Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*

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Genetic mother–offspring analyses based on six enzyme gene loci show that about 60 per cent of the females of the mound-building red wood ant *Formica aquilonia* mate with several males. The number of matings inferred from the offspring genotypes range from one to six, the arithmetic mean being 1.94. The mates do not contribute equally in the inseminations; in the case of two matings, one male is estimated to inseminate on average 77 per cent of the offspring. The average relatedness among the offspring of a single female is 0.6, corresponding to the effective number of matings of 1.43. Three of the six loci show remarkable allele frequency differences between the sexes. At each of these loci the males virtually lack the alleles present in females with frequencies 0.18 at *Me*, 0.25 at *Pgk* and 0.27 at *Gpi*. Segregation analyses indicate normal Mendelian inheritance at these loci and the difference between the sexes seem likely to result from selection.

Keywords: colony-level selection, *Formica aquilonia* polyandry, relatedness, sex-specific allele frequencies, social insects.

Introduction

It is commonly thought that multiple mating can be disadvantageous to females owing to increased risks (e.g. Moritz, 1985), although such effects have rarely been documented (Gwynne, 1989). The major result of polyandry is a genetically diverse progeny. In sexually reproducing organisms, genetic diversity among the offspring may help some of them to survive in a patchy environment (Williams, 1975). In eusocial insects the same applies to the sexual offspring that disperse whereas the sterile workers stay together in the parental nest and the genetically diverse colony faces the environment as a collective unit. The hypotheses explaining polyandry in eusocial insects must therefore take into account its possible consequences for the whole colony. The hypotheses can be classified depending on whether polyandry is considered adaptive to the individual females (queens), to the whole colony, to the population, or whether the hypothesis refers to the queen–worker conflict over colony reproduction (Crozier & Page, 1985; Pamilo, 1991a). Two hypotheses, both based on colony-level phenomena, have been particularly favoured in recent literature: they are referred to here as a load hypothesis and a diversity hypothesis.

The load hypothesis is based on the distribution of diploid males among nests (Page, 1980; Crozier &

Page, 1985). Individuals homozygous for the sex-determining locus (or loci) develop into diploid males in the honeybee (Mackensen, 1951), in the fire ant (Ross & Fletcher, 1985a) and probably in many other, normally male haploid, hymenopteran species (Crozier, 1975). Such diploid males are non-viable or sterile and cause a burden to the colony. As frequency-dependent selection should lead to a large number of equally freqent sex alleles, the frquency of diploid males in the population remains low. Multiple mating by the females affects the distribution of diploid males among the colonies, but the total frequency of diploid males in the population remains unaffected. Polyandry increases the proportion of colonies suffering from the load and at the same time decrease the average load in those colonies that are affected. The fitness effects depend on how the colony success changes as the function of the proportion of diploid offspring developing into males (Crozier & Page, 1985).

The diversity hypothesis emphasizes the adaptive significance of the genetic variation among the worker force (Crozier & Page, 1985). A special aspect of that hypothesis was proposed by Hamilton (1987) and Sherman *et al.* (1988) who suggested that genetic diversity of the worker force can improve the resistance of the whole colony to diseases. Some support for this idea comes from the kin-biased transmission of

trypanosome parasites in bumblebees (Shykoff & Schmid-Hempel, 1991a,b).

The diversity hypothesis predicts that multiple mating is particularly adaptive in species with monogynous colonies (i.e. colonies with a single queen). Increasing the number of coexisting reproductive queens in a colony (polygyny of colonies) raises the genetic diversity among nest members to an extent that quickly dilutes the effects of polyandry. The predictions of the load hypothesis depend on how sensitive the colonies are to the existence of diploid males. The observational evidence suggests that queens founding new nests independently should be monandrous because multiple mating decreases the success of colony foundation by increasing the chance of having diploid males among the offspring (Ross & Fletcher, 1986). The level of polyandry in species with polygynous colonies is expected to depend on selection at the individual level as polyandry does not add much to the genetic diversity of such colonies.

