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Genetic population structure and sociogenetic organisation in *Formica truncorum* (Hymenoptera; Formicidae)

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Summary. The genetic population structure and the sociogenetic organization of the red wood ant *Formica truncorum* were compared in two populations with monogynous colonies and two populations with polygynous colonies. The genetic population structure was analysed by measuring allele frequency differences among local subsets of the main study populations. The analysis of sociogenetic organisation included estimates of nestmate queen and nestmate worker relatedness, effective number of queens, effective number of matings per queen, relatedness among male mates of nestmate queens and relatedness between queens and their male mates. The monogynous populations showed no differentiation between subpopulations, whereas there were significant allele frequency differences among the subpopulations in the polygynous population. Workers, queens and males showed the same genetical population structure. The relatedness among nestmate workers and among nestmate queens was identical in the polygynous societies. In three of the four populations there was a significant heterozygote excess among queens. The queens were related to their male mates in the polygynous population analysed, but not in the monogynous ones. The data suggest limited dispersal and partial intranidal mating in the populations with polygynous colonies and outbreeding in the populations having monogynous colonies. Polyandry was common in both population types; about 50% of the females had mated at least twice. The males contributed unequally to the progeny, one male fathering on average 75% of the offspring with double mating and 45–80% with three or more matings.

Key words: Population structure – Genetic relatedness – Sociogenetic organisation – Queen number – Social insects

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Introduction

Hamilton's (1964) principle of inclusive fitness is a central concept in explanatory models of the evolution of eusociality among insects. The model incorporates genetic relatedness as a key factor in the general cost-benefit formula for helping behaviour and is based on the probability that two individuals carry genes that originate from the same ancestor. The principle has been used most extensively to understand the evolution of eusociality among Hymenoptera and in developing theories about social evolution within this group (Hamilton 1964; Trivers and Hare 1976; Rosengren and Pamilo 1983; Nonacs 1988; Ratnieks 1988; Pamilo 1991b, c). Originally Hamilton (1964) used the eusocial Hymenoptera as an example of his theory because the haplodiploid sex determining system of this group can cause higher relatedness between sisters than between sisters and brothers or mother and offspring. Females may therefore maximise their inclusive fitness by raising female siblings instead of their own offspring. This asymmetry in relatedness also causes potential conflicts between workers and queens (Hamilton 1964; Trivers and Hare 1976). This conflict has become important for evaluating kin selection arguments (Trivers and Hare 1976; Boomsma and Grafen 1990, 1991; Pamilo 1990a, 1991b, c). Genetic relatedness is therefore a key parameter for understanding the consequences of inclusive fitness for social evolution.

The arguments presented above often assume that social insect colonies have one singly mated queen. However, the social structure and consequently the genetic relationships among colony members in social insect colonies may vary both inter- and intraspecifically (Hölldobler and Wilson 1990, pp. 209–211). Ant colonies may have several reproducing queens (polygyny), the queens may mate several times (polyandry), the queens may contribute unequally to the brood and the males may contribute unequally to the brood produced by their mates (Elmes 1980; Pamilo 1982a, 1993; Ross 1988; Stille et al. 1991; Heinze et al. 1992). Changing the genetic relationships among workers within a colony may change

the relatedness asymmetries within the colony and hence the nature of conflict between workers and queens. Therefore, the social organisation of colonies is a critical factor when sex allocation under worker control is evaluated (Trivers and Hare 1976; Boomsma and Grafen 1990, 1991; Pamilo 1990a, 1991b,c) and when analysing predictions about the evolution of colony characteristics (Pamilo 1991c).

Despite the disadvantages decreased nestmate relatedness causes to workers, both polygyny and polyandry can be favoured by colony-level selection if genotypic diversity enhances efficiency (Crozier and Consul 1976; Moritz and Southwick 1987; Page et al. 1989). Polygyny may also be favoured if dispersal risks are high (Pamilo 1991c) or if queens are shortlived and brood development takes a long time (Nonacs 1988; Seppä 1993; Rosengren et al. 1993). Kin selection arguments have been shown to support the evolution of polygyny both from the workers' and the queens' point of view under specific conditions (Pamilo 1991c). The relatedness among coexisting queens reflects the way colonies become polygynous, by recruiting daughters or by recruiting randomly from the population. Hölldobler and Wilson (1990, pp. 187–188) cite only two relatedness records for coexisting queens and Rosengren et al. (1993) cite eight. The studies available on nestmate queen relatedness have shown that coexisting queens in moderately polygynous ants often are related (Craig and Crozier 1979; Pamilo 1981, 1982b; Pearson 1982; Pamilo and Rosengren 1984; Douwes et al. 1987; Seppä 1993). In highly polygynous species, such as *Formica aquilonia* and *Solenopsis invicta* (Pamilo 1982b; Rosengren et al. 1993; Ross 1993) queen relatedness is close to zero.

Polyandry may be favourable for workers under a number of conditions; increased genetic diversity may be adaptive for colonies (Crozier and Page 1985; Hamilton 1987; Sherman et al. 1988) and it can be facilitated by the sex allocation conflict (Starr 1984; Moritz 1985; but see Woyciechowski and Lomnicki 1987; Ratnieks 1990). In monogynous colonies polyandry may be either favoured or selected against, depending on the colony life history pattern and the net effect the production of diploid males has on colony mortality (Page 1980; Crozier and Page 1985; Ratnieks 1990). The occurrence of diploid males cause high mortality of monogynous colonies in *Solenopsis invicta* (Ross and Fletcher 1986). If a small proportion of diploid males in the brood cause a high mortality risk for a colony, polyandry should be selected against. However, if the queen-worker conflict is a major component of selection, and the occurrence of diploid males is not, then polyandry is expected to evolve in monogynous societies (Nonacs 1992). In contrast polyandry is not expected in polygynous societies unless it confers some selective advantage for individual queens.

Estimates of genetic relatedness are also important when testing kin-selection arguments for the evolution of polygyny. The effective numbers of coexisting queens and the effective numbers of matings derived from relatedness estimates are, however, significantly affected by the genetic relationships among queens, among the male mates of queens and between a queen and her mate. The term *sociogenetic organization* (Seppä 1993) includes the effects of these factors and is here used to describe the genealogical relationships among nestmates relative to the whole breeding population.

