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Paternity in eusocial Hymenoptera

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SUMMARY

Variation in paternity frequency in colonies of eusocial insects has profound effects on the relatedness among offspring and on the genetic diversity of colonies. Data on queen 'mating-frequency' in eusocial Hymenoptera vary in both quality and the phase of the 'mating' process they address. Some are observational studies of the range or maximum number of copulations; others are derived from estimates of the number of sperm in males and queens; others use genetic techniques to determine the paternity of different males among female offspring. Only the latter data can be used to calculate relatedness among offspring females. Previous reviews drew attention to these problems, but their results have established the impression that high paternity frequencies are common, largely because multiple copulations are frequently observed. For ants, we show that: (i) the range of observed copulations overestimates effective paternity frequency; and (ii) the mean effective paternity frequency in 19 species, for which accurate data based on allozyme analysis of mother – offspring combinations are available, is only 1.16 (range 1–1.48). Over one third of these species have queens in which only one male contributes to paternity. Data from 34 species, which include less detailed genetic studies and four species studied using sperm counts, give similar results. Only two species, both *Atta* leaf cutter ants and both studied using data on sperm stored in queen spermathecas, appear to have effective insemination frequencies above two. Data on bees and wasps show a similar trend. We conclude that reliably documented high paternity or insemination frequencies (> 2) are currently restricted to one phylogenetically isolated and highly eusocial taxon each in ants, eusocial bees and wasps (*Atta*, *Apis* and *Vespula*, respectively). This pattern justifies the working hypothesis that multiple mating, by lowering the relatedness between female offspring and thereby the benefits of reproductive helping behaviour, has not been a general constraint for the evolution of eusociality in the Hymenoptera.

Using reliable data on paternity frequency and insemination, we re-analyse two factors that it has been suggested correlate with mating frequency: colony population and number of egg-laying queens per colony. We find the following.

1. There is a significant positive correlation between paternity/insemination frequency and colony size for monogynous ants, but not for polygynous ants. This result seems to support the 'sperm limitation' hypothesis, that queens which need to be highly fecund copulate multiply to store sufficient sperm. We note, however, that the same trend is expected when large and/or long-lived colonies profit more from having genetically diverse offspring.

2. There is no significant negative correlation between paternity/insemination frequency and number of queens per colony. However, when the analysis is restricted to species with large colonies and no intranidal mating, the correlation between paternity frequency and queen number becomes marginally significant. Several previous reviews have addressed the possible adaptive significance of multiple paternity. In contrast, and in keeping with the data that show single paternity to be frequent, we discuss selective reasons for single or low paternity. We compare the relative effects of multiple paternity and multiple maternity on genetic diversity within colonies and show that they are not equivalent, and we also discuss directions for future research on paternity issues in social insects.

1. INTRODUCTION

Studies of mating systems and reproductive altruism have played major roles in testing Darwinian and Hamiltonian theories of natural selection, sexual selection, and kin selection (Cronin 1991). A major goal in the study of mating systems is to understand the evolution of particular mating strategies, such as the number of partners mated with (Thornhill & Alcock 1983; Ridley 1988; Keller & Reeve 1995). Males will almost always benefit from having multiple partners because this readily leads to their siring more offspring, but the benefits for females are less obvious.

Variation in queen mating-frequency in the eusocial Hymenoptera is of particular biological interest for two main reasons (for definitions of mating, insemination, paternity and other terms see table 1). The first is the effect of paternity frequency on the kin structure of colonies, and hence on the evolution of social behaviour. In particular, effective paternity frequency is significant in the origin, maintenance, and modification of worker behaviour in the Hymenoptera.

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Table 1. *Definitions referring to copulation, insemination, and paternity of females*

copulation frequency	the number of males that copulate with a queen whether or not sperm are transferred or stored
insemination frequency	the number of males with sperm stored in the spermatheca regardless of relative contributions
effective insemination frequency	the reciprocal of the sum of the squared proportional sperm contributions
paternity frequency	the number of males that father offspring of a queen regardless of relative contributions
effective paternity frequency[a]	the reciprocal of the sum of the squared proportional paternities (Pamilo 1993; Queller 1993*b*); deviations from equal paternity are also referred to as 'sperm bias' (Cole 1983)
mating frequency	a general term which might, depending on context, refer to any or all of the above
monandry, polyandry	general terms having the meaning single mating and multiple mating by queens

[a] Effective paternity frequencies based on a single sample of females (i.e. one sampling time) are sometimes referred to as 'short term'. Here the duration for which the estimate applies is approximately the duration of the stage sampled (i.e. adult worker longevity when adult workers are sampled, unless a particular cohort is sampled). Samples can be combined to calculate an effective paternity frequency (i.e. 'long term' effective) over any specified period up to the life of the queen. Effective paternity frequencies are often calculated as population-wide estimates, but can be calculated for a single queen or any set of queens.

Theoretical studies show that single paternity, or low frequencies of multiple paternity, favour the evolution of eusociality, especially when combined with female-biased sex ratios (Hamilton 1964, 1972; Trivers & Hare 1976; Cole 1983; Frank & Crespi 1989) or worker production of males (Charnov 1978). High paternity frequencies could prevent or reverse eusociality (Hamilton 1964; Wilson 1971; Pamilo 1991*a*). High paternity frequencies may also lead to the evolution of less female-biased sex-allocation (Trivers & Hare 1976; Benford 1978; Boomsma & Grafen 1990, 1991), and worker-policing (the mutual inhibition of male production by workers) (Starr 1984; Ratnieks 1988; Ratnieks & Visscher 1989).

The second main reason is the contrast between the eusocial Hymenoptera and non-social animals concerning the causes of multiple paternity. Of eight general advantages hypothesised for females that mate multiply (Ridley 1988; Keller & Reeve 1995), several cannot apply to the eusocial Hymenoptera, others are likely to be relatively less important, whereas one – increased genetic diversity among offspring females – is hypothesised to be of much greater relevance (table 2). These differences in relevance can be attributed to two main causes. First, idiosyncracies of the social Hymenoptera, such as queens not remating after beginning to lay eggs (or even of the Hymenoptera; there is a general absence of nuptial gifts by hymenopteran males) but which are probably not consequences of social life. These differences are mentioned in table 2. Second, and perhaps of greater interest, are the ways in which social life itself can influence the costs and benefits of additional matings by queens.

Concerning the influence of social life on queen mating-frequency, the most important hypothesis in table 2 'increased genetic variation among offspring' has given rise to five distinct subhypotheses which differ largely in the mechanism by which variation in worker diversity may influence colony performance, survival, or reproduction, and hence queen fitness. For example, greater diversity is variously hypothesised to increase the queen's inclusive fitness by: (i) enhancing colony division of labour (Crozier & Page 1985; Page & Robinson 1991); (ii) enhancing colony tolerance to environmental conditions (Crozier & Page 1985); (iii) reducing the deleterious effect of diploid male production on colony performance (for details see Crozier & Page 1985; Ratnieks 1990*a*; Pamilo *et al.* 1994); (iv) increasing colony survival against diseases (Hamilton 1987; Sherman *et al.* 1988; Shykoff & Schmid-Hempel 1991; Schmid-Hempel 1994); and (v) causing the workers to produce a more male-biased colony sex allocation ratio (Moritz 1985; Queller 1993*a*; Ratnieks & Boomsma 1995). The only other hypothesised advantage for multiple mating by queens that is a consequence of social life is a modified version of the sperm replenishment hypothesis (table 2): queens in species with large colonies copulate with multiple males to store sufficient sperm to ensure high fertility (Cole 1983). This hypothesis does not require that genetic diversity be increased, although this will happen to a greater or lesser extent, depending on sperm clumping, as an unselected consequence.