In this paper, I present results from genetic mother–offspring analyses in an ant *Formica aquilonia* which typically was highly polygynous colonies with tens or hundreds of reproductive queens in the same nest (Pamilo, 1982; Rosengren & Pamilo, 1983). The aims of the analyses are to estimate the level of polyandry in order to evaluate the above mentioned hypotheses, to estimate the proportion of offspring inseminated by each of the mates, to estimate the mating types, and to confirm the Mendelian inheritance of the observed allozyme variation.

Materials and methods

Laboratory methods

The study is based mainly on laboratory cultures of *Formica aquilonia*. The ants for these cultures were collected from two nests at the Tvärminne Zoological Station, Hanko in southwestern Finland. There were 200 old queens (100 from each nest) and a few thousand workers from the respective nests were collected in early May 1987 and brought to the laboratory. The laboratory nests were constructed from two ceramic flower pots, one within the other, and the ants placed their nest chambers in the space between the bottoms (Pamilo, 1982). One queen and about 100 workers were placed in each such nest and the offspring were removed from the successful nests in July as pupae. The genotypes of the offspring and the mother queen were analysed at six loci. In all, 113 laboratory nests produced offspring, all of which were workers. The mean number of offspring used in the electrophoretic study was 22.4 per nest (s.d. = 15.5) (Table 1).

The nests used as the source of the old queens belong to a group of nests within a two hectare area. To characterize the population structure, 10–30 workers from each of 14 nests and 131 males from three nests (collected in 1990) in the same population of *F. aquilonia* were also studied electrophoretically.

The genotypes were determined using enzyme electrophoresis. The pupae were individually homogenized in 30 μl of distilled water for the analysis. The gaster of adult workers was removed and the head and thorax were first rinsed in water before homogenizing them in 30 μl of water (60 μl for sexuals). The enzyme phenotypes were resolved by horizontal starch gel electrophoresis. The buffers used were (i) tray: 135 mM tris-citrate at pH 7.1, and gel: 43 mM tris-citrate at pH 8.4 (Varvio-Aho & Pamilo, 1980); (ii) tray: 135 mM tris-citrate-EDTA at pH 7, and gel: diluted 1:16 from the tray buffer (Ayala *et al.*, 1974). Standard histochemical stainings were used for glucosephosphate isomerase (GPI), malic enzyme (ME) and peptidase (PEP, using glycyl-leucine as substrate) from buffer i, and phosphoglucomutase (PGM), esterase (EST) and phosphoglycerate kinase (PGK) from buffer ii. EST and PGK were stained using fluorescent stains. Other enzymes examined were either monomorphic or did not give scorable results. The abbreviations for the

Table 1 Distribution of brood sizes used in the electrophoretic analyses. The number of mates is the mean of the minimum numbers required to explain the offspring genotypes

Number of offspring	Number of broods	Minimum number of mates
0–10	26	1.54
10–19	24	1.96
20–29	40	1.83
30–39	9	3.11
40–49	5	2.60
50–59	5	2.20
60+	4	1.75

enzymes are given in capital letters and those for the respective loci and alleles in italics.

Statistical methods

The level of polyandry and the paternal genotypes can be directly inferred from the observed genotypes of the mother and her offspring. The inference is made simple by haploidy of the ant males. The problem is that multiple mating cannot be detected when the mates have identical genotypes, and there is uncertainty about the mate genotype when a heterozygous female has a diploid offspring with an identical heterozygous genotype.

If the proportion of the offspring fathered by the ith mate is p_i, the relatedness g within a brood is

$$g = 0.25 + \tfrac{1}{2}\Sigma p_i^2 = 0.25 + 1/(2m_e), \tag{1}$$

where m_e is the effective number of mates (Starr, 1984). When the mate contributions Σp_i^2 are estimated from a finite sample of N offspring, the estimate includes also a sampling error. If the observed contributions by the mates are y_i, the estimate corrected for the sampling error is

$$\Sigma p_i^2 = (N\Sigma y_i^2 - 1)/(N - 1). \tag{2}$$

The relatedness among siblings is also affected by departures from random mating. I have earlier examined the case of regular inbreeding (Pamilo, 1985). When the inbreeding coefficient due to intra-nidal mating is F, the relatedness is, at least approximately,

$$g = 0.25 + \tfrac{1}{2}\Sigma p_i^2 + \frac{2F}{1+F}[0.75 - \tfrac{1}{2}\Sigma p_i^2]. \tag{3}$$

