploid males that arise from fertilized eggs that are homozygous at the sex-determining locus (or loci) are inviable or infertile. Models of the load hypothesis analysed in this study suggest that slow growth and high mortality of colonies with diploid males favour single mating by queens. The longer the period of colony growth (the period with selective differences) and the heavier the mortality, the stronger is the selection for monandry. The load hypothesis also predicts an association between monogyny and monandry. In contrast, the diversity hypothesis predicts an association between monogyny and polyandry, as multiple mating offers a way by which a monogynous colony could increase its genetic heterogeneity.

Up to 10% of all males are diploid in species and populations of *Formica* ants with highly polygynous colonies (*F. aquilonia, F. polyctena, F. truncorum*). No diploid males were found in two mainly monogynous species (*F. exsecta, F. pratensis*) which also have a high level of monandry. This agrees with the prediction of the load hypothesis. A surprisingly high frequency of nests in three other species (*F. rufa, F. lugubris, F. truncorum*) with monogynous/weakly polygynous colonies produce diploid males, although the frequency varies among their populations. In extreme cases half of the diploid sexuals within a colony develop into males. Diploid males have been observed only at the time of normal sexual production. It seems that at other times they are eliminated at early developmental stages, so as to minimize the load on the colony.

KEY WORDS: ants, diploid males, *Formica*, genetic load, polyandry, polygyny, social insects.

INTRODUCTION

Multiple mating by females is not uncommon in insects; several hypotheses to explain the evolution of female receptivity are discussed by THORNHILL & ALCOCK (1983). Polyandry in eusocial Hymenoptera is fairly common (PAGE & METCALF 1982, STARR 1984, PAGE 1986) and has a special interest because it is connected to the kin structure of colonies. In particular, multiple insemination leads to increased genetic diversity among the workers. This diversity can affect the performance of the colony, and it also affects the genetic relatedness among colony members.

The hypotheses concerning polyandry in eusocial Hymenoptera can be classified as those referring to selection at the level of either individuals, colonies, or populations, or to queen-worker conflict over reproductive allocation (PAMILO 1991a). In their review CROZIER & PAGE (1985) compared alternative hypotheses presented in earlier literature and considered colony-level selection to be perhaps the most important single factor selecting for polyandry. They considered two hypotheses of colony-level selection: one suggesting that genetic heterogeneity of the worker force might be adaptive to the colony and one based on the distribution of non-functional diploid males in colonies. We refer to them as the *diversity hypothesis* and the *load hypothesis*, respectively. CROZIER & PAGE particularly emphasized the relationship between mating frequency and diffences in the effect of diploid male production in slowing the colony growth rate. This study extends the load hypothesis by considering the effect of diploid males on colony mortality. The results show that the load hypothesis and the diversity hypothesis give different predictions concerning the connection between the number of matings and the number of queens in a colony. We also examine the occurrence of diploid males in natural colonies of *Formica* ants in order to estimate the load caused by them to the colonies. Genetic studies in the same species are used for estimating polyandry and polygyny and the results are used to evaluate the diversity and load hypotheses.

MATERIALS AND METHODS

Seven species of mound-building *Formica* ants were included in the study: *F. aquilonia, F. polyctena, F. rufa, F. lugubris, F. pratensis, F. truncorum* and *F. exsecta*. Genetic colonial structures and the occurrence of diploid males were studied with enzyme electrophoresis in populations from southern Finland. Electrophoresis is performed as described by PAMILO (1993) and SUNDSTRÖM (1994). The allelic designations are based on the electrophoretic mobilities of the respective allozymes (PAMILO 1993). The material and the enzyme loci used in each species are as follows.

F. aquilonia. Males and females were sampled from several localities around the city of Helsinki in southern Finland. The sampling localities are Paloheinä in Helsinki (males from 2 nests and females from 11 nests), Träskända (8 and 10 nests) and Solkulla (6 and 40 nests) in Espoo and Martinlaakso in Vantaa (3 and 12 nests). All the localities are within 10 km of each others; the results are pooled and referred to as the Helsinki population. The samples were screened for the enzymes glucosephosphate isomerase (GPI), malic enzyme (ME), peptidase (PEP), phosphoglucomutase (PGM), esterase (EST), 6-phosphogluconate dehydrogenase (PGD), cathodal malate dehydrogenase (MDH-1) and phosphoglycerate kinase (PGK).

F. polyctena. Males from four nests and females from 21 nests were sampled from a population in Kauniainen, close to Helsinki. The same enzymes as in *F. aquilonia* were studied.