Several reviews (Page & Metcalf 1982; Cole 1983; Starr 1984; Page 1986; Keller & Reeve 1994) indicate that multiple mating occurs in many species of eusocial Hymenoptera. However, the extent to which multiple copulation leads to multiple paternity among offspring females has been questioned (Page & Metcalf 1982; Starr 1984; Page 1986) and remains uncertain to date. In this review we assess the evidence for multiple paternity, and to a lesser extent multiple insemination, in the eusocial Hymenoptera. First, we show that most studies of mating frequency in ants have been observations of copulation and that the number of copulations is greater than insemination or paternity frequencies, as estimated by studies of sperm or the genetic analysis of female offspring. Second, we review the reasons why observations of copulation are likely to overestimate paternity frequency. We also review the theory on effective mating frequency and variance in paternity. With the aim of making the theory accessible to a wider audience, we provide geometric illustrations of the main points. Third, we review the reliable comparative data (i.e. based on genetic analyses or sperm counts) on paternity and show that population-wide effective paternity frequencies in most ant species are close to one. A similar pattern occurs in eusocial

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Table 2. *Hypothesised general advantages of multiple mating for female animals and their probable relevance for queens of eusocial Hymenoptera*

(Data based on previous listings in Ridley 1988 and, particularly, Keller & Reeve 1995. Note that we do not address the relative merit of these hypotheses for female animals, but only suggest their comparative relevance for queen Hymenoptera.)

advantages for non-social females	comparative relevance for queen Hymenoptera	reasons for difference in relevance
sperm replenishment	not relevant or relevant with modification	Queens cannot remate to replace sperm because mating takes place only during a short period early in adult life before egg-laying begins. Queens store a lifetime supply of sperm in their spermathecas. Selection has resulted in some species having extremely large spermathecas (e.g., *Apis*, *Atta*). The need to store large numbers of sperm may have caused multiple mating in species with highly fecund queens (Cole 1983, see text). Note however, that in *Apis mellifera* queens only store one male's worth of sperm despite mating with many males (see text). Sperm replenishment by remating occurs in termites.
nutrient gifts from males	less relevant	Extra nutrients might be advantageous during the colony founding phase in species with independent colony founding, but would be insignificant in swarm-founding species (e.g., *Apis*), or in situations where queens are accepted into an established nest (dependent colony founding). Ant queens normally mate directly after leaving their natal nest, and they only take part in nuptial flights when fully mature, i.e. after having acquired all the resources needed for successful colony-founding from their nestmate workers. Male gifts have not been documented or inferred for any eusocial hymenopteran species, and are also not reported in non-social Hymenoptera.
extra-pair copulation with high quality males	not relevant	Eusocial Hymenoptera only pair briefly during mating and females do not remate later in life.
bet-hedging against sterility of the first male	equally relevant	In exceptional mating systems such as that of the honey bee, this advantage may become negligible.
male offspring have more competitive sperm (because they are descended from sperm that was subject to competition within the mother)	less relevant in general; possibly of great relevance in some species	Because a male has no father in the Hymenoptera, any advantages due to more competitive sperm accrue to grandsons not to sons. It is likely that this will reduce any advantage from multiple mating to female Hymenoptera. Some social insects may provide excellent opportunities for sperm competition, such as *Apis mellifera* in which the sperm from many males are deposited in the oviduct during a single mating flight, and then subsequently enter the spermatheca (see text).
increased genetic variation among offspring	more relevant	This hypothesis is probably the one with the greatest relevance to eusocial Hymenoptera because the queen's fitness in single-queen species depends almost totally on her worker offspring, and there are potentially many ways in which worker diversity can affect colony performance or reproduction. Important subhypotheses are: (i) enhancing division of labour; (ii) tolerance to a wider range of environmental conditions; (iii) reducing the deleterious effect of diploid male production; (iv) enhanced disease tolerance; (v) more male-biased production of sexuals. (see text). In *Apis mellifera* there is evidence that multiple mating reduces the deleterious effect of diploid males.
phenotypic correlation between the sexes (Halliday & Arnold 1987)	less relevant in many species	In many eusocial Hymenoptera males give all their sperm to the first queen they mate with (e.g., the argentine ant, Keller & Passera 1992) or die after mating once. In these species selection has favoured single mating in males so any genetic correlation to females would cause single mating by queens. In particular, multiple mating by honey bee queens cannot be caused by a genetic correlation because males die on their first mating (Sherman & Westneat 1988).
to stop being pestered by a male	less relevant	Because queens only mate on one or a few occasions they are only susceptible to male pestering for a relatively short period.

bees and wasps. Fourth, using only data on effective paternity and insemination frequencies, we re-evaluate two cross-species correlations with mating frequency that have been reported for ants: (i) a positive correlation between mating frequency and colony population (Cole 1983); and (ii) a negative correlation between mating frequency and the number of egg-laying queens per colony (Keller & Reeve 1994).

Although both trends are in the predicted direction when using paternity data, only the former is highly significant. This implies that monogynous (single queen per colony) ant species with large colonies have a higher genetic diversity among nestmate workers than species with small colonies. Finally, in concluding, we discuss the selective advantage of single or low paternity to queens, compare the relative effects of multiple paternity and multiple maternity on nestmate relatedness, stress the importance of taking the cost of copulations into account, and make suggestions for further study.

2. COMPARATIVE DATA ON QUEEN MATING-FREQUENCY

Page & Metcalf (1982) and subsequent reviewers have categorized four types of data on queen mating-frequency: observations of copulations (O); sperm counts of newly inseminated queens and virgin males (D); estimates of paternity, based on visible genetic markers (V); estimates of paternity based on allozyme markers (A). The genetic methods (A,V) allow the relative paternity of a queen's mates to be assessed (but not the number of copulations if some copulations do not result in offspring), so that accurate estimates of effective paternity frequency can be made. If sperm clumping (see below) occurs then method (D) can overestimate effective paternity frequency. If sperm clumping is negligible, method (D) can underestimate the effective paternity frequency when queens store only part of the sperm they receive during copulation or when males contribute less than all of their sperm. Tschinkel & Porter (1988) report that in exclusively single mating fire ants, only about two thirds of a male's sperm gets stored in the queen's spermatheca. On the other hand, 97% of the sperm in the queen's oviduct was found to enter the spermatheca in single-mating *Melipona* bees (Kerr *et al.* 1962). An extreme example of non-storage is found in the honey bee, *Apis mellifera*, where the number of sperm in a queen's reproductive system directly after mating (reflecting the number of inseminations) is 5–25-fold higher than the number eventually stored in the spermatheca (Kerr *et al.* 1962; Page & Metcalf 1982; Koeniger 1991; Moritz & Southwick 1992). A similar but less extreme case was recently documented for the leaf-cutter ant *Acromyrmex versicolor* (Reichardt & Wheeler 1996).