Gene frequency differences between the sexes lead to an excess of heterozygotes, and the value of F among the offspring is negative. Such a situation is easily transient in the population. However, if the only factor affecting the F value is the gene frequency difference (i.e. the female genotypes have Hardy–Weinberg frequencies and there is no assortative mating), the expected relatedness among siblings is

$$g = 0.25 + \tfrac{1}{2}\Sigma p_i^2 + \frac{2F}{1+F}[0.25 - \tfrac{1}{2}\Sigma p_i^2]\frac{1 - x_f - x_m}{x_m - x_f}, \tag{4}$$

where x_f and x_m are the gene frequencies in females and males. When $x_f = x_m$, the last term is zero ($F = 0$) and the relatedness is as given in eqn (1).

It is also possible to analyse the distribution of the offspring genotypes among the broods and estimate the

average relatedness of siblings (Pamilo, 1984). The relatedness is a function of the number and contributions of different mates, and it avoids the problem caused by indistuinguishable mates with identical genotypes. The problem with this approach is that it gives an average relatedness that neglects the variation among the broods. Furthermore, it is not possible with this approach to estimate separately the number of mates and the proportional contributions by them, the estimate depends on the effective number of mates. Departures from random mating also affects the estimate. However, it is useful to compare these estimates with those obtained from the above equations. The relatedness within a brood j can be written from eqn (1) as

$$g_j = 0.25 + \tfrac{1}{2}(m_j V_j + 1/m_j), \tag{5}$$

where m_j is the number of mates and V_j the variance of their relative contributions. The mean relatedness over all broods is then

$$g = 0.25 + \tfrac{1}{2}\overline{mV} + 1/(2H), \tag{6}$$

where H is the harmonic mean number of mates per female. Combining eqn (1) and (6) gives the effective number of mates as

$$m_e = H/(1 + H\overline{mV}). \tag{7}$$

Equations (6) and (7) correct a small error in the earlier derivations of Strassmann *et al.* (1991) which had a product of means $(\overline{m}\,\overline{V})$ instead of the mean of the products $(\overline{mV})$.

The average relatednesses among siblings and nest mates is estimated from the observed genotype frequencies using the method of Pamilo (1984) as modified by Queller & Goodnight (1989) and Pamilo (1990).

Results

Mendelian segregation

Three allozyme variants were found segregating in PGM and two variants in the other five enzymes. The most common allozyme of each enzyme is called '100' and the others are named according to the relative mobility of the allozymes. Based on the banding pattern, PGK and PGM are monomeric enzymes, GPI, PEP and EST are dimers and ME is a tetramer.

The interpretation that the observed allozyme variation is genetically determined is supported by a comparison of the maternal and offspring phenotypes (Table 2a). All the offspring are legal, i.e. they carry at least one maternal allele, when the enzyme phenotypes are interpreted to represent genotypes of codominant

Table 2 Observed mother genotypes and (a) total numbers of offspring genotypes for each mother genotype, and (b) inferred frequencies of mate genotypes separately for each locus. Mate frequencies are calculated by counting each brood once and assigning each male genotype the frequency of offspring derived from the genotype within the brood

Locus	Mother Genotype	Number	(a) Diploid offspring						(b) Mates		
Gpi			*100/100*	*100/70*					*100*	*70*	
	100/100	50	1118	12					49.6	0.4	
	100/70	59	670	663					58.0	0	
Me			*100/100*	*100/95*					*100*	*95*	
	100/100	51	1074	43					48.6	2.4	
	100/95	28	328	279					28.0	0	
Pep			*100/100*	*100/80*	*80/80*				*100*	*80*	
	100/100	79	1349	375	—				62.4	16.6	
	100/80	22	156	254	86				15.3	6.7	
Pgm			*120/120*	*120/100*	*120/70*	*100/100*	*100/70*	*70/70*	*120*	*100*	*70*
	120/120	13	82	176	74	—	—	—	2.7	7.6	2.6
	120/100	49	188	317	44	275	25	—	17.9	25.5	5.7
	120/70	15	53	44	84	—	69	22	4.5	8.4	2.1
	100/100	22	—	96	—	311	90	—	4.4	13.6	4.0
	100/70	10	—	37	42	53	33	18	4.6	4.7	0.7
Est			*120/120*	*120/100*	*100/100*				*120*	*100*	
	120/120	23	52	492	—				2.8	20.2	
	120/100	57	80	622	532				7.9	49.1	
	100/100	27	—	157	330				9.4	17.6	
Pgk			*100/100*	*100/80*					*100*	*80*	
	100/100	54	1075	4					53.7	0.3	
	100/80	55	706	659					55.0	0	