F. rufa. Two populations are included, one in Siuntio and one in Hanko, both in southern Finland. The Hanko population is scattered on small islands within a 15 km^2 area close to Tvärminne Zoological Station of Helsinki University; these are the same islands used earlier in a genetic study of *F. exsecta* and *F. fusca* (PAMILO 1983). Males from 13 and workers from 26 nests were sampled in 1988-1991. The Siuntio population is about 50 km east of the Hanko population. Males from 21 and workers from 23 nests were sampled. The same enzymes as in *F. aquilonia* were studied.

F. lugubris. Two populations were studied. Males from 11 and workers from 19 nests were sampled in Hanko and males and workers from six nests in Sipoo (southern Finland). The same set of enzymes as in *F. aquilonia* were screened.

F. pratensis. Males from 12 nests (10 males per nest) and workers from 37 nests (10 workers per nest) were sampled in 1991 in the population in Hanko (used earlier for sex ratio studies by PAMILO & ROSENGREN 1983). Variations in GPI and PEP were used.

F. truncorum. The data are based on genetic studies of 95 nests in Denmark (NE Jutland), 23 nests in Hanko, and 72 nests from three islands in Inkoo, southern Finland. The sociogenetic organization has been analyzed elsewhere (SUNDSTRÖM 1994) and we include here results on the occurrence of diploid males. The populations in Denmark and Hanko have monodomous and most likely monogynous colonies, whereas the Inkoo population consists of polydomous and polygynous colonies on all of the islands studied (SUNDSTRÖM 1989, 1994). The enzymes used are isocitrate dehydrogenase (IDH), PGK and aconitase (ACO).

F. exsecta. The study population is located on a 10 ha island at Tvärminne in Hanko. The genetic data on colonial structures based on worker genotypes is taken from a previous study (PAMILO 1991b). Males were sampled from a total of 28 nests over several years and on average 10 males per nest were screened electrophoretically for anodal malate dehydrogenase (MDH-2).

Diploid males are inferred from heterozygous genotypes at at least one enzyme locus. As diploid males can be recognized only when heterozygous, we use the observed frequency of heterozygous diploid individuals (HET = frequency of females and workers heterozygous for at least one of the loci studied) as the probability of detecting male diploidy. The genetic relatedness of individuals within and between the sexes in a nest are estimated by means of the method of PAMILO (1984) as modified by QUELLER & GOODNIGHT (1989) and PAMILO (1990).

MODELS

Sex determination and colony growth

In the honeybee, sex is determined by a single locus: heterozygotes develop into females and homozygotes and hemizygotes into males (MACKENSEN 1951). The same has been shown to be the case in *Melipona* bees (KERR 1987), as well as in other bees (MORITZ 1986) and in the ant *Solenopsis invicta* (ROSS & FLETCHER 1985), but more than one locus may be involved. Diploid males are a burden to the colony

because they consume resources and develop neither into functional reproductives nor into workers contributing labour (MORITZ 1986, ROSS & FLETCHER 1986, WOYKE 1986).

When sex is determined by one locus, the proportion (D) of males among the diploid offspring of a single pair is either $D = 0.5$ or 0.0. With double mating, the proportion of diploid males depends both on the paternal genotypes and on relative sperm use. Assuming that the two males contribute equally, the proportion of diploid males is either $D = 0.5$, 0.25 or 0.0 depending on whether both, one or none of the males carries a sex allele identical to one of the queen's two alleles. When the number of matings increases, the number of colonies producing diploid males increases but at the same time the expected value of D in each affected colony becomes smaller. If there is a linear relationship between D and the colonial fitness (line *a* in Fig. 1), the distribution of diploid males among colonies does not affect the expected mean fitness. If fitness is reduced only slightly by small values of D but drastically by large values (curve *b* in Fig. 1), a queen would increase expected fitness by mating many times. If the fitness is not much affected by a reduction of D below 0.5 (curve *c* in Fig. 1), single mating by queens would be expected.

PAGE (1980) showed that colony productivity need not be a linear function of D but depends on colony growth and mortality, both of which can be affected by D in a non-linear way. CROZIER & PAGE (1985) slightly modified PAGE's original model and connected selection for polyandry with the timing of sexual production; single mating being favoured when sexuals are produced during the exponential phase of colony growth, and multiple mating when sexuals are produced in the asymptotic phase [these phases have been termed increasing returns to scale and decreasing returns to scale by OSTER & WILSON (1978)]. The assumptions in their model were that colonies grow logistically, that a colony's survival does not depend on its size, and that the number of sexuals is determined by colony size at the time of sexual production. They further assumed that the initial size at $t = 0$ (N_0) and the maximum possible size at the end of colony growth (N_{max}) are constant and independent of the value of D.