Method (D) can thus reliably prove multiple insemination, when queens store more sperm than is produced by a single male, but not single insemination (Page & Metcalf 1982). We contrast data of the (O) category, which are hypothesised to overestimate effective paternity frequency, with the (D, V, A) data which are unbiased (A, V) or may overestimate or (more likely) underestimate paternity (D). Five increasing and overlapping datasets were available (Page & Metcalf 1982; Cole 1983; Starr 1984; Page 1986; Keller & Reeve 1994), plus additional material published subsequently.

More species of ants have been studied than eusocial bees and wasps (table 3). For bees and wasps the proportions of records based on observations (O)

Table 3. *Number of records on species specific queen mating-frequency, subdivided according to method to contrast observations of copulation (O) with other methods: sperm counts (D), visual genetic markers (V), and allozyme genetic markers (A)*

(Where more than one method was used per species, a score was made in both method categories. The results of 2×2 G-tests (with Williams correction) for independence of mating frequency category and method category are given in the final two columns. N.S. means $P > 0.1$.)

	queen mating-frequency					
	single		multiple		significance	
method	D, V, A	O	D, V, A	O	G_w	P
eusocial wasps						
Page & Metcalf 1982	0	0	2	0	—	—
Cole 1983	0	3	3	3	2.56	N.S.
Starr 1984	3	3	2	3	0.10	N.S.
Page 1986	1	2	4	3	0.41	N.S.
total	4	3	5	5	0.08	N.S.
eusocial bees						
Page & Metcalf 1982	1	4	2	4	0.21	N.S.
Cole 1983	2	4	3	6	0.00	N.S.
Starr 1984	1	0	1	0	—	—
Page 1986	3	4	5	7	0.02	N.S.
total	5	4	5	7	0.37	N.S.
ants						
Page & Metcalf 1982	6	1	1	15	13.7	< 0.001
Cole 1983	6	4	1	21	11.0	< 0.001
Starr 1984	3	1	2	10	3.92	< 0.05
Page 1986	6	6	6	26	3.81	$= 0.05$
Keller & Reeve 1994	17	9	8	30	12.6	< 0.001
total	20	10	8	32	15.6	< 0.001

versus other methods (D, V, A) are approximately equal, and within each category (O versus D, V, A) the proportions of species with multiple mating are similar. This equality holds when the bee and wasp data are combined ($G_w = 0.42$, not significant). However, the more abundant data on ants are highly unbalanced, both with respect to method and conclusions. There are about 1.5-fold as many species in the (O) category than in the (D, V, A) categories. More importantly, observational data (O) suggest that multiple copulation is significantly more common than single copulation, whereas the (D, V, A) data suggest paternity or insemination frequencies close to 1 are most common (table 3). The preponderance of (O) data in ants, compared to the more even distributions in social wasps and bees, may reflect the ease of observing mating aggregations of ants, at least in species that mate on the ground or on vegetation. However, this does not explain the discrepancy between the (O) and (D, V, A) data. The most likely explanation for this discrepancy is that copulation frequency is, in fact, a poor predictor of paternity frequency. The discrepancy has several possible causes, as is shown next.

3. DISCREPANCY BETWEEN COPULATION AND PATERNITY FREQUENCY

(*a*) *Small datasets and other problems*

Many data on copulation were not collected for the purpose of estimating queen copulation frequency. In some cases meaningful estimates of mean copulation frequency are impossible as sample sizes are either very small, or because only the maximum observed number of copulations is reported. This is illustrated by the following examples. The ant *Myrmica ruginodis* (previously called *Myrmica rubra*, whereas the current *Myrmica rubra* was previously called *Myrmica laevinodis* ; see Yarrow 1955) was classified as having 5–6 matings per queen (Page 1986; Keller & Reeve 1994). This is based on the statement by Forel (1930) that 'five or six males can be seen mating with the same female'. However, allozyme analysis of 34 mother – offspring combinations of this species, but of another population (Seppä 1994*a*), showed that multiple paternity occurred in only about 21% of broods, and that the effective population-wide paternity frequency was only 1.07 (table 5). Similarly, Woyciechowski (1990) stated that queens of the related species *Myrmica rubra* are likely to copulate singly under natural conditions, although multiple copulation (range 1–7; arithmetic mean 2.15; harmonic mean 1.46) could be induced when queens were confined in a tube and presented with a sequence of males. Allozyme data from another population of *Myrmica rubra* show that double paternity occurs frequently, but that the effective population-wide paternity frequency is $\leqslant 1.35$ (Seppä 1994*b*; table 5). Another ant datum (Page & Metcalf 1982; Page 1986; Keller & Reeve 1994), the mating frequency of 4 for *Mycocepurus goeldii* (Kerr 1961), is based on a single queen. Observations of multiple copulations in *Formica rufa* (Marikovsky 1961), *Brachymyrmex depilis* (Page 1982), *Formica opaciventris* (Scherba 1961) and *Formica bradleyi* (Halverson *et al.* 1976) also have problems: sample sizes are either very small or not given at all, and the only justifiable conclusion is that some queens were seen to copulate with several males. If the majority of queens copulated only once or if unequal sperm transfer, unequal sperm storage, or sperm clumping occurred, these data are compatible with population-wide effective paternity frequencies close to 1. Such 'multiple mating' records may in fact represent a similar mating system as those of *Pheidole sitarches* (Wilson 1957) and *Formica dakotensis* (Talbot 1971) where double mating was observed as a more or less rare event, such that they were later categorized as single mated (Page & Metcalf 1982; Cole 1983; Starr 1984; Page 1986; Keller & Reeve 1994).

Even well-replicated quantitative studies on copulation frequency in queens, such as those on *Pogonomyrmex* ants (Hölldobler 1976; Mintzer 1982) may be unsuitable for inferring effective paternity frequency in the absence of confirmation from genetic studies. In both studies *Pogonomyrmex* queens were observed to mate with 1–6 males. However genetic studies of *Formica aquilonia* have shown that queens inseminated by this range of males have an effective mean paternity frequency of only 1.48 (Pamilo 1993; Fortelius 1994; see also table 5).

(*b*) *Causes of unequal male contributions*

Copulation frequency and effective paternity frequency are equal when every male that copulates with a queen has an equal and constant share in paternity. However, paternity is frequently unequal (e.g. Page & Metcalf 1982; Ross 1986; Pamilo 1993; Sundström 1993*a*). Inequality may result from many factors (table 4).