The genetic structure within ant populations can be divided into two parts, that due to subdivision of populations and that due to relatedness among individuals within colonies. The population structure reflects patterns of gene flow between subpopulations and can be measured by allele frequency differences. The distribution of individuals within a subpopulation is reflected by the genotype distributions within groups in relation to that between groups. Population viscosity, as defined by the tendency to reproduce in the natal patch, also affects the relatedness estimates, increasing them in genetically subdivided populations. Genetic subdivision of populations is often considered favorable for evolutionary change, because it facilitates genetic change (Wright 1931, 1940). Social behaviour may thus be a result of selection in viscous populations (Hamilton 1964; Wilson 1980; Goodnight 1992; Taylor 1992). Secondary polygyny, the coexistence of several reproductive queens, is frequently linked to limited dispersal by sexuals and colony reproduction by budding (Cherix et al. 1991; Fortelius et al. 1993). Such limited dispersal is expected to be expressed also in the genetic population structure.

Both the population structure and the mating pattern among the individuals within that population can be determined by allozyme analysis. In this paper I will focus on the genetical population structure and its relationships to the sociogenetic organisation of colonies by comparing monogynous and polygynous societies of a single species. I will present data on genealogical relationships among workers, males, queens and their mates, including the degree of polyandry among queens and the occurrence of unequal sperm contribution of the queen's mates. This procedure allows a comparison of the mating structure in monogynous and polygynous societies, hence producing information necessary to analyse factors affecting the evolution of polyandry and to test the arguments for kin selection in the evolution of polygyny.

Methods

Population and colony samples. Formica truncorum is generally considered as a species of *Formica* s.str., although it differs ecologically, morphologically and genetically from the group of mound-building red wood-ants of the *F. rufa* group (Yarrow 1955; Collingwood 1979; Pamilo et al. 1979). The colony structure is very flexible, ranging from clearly monodomous to highly polydomous (Rosengren et al. 1985, 1986). Moreover, the monodomous colonies are generally monogynous, whereas the polydomous ones tend to be polygynous (Sundström 1989). The two different colony types are polymorphic at the same loci and show no systematic allele frequency differences between major populations.

Ants were collected from four different locations in south-west Finland and east Jutland (Mols), Denmark (Fig. 1). All of these main populations except Espoo consisted of several sites separated by either unsuitable terrain (Denmark) or water (Inkoo, Hanko). The average distances between sites were much greater in Denmark than in the Finnish populations. Table 1 gives the number of colonies sampled in each population and the type of material collected (workers, reproductive queens, males). Sundström (1989) has

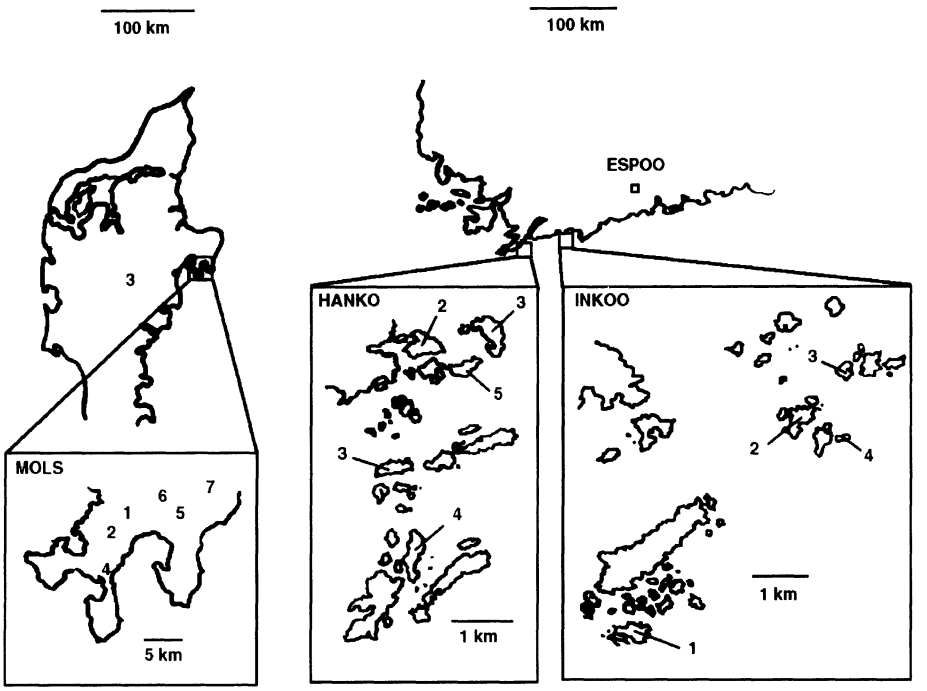

Fig. 1. The sampling sites in Denmark and Finland. The *numbers in the insets* correspond to the different sites in each population. *Insets* show the details of the Danish population in Mols, the Hanko population and the Inkoo population

shown that the colonies in Inkoo are polygynous and that the ones in Hanko are monogynous. The other populations have not previously been analysed.

Laboratory colonies. To estimate the number of matings by queens and the allele frequencies of male mates, the progeny of single queens collected from polygynous colonies in Inkoo were analyzed. The colonies sampled frequently contained well over 20 wingless, mated and functional queens. The queens were housed in ceramic flowerpots (see Pamilo 1982a) with approximately 100 workers and fed maple syrup and standard diet (Keller et al. 1989) and kept at low but constant humidity. Of 163 colonies 95 produced worker progeny and 10 produced both male and female sexuals. The corresponding data were obtained for the monogynous colonies by deducing the genotype of the queen and her mate(s) using both worker and male genotype information.

Electrophoresis. Generally 10–30 workers or worker pupae, 10 males and 10 old queens collected from the natural colonies (Table 1), and all or a maximum of 30 pupae from the laboratory colonies, were analysed by horizontal starch gel electrophoresis. If adult workers were used, the abdomens were removed and the ants rinsed in water before deep-freezing (–70°C). This procedure was not necessary for males or old queens, but the abdomens of the old queens were removed for dissections. The electrophoretic procedure is described in Seppä (1992). Three loci were studied: isocitrate dehydrogenase (*Idh*, two alleles), phosphoglycerate kinase (*Pgk*, two alleles) and aconitase (*Aco*, three alleles). All stainings used buffer system II described in Seppä (1992).