If colonies grow logistically, colony size at any time is not linearly proportional to the frequency of diploid males. The existence of diploid males will slow down

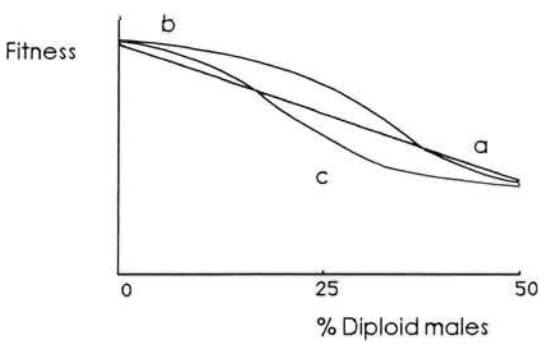

Fig. 1. — Fitness of a colony as a function of the proportion of diploid offspring developing into males (D). The three curves predict different selection pressures leading either to monandry (*c*) or polyandry (*b*) or being indifferent for the number of matings (*a*).

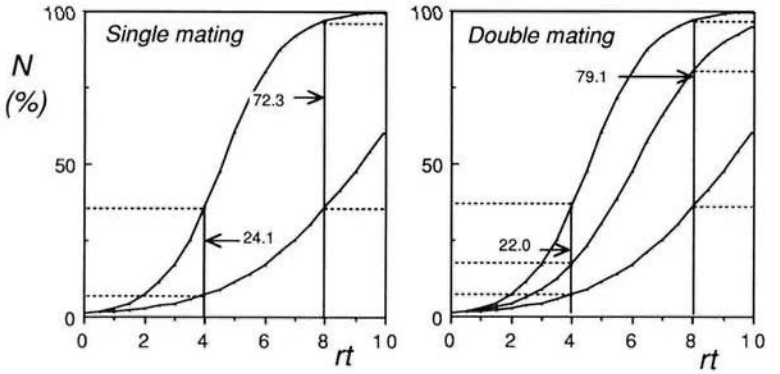

Fig. 2. — Colony growth curves based on equation (1) as functions of the product rt. (Colony size N is given as the percentage of the maximum size N_{max}). *Left*: single mating; curves are (from left to right) for colonies with 0% and 50% diploid males. *Right*: double mating; curves are (from left to right) for colonies with 0%, 25% and 50% diploid males. The arrows indicate the mean colony size at time points $rt = 4$ and $rt = 8$. The means are based on the expected genotype frequencies when the sex-determining locus has five equally frequent alleles.

early growth, whereas all colonies in this model converge to the same size when they reach the asymptotic stage. So, if we compare colonies with zero and proportion D diploid males ($D > 0$), the size ratio $N(t,D)/N(t,0)$ is smaller than $1\text{-}D$ early in colony growth but becomes greater than $1\text{-}D$ later [$N(t,D)$ denotes the size at time t of a colony in which a proportion D of diploid offspring is males].

Colonies with different proportions of diploid males have different growth curves. If the growth rate is r, colony size at time t is, from the logistic model,

$$N(t,D) = \frac{N_{max}}{1 + [(N_{max}-N_0)/N_0] \exp[-(1-D)rt]} \qquad (1)$$

The relative fitness of females with different numbers of mates depend on both the proportions of colonies with different values of D and the expected colony sizes given by (1).

PAGE (1980) examined this model assuming one sex locus. He chose a logistic fitness function, although he did not derive it directly from the colony growth curve. CROZIER & PAGE (1985) based the fitness function on the colony size given by equation (1) and extended the model to multiple loci. However, their formulae for the frequencies of colony types (their symbols D and Z) are slightly incorrect when the number of loci is greater than one. They further used the mean values of D from different colony types for calculating the expected colony sizes from (1). However, because each value of D gives a different growth curve, one should first calculate the growth curves separately for each colony type and then calculate the mean colony size for a given number of matings. For these reasons, we have reanalyzed the model numerically for various situations.