alleles. Furthermore, the segregation of offspring genotypes follows relatively well the Mendelian expectation. At a biallelic locus, a heterozygous mother should produce heterozygous and homozygous offspring in a 1:1 ratio. There is a slight excess of homozygotes at *Me* ($\chi^2 = 4.2$, 1 d.f., $P < 0.05$), but a very close fit at *Gpi*, *Pep*, *Est* and *Pgk* (the values of χ^2 are 0.1, 0.2, 0.0 and 1.6, respectively).

The *Pgm* genotypes among the offspring of heterozygous mothers systematically depart from the 1:1 ratio. Focusing on two alleles at a time, the ratio of homozygotes to heterozygotes is 463:317 for the mother genotype Pgm^{120}/Pgm^{100} ($\chi^2 = 27.3$, $P < 0.001$), 75:84 for Pgm^{120}/Pgm^{70} ($\chi^2 = 0.5$, $0.001 < P < 0.01$) and 71:33 for Pgm^{100}/Pgm^{70} ($\chi^2 = 13.9$, $P < 0.001$). We can also examine the segregation of the alleles of a heterozygous mother when the male has carried the third allelic type, e.g. when the mother is Pgm^{120}/Pgm^{100} and the male has the allele Pgm^{70}. This comparison shows that the allele Pgm^{120} is transmitted slightly more frequently than Pgm^{100} (44:25, $\chi^2 = 5.2$, $0.01 < P < 0.05$), Pgm^{70} more frequently than Pgm^{120} (69:44, $\chi^2 = 5.5$, $0.01 < P < 0.05$), but there is no difference between Pgm^{100} and Pgm^{70} (37:42, $\chi^2 = 0.3$, ns).

I also tested for the heterogeneity among families for the segregation of the *Pgm* genotypes (the same six cases described in the previous paragraph). There appears to be a slight heterogeneity in the segregation of the alleles Pgm^{120} and Pgm^{70} ($\chi^2 = 23.6$, 9 d.f., $0.001 < P < 0.01$) but not in the other cases. The value of χ^2 is 10.4 (10 d.f.) for the segregation of the alleles Pgm^{120} and Pgm^{100} of the heterozygous mothers and 2.9 (4 d.f.) for the alleles Pgm^{100} and Pgm^{70}. The values of χ^2 when testing the heterogeneity of heterozygous and homozygous offspring among the progenies of heterozygous mothers are 59.9 (46 d.f) for the mother genotype Pgm^{120}/Pgm^{100}, 16.2 (9 d.f.) for Pgm^{120}/Pgm^{70} and 8.2 (8 d.f.) for Pgm^{100}/Pgm^{70}.

Although the observed segregation of the alleles at *Pgm* do not strictly obey the expected Mendelian ratios, the departures are not dramatic enough to reject the hypothesis of genetic determination of the allozyme variation. In support of the hypothesis we can note that not a single offspring represents an illegal genotype, and 130 of 131 males electrophoresed in 1990 showed a haploid pattern. Only one male had a heterozygous genotype at *Pgm*, indicating that this male was diploid.

The offspring genotypes also suggest that the six loci segregate independently of each other. This is tested by

examining the segregation of alleles when the mother is heterozygous for two loci at the same time (Table 3). Only one of 15 tests shows a significant association (between *Gpi* and *Pgk*, $P < 0.05$).

Number of matings

Because the ant males are haploid, it is possible to use the mother–offspring genotypic data to infer the genotypes of the mates. The only case where the male genotype cannot be inferred with certainty arises when the mother and her daughter have an identical heterozygous genotype. In that situation we cannot say which of the two alleles comes from the mother and which from the father.