Genetic relatedness and mating structure. Genotype frequency data were used to test both the relatedness (r) among nestmates and subdivision of hierarchical populations. The population structure was analysed using F-statistics (Wright 1951). A deviation from panmixis is determined by comparing the observed (H_{obs}) and expected (H_{exp}) amounts of heterozygosity over all loci and is described by the inbreeding coefficient $F = 1-(H_{obs}/H_{exp})$. Subdivision of the population is determined as $F_{st} = (H_t-H_s)/H_t$, where H_s is the mean expected amount of heterozygosity in the subpopulations and H_t is total expected amount of heterozygosity in the whole population (Nei 1987, p. 190). The inbreeding coefficient within a population (F_{it}) can be decomposed into effects due to inbreeding within subpopulations (F_{is}) and due to allele frequency differences among subpopulations (F_{st}) where $(1-F_{it}) = (1-F_{st})(1-F_{is})$ (Wright 1943). All these parameters were calculated weighting the colonies equally. The reason for this is that sample sizes should not affect the contribution each colony makes to the population average.

Sample sizes for statistical testing must in the case of social insects be defined using the effective population size. For worker data this is the number of reproductive individuals each colony represents, corrected by the number of colonies sampled. For queen data the number of individuals sampled can be used, because in their case every individual potentially contributes to the gene pool.

The test variable and the effective sample sizes for testing the F-values were thus determined as follows (Li and Horwitz 1953):

Workers	$F_{st}\ \chi^2 = 3NF$	$N = (0.75/rn)$, where n = number of colonies analysed and r = estimated worker nestmate relatedness
	$F_{is}\ \chi^2 = NF^2$	$N = (0.75/rn)$
Queens	$F_{st}\ \chi^2 = 2NF$	N = number of individuals analysed
	$F_{is}\ \chi^2 = NF^2$	

The product NF is multiplied by 3 for workers because they represent at least three independent haploid genomes (two from the mother and one from the father) from the previous reproductive generation (Pamilo 1983).

The relatedness was measured from allele frequency data as a regression coefficient (Pamilo and Crozier 1982; Pamilo 1989, 1990b; Queller and Goodnight 1989) using the algorithm of Pamilo (1989) and the weighting scheme of Pamilo (1990b). The standard errors of the means were obtained by jackknifing over the colonies. The standard errors obtained correspond to the standard error of the mean. The relatedness estimates thus obtained were tested for deviations from zero, 0.75 or 0.25 using Student's t-distribution. The relatedness estimates are affected by both genetic differentiation of subpopulations and by local inbreeding (Pamilo 1989). Therefore the relatedness values used for estimating the effective number of reproductive females per colony were corrected for inbreeding as suggested by Pamilo (1990b). In the Inkoo population this correction yielded negative relatedness estimates both for queens and workers because the differentiation among sites was considerable. Therefore the relatedness was estimated separately for each site in

Inkoo and the mean of those estimates is used. This is in contrast to the procedure used by Seppä (1993) where inbreeding within local sites boosted the relatedness estimates. Diploid males, detected by heterozygous genotypes in any of the three loci, were found in the Inkoo population and omitted from all analyses.

If the queens or their mates are related to each other or do not contribute equally to the brood the relatedness estimates alone will not give the correct estimate of the number of reproducing individuals within colonies. Therefore, the effective number of queens is estimated using the formula of Ross (1993)

$$N_e = (4r_s - r_q - 2r_{m1})/(4r_f - r_q - 2r_{m1}) \quad (1)$$

where r_s is the average relatedness of progeny of a single queen, r_q is the average relatedness of nestmate queens, r_f is the average relatedness of nestmate workers and r_{m1} is the average relatedness among the male mates of coexisting queens in single polygynous colonies (Ross 1993). This estimate corresponds to the harmonic mean number of queens per nest if all nestmate queens reproduce equally. The present data allow the estimation of all these parameters.

Similarily, because all males may not contribute equally to the progeny of their mates and there may be queen-to-queen variation in the number of matings, the effective number of mates of a single queen is:

$$M_e = (2r_{fs} - 0.5 - r_{m2})/(2r_s - 0.5 - r_{m2}) \quad (2)$$

where r_{fs} is the average relatedness of progeny belonging to the same patriline (0.75), r_s is the average relatedness of single queen progeny and r_{m2} is the average relatedness among mates of single queens (Ross 1993). Again all the parameters needed can be estimated from the current data. This estimate corresponds to the harmonic mean number of mates per queen if these mates always share equally in paternity (Pamilo 1982a).

The relatedness between nestmate workers and males and the relatedness between queens and their mates were calculated as the regression between the two groups (Pamilo 1990b) and expressed as the regression of workers or queens on males. For the progeny of a singly mated queen the expected regression of workers on males is 0.25, but 0.5 for the regression of males on workers. Workers thus have 25% of their genes in common with their brothers, whereas males have 50% of their genes in common with their sisters. The genotypes of the male mates of queens were deduced by combining the information from worker and male and queen genotypes.

The observed number of mates of each queen was determined from the progeny by combining the genotype information from all three loci for each individual. In the laboratory colonies the queen genotype was also available. The proportion of offspring fathered by each male in double and triple matings was determined using formulas 1 and 2 from Pamilo (1993). In the formula the proportion of offspring fathered by the ith male is p_i and the relatedness $g = 0.25 + 1/2\Sigma p_i^2$. I use the parameter g to distinguish this measure of pedigree relatedness from the estimated average relatedness r among colonies. The paternity contributions of each male were corrected for a finite sample size giving $\Sigma p_i^{2'} = (N\ \Sigma p_i^2 - 1)/(N-1)$, where N is the number of individuals analysed within each colony. In this way relatedness was estimated separately for each colony with one multiply mated queen. From these data the means and variances over all colonies were calculated. Corrections for inbreeding and allele frequency differences were not made because there was no significant inbreeding in the colonies included and no allele frequency differences between mates. The relatedness for progenies of singly mated queens was calculated the same way as the average intracolony relatednesses above (Pamilo 1989, 1990b) using a subset of colonies determined as having a singly mated queen on the basis of genotype distributions. Some double (or triple) matings are not detected by this method because multiple male mates of single queens may have identical genotypes. This was corrected by calculating the probability that two males have the same genotype using the allele frequencies in the local population. Thus, if the proportion of males with an identical genotype is p and the observed proportion of double matings D_o, then the corrected proportion of double matings is: $D_c = D_o/(1-p)$.