As it happens, these corrections do not markedly change the qualitative results of the model. When comparing single mating with double mating, the model predicts that single mating is favoured if sexual production takes place dur-

ing the exponential phase, whereas double mating is favoured if sexual production is postponed to the asymptotic phase of colony growth. The reason for this shift is that when the colonies approach the maximum size N_{max}, size differences between colonies with zero or 25% diploid males gradually diminish, and colonies with a single-mated queen have the disadvantage that a large fraction produce 50% diploid sons. This can be seen from the colony growth curves and mean colony sizes shown in Fig. 2 for a case where sex is determined by a single locus with five equally common alleles.

The same conclusion could be reached directly from the formula (1). When the second derivative of (1) with respect to 1-D is negative, it is advantageous to distribute the diploid males more evenly in the colonies; multiple mating leads to such a distribution. The condition for this becomes $\exp[rt(1\text{-}D)] < N_{max}\text{-}N_0$, from which we see that multiple mating is favoured when the product rt is large (sexual production is postponed to the asymptotic phase of colony growth) or N_{max} is small (the colony reaches maximum size quickly).

There are two additional factors, both of which can modify the result of the model. First, final colony sizes may be affected by diploid male production. This was modelled by RATNIEKS (1990) who showed that the timing of diploid male elimination is important. Second, diploid males may affect colony mortality. CROZIER & PAGE (1985) assumed that the survival rate is the same for all colonies, and that fitness only depends on final colony sizes. This means that there is no disadvantage from diploid males if sexual production takes place late in the asymptotic phase of colony growth, because all colonies survive and eventually reach the maximal size. It is, however, likely that colonies with a high proportion of diploid males suffer not only from a low growth rate but also from higher mortality due to smaller size. It thus seems reasonable to include colony mortality in the above model, as suggested by PAGE (1980).

Sex determination and colony mortality

The problem is that we do not know how mortality depends on colony growth. Many colonies die young; some colony deaths are independent of the growth rate but there is also good reason to think that survival depends on how quickly a colony passes from its founding stage to an ergonomic stage (OSTER & WILSON 1978). In the absence of empirical data, we use two functions for survival rate

$$s_1(N) = 1\text{-}\exp[-\alpha N] \quad (2a)$$

$$s_2(N) = \exp[\alpha(N\text{-}N_{max})] \quad (2b)$$

of which s_1 is a convex and s_2 is a concave function of colony size N. For function s_1 very small colonies suffer from high mortality, but a moderate increase in the size of small colonies greatly increases the survival rate. For function s_2 small decreases in the colony size below the maximal size severely reduce the survival rate but differences in the colony size of small colonies have little effect on survival. The constant α affects the steepness of the curves. A high frequency of diploid males results in slow colony growth (Fig. 2) and high mortality. As the survival rates s_i were defined in (2) as functions of N, we use a discrete time model to calculate the probabilities that a colony has survived to time t. This probability is given as a product of the probabilities of surviving from one time point to the next.

Slow-growing colonies are punished more if this product includes many short periods instead of a few long periods.

With single mating and one sex-locus, a large proportion of nests has $D = 0$ and a small proportion have $D = 0.5$. These proportions depend on the number of sex alleles at the locus. With double mating, the frequencies of these two colony types decrease equally, and additional colonies with $D = 0.25$ occur. The relative successes of single and double mating depend on whether the fitness of colonies with $D = 0.25$ is above or below the mean of the two other colony types. Other values of D are possible with three or more matings, or if males do not contribute equally to paternity.

Applying the logistic growth model of equation (1) and the above mortality models, a number of generalizations are possible. The longer the period of colony growth and the heavier the mortality, the stronger is selection for monandry. The reason for this is that monandrous females have the highest frequency of broods with $D = 0$.

If sexual production is postponed to the asymptotic phase of colony growth, and all colonies finally reach the same size, then fitness differences depend on how well the colonies have survived. That is, fitness differences depend only on mortality effects. When mortality does not depend on colony size, postponing sexual production can give an advantage to multiple mating, but fitness differences are small because colonies all converge to the same size. In situations where multiple mating is selected for, fitness differences between single- and multiple-mated females tend to be small, but when multiple mating is selected against, mortality effects can easily lead to a severe reduction in the fitness of multiply mated queens. If colony survival is a diminishing function of the frequency of viable offspring ($1-D$), multiple mating can be selected for because it reduces the proportion of nests with high D.

Increased genetic diversity, i.e. a large number of sex alleles, leads to smaller fitness differences. This is because genetic diversity reduces the overall proportion of diploid males and makes mortality effects less significant. Numerical calculations also show cases in which two or four matings are favoured over single mating but triple mating is not. Such results must depend on the frequencies of colonies with specific D values; the fitness differences in those cases are generally small and stochasticity in the values of D (including different contributions of the mates) may wipe them out.