Table 4. *Successive stages in the 'mating' process of queens and factors that may cause changes in the proportional representation of males at each stage*

(Definitions were modified and extended from Page 1986.)

stage 1	copulation to insemination (sperm acquisition)	non-transfer of sperm during copulation; unequal transfer of sperm during copulation; sperm removal or displacement by later males; competition among sperm from different males; queen mechanisms promoting equal or unequal sperm storage
stage 2	insemination to fertilization of eggs (sperm utilization)	sperm clumping; differential sperm death; sperm competition in spermatheca; sperm competition after release from spermatheca
stage 3	fertilization to production of immature or mature offspring	differential mortality among patrilines; differential caste distribution among patrilines (queen, worker)

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Table 5. *Population-wide effective paternity frequency* (m_e) *and relatedness among offspring females* (g *and* b_{ff}) *in 19 ant species with complete allozyme data*

species of ant (*specified population*)	no. of queens[b]	mating[c]	no. broods	no. off-spring	no. loci variable	H[d]	sperm bias[e]	proportion double paternity: observed	detectable[f]	expected[g]	Σp_i^2[h]	paternity m_e[i]	paternity category	relatedness g[j]	relatedness b_{ff}[k] ± S.E.	references
Aphaenogaster rudis	s	no data	47	12.0	1	0.404	—	0	0.41	⩽ 0.05	—	⩽ 1.02	s	⩾ 0.74	0.83 ± 0.06[l,p]	Crozier 1973,1974
Colobopsis nipponicus	s	no data	59	20.1	1	0.264	—	0	0.21	⩽ 0.08	—	⩽ 1.03	s	⩾ 0.73	0.75 ± 0.05[p]	Hasegawa 1994
Conomyrma insana	s	no data	34	8–32	1	0.206	—	0	~0.15	⩽ 0.19	—	⩽ 1.09	s or s–d	⩾ 0.71	cannot be estimated	Berkelhamer 1984
Formica aquilonia[a]	m	l, i	113	22.4	6	0.351	0.77	0.57	0.84	0.67	0.643	1.48[m]	s–m	0.61	0.60 ± 0.02[n,o]	Pamilo 1993
Formica exsecta (Joskar)	f	l	19	22.3	1	0.283	0.78	0.11	0.28	0.37	0.637	1.16	s–d	0.68	0.62 ± 0.13[p]	Pamilo & Rosengren 1983, 1984; Fortelius *et al.* 1987
Formica pressilabris	m	no data	46	10.8	2	0.377	0.74	0.15	0.48	0.32	0.678	1.12	s–d	0.70	0.66 ± 0.06[n,o]	Pamilo 1982*a*
Formica sanguinea	f	l	24	7.1	1	0.310	0.73	0.08	0.15	0.55	0.564	1.31	s–d	0.63	0.56 ± 0.14[n,o]	Pamilo 1982*a*; Pamilo & Varvio Aho 1979
Formica transkaucasica	f	no data	60	9.9	1	0.285	0.93	0.03	0.13	0.25	0.865	1.03	s–d	0.73	0.74 ± 0.08[n,o]	Pamilo 1982*a*
Formica truncorum[a,q]	s or m	l	116	24.0	3–4	0.360	0.67	0.43	0.77	0.56	0.560	1.43[m]	s–m	0.60	0.60 ± 0.05[p]	Rosengren *et al.* 1986; Sundström 1989, 1993*b*
Harpagoxenus sublaevis	s	k	49	20.0	2	0.281	—	0	0.43	⩽ 0.05	—	⩽ 1.02	s	⩾ 0.74	0.74 ± 0.04[o]	Bourke *et al.* 1988
Lasius niger (Amsterdam)[a]	s	m, l	31	7.9	2	0.327	0.74	0.29	0.37	0.79	0.599	1.46	s–d/s–m	0.59	0.61 ± 0.10[o]	Van der Have *et al.* 1988
Lasius flavus	f	m, l	25	8.5	1	0.127	—	0	0.10	⩽ 0.39	—	⩽ 1.20	s or s–d	⩾ 0.67	0.71 ± 0.11[n,o]	Boomsma *et al.* 1993
Lasius neglectus	m	i	11	6.1	1	0.299	—	0	0.13	⩽ 0.63	—	⩽ 1.36	s or s–d	⩾ 0.62	0.66 ± 0.18[n,o]	Boomsma *et al.* 1990
Leptothorax pergandei	s	l	12	10.1	2	0.262	0.92	0.08	0.31	0.27	0.858	1.04	s–d	0.73	0.76 ± 0.04[n,r]	Heinze *et al.* 1995
Myrmica rubra	f or m	m, l	22	7.3	3	0.124	0.73	0.18	0.28	0.65	0.733	1.21	s–d	0.66	0.62 ± 0.12[n,o,s]	Seppä 1994*b*, unpublished
Myrmica ruginodis	f	m, l	34	8.1	4	0.400	0.71	0.15	0.71	0.21	0.678	1.07	s–d	0.72	0.72 ± 0.03[n,o]	Seppä 1994*a*
Solenopsis invicta	s or m	m, l	55	26.5	2	0.460	—	0	0.74	⩽ 0.02	—	⩽ 1.01	s	⩾ 0.74	0.71 ± 0.05[p]	Morrill 1974; Ross & Fletcher 1985
Solenopsis richteri	f	m, l	58	16.7	2	0.254	—	0	0.49	⩽ 0.03	—	⩽ 1.02	s	⩾ 0.74	0.74 ± 0.04[p]	Markin *et al.* 1971; Ross *et al.* 1987, 1988
Solenopsis geminata	f	m, l	30	17.3	5	0.246	—	0	0.77	⩽ 0.04	—	⩽ 1.02	s	⩾ 0.74	0.79 ± 0.03[p]	Ross *et al.* 1987, 1988; Ross, pers. com.
mean			44	14.3	2.1	0.296	0.77	0.11	0.41	⩽ 0.32	0.68	⩽ 1.16		⩾ 0.69	0.69 ± 0.02	

[a] Species where paternity frequencies higher than two were also observed; these were considered as double paternity in the calculations for the columns 'observed', 'detectable', and 'expected'.
[b] s: Always one queen per nest (monogyny); f: usually one, but occasionally several queens per nest; m: always many functional queens per nest (see text).
[c] Massive (m), local (l), intranidal mating (i) and pheromone-calling females (k) (Hölldobler & Bartz 1985).
[d] Average heterozygosity over the variable loci used in the calculations.
[e] The mean sperm bias given is equivalent to the variable c, used in the text; it is calculated as the arithmetic mean of the largest male's paternity in colonies where two patrilines occurred.
[f] Detection efficiencies calculated according to Pamilo (1982*a*), using either the average sperm bias observed, or an estimated fixed value of $c = 0.7$ for the cases in which no double matings were observed.
[g] For the cases where no double matings were observed, an upper limit was estimated by assuming that the next sample (nest $n+1$) would have been a recognizable two-father nest.
[h] Calculated from offspring samples ⩾ 5, applying the correction for finite sample size given by Pamilo (1993; our eq. 6).
[i] m_e, the effective paternity frequency, is calculated from the expected proportion of double matings and Σp_i^2, i.e. by averaging equation 2 over all colonies.
[j] g, the relatedness among offspring females, is directly derived from equation 1, and can maximally be 0.75 under random mating (see text).
[k] b_{ff}, the regression relatednesses, is based on offspring data alone, estimated according to Pamilo (1984).
[l] Calculated from the data for the Turnerville and Greensboro populations.
[m] Estimates taken directly from the source papers, taking also into account Σp_i^2 values for queen-matings higher than 2.
[n] Estimate based on offspring reared from single queens.
[o] Estimates from mature field colonies are considerably lower, due to polygyny (see also Pamilo 1981, 1982*b*; Rosengren *et al.* 1993).
[p] Estimate based on monogynous field colonies.
[q] Data are only from monogynous populations at Tvärminne and Mols.
[r] Relatedness estimate equals 0.886, but inbreeding was also significant ($F = 0.312$). The estimate corrected for inbreeding (cf. Pamilo 1993) is given here.
[s] Data taken only from the largest of two neighbouring sites, so that complications due to inbreeding and population substructuring were avoided.