Results

Genetic relatedness among nestmate workers, queens and males

The worker relatedness estimates from natural colonies and laboratory cultures are shown in Table 1. The variation in relatedness among populations is very high and all the populations have a relatedness estimate lower than 0.75, indicating either multiple mating by queens, polygyny or both. In the populations with high relatedness values each colony usually has only one or two offspring genotypes at each locus and in the latter case in roughly equal proportions. If there are more than two worker genotypes they can all be explained by multiple mating. This fits the assumption of monogyny and occasional polyandry. In the populations with low relatedness values single colonies frequently show several or all possible worker and male genotypes, which can only be explained by the presence of several matrilines. The relatedness estimates in the monogynous colonies correspond well to those from the laboratory cultures, while the estimates from the polygynous colonies are significantly lower ($t = 9.7$, $P < 0.0001$) and hence cannot be explained by polyandry alone.

The average relatedness estimates for both workers and queens in the Inkoo population are equal, but boosted by differentiation among islands and give negative relatednesses when corrected for differentiation among sites (islands). When each site is analysed separately, worker, queen and male relatednesses are positive but lower than the ones in the pooled data (Table 1). The effective numbers of queens are calculated based on the latter values and are presented in Table 1. Because the monogynous populations in Denmark and Hanko do not show any such subdivision of populations the inbreeding correction can be applied to the pooled data. All queens are wingless and most of them are functional, with filled spermatheca (105 filled, 14 empty) and corpora lutea scars on the ovaries (108 positive, 11 negative) indicating egg laying.

Genetic population structure

Table 2 shows the inbreeding coefficients relative to the total population (F_{it}) and decomposed into effects due to inbreeding within subpopulations(F_{is}) and due to genetic subdivision of populations (F_{st}). where $(1-F_{it}) = (1-F_{st})(1-F_{is})$. When population differentiation (F_{st}) increases the F_{it} value will also increase. An excess of heterozygotes (negative F_{is}) may mask the effects of genetic subdivision of populations. This has consequences for the relatedness estimates as they are calculated assuming that populations are in Hardy-Weinberg equilibrium, as will be explained below.

The negative F_{is} values indicate a significant excess of heterozygosity in two of the three loci among queens in both monogynous populations (Denmark and Hanko) ($\chi^2 = 13.6$, $P < 0.001$, $\chi^2 = 3.9$, $P < 0.05$). Neither the queens nor the workers show any genetic subdivision of either monogynous population. There is also a slight but

Table 1. Within-caste relatedness estimates of workers, reproductive queens and males and effective number of queens in different populations of *Formica truncorum*

Population	N	r	SE	r^*	SE	N_E	
Monogynous populations							
Denmark	Workers	91	0.60***	0.03	0.60***	0.03	1
	Males	33	0.37**	0.04			
Hanko	Workers	23	0.60***	0.05	0.59***	0.05	1
	Males	18	0.36*	0.05			
Polygynous populations							
Inkoo	Workers	89	0.25***	0.02	0.10***	0.06	16
	Queens	76	0.24***	0.02	0.07***	0.03	
	Males	52	0.14***	0.02	0.07**	0.03	
Espoo	Workers	21	0.11**	0.04	–		5.6
	Queens	11	0.00	0.04			
Laboratory colony	Workers	95	0.60***	0.05	0.59***	0.05	

N = number of colonies analysed, r = relatedness, r^* = relatedness corrected for inbreeding (Denmark, Hanko, Espoo, laboratory colony) and average relatedness on islands (Inkoo)
N_E = effective number of queens, determined using the corrected estimates (r^*)
Other asterisks indicate significant deviations from zero (reproductive queens, workers and males from polygynous colonies), 0.75 (workers from monogynous colonies and laboratory colonies) or 0.5 (males from monogynous colonies): * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 2. Genetic population structure and inbreeding coefficients

		N	F_{it}	F_{st}	F_{is}
Monogynous populations					
Denmark	Workers	91	0.08	0.03	−0.12
Denmark	Queens	85	−0.33***	0.05	−0.40***
Hanko	Workers	24	0.06	0.03	−0.09
Hanko	Queens	23	−0.34	0.05	−0.41*
Polygynous populations					
Inkoo	Workers	89	0.21***	0.19***	0.02
Inkoo	Queens	76	0.08	0.19***	−0.13***
Espoo	Workers	21	0.05		
Espoo	Queens	11	0.09		

N = number of colonies analysed
Asterisks indicate significant deviations from zero * $P<0.05$, ** $P<0.01$, *** $P<0.001$

significant heterozygote excess among the queens in the polygynous Inkoo population (χ^2 = 13.2, $P<0.001$), but not among the queens in the Espoo population (F_{it} positive) (Table 2).

The estimates of relatedness between workers and nestmate males are lower in the monogynous colonies than expected for a brother-sister relationship. This may be caused by the high excess of heterozygotes among queens in two of the three loci (Denmark: *Pgk*: $F = -0.2 \pm 0.1$, *Aco*: $F = -0.18 \pm 0.08$, *Idh*: $F = -0.03 \pm 0.1$; Hanko: *Pgk*: $F = -0.44 \pm 0.11$, *Aco*: $F = -0.30 \pm 0.13$, *Idh*: $F = -0.02 \pm 0.03$). The corresponding regression coefficients of nestmate workers on males is for the Danish population 0.10 ± 0.06, 0.11 ± 0.05 and 0.25 ± 0.08 and for the Hanko population 0.17 ± 0.1, 0.20 ± 0.06 and 1.00 ± 1.00 respectively. In Hanko *Idh* is monomorphic for the colonies that produced males. An excess of heterozygotes among queens reduces the between-nest variance and increases the intracolony diversity causing lower relatedness estimates. The genotype distributions in all loci correspond to those expected for brother-sister pairs, and also for one female (the queen) producing them. The worker-male regression is slightly lower in the polygynous colonies, but this estimate is probably boosted by the allele frequency differences among islands (positive F_{it} value).