RESULTS

Highly polygynous colonies

The species *F. aquilonia* and *F. polyctena* are known to have highly polygynous colonies (ROSENGREN & PAMILO 1983). This was confirmed by our observations in the course of the present study and by our earlier low estimates of genetic relatedness of nest mates (PAMILO 1987, 1993; PAMILO et al. 1992).

Diploid males were found with a low frequency in both species (Table 1), which agrees with our earlier observations in the Tvärminne population of *F. aquilonia* (PAMILO 1993) and in the similarly polygynous *F. pressilabris* (PAMILO & ROSENGREN 1984). About 90% of diploid individuals in *F. aquilonia* and *F. polyctena* have at least one heterozygous marker locus, so diploid males can be easily detected if

Table 1.

Frequency of diploid males in *Formica* ants with polygynous nests. HET is the average frequency of diploid individuals that have at least one heterozygous locus.

Species	HET	Number of males	
		total	diploid
F. aquilonia			
Hanko[a]	0.83	131	1
Helsinki	0.92	203	3
F. polyctena	0.81	72	5
F. truncorum			
Inkoo	0.85	1120	110
F. exsecta[b]	0.09	253	0
F. pressilabris[b]	0.48	199	6

[a] Based on PAMILO (1993); [b] based on PAMILO & ROSENGREN (1984).

they exist (Table 1). The population sex ratios in these two species tend to be female-biased (PAMILO & ROSENGREN 1983), and the frequency of diploid males among all diploid offspring is smaller than that among all males. We have found diploid males only at the time of normal sexual production.

The frequency of diploid males in the polygynous type of *F. truncorum* is high, about 10% of all males are diploid, and diploid males were detected in most nests that were sampled. The sexual brood in this species develops simultaneously with a worker brood, and we have not estimated what proportion of all diploid offspring develop into males.

No diploid males were detected in our earlier study of the highly polygynous *F. exsecta* (PAMILO & ROSENGREN 1984), but the probability of detecting them was small because of the low genetic variability (Table 1).

Monogynous and weakly polygynous colonies

The worker nest mates are highly related in the populations studied in *F. rufa*, *F. lugubris*, *F. pratensis* and monodomous forms of *F. truncorum* and *F. exsecta*, relatedness being within the range of 0.55-0.75 (Table 2). The populations of *F. rufa* and *F. lugubris* are scattered on different islands with clear genetic differentiation as shown by the positive fixation indices (Table 2). It is therefore useful to partition the relatedness into components reflecting this geographical differentiation on one hand and the immediate pedigree links among nest mates on the other (PAMILO 1984, 1989). Relatedness, which depends on the number of reproducing individuals, $g^* = [g\text{-}2F/(1+F)]/[1\text{-}2F/(1+F)]$, drops below 0.5 in these two species (Table 2).

Restricted gene flow within the study areas in *F. rufa* and *F. lugubris* results in positive fixation indices and is also seen as a spatial clustering of alleles. For example, four alleles segregate at *Pep* in *F. rufa* from Hanko. The allele Pep^{70} is found in seven nests in Hanko, four of these being on the same island. Similarly, six of the 11 *F. lugubris* nests having the allele Gpi^{70} in Hanko are on the same island, and the allele was not found at Sipoo. *F. truncorum* from the same islands show little spatial differentiation, whereas the population of *F. exsecta* on a single island shows

Table 2.

Estimates of the coefficient of relatedness (g), fixation index (F) and the component of relatedness attributable to the number of reproductive individuals in a colony (g*) in the five monogynous/weakly polygynous species; n is the number of colonies sampled.

	No. of loci	Workers						Males			Males to workers	
		n	g_w	(SE)	F	g_w*	(SE)	n	g_m	(SE)	g_{mw}	(SE)
F. rufa												
Hanko	6	26	0.68	(0.06)	0.27	0.44	(0.11)	13	0.58	(0.07)	0.59	(0.10)
Siuntio	5	23	0.55	(0.06)	0.01	0.55	(0.06)	21	0.41	(0.05)	0.32	(0.05)
pooled	6	49	0.63	(0.04)	0.16	0.49	(0.06)	34	0.48	(0.05)	0.45	(0.06)
F. lugubris												
Hanko	7	19	0.61	(0.05)	0.17	0.45	(0.09)	11	0.45	(0.06)	0.55	(0.09)
Sipoo	4	6	0.75	(0.22)	0.52	0.22	(0.41)	6	0.71	(0.13)	0.66	(0.22)
F. pratensis	2	35	0.66	(0.08)	−0.04			12	0.53	(0.14)	0.52	(0.20)
F. truncorum[a]												
Denmark	3	91	0.60	(0.03)	−0.06			33	0.37	(0.04)	0.24	(0.05)
Hanko	3	23	0.60	(0.05)	0.07	0.59	(0.05)	18	0.36	(0.05)	0.31	(0.07)
F. exsecta[b]	1	63	0.71	(0.07)	0.14	0.62	(0.06)	29	0.47	(0.19)	0.53	(0.14)