Using the genetic data, the genotypes of the mates are inferred separately for each offspring (Table 4). Less than half of the broods (49, or 44 per cent) can be explained with a single mate type and the highest number of detectable mate types per female is six. Two progenies were fathered by six and one by five male genotypes. The arithmetic mean number of detectable mate types is 1.94. Ignoring the progenies with less than 10 individuals, the mean is 2.06. As there is a

probability of about 15–17 per cent (depending whether one uses the male allele frequencies deduced from the laboratory cultures or those observed in 1990) that two randomly chosen males have shared the same multilocus genotype, this method does not estimate the true number of mates, only the number of mate types. The detected number of double matings is 38 (Table 4), so the probable number of broods with two identical fathers is about seven. Other corrections for undetected mates are difficult because of the small proportion of highly polyandrous queens and because the detected number of matings also depends on the number of offspring analysed (Table 1).

The observed mating types are presented separately for each locus in Table 2b. Note that these numbers do not represent the real mating frequencies because not all matings can be separated from each other. However, it is clear that there are some big allele frequency differences between the sexes. Most notably, the loci *Gpi*, *Me* and *Pgk* are almost monomorphic in the males, although all of them are clearly polymorphic in the queens. This means that these loci give practically no information concerning the mating frequency of queens.

From the mating types inferred from the offspring genotypes it is also possible to estimate the relative contributions of each mate and the variance of the contributions using eqn (2). It turns out that one male has normally fathered well over 50 per cent of the offspring. It seems fair to conclude that the males contribute unequally, although their precise contributions can be masked by several mates having an identical genotype. The variances (or Σp_i^2, Table 4) indicate that in progenies with two fathers, one of them produces about 77 per cent of the offspring.

When the estimates of Σp_i^2 are used to calculate relatedness among the offspring using eqn (1), the estimates of relatedness thus obtained are 0.57 for progen-

Table 3 Independent segregation of the six loci calculated from offspring of mothers heterozygous for the two loci in question. Above the diagonal are chi-squared values and below diagonal are degrees of freedom

	Gpi	Me	Pep	Pgm	Est	Pgk
Gpi	—	5.3	7.5	11.7	9.7	36.8
Me	7	—	0.3	1.2	0.8	9.8
Pep	8	1	—	3.0	3.2	10.8
Pgm	12	4	5	—	17.8	22.3
Est	10	3	6	11	—	7.7
Pgk	24	9	8	16	8	—

Table 4 Contributions of different mates as detected from offspring genotypes. Corrected values for the number of broods takes into account undetected double matings by two males of the same genotype. Corrected estimates of Σp_i^2 are based on the eqn 2 and the relatedness estimates g are from eqn 1

Number of mates	Number of broods		Σp_i^2		
	Observed	Corrected	Observed	Corrected	g
1	49	42	1	1	0.75
2	38	45	0.696	0.643	0.57
3	15	15	0.501	0.443	0.47
> 3	11	11	0.443	0.413	0.46
Total	113				0.61

ies with two identifiable mates, 0.47 for three mates and 0.46 for more than three mates (Table 4). These values are clearly higher than those expected if the mates had contributed equally (the expected values are 0.50, 0.42 and 0.25–0.38, respectively). The estimates of Σp_i^2 (Table 4) suggest that the effective number of matings is 1.56 for two actual mates, 2.26 for three mates and 2.42 for more than three mates. Averaging over all broods, eqn (7) gives an average estimate of $m_e = 1.48$. The mean relatedness over all progenies, taking into account the correction for undetected double matings (Table 4), is 0.61.

The level of polyandry can also be estimated directly from the genetic heterogeneity of the offspring. That heterogeneity depends both on the number of mates and on the relative contributions of each of the mates and it can be measured as the genetic relatedness of the offspring within single broods. The estimates for single loci vary greatly, from 0.29 for *Gpi* to 0.70 for *Pep* (Table 5). As noted above, almost all the mates had been of the same type at *Gpi*, *Me* and *Pgk*. The relatedness estimates based on these loci therefore only reflect the proportions of the queen genotypes and should not be used for estimating polyandry. The other three loci are informative and the mean relatedness from them is 0.60. Using eqn (1), the relatedness estimate $g = 0.60$ corresponds with the effective number of mates being 1.43, on average.

Population structure

As noted above, the six loci give conflicting information concerning the genetic heterogeneity within broods. Similarly, the loci give conflicting results concerning the inbreeding coefficient (Table 5). Low estimates of relatedness are connected with an excess of heterozygous individuals.