The F_{st} values describing the genetic subdivision of the Inkoo population are similar for queens and workers and indicate considerable allele frequency differences among subpopulations. The F_{is} value is effectively zero for the workers and shows that worker allele and genotype frequencies are in Hardy-Weinberg equilibrium within subpopulations. Therefore the positive F_{it} value among workers only reflects population subdivision, not inbreeding. The allele frequencies do not differ between queens and their mates ($\chi^2 < 1.8$, $P > 0.05$ for all loci and all populations). The Espoo population does not show any inbreeding.

Number of matings

The results from the laboratory cultures show multiple mating of the queens (Table 3). The effective number of matings (M_e) is 1.43 in all populations assuming that the male mates of single queens are unrelated. The estimated relatedness among male mates of single queens (r_{m2}) is zero in all cases, whereas the average relatedness of male mates of nestmate queens is higher than zero in the polygynous Inkoo population (Table 4). This difference occurs because relatedness estimates use the background population as a reference for the degree of similarity within groups (Pamilo 1990b). The average estimate of relatedness among male mates of nestmate queens uses the other colonies in the population as reference, whereas

Table 3. The number of matings and paternity contribution of males as detected from offspring genotypes

Population	N	%	Number of mates						
			1		2		$\Sigma p_i^{2'}$	≥ 3	$\Sigma p_i^{2'}$
			Corr	Obs	Corr	Obs		Obs	
Inkoo	95	12	57	58	13	12	0.77 ± 0.05	25	0.42 ± 0.09
Denmark	93	27	70	75	20	15	0.53 ± 0.05	3	0.37 ± 0.15
Hanko	23	19	11	13	10	8	0.56 ± 0.05	2	0.65 ± 0.25

The observed values (obs) are corrected (corr) for undetected matings by males with identical genotypes. The paternity contribution is expressed as the variance in the proportion of offspring fathered by each male ($\Sigma p_i^{2'}$) with a correction for sample sizes,
% = percentage of male mates with identical genotypes calculated using the populationwise allele frequencies

Table 4. Relatedness ($r \pm$ SE) of queens to their male mates, among the male mates of nestmate and single queens and between nestmate workers and males

Population	N	n	Queen to male mate	Among the male mates of		Workers to males
				Nestmate queens	Single queens	
Inkoo	10	95	0.16 ± 0.04***	0.11 ± 0.04*	0.01 ± 0.03	0.08 ± 0.02 (23)***
Denmark	5	85	0.02 ± 0.02	0.002 ± 0.02	0.02 ± 0.07	0.14 ± 0.03 (33)***
Hanko	5	23	0.10 ± 0.06	0.005 ± 0.11	0.03 ± 0.13	0.18 ± 0.05 (18)

The number of male-producing colonies analysed is shown in parentheses
N represents the number of colonies analysed in Inkoo and the number of sites in the monogynous populations from Denmark and Hanko (the queen genotypes within each site have been pooled), n represents the number of queens
Significance levels (t-tests) are shown for deviations of r from zero (queen to male mate, among male mates of queens) or deviations from 0.25 (workers to males): *** $P<0.001$

the estimate of male mates of single queens also includes the male mates the other queens in the same colony. Because the male mates of nestmate queens on average are related this will mask any relatedness among male mates of single queens. Assuming that the relatedness of male mates of single queens is approximately the same as the average relatedness among male mates of nestmate queens in a polygynous colony ($r = 0.11$, Table 4), the effective number of mates rises only slightly ($M_e = 1.5$).

The pedigree relatedness (g) was estimated for progenies of multiply mated single queens. The expected relatedness among progenies of doubly mated queens is 0.5 if paternity contribution is equal, but this estimate will be affected by how males contribute to the offspring. The variance of paternity contribution ($\Sigma p_1^{2'}$) (Table 3) shows that paternity is unequal, corresponding to one male fathering 60–90% of the offspring with double mating and 40–80% with three or more matings. The g values were tested separately on the progeny from the laboratory colonies for 2, 3, 4 and 5 mates using $t = (x-\mu)/s_x$, where μ is the expected relatedness with equal contribution, x is the observed mean value and s_x is the standard error of the mean (Fig. 2). Only data from the laboratory colonies were used for this purpose. They are more reliable both because the queen genotypes were known and because the expected proportion of male mates with identical genotypes was low. The observed values are in the tested cases significantly higher than the ones expected at equal contribution (Fig. 2).

The relatedness between queens and their mates is

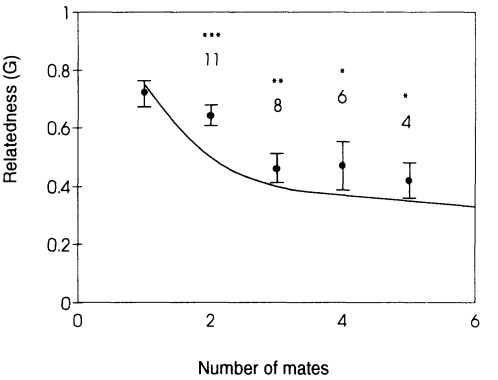

Fig. 2. Pedigree relatedness estimates (g) and 95% confidence intervals for progenies from laboratory colonies, with a queen mated one, two, three, four and over four times. The value of g is estimated separately for each colony using information on the proportion of offspring fathered by each male. The relatedness estimate for progenies of singly mated queens corresponds to the average relatedness (r). The *number of colonies* included in the analyses and *significance levels* are shown (* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$). The *solid line* shows the expected relatedness at equal contribution of males

higher than zero in the polygynous population ($t = 4.3$, $n = 10$, $P<0.01$), but not in the monogynous ones (Denmark: $t = 2$, $n = 5$, $P>0.05$; Hanko: $t = 1$, $n = 5$, $P>0.05$) (Table 4). The relatedness among the male mates of nestmate queens also differs from zero in the polygynous colonies ($t = 2.8$, $n = 10$, $P<0.05$), but not in the monogynous ones ($t = 0.1$, $n = 5$, $P>0.05$). The

queen to male mate regression is higher than that for workers to males, but the difference is not significant. The two means fall within the 95% confidence intervals of the means (Table 4).