[a] Based on SUNDSTRÖM (1994); [b] based largely on PAMILO (1991b).

clear signs of inbreeding (Table 2). The average number of reproductives in the nests of these species should be better indicated by g^* than by g.

The values of worker relatedness suggest that the number of reproducing individuals is low in the five species but that the workers are not always full sisters. It is not possible to infer from the worker relatedness values alone whether colonies are weakly polygynous, polyandrous or both. The analyses including males result in relatedness estimates around 0.5 both among male nest mates and of males to worker nest mates (Table 2). This is the value expected in monogynous colonies. However, the present estimates are somewhat boosted by spatial differentiation in *F. rufa* and *F. lugubris*, as discussed above in the case of worker relatedness, and the estimates including males have rather large standard errors.

The male genotypes should directly reflect the genotype of the queen if a colony is monogynous and no worker reproduction exists. A comparison of male and worker genotypes from the same nest therefore indicates colonies that must have more than one queen or a queen with more than one mate. Such a comparison shows that the colonies fitting the assumptions of both monogyny and monandry include 26 out of 29 colonies of *F. exsecta*, and a little less than half of the colonies in the *F. rufa* group species: four of 11 colonies in *F. pratensis*, 15 of 35 in *F. rufa* and seven of 17 in *F. lugubris*. The genotypes in most of the remaining colonies can be explained by monogyny coupled with polyandry, but it is difficult to rule out polygyny. A few nests require polygyny to explain the observed genotypes (one in *F. pratensis*, six in *F. rufa* and two in *F. lugubris*). In some cases, the contribution of different queens or their mates are uneven, e.g. in a nest of *F. lugubris* where only one of the 45 males carried a deviating allele. Similar observations have been made in *F. truncorum* (SUNDSTRÖM 1994)

One nest of *F. pratensis* shows somewhat unexpected genotype frequencies. The male sample included two alleles of *Gpi* with about equal frequencies, indicating a

Table 3.

Frequency of nests producing diploid males in *Formica* species with monogynous/oligogynous nests. HET is the frequency of diploid individuals that have at least one heterozygous locus (in nests producing both haploid and diploid individuals at the same time).

Species	HET	Number of nests producing			
		males	females	both sexes	diploid males
F. rufa					
Hanko	0.74	9	1	5	2
Siuntio	0.92	24	5	7	0
F. lugubris	0.83	7	1	4	2
F. pratensis	0.54	6	4	6	0
F. truncorum					
Denmark	0.73	13	21	29	0
Hanko	0.76	6	5	12	3
F. exsecta	0.39	3	3	12	0

heterozygous queen, but all the workers (10 examined) were also heterozygous.

Diploid males were found in neither *F. exsecta* nor *F. pratensis* (Table 3). Males from two *F. exsecta* nests were clearly polymorhic including both large macraners and small micraners (see FORTELIUS et al. 1987). These were also the only *F. exsecta* nests that produced no females among the sexuals (they did produce workers). All the workers in one of these nests were heterozygous at *Mdh-a*, but heterozygotes were detected in neither male morph.

Two nests of both *F. rufa* and *F. lugubris* produced diploid males in Hanko (Table 3). Both sexes were produced in large numbers in these nests and the frequency of males was roughly estimated to be 25-50%. The males and females had the same frequency of heterozygotes in the two *F. rufa* nests (the frequencies of individuals with at least one heterozygous locus were 10/20 and 12/18 in females and 11/20 and 15/16 in males). The frequency of heterozygotes was lower among males than among females in the two *F. lugubris* nests (the frequencies being 8/10 and 20/20 in females and 7/42 and 4/17 in males). The two populations of *F. truncorum* with monogynous colonies (Denmark and Tvärminne) showed different patterns. No diploid males were found in the Danish population, whereas three nests at Tvärminne produced them. Not all males in these nests were diploid. (Possible diploid males were detected in two additional nests, but these observations were not confirmed and are not included in Table 3).