The transfer of different amounts of sperm may be due to differences in inseminating ability among males. Some males may transfer less because their reproductive organs are less well developed, because they have mated before, because they are saving sperm for future matings, or because the copulation is terminated early. In this context it is important to note that males of eusocial Hymenoptera may be severely sperm limited. With only one known exception (Heinze & Hölldobler 1993), testes of ant males degenerate shortly after eclosion. This implies that their total amount of sperm is fixed and that they cannot develop more as adults (Passera & Keller 1992). This also applies to honey bees (Winston 1987), but it is unclear to what extent the same is true for other eusocial bees and wasps. In ants, copulation duration tends to decrease with increasing number of matings for queens, but to increase with increasing number of matings for males (Hölldobler 1976; Woyciechowski 1990; Fortelius 1994). Multiple matings (and sperm-saving tactics) by individual males are perhaps most likely when males are relatively long-lived and can forage or return to their natal nests between mating flights to feed. This seems to be the normal situation for many social wasps (Alcock *et al.* 1978; Ross & Carpenter 1991) and bumble bees (Alford 1975, p. 76; Jennersten *et al.* 1991), but is rare in ants, except when mating takes place in or close to the nest (Buschinger & Alloway 1979; Passera & Keller 1992; Fortelius *et al.* 1993). Honey bee males return to the nest after unsuccessful mating flights (Howell & Usinger 1933; Free & Williams 1975) but can only mate once. Many ants have mating flights in which males are only capable of mating for a few hours and cannot return to their natal nests (Starr 1984). In addition, in many ants (e.g. *Lasius*; see Boomsma *et al.* 1982) males are much smaller than queens, resulting in a male-biased numerical sex ratio. These factors would render multiple copulation opportunities unlikely for males, thereby removing most of the benefits of conserving sperm (but see Boomsma 1996). The most extreme cases of male-biased numerical sex ratios are found in species which reproduce by colony fission, as in honey bees, stingless bees, and army ants (reviewed in Page & Metcalf 1984), or budding, as in argentine ants (Keller & Passera 1992). In the first three cases males die as a result of their first and only mating (Page 1986). The opposite situation may occur in species that have relatively robust males that are similar in size to queens (e.g. *Pogonomyrmex*). Here numerical sex ratios are more equal or even female biased (see figure 2, Boomsma 1989) and males may be able to survive outside the nest for several days, thereby making additional copulation possible, as observed (Nagel & Rettenmeyer 1973; Hölldobler 1976; Davidson 1982; Mintzer 1982). Finally, it is important to note that the mating swarm sex ratio may vary in time (Talbot 1972; Nagel & Rettenmeyer 1973). When males predominate, especially at the beginning of a mating flight, they are perhaps more likely to save sperm in anticipation of subsequent mating opportunities.

Queens may also influence paternity by their willingness to copulate multiply (O'Neill 1994) and by post-copulation factors which affect sperm storage. Insect sperm competition and storage have mostly been studied in non-social insects (e.g. Parker 1970; Walker 1980; Thornhill & Alcock 1983; Ridley 1989; Zeh & Zeh 1994) or honey bees (Page 1986). In *Apis mellifera* and *A. cerana*, torn-off male genitalia function as mating plugs (Koeniger 1991), which prevent sperm from leaking out of the queen (Woyciechowski *et al.* 1994). The mating plug does not prevent additional matings, and even makes queens more attractive for subsequent drones (Koeniger 1991). Males do not fight for access to queens (Koeniger 1986) and they seem to leave each others' ejaculates in place (Walker 1980). In the two species of little honey bee, *A. florea* and *A. andreniformis*, male morphology suggests that sperm are injected directly into the spermatheca, leading to the possibility of sperm displacement. In these two species males produce less sperm and mating signs are lacking (Koeniger *et al.* 1989, 1990). After copulation *A. mellifera* queens gradually expel the sperm in their reproductive tract while slowly (40 h) filling the spermatheca, which seems to promote equal storage of sperm from each male (Page 1986; Koeniger & Ruttner 1989). There is no evidence for sperm competition after instrumental insemination (Page 1986). Mating plugs, preventing further insemination, occur in *Melipona* stingless bees (Kerr *et al.* 1962). However, in *Melipona* it is possible that queens remate later in life (Campos & Melo 1990), as has also been suggested for *Eciton* army ants (Rettenmeyer 1963).

In non-social insects the absence of mechanisms promoting equal sperm storage may act as a constraint against multiple insemination and ultimately multiple paternity, often benefitting the last male to mate (Birkhead & Hunter 1990). In contrast, in the honey bee it is likely that mechanisms under queen control actively promote sperm mixing in the oviduct (see Koeniger & Ruttner 1989 and references therein). In the absence of such mechanisms, multiple copulations may result in unequal sperm storage when males provide queens with more sperm than is eventually stored (Garófalo 1980; Page & Metcalf 1982; Starr 1984).

A potentially important but less well-studied factor that can cause unequal paternities is sperm clumping, manifested as temporal changes in paternal contributions due to non-random sperm use. Depending on the timescale, clumping can have various effects on the genetic diversity of and relatedness among the female offspring (figure 1). Trivers & Hare (1976), referring to Taber's (1955) study of the honey bee (see also Martinho & Goncalves 1978), suggested that sperm clumping in the eusocial Hymenoptera would result in effective paternity frequencies close to 1, even when multiple insemination occurred. Later authors argued that, in spite of fluctuations in paternity, patrilines are approximately constant in their long-term representation in honey bees (Crozier & Brückner 1981; Page & Metcalf 1982; Laidlaw & Page 1984; Page *et al.* 1984; Estoup *et al.* 1994). Page & Metcalf (1982) caution that this does not mean that sperm use is completely random. Laidlaw & Page (1984) show that the average short-term effective mating frequency

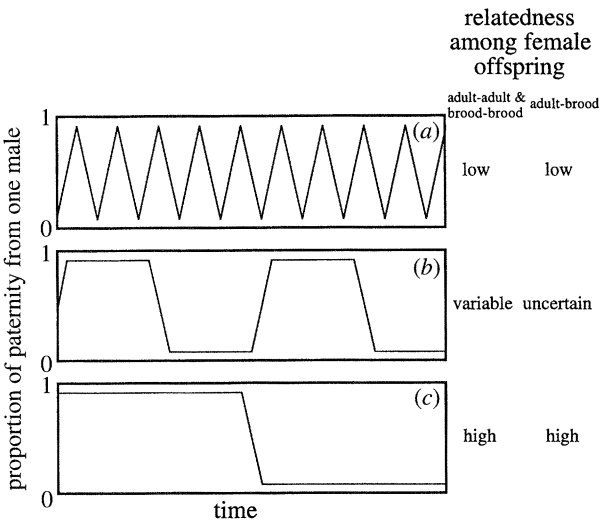

Figure 1. Effect of sperm clumping on genetic diversity of, and relatedness among, female offspring in a colony headed by a single queen inseminated by two unrelated males. In all three cases the long term effective paternity frequency is the same (ca. 1.5). Differences in the timescale of clumping, however, cause various effects on diversity and relatedness. In (*a*) the timescale of clumping is short so diversity is high and relatednessis low. Short term effective paternity frequency is close to 1.5. In (*c*) the timescale of clumping exceeds the duration of the adult+brood lifespan, giving high relatedness and low diversity over most of the colony life cycle. Short-term effective paternity frequency is close to one, except where sperm use changes from one male to the other. In (*b*) the timescale is intermediate. This leads to higher relatedness within the specified cohorts of adults (i.e. workers) or brood than the relatedness betweeen brood and adults. Diversity and relatedness of the specified adult cohort is the same as in (*c*).

in honey bee queens is lower than the lifetime effective mating frequency, which is in turn lower than the insemination frequency. In their study, two thirds of the difference between the number of inseminations and the short-term effective number of matings appeared to be due to events occurring before the sperm are stored in the spermatheca, whereas the remaining one third primarily reflected weekly and seasonal fluctuations that were probably caused by sperm clumping in the spermatheca (see table 9 in Laidlaw & Page 1984).