Discussion

Genetic population structure and intracolony relatedness

The results show considerable variation in estimates of genetic relatedness among populations. This variation in relatedness patterns correspond to differences in the genetic structure of the populations. Low intracolony relatedness due to polygyny correlates with considerable genetic differentiation between subsets of main populations, whereas no such differentiation was detected in those showing high intracolony relatedness. This suggests limited dispersal and partial intranidal mating of sexuals in the polygynous population and outbreeding in the monogynous populations.

The small variation in the relatedness estimates within each population moreover indicates sociogenetically relatively uniform colonies within populations. The decrease of relatedness below 0.75 can be explained by polyandry in the populations from Denmark and Hanko, which is confirmed by the genotype distributions among known worker and male offspring of single queens. This is in correspondance with earlier results for this species (Sundström 1989). The Inkoo and Espoo populations show a clearly lower within-nest relatedness estimate and the worker and male genotypes can in most cases not be explained as being produced by a single multiply mated queen. The expected relatedness among nestmate workers decreases to 0.25 with increased polyandry. All the empirical estimates for polygynous nests are well below this value, when corrected for genetic differentiation among subpopulations. Therefore two different colony types, monogynous and polygynous, can be distinguished. The effective number of queens per nest in the Inkoo population varies between 2 and 16, assuming either zero or the observed (0.1) relatedness among queens. However, most colonies sampled for old queens contained more than 20 wingless queens, most of which were inseminated and egg-laying. The effective number of queens is very sensitive to small differences in the relatedness estimate among queens when the worker relatedness is close to zero. Therefore estimates of the effective number of queens should be treated with caution when worker relatedness is low. The relatedness estimates may be further decreased by queens being superseded and replaced. Queens of monogynous *Formica* species appear to live on average 20 years (Pamilo 1991a), but no estimates on average life span of polygynous *Formica* queens are available.

The relatedness estimates for nestmate males in the monogynous populations are significantly lower than 0.5. Similarily the worker-male regression coefficients are lower than 0.25. This is largely due to the excess heterozygosity among the queens in these populations, as the expectations of 0.5 and 0.25 assume that the populations are in Hardy-Weinberg equilibrium. Excess heterozygosity will decrease the intercolony genetic variance and increase the intracolony variance, resulting in lower relatedness estimates. Males are particularily affected by this, because they inherit their entire genome from the queen. On the basis of worker and male genotype distribution the males are likely to be produced by the queen. Worker reproduction cannot be completely ruled out, but is not likely to occur as this would, rather, increase the relatedness between workers and males.

The parameter F includes all factors that reflect genetic subdivision of populations – both increased homozygosity due to inbreeding and the Wahlund effect (Wahlund 1928) resulting from allele frequency differences among subpopulations. There was a significantly positive F_{it} value only for workers in the polygynous Inkoo population consisting of several subpopulations. Because the effective population size is very large the polygynous populations, inbreeding is expected to be low and therefore the decline in heterozygosity is expected to be slow. Decomposing the F_{it} value into its components shows that it consists almost entirely of allele frequency differences among subpopulations. This suggests limited dispersal in the polygynous population. In the monogynous populations the lack of allele frequency differences between subpopulations suggests either continuous gene flow between them, or that those populations are young and represent several independent colonizations.

Similar correlations between sociogenetic organisation and population structure have been shown in *Formica exsecta* (Pamilo and Rosengren 1984) and *Formica sanguinea* (Pamilo 1981; Pamilo and Varvio-Aho 1979), although the pattern is different. *F. exsecta* shows signs of inbreeding in monogynous populations. In *F. sanguinea* the variation in queen number is continuous and higher numbers of queens correlate with higher population viscosity (Pamilo and Varvio-Aho 1979).

Environmental factors such as habitat structure have been proposed as agents affecting the evolution of polygyny (Hölldobler and Wilson 1977; Rosengren et al. 1985, 1986). In the present case the archipelago islands represent a very uniform habitat structure both in size, longevity and quality, but still contain both monogynous and polygynous populations. Such differences in colony structure may reflect different ages of the populations studied. Monogyny is likely to preceed polygyny in *Formica* ants (Rosengren and Pamilo 1983) and the polygynous colonies may originally be founded by several independent colonizations. Thus the now polygynous populations may have had much the same population structure as the monogynous ones have now. This is partly supported by the results for the Espoo population, where the relatedness among queens is lower than that among queens in the Inkoo population. The Espoo population may represent a transition period when young females disperse, but join existing colonies yielding nonrelated queens, but related workers if the number of queens is relatively low. Subsequent intranidal mating and adoption of daughter queens will increase the relatedness among queens, as is observed in the Inkoo population.

The similarity in genetic population structure and genetic relatedness estimates between workers and queens in polygynous colonies suggest that daughter queens are recruited back to the colony. Queens were related to their mates in the polygynous societies, but not in the monogynous ones. This also suggests intranidal mating and limited dispersal in the polygynous populations, whereas nuptial flights and outbreeding is the rule in the monogynous populations. This connection between polygyny and intranidal mating and monogyny and dispersal has been suggested by behavioural studies on the present populations and several other *Formica* species (Fortelius 1987; Fortelius et al. 1993; Rosengren et al. 1993). However, the slightly lower relatedness estimates among queens than among workers in the Espoo populations indicate relatively few but unrelated queens or reproductive competition among queens.

Although there were no consistent allele frequency differences among workers, queens and males within subsets of the polygynous societies or among monogynous populations there was an excess of heterozygotes among queens, but not among workers. Such a pattern has earlier been observed in *Formica exsecta* (Pamilo and Rosengren 1984). High levels of heterozygosity may be associated with heterosis effects and may give a selective advantage during the nestfounding stage or yield faster larval development rates selecting highly heterozygous females to develop into queens.

Mating structure

Polyandry is common, with about 50% of the females being at least twice mated both in monogynous and polygynous colonies. The frequency of triple matings is higher in the polygynous societies than in the monogynous ones. This may be due to a higher availability of males because of the male-biased sex ratio (Rosengren et al. 1986; own observations). It may also be a methodological artifact because the queen genotypes in the monogynous colonies were only indirectly inferred from the worker and male genotypes and are therefore not as reliable as for the laboratory colonies. The number of matings is variable in other *Formica* ants; some are polyandrous while others are largely monandrous irrespective of the number of queens (Pamilo 1993; Pamilo et al. submitted). In general, earlier genetical studies have shown monandry or low levels of polyandry in ants (Page 1986; Ross et al. 1988; van der Have et al. 1987; Seppä 1993), although high numbers of matings have been observed in behavioural studies (Page 1986).