No diploid males were detected in the Siuntio and Sipoo populations of *F. rufa* and *F. lugubris*.

DISCUSSION

As shown by the above models, production of diploid males can readily affect both the mortality and growth rate of colonies, leading to selection on the mating behaviour of the females. CROZIER & PAGE (1985) interpreted their results from the demographic model in terms of fitness variances, selection favouring the genotype

with the lower variance. Actually, their model can create substantial differences in the expected fitness of females as a function of the number of matings (Fig. 2). Evolution in such a deterministic model is directed by the mean fitness rather than by its variance.

CROZIER & PAGE (1985) concluded that single mating is favoured when sexual offspring are produced during the exponential phase of colony growth, but multiple mating is favoured when sexual production is postponed to the asymptotic phase. Adding mortality in the model can diminish the advantage of polyandry attached to delayed reproduction, favouring single mating. The advantage of single mating is strongly suggested by experimental data from the fire ant *Solenopsis invicta* (ROSS & FLETCHER 1986, ROSS et al. 1988). The reason for this is that double mating increases the proportion of monogynous colonies that produce diploid males. If a colony with 25% diploid males has a high probability of dying in its founding stage, double mating simply leads to a higher proportion of colonies dying and to a lowered mean fitness of double-mated queens.

RATNIEKS (1990) discussed the advantages of single or multiple mating with respect to the timing of the removal of diploid males. If these males are removed early in development, as in the honeybee, they cause little damage to the colony and a low frequency of diploid males can be tolerated. This allows multiple mating to evolve. If diploid males develop into adults, as in the fire ant and in *Melipona*, or are removed in late developmental stages, they are a heavy burden to the colony even in small numbers, and single mating is favoured. RATNIEKS' model was based on the assumption that colony size is limited by the available space in the brood combs. The same principle applies to ant colonies, although they have no brood combs that would limit egg laying.

The present observations differ from those in *Solenopsis invicta* in two respects. Multiple mating seems to be common in several species, and diploid males are found in natural, viable monogynous (or weakly polygynous) colonies. Mother-offspring analyses of laboratory cultures have clearly shown polyandry in three *Formica* species: *F. sanguinea* (PAMILO 1982), *F. truncorum* (SUNDSTRÖM 1989, 1994) and *F. aquilonia* (PAMILO 1993). Two other species (*F. pressilabris* and *F. transkaucasica)* have proved to be mainly monandrous (PAMILO 1982). The estimates of relatedness in the mono/oligogynous species in the present study fall mainly within the range observed among siblings of the polyandrous *Formica*.

The hypotheses of polyandry and polygyny can be separated by male relatedness. The males produced by the queen in monogynous colonies have a relatedness of 0.5. If there are several queens per nest, the relatedness among males depends on the queen-to-queen relatedness. When the relatedness among workers ranges from 0.5 to 0.6, the relatedness among males should range from 0.44 to 0.47 if the queens are full sisters, from 0.4 to 0.45 if the queens are as related as the worker nest mates are, and from 0.33 to 0.40 if the queens are unrelated. Most observed values of male relatedness (Table 2) are close to 0.5, but the differences in the expected values are too small compared to the standard errors to allow a distinction to be made between monogyny and polygyny.

High relatednesses in *F. pratensis* and monodomous *F. truncorum* and *F. exsecta* and our earlier observations (PAMILO & ROSENGREN 1983; PAMILO 1991b; SUNDSTRÖM 1989, 1994) suggest that their colonies are mainly monogynous and the relatedness below 0.75 can be explained by polyandry. The relatedness estimates in *F. rufa* and *F. lugubris* drop well below 0.5 when corrected for population subdivision, and this seems to be due to both polyandry and polygyny. Colonies of *F. rufa*

probably tend to be monogynous, and a large proportion can be explained even with monogyny and monandry (this simple colonial structure could also explain the high frequency of diploid males in two colonies). It is more difficult to explain the genetic diversity in *F. lugubris* colonies by monogyny.