The clear indications of sperm clumping in the honeybee (Taber 1955; Martinho & Goncalves 1978; Laidlaw & Page 1984; see also Kerr *et al.* 1980) seem in contrast with the more constant paternities in the offspring of single queens across monthly samples in *Vespula* yellowjackets (Ross 1986) and in early versus late season samples in *Polistes metricus* (Metcalf & Whitt 1977). Although some theoretical predictions on sperm clumping were recently derived (Boomsma 1996), it is at present impossible to draw general conclusions about sperm clumping in eusocial Hymenoptera. Future work may find species in which lifetime effective paternity frequencies are considerably higher than short-term effective paternity frequencies, or that some males' sperm never enter or leave the spermatheca. Sperm clumping may also be affected by the number of matings of a queen. In a pseudoscorpion, approximately equal paternities were found in females that had mated with three males, whereas significant last male precedence occurred when females mated twice (Zeh & Zeh 1994). In this review, we concentrate on short-term effective mating frequencies (duration approximately the lifetime of the stage studied) as too few studies have explicitly looked at the composition of the brood of single queens at more than one time. Unless specified otherwise, we use effective paternity frequency as shorthand for short-term effective population-wide paternity frequency.

(*c*) *Consequences of unequal paternity for female offspring relatedness*

Unequal paternity results in the harmonic mean paternity frequency being lower than the arithmetic mean (i.e. effective paternity frequency is less than paternity frequency). Starr (1979, 1984), Cole (1983), Laidlaw & Page (1984), and Page (1986) showed that unequal paternity can substantially reduce the extent that multiple insemination reduces relatedness among the female offspring of a queen. However, because of the problems with the empirical data discussed earlier, these and other reviewers (Page & Metcalf 1982; Page 1986; Keller & Reeve 1994) did not present paternity data as effective paternity frequency. As mentioned above, uncritical later use of (O) data has contributed to the idea that relatedness among offspring females is frequenly low in eusocial Hymenoptera owing to multiple mating (e.g. Shykoff & Schmid-Hempel 1991; Keller & Reeve 1994). In this section we evaluate this idea by reviewing both the theory on effective paternity and the most reliable empirical (A) data for paternity in ants. The theory shows that the observed inequalities in paternity among and within queens of the same population can easily cause a substantial discrepancy between paternity and effective paternity frequencies. Our review of the (A) data shows that the mean effective paternity frequency across ant species is only slightly above 1.

To appreciate the value of the genetic data (A, V), it is necessary to understand the effect of paternity on the relatedness among offspring females. Several authors (Pamilo 1993; Queller 1993*b*; Ross 1993) have given general equations for this relationship, based on Wade (1982), Starr (1979, 1984) and Laidlaw & Page (1984). Despite this thorough theoretical framework, in our experience the theory is unclear for many social insect biologists. Our treatment, therefore, focuses on graphical illustrations of the basic equations. To do this, we start from the following equation (Pamilo 1993), which gives the relatedness, g, among female offspring of a queen as,

$$g = 0.25 + 0.5(1/m_e), \qquad (1)$$

where m_e is the effective number of the queen's mates. This equation shows that relatedness is a linear function of the reciprocal of the effective number of mates (figure 2, line a). For comparison (see also later sections) the other lines in figure 2 show the effects of increasing queen number with single mating (lines b

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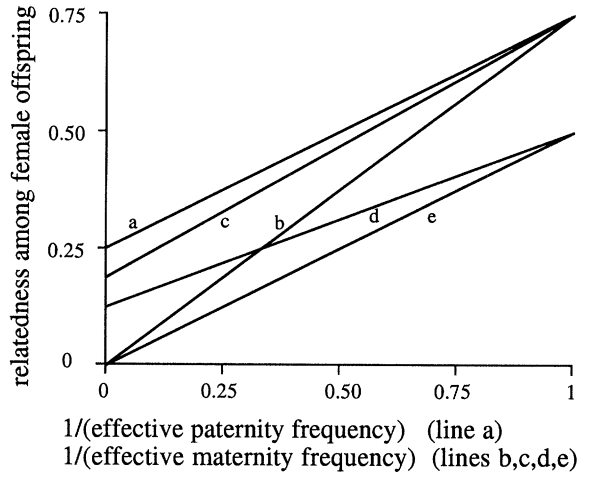

Figure 2. The relatedness among female offspring in a colony (i.e. workers and virgin queens) as a function of the reciprocal of effective paternity frequency (a) or effective maternity frequency (b, c, d, e). For c, d colony queens are assumed to be the offspring of a single mother-queen, who is either single-mated (c), or double-mated (d). The offspring queens have the same mating frequency as their mother queen. Males inseminating the same or different queens within a colony are assumed to be unrelated. a: single maternity, multiple paternity (males unrelated); b: single paternity, multiple maternity (queens unrelated); c: single paternity, multiple maternity (full-sister queens); d: double paternity (males unrelated), multiple maternity (full and half-sister queens); e: double paternity (males unrelated), multiple maternity (queens unrelated).

and c) and with double mating (lines d and e) on offspring relatedness. Figure 2 shows that in situations where multiple males or queens contribute to colony offspring, the relatedness effect is linearized by taking the reciprocal of the effective paternity or maternity frequencies. In addition, figure 2 shows that both the gradient and the intercept of the relationship between relatedness and effective number of parents are affected by factors such as the relatedness among multiple queens or the number of males that queens mate with. The gradients of lines (a–e) determine the rate at which relatedness is reduced by changing effective (harmonic) paternity or maternity frequencies.

As shown by Starr (1984) and other references above, the effective paternity frequency equals the reciprocal of the sum of the squared paternities, p_i, where

$$m_e = 1/\Sigma p_i^2. \quad (2)$$

When the offspring have a single father, Σp_i^2 equals 1, and when there are two fathers Σp_i^2 equals $c^2+(1-c)^2$, where c is the proportion of offspring from one of the two males (for consistency, we will let c denote the contribution of the majority male). $c^2+(1-c)^2$ is the probability that two randomly chosen female offspring have the same father, and $[1-c^2-(1-c)^2] = [2c(1-c)]$ is the probability that they have different fathers. Because full sisters are related by 0.75 and half sisters by 0.25 the mean relatedness among the offspring is

$$g = 0.75\ [c^2+(1-c)^2]+0.25\ [2c(1-c)], \quad (3)$$

which simplifies to $0.25+0.5\ [c^2+(1-c)^2]$, which is m_e from equation (2) substituted into (1).