The allele frequencies in queens are estimated by direct counting of the queen genotypes. The frequencies in the males are estimated in two ways. First, the frequencies can be estimated from the inferred mating types of Table 2b. Second, I examined new males produced in the population in 1990; they were obtained from only three nests. The allele frequencies in these two cohorts of males are very similar to each other and clearly different from those in the queens (Table 5).

The loci *Gpi*, *Me* and *Pgk* are practically monomorphic in the males, yet each of them has a high frequency of heterozygous queens. The allele frequencies of *Est* also differ in the two sexes, but the genotype frequencies in the queens agree well with the Hardy–Weinberg expectation. As a result of the gene frequency difference, the *Est* locus gives a negative estimate of inbreeding, *F*, and the relatedness estimate

Table 5 Estimates of genetic relatedness (g with 95 per cent confidence limits) and inbreeding (F) calculated from the offspring genotypes, and the frequencies of the rare alleles among the queens, their mates and males samples in 1990

| Locus | g (95% C.L.) | F | Allele frequencies | | |
| | | | Queens | Males | |
				Mates	1990
Gpi	0.30 (0.24–0.36)	−0.15	0.27	0.004	0
Me	0.37 (0.24–0.50)	−0.07	0.18	0.03	0
Pep	0.71 (0.64–0.78)	0.01	0.11	0.23	0.16
Pgm	0.61 (0.55–0.67)	0.10			
Pgm[120]			0.41	0.31	0.32
Pgm[70]			0.12	0.14	0.23
Est	0.48 (0.38–0.58)	−0.19	0.48	0.19	0.29
Pgk	0.29 (0.24–0.34)	−0.15	0.25	0.003	0

is also lower than that obtained from *Pep* and *Pgm* (Table 5). If we use eqn (4) to estimate Σp_i^2 by inserting the values of F, g and the gene frequencies x_f and x_m of the *Est* locus (Table 5), we obtain $\Sigma p_i^2 \approx 0.5$. The reason for this is that the value of $g = 0.5$ requires that $\Sigma p_i^2 = 0.5$ and the correction term of eqn (4) becomes zero independently of the allele frequencies. We should note, however, that eqn (4) may not be applicable in this situation, because the inferred mating types (Table 2) indicate assortative mating. The frequency of Est^{120} male is 12 per cent among the mates of Est^{120}/Est^{120} queens but 35 per cent among the mates of Est^{100}/Est^{100} queens.

The inbreeding coefficient and the genetic relatedness of worker nest mates are also estimated from the data collected from 14 nests of the *F. aquilonia* population. The estimate of relatedness is 0.056 (s.e. = 0.023).

Discussion

Although repeated matings by females have been observed in many ants (Page, 1986), genetic studies have often indicated monandry or a low level of polyandry (Page, 1986; van der Have *et al.*, 1988, Ross *et al.*, 1988. Similar mother–offspring analyses as performed here proved *Formica pressilabris* and *F. transkaucasica* practically monandrous (Pamilo, 1982). The present results clearly demonstrate multiple insemination in *F. aquilonia*. A similar level of polyandry has been detected in two other *Formica* species, *F. sanguinea* (Pamilo, 1982) and *F. truncorum* (Sundström, 1989). In all three species, the genetic relatedness among siblings falls within the range 0.56–0.63.

Genetic studies of natural colonies of *F. truncorum* suggest that the level of polyandry is similar for queens in monogynous and polygynous colonies (Sundström, 1989). The estimates for *F. sanguinea* and *F. aquilonia* come from polygynous colonies, the level of polygyny in the latter being exceptionally high for any ant. Polyandry does not contribute significantly in the genetic diversity of the worker force in these ants. The advantage, if any, of polyandry should therefore be at the individual rather then at the colonial level.