Multiple matings caused a smaller decrease in the relatedness estimates of single queen progenies, than expected if all males contribute equally to the progeny. The data show that the males contribute unequally. This is the rule also among other polyandrous *Formica* ants (Pamilo 1993). A problem here is that seemingly unequal contributions of the males may in fact be due to two males having the same genotype. This number is likely to be quite small because the expected proportion of males having identical genotypes is less than 12% in the laboratory colonies. However, with three or more matings the proportion of undetected matings increases. Therefore the estimates of proportion of offspring fathered by each male are less reliable when the number of matings is high. The proportion of offspring fathered by each male is, however, clearly unequal also when there are only two mates. The relatedness estimates obtained for two species of social wasps (*Vespula squamosa* and *Paravespula maculifrons*) were considerably lower than the ones obtained in this study. The females had mated with two to seven males and relatedness among the progenies were 0.40 and 0.32, sperm being used randomly for fertilizations (Ross 1986).

Polyandry is considered a key factor in increasing genetic diversity in monogynous societies (Crozier and Page 1985) emphasizing the adaptive significance of diversity. It may also be selected against if multiple mating leads to a larger fraction of colonies producing diploid males and any proportion of diploid males causes a load to the colony (Page 1980; Crozier and Page 1985). This prediction thus depends on the effect diploid males have on monogynous colonies, while polygynous ones should not show any distinction. In *Solenopsis invicta* the occurrence of diploid males caused high mortality of incipient monogynous monandrous colonies (Ross and Fletcher 1986). The monogynous and polygynous colonies of *F. truncorum* are equally polyandrous so neither scenario is unequivocally supported. With the evolution of polygyny, mating frequencies may also be expected to decline (Nonacs 1992), but this is not supported by the present results. However, if the average mating frequency is less than two, as is the case in this species, the population is expected to share more characteristics with a monandrous society than with a polyandrous society (Nonacs 1992).

Although polyandry has a minor effect on increasing diversity in polygynous societies, it may be advantageous for individual queens. If the frequency of diploid males in the population is high, females may benefit by mating multiply to ensure reception of viable sperm. A larger genetic heterogeneity among the offspring may in itself be adaptive and may also increase the chance of having some highly heterozygous individuals among the offspring. Considering the observed excess of heterozygotes among queens this may have important fitness consequences for the reproducing individuals.

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References

Boomsma JJ, Grafen A (1990) Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. Evolution 44:1026–1034

Boomsma JJ, Grafen A (1991) Colony-level sex ratio selection in the eusocial Hymenoptera. J Evol Biol 3:383–407

Cherix D, Chautems D, Fletcher DJC, Fortelius W, Gris G, Keller L, Passera L, Rosengren R, Vargo EL, Walther F (1991) Alternative reproductive strategies in *Formica lugubris* Zett. (Hymenoptera: Formicidae). Ethol Ecol Evol 1:61–66

Collingwood CA (1979) The Formicidae (Hymenoptera) of Fennoscandia and Denmark. Fauna Entomol Scand 8:1–174

Craig R, Crozier RH (1979) Relatedness in the polygynous ant *Myrmecia pilosula*. Evolution 33:335–341

Crozier RH, Consul PC (1976) Conditions for genetic polymorphism in social Hymenoptera under selection at the colony level. Theor Pop Biol 10:1–9

Crozier RH, Page RE Jr (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. Behav Ecol Sociobiol 18:105–115

Douwes P, Sivusaari L, Niklasson M, Stille B (1987) Relatedness among queens in polygynous nests of the ant *Leptothorax acervorum*. Genetica 75:23–29

Elmes GW (1980) Queen numbers in colonies of the ants of the genus *Myrmica*. Insectes Soc 27:43–60

Fortelius W (1987) Different patterns of female behaviour in mono- and polydomous *Formica* populations. In: Eder J, Remboldt H (eds) Chemistry and biology of social insects. Peperny, München, pp 293–294

Fortelius W, Rosengren, R, Cherix, D Chautems, D (1993) Queen recruitment in a highly polygynous supercolony of *Formica lugubris* (Hymenoptera; Formicidae). Oikos 67:193–200

Goodnight K (1992) The effect of stochastic variation on kin selection in a budding viscous population. Am Nat 6:1028–1040

Hamilton WD (1964) The genetical evolution of social behaviour. J Theor Biol 7:1–52

Hamilton WD (1987) Kinship, recognition, disease, and intelligence: constraints of social evolution. In: Itô Y, Brown JL, Kikkava J (eds) Animal societies: theories and facts. Japan Scientific Societies, Tokyo, pp 81–102

Have T van der, Boomsma JJ, Menken SBJ (1988) Sex investment ratios and relatedness in the monogynous ant *Lasius niger* (L.). Evolution 42:160–170

Heinze J, Lipski N, Hölldobler B (1992) Reproductive competition in colonies of the ant *Leptothorax gredleri*. Ethology 90:265–278

Hölldobler B, Wilson EO (1977) The number of queens: an important trait in ant evolution. Naturwissenschaften 65:8–15

Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin Heidelberg New York

Keller L, Cherix D, Ulloa-Chacon P (1989) Description of a new artificial diet for rearing ant colonies as *Iridomyrmex humilis*, *Monomorium pharaonis* and *Wasmannia auropunctata* (Hymenoptera: Formicidae). Insectes Soc 36:348–352

Li CC Horwitz DG (1953) Some methods of estimating the inbreeding coefficient. A. J Hum Genet 5:107–117

Moritz RFA (1985) The effects of multiple mating on the worker-queen conflict in *Apis mellifera* L. Behav Ecol Sociobiol 16:375–377

Moritz RFA, Southwick EE (1987) Phenotype interactions in group behaviour of honey bee workers (*Apis mellifera* L.). Behav Ecol Sociobiol 21:53–57

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nonacs P (1988) Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution 42:566–580