Extensions to additional males work in the same way, as shown in figure 3*a*. If the number of patrilines is j, then Σp_i^2 varies between $1/j$, when male contributions are equal, and 1, when the contributions of all but one male approach 0. The combined areas of the white rectangles in figure 3*a* represent the probability of two randomly chosen individuals being half-sisters, whereas the black squares are the full-sister probabilities. The effectiveness of multiple insemination in causing multiple paternity depends on the relative proportions of full-sisters and half sisters (i.e. on the proportional area of all white squares in figure 3*a*).

Additional factors, such as non-zero relatedness between males mating with the same queen or non-zero relatedness between a queen and her mates can be included in equation (1) (Pamilo 1993; Queller 1993*b*; Ross 1993) and would produce different lines in figure 2. Here, we focus on equation (2) and show how m_e is affected by variation in paternity within and among queens. We use figures like figure 3*a* to show that Σp_i^2 can be written as the sum of the harmonic mean number of matings and the sum of squared paternity deviations from equality. Second, we use a similar figure to show that in calculating Σp_i^2 over queens within a population, the squared deviations can be partitioned into a mean sperm bias effect and squared deviations from that mean.

Pamilo (1993, equation 5), states that relatedness within a female brood j can be written as:

$$g = 0.25+0.5(1/m_j+m_jV_j) \quad (4)$$

where m_j is the number of fathers (paternity frequency) and V_j is the paternity variance. Thus, a total sum of squares (Σp_i^2) has been written as a mean ($1/m_j$) plus a residual sum of squares (m_jV_j). The logic of this is shown in figure 3*b*, for a situation where paternities are 70% and 30%. Both deviate 20% from equality to give the following equivalence:

$$\Sigma p_i^2 = 0.7^2+0.3^2 = 0.58,$$

$$1/m_j+m_jV_j = 1/2+2(0.2^2) = 2(0.5^2)+2(0.2^2) = 0.58.$$

When a relatedness estimate is required over more than one brood, either offspring of different queens or different samples from offspring of the same queen, this can be calculated by using the mean of Σp_i^2 across broods or the mean of the sums of the two terms in brackets in equation (4) (Pamilo 1993).

Paternity partitioning can be extended to include additional sources of variation. For example, if we are interested in the population-wide relatedness consequences of variable paternities among k broods that have two fathers, equation (4) can be modified to

$$g = 0.25+0.5[2(0.5)^2+2(\bar{c}-0.5)^2+2V_k]. \quad (5).$$

Here, $(\bar{c}-0.5)$ is the mean deviation from 0.5 (mean sperm bias) across all broods with two fathers, and V_k is the residual variance caused by brood-specific variations in paternity from this mean. The equivalence of equation (5) with the earlier equations is

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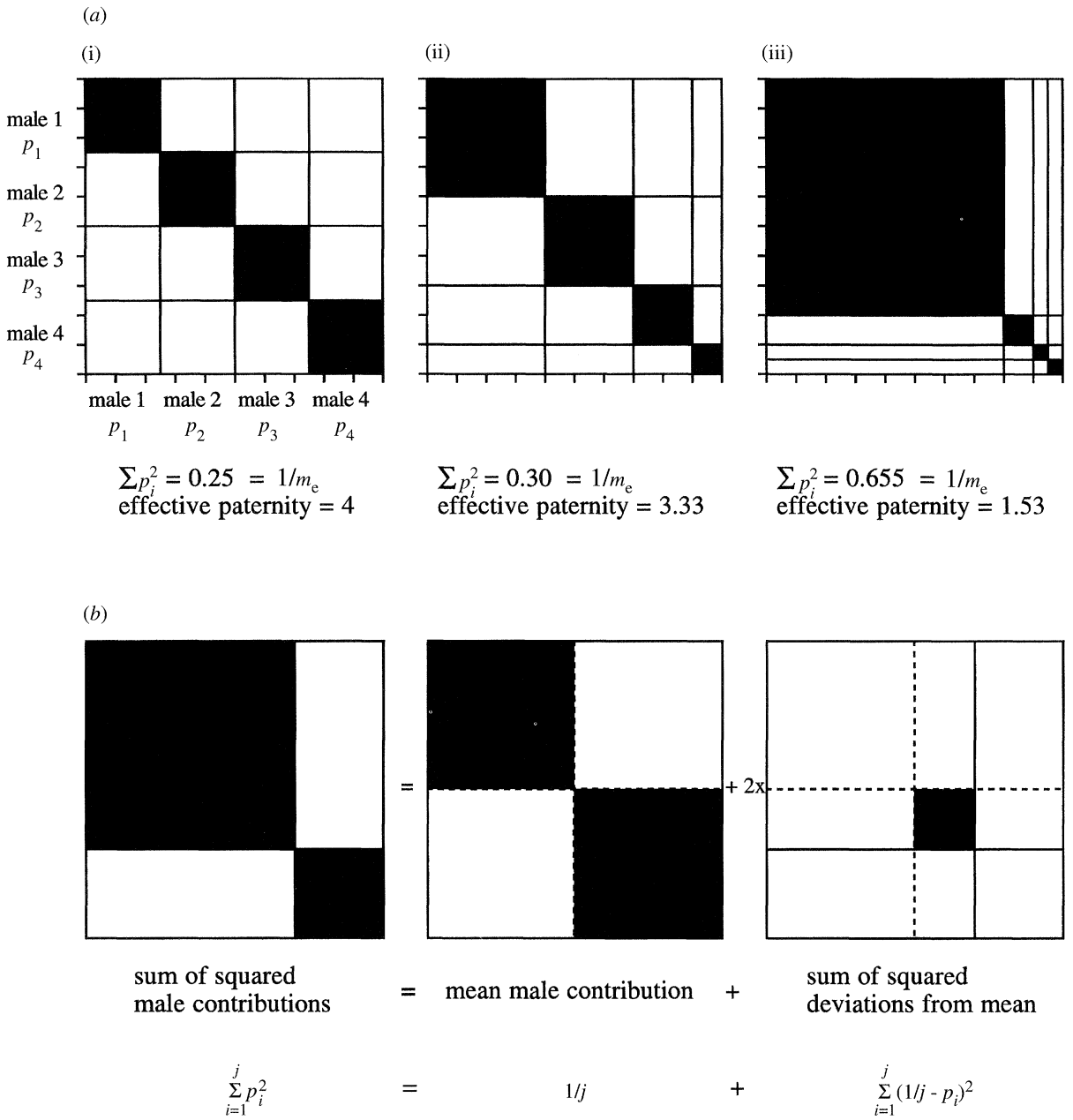

Figure 3. Effect of paternity variation on effective paternity frequency. (*a*) Paternity frequency of four with increasing inequality in paternal contributions giving diminishing effective paternity frequency. (i) Zero paternity variation; (ii) small paternity variation; and (iii) great paternity variation. (*b*) Sum of squared paternal contributions Σp_i^2 represented as mean plus variance (see text for details).

illustrated in figure 4, for the situation where half of the two-father broods have paternities of 0.7 and 0.3, and the other half have paternities of 0.9 and 0.1.