High levels of polyandry have been detected in some social wasps and bees that have monogynous colonies. Ross (1986) monitored progenies of two species of *Vespula* wasps (*V. squamosa* and *Paravespula maculifrons*). The females had mated with two to seven males, the relatedness among worker siblings being 0.40 and 0.32. Honeybee females are also known to mate with many males (Page, 1986). However, the ants with monogynous colonies seem to show a tendency towards monandry or a low level of polyandry, including *Aphaenogaster rudis* (Crozier, 1973), *Solenopsis invicta* (Hung & Vinson, 1976; Ross & Fletcher, 1985b), *S. geminata* and *S. richteri* (Ross *et al.*, 1988), *Rhytidoponera confusa* and *R. chalybaea* (Ward, 1983), *Lasius niger* (van der Have *et al.*, 1988), *Formica exsecta* (Pamilo, 1991b), *Myrmica puncti-ventris* (Snyder & Herbers, 1990), *M. lobicornis* and *M. ruginodis* (P. Seppä, unpublished data). This agrees with the prediction of the load hypothesis.

While one diploid male of *F. aquilonia* was found in the present study, others have been detected earlier with a low frequency in *F. pressilabris* (Pamilo & Rosengren, 1984). These observations show that at least some diploid males grow to adults and, hence, can waste the resources of the colony. Both species have highly polygynous colonies and the production of some diploid males may therefore not cause a great burden to the colonies. In the fire ant *S. invicta* monogynous colonies producing diploid males suffer severely and become eliminated at early stages of colony growth, whereas diploid males can arise in polygynous colonies without causing much harm (Ross & Fletcher, 1986).

In addition to the original question concerning polyandry, a new problem concerning the allele frequency differences between the sexes arose during the study. Such differences could arise in small ant populations where the nests specialize in producing either females or males (Pamilo & Rosengren, 1983; Nonacs, 1986). If the queens live long, as seems to be the case in the *Formica* ants (Pamilo, 1991b), the mating types reflect the past population history. However, the new males studied in *F. aquilonia* lacked the same alleles as did the males inferred from the offspring genotypes. This points to a systematic bias in the male genotypes.

There are two explanations for the extremely low variation at *Gpi*, *Me* and *Pgk* in males. First, it may be that only one type of haploid eggs is formed. That would be the case if the rare allele is linked to a dominant factor which causes the queen always to fertize the eggs: males would be produced only by females homozygous for the common allele. Second, haploid eggs carrying the rare allele could be produced but eliminated during larval development. This would be the case if the electrophoretic marker is linked with a recessive lethal allele at another locus in the same chromosome.

There are no data to distinguish between these hypotheses. The first of them can be doubted because it is very speculative although sex ratio specialization by queens, observed in many ants (Pamilo & Rosengren, 1983; Nonacs, 1986), fits this hypothesis. Genetic variation for sex ratio has not been demonstrated in any ants, and the present hypothesis would require specific genes in three chromosomes. Extrachromosomal sex ratio distorters have been observed in Hymenoptera (Werren *et al.*, 1987) as well as in several other organisms (Hurst, 1991) but it is difficult to believe in a linkage of such a factor with three different chromosomal markers in *Formica aquilonia*.

The second hypothesis can be considered to be more likely because harmful mutations are known to occur and accumulate in the genomes (Pamilo *et al.*, 1987). However, if the alleles are lethal and expressed in the haploid males, we would also expect the frequency to be low in females. The observed high frequencies of the alleles in females cast some doubt over this hypothesis unless there is strong overdominance in the females.

It would be possible to test these two hypotheses by letting single queens produce sexual offspring in laboratory colonies. The hypotheses make clearly different predictions concerning the offspring of heterozygous queens. It should be noted that both of the hypotheses assume linkage disequilibrium between the electrophoretic marker loci and other genes. Such a disequilibrium is population-specific and need not be observed elsewhere; in fact, workers homozygous for the rare alleles have been found regularly in other populations.

Two of the other loci, *Pgm* and *Est*, also show features indicating possible selection at them or linked loci. Transmission of the alleles at *Pgm* depart from normal Mendelian expectation and the mating types inferred from the *Est* genotypes indicate assortative mating. A recent study of the fire ant *S. invicta* showed allele and genotype frequency differences between both castes and sexes as one homozygous *Pgm* genotype is consistently eliminated from the queens of poly-

gynous, but not of monogynous, colonies, apparently because of reproductive competition among the coexisting queens (Ross, 1992). There is no direct evidence suggesting that the observed allele frequency differences in *F. aquilonia* would be similarly mediated by social behaviour.

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