We have detected diploid males in the nests only at the times when the colonies normally produce sexual offspring. The model of RATNIEKS (1990) is therefore applicable to this situation and it seems probable that diploid brood developing into males at other times of the season is eliminated early and does not cause a heavy load on the colonies. In addition to the highly polygynous colonies of *F. aquilonia, F. polyctena, F. truncorum* and *F. pressilabris*, diploid males were found in very sparse populations of monogynous/weakly polygynous *F. rufa, F. lugubris* and *F. truncorum*, the nests being scattered on islands with some clear genetic differentiation. The number of sex alleles is drastically reduced with such a population structure. Diploid males were not detected in the other species with monogynous colonies, *F. exsecta* and *F. pratensis*. It is possible that their populations have more sex alleles, as the nest densities are higher than in the island populations of *F. rufa, F. lugubris* and *F. truncorum*, although the populations are not large. It is also possible that the colonies of these two species suffer more from diploid males, and colonies producing them are eliminated at a founding stage as in *S. invicta*. The association of monogyny and monandry, commonly seen in these species, fits the prediction of the load hypothesis.

The populations with highly polygynous colonies show up to 10% diploid males (Table 1). These populations have locally high nest densities and, because of polygyny, large population sizes. Our studies, however, suggest that this social type is commonly connected to restricted gene flow and genetic differentiation among subpopulations (SUNDSTRÖM 1989, 1994; PAMILO et al. in prep.). This is expected to lead to the loss of sex alleles and the production of diploid males.

The diversity hypothesis for polyandry has been developed by HAMILTON (1987) and SHERMAN et al. (1988), who proposed that intracolony variability could increase colony resistance to pathogens and parasites (see SHYKOFF & SCHMID-HEMPEL 1991 for empirical data). It is also supported by the evidence for genetic variance in the task preferences and the age-dependent task profile of workers (CALDERONE & PAGE 1988, FRUMHOFF & BAKER 1988, ROBINSON & PAGE 1988, STUART & PAGE 1991).

The hypothesis put forward by SHERMAN et al. (1988) assumes that the variance of disease resistance is larger among monandrous than among polyandrous colonies. Polyandrous colonies have intermediate resistance because often some, but seldom all, of the workers are resistant. Because of host-parasite co-evolution, selection in the population tends to create genetic diversity rather than a directional change toward a specific resistance (SHERMAN et al. 1988), and resistance has a stable density distribution. If there is behavioural dominance, i.e. a small group of resistant workers gives a good resistance to the whole colony, the density distribution will be skewed. This can be expected under polyandry. If selection is of the truncation type, colonies below a certain resistance level will be eliminated while those above it will survive. Depending on the position of the truncation point and on behavioural dominance, the consequences of selection are quite different. Polyandry is favoured if the truncation point is below the population mean resistance and if there is behavioural dominance.

Both the load hypothesis and the diversity hypothesis refer to colony-level selection in the sense that the success of a colony depends on the combined genotypes of the workers (PAMILO 1991a). Interestingly, these hypotheses seem to predict different associations between polyandry and polygyny.

If genetic diversity of the worker force is adaptive, the benefits of polyandry should be clear in monogynous colonies. The level of polyandry should decrease with polygyny if multiple mating is costly to the queen (e.g. increases chance of death on mating flight). The diversity hypothesis therefore predicts an association between polyandry and monogyny.

The load hypothesis predicts selection for monandry when colonies with moderate frequencies of diploid males have high mortality. This result was here derived for monogynous colonies; in polygynous colonies the frequency of diploid males is diluted by the progeny of other queens, and selection against polyandry becomes weak. The load hypothesis therefore predicts an association between monandry and monogyny. There is no reason to expect the level of polyandry to change with polygyny, unless there are other factors favouring polyandry at the individual level. Monogyny could allow multiple mating when the load caused by diploid males is insignificant. This could be the situation when the frequency of diploid males is very low, they are eliminated at early developmental stages, or when new colonies are produced by fission. Fissioning colonies, such as those in the honeybee, lack the incipient stage of very small colony size when the selective differences are predicted to be large (Fig. 1).

The *Formica* species do not give unequivocal support to either hypothesis. The occurrence of diploid males with a high frequency in some apparently monogynous colonies indicates that they do not form an unbearable load. This speaks against the load hypothesis. It is not always possible to conclude whether the intracolonial genetic diversity is due to polygyny or polyandry, but the estimates of worker relatedness in apparently monogynous colonies are very close to estimates obtained within single-queen progeny in some highly polygynous species. This would suggest that the number of matings in this group of ants does not correlate with the degree of polygyny and that colony-level selection is not a major factor in the evolution of polyandry. Two species still fit the prediction of the load hypothesis; no diploid males were detected in the populations of the species with highest intracolonial estimates of relatedness. Whether this means that the colonies producing diploid males die early or that the frequency of diploid males is very low, remains to be examined.

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