Nonacs P (1992) Male parentage and sexual deception in the social Hymenoptera. In: Wrensch DL, Ebbert MA (eds) Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York

Page RE Jr (1980) The evolution of multiple mating behaviour by honey bee queens (*Apis mellifera* L.). Genetics 96:263–273

Page RE Jr (1986) Sperm utilization in social insects. Annu Rev Entomol 31:297–320

Page RE Jr, Robinson GE, Calderone NW, Rothenbuhler WC (1989) Genetic structure, division of labor and the evolution of insect societies. In: Page RE, Breed MD (eds) The genetics of social insects. Westview, Boulder, pp 15–29

Pamilo P (1981) Genetic organization of *Formica sanguinea* populations. Behav Ecol Sociobiol 9:45–50

Pamilo P (1982a) Multiple mating in *Formica* ants. Hereditas 97:37–45

Pamilo P (1982b) Genetic population structure in polygynous *Formica* ants. Heredity 48:95–106

Pamilo P (1983) Genetic differentiation within subdivided populations of *Formica* ants. Evolution 37:1010–1022

Pamilo P (1989) Estimating relatedness in social groups. Trends Ecol Evol 4:353–355

Pamilo P (1990a) Sex allocation and queen-worker conflict in polygynous ants. Behav Ecol Sociobiol 27:31–36

Pamilo P (1990b) Comparison of relatedness estimators. Evolution 44:1378–1382

Pamilo, P (1991a) Life span of queens in the ant *Formica exsecta*. Insectes Soc 38:111–120

Pamilo P (1991b) Evolution of colony characteristics in social insects 1. Sex allocation. Am Nat 137:83–107

Pamilo P (1991c) Evolution of colony characteristics in social insects 2. Number of reproductive individuals. Am Nat 138:412–433

Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica* aquilonia. Heredity 70:472–480

Pamilo P, Crozier R (1982) Measuring genetic relatedness in natural populations: methodology. Theor Pop Biol 21:171–193

Pamilo P, Rosengren R (1984) Evolution of nesting strategies of ants: genetic evidence from different population types of *Formica* ants. Biol J Linn Soc 21:331–348

Pamilo P, Varvio-Aho S (1979) Genetic structure of nests in the ant *Formica* sanguinea. Behav Ecol Sociobiol 6:91–98

Pamilo P, Vepsäläinen K, Rosengren R, Varvio-Aho S-L, Pisarski B (1979) Population genetics of *Formica* ants II. Genetic differentiation between species. Ann Entomol Fenn 45:65–76

Pearson B (1982) Relatedness of normal queens (macrogynes) in nests of the polygynous ant *Myrmica rubra* Latreille. Behav Ecol Sociobiol 12:1–4

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat 132:217–236

Ratnieks FLW (1990) The evolution of polyandry by queens in social Hymenoptera: the significance of timing of removal of diploid males. Behav Ecol Sociobiol 26:343–348

Rosengren R, Pamilo P (1983) Sex ratio strategy as related to queen number, dispersal behaviour and habitat quality in *Formica* ants (Hymenoptera: Formicidae). Entomol Gen 11:139–151

Rosengren R, Cherix D, Pamilo P (1985) Insular ecology of the red wood ant *Formica truncorum* Fabr. 1. Polydomous nesting, population size and foraging. Mitt Schweiz Ges 58:147–175

Rosengren R, Cherix D, Pamilo P (1986) Insular ecology of the red wood ant *Formica truncorum* Fabr. 2. Distribution, reproductive strategy and competition. Mitt Schweiz Ges 59:63–94

Rosengren R, Sundström L, Fortelius W (1993) Monogyny and polygyny of Formica ants – a result of different dispersal tactics? In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford

Ross KG (1986) Kin selection and the problem of sperm utilization in social insects. Nature 323:798–800

Ross KG (1988) Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera; Formicidae). Behav Ecol Sociobiol 23:341–355

Ross KG (1993) The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. Am Nat 141:554–576

Ross KG, Fletcher DJC (1986) Diploid male production – a significant colony mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 19:283–291

Ross KG, Vargo, EL, Fletcher DJC (1988) Colony genetic structure and queen mating frequency in fire ants of the subgenus *Solenopsis* (Hymenoptera: Formicidae). Biol J Linn Soc 34:105–117

Seppä P (1992) Genetic relatedness of worker nestmates in *Myrmica ruginodis* (Hymenoptera: Formicidae) populations. Behav Ecol Sociobiol 30:253–260

Seppä P (1993) Sociogenetic organization of *Myrmica ruginodis* and *Myrmica lobicornis* (Hymenoptera: Formicidae) colonies and populations: number, relatedness and longevity of reproducing individuals. J Evol Biol, in press

Sherman PW, Seeley TD, Reeve HK (1988) Parasites, pathogens, and polyandry in social Hymenoptera. Am Nat 131:602–610

Starr CK (1984) Sperm competition, kinship and sociality in the aculeate Hymenoptera. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic, New York, pp 427–464

Stille M, Stille B, Douwes P, (1991) Polygyny, relatedness and nest founding in the polygynous myrmicine ant *Leptothorax acervorum* (Hymenoptera; Formicidae). Behav Ecol Sociobiol 28:91–96

Sundström L (1989) Genetic relatedness and population structure in *Formica* truncorum Fabr. (Hymenoptera: Formicidae). Actes Coll Ins Soc 5:93–100

Taylor P (1992) Altruism in viscous populations – an inclusive fitness model. Evol Ecol 6:352–356

Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. Science 191:249–263

Wahlund S (1928) Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre ausbetrachtet. Hereditas 11:65–106

Wilson DS (1980) The natural selection of populations and communities. Benjamin/Cummings, Menlo Park

Woyciechovski M, Lomnicki A (1987) Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. J Theor Biol 128:317–327

Wright S (1931) Evolution in Mendelian populations. Genetics 97–159

Wright S (1940) Breeding structure of populations in relation to speciation. Am Nat 74:232–248

Wright S (1943) Isolation by distance. Genetics 28:114–138

Wright S (1951) The genetical structure of populations. Ann Eugenics 15:323–354

Yarrow IHH (1955) The British ants allied to *Formica rufa* L. (Hym; Formicidae). Trans Soc Br Entomol 12:1–48