Each additional variance component increases relatedness. In the example of figure 4, m_e equals 1.43 (1/0.70) and relatedness 0.60 (equation 1), but if all colonies had the population-mean sperm bias (0.8), m_e would have been 1.47 (= 1/0.68) and relatedness 0.59. A more extreme difference among broods with the same population mean sperm bias (e.g. half the queens having 0.65:0.35 paternities and half 0.95:0.05) would have given an m_e of 1.38 (1/0.725) and an average relatedness of 0.61.

The above shows that variation in paternity among males both within and among broods can cause a substantial discrepancy between paternity frequency and effective paternity frequency. Early studies have indicated that paternity is variable and occasionally highly skewed (Metcalf & Whitt 1977; Metcalf 1980; Pamilo 1982*a*). These results are corroborated by many recent studies, as shown in the next section, where we determine effective paternity (A, V data) and insemination (D data) frequencies based on reliable data.

(*d*) *Allozyme estimates of paternity*

For ants, the genetic data that allow the estimation of effective paternity frequency by providing data on the genotypes of mothers and daughters are all based

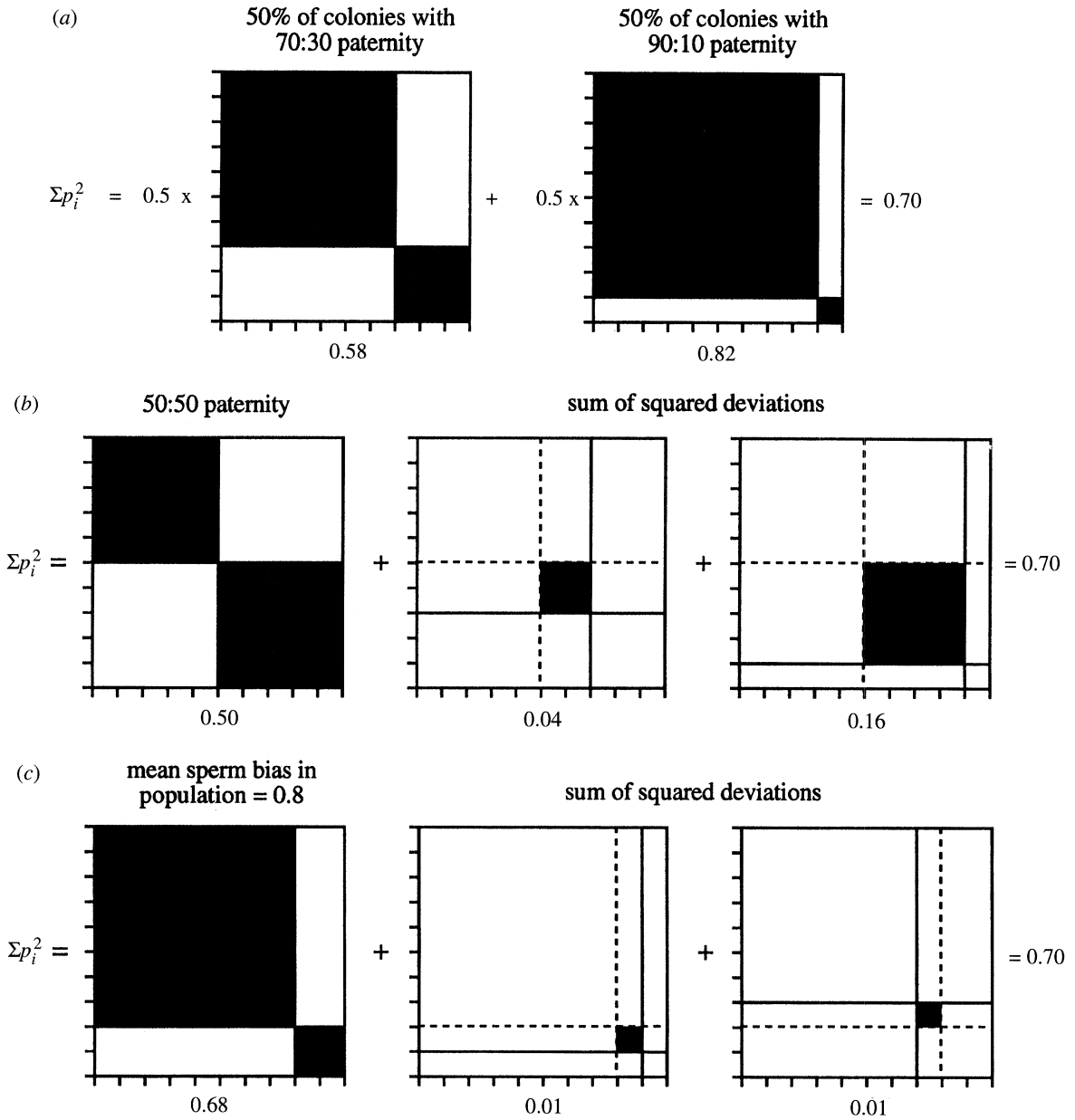

Figure 4. Three equivalent methods of determining Σp_i^2 for a situation where all colonies have double paternity but fall into two classes: half with 70:30 and half with 90:10 paternities. (*a*) Σp_i^2 calculated as the sum over all colonies of the colony-class-specific Σp_i^2. (*b*) Σp_i^2 calculated as mean paternity assuming all colonies have equal paternity (50:50) plus squared deviations from 50%. (*c*) Σp_i^2 calculated as mean paternity assuming all colonies have population-wide paternity (80:20) plus squared deviations from 80%.

on allozymes (table 5). This is also largely true for the more sparse data on bees and wasps (see below). In general, there are two main difficulties in using genetic data to assess paternity frequency: errors caused by non-sampling and errors caused by non-detection.

The non-sampling error is simply the probability that the offspring of a given male will not be represented in the sample of females collected for genetic analysis. With large samples this error approaches zero. However when small samples are made, as they often are, the possibility that one or more males are unrepresented in the sample cannot be ignored. Paternity contribution also affects the sampling probability. Obviously, if a male fathers only a small proportion of the female offspring then a larger sample is needed to be reasonably sure of including at least one of his offspring. Figure 5 shows the probability, based on the binomial theorem, that at least one daughter of a given male will be sampled as a function of sample size and paternity contribution. There can be no definite rule for what constitutes an adequate sample size, because this depends on the question being addressed, but, as an example, a sample of 20 will include a daughter of a male with $> 14\%$ paternity with $> 95\%$ probability (figure 5).

The non-detection error is the probability that the offspring of one male are genetically indistinguishable from the offspring of a second male. Male haploidy, as in the Hymenoptera, is an advantage in assigning paternity because the gametes produced by a male are identical and are inherited by all his daughters. Countering this advantage is the generally low

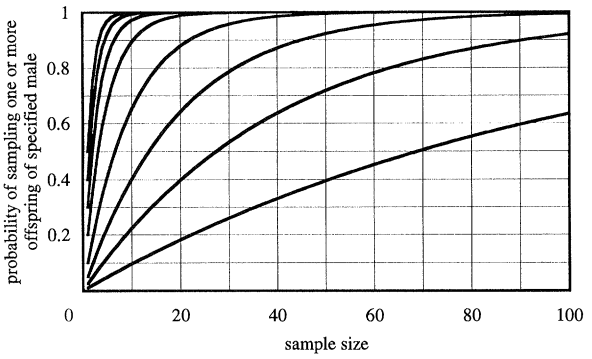

Figure 5. Sampling efficiency. The probability that at least one offspring of a specified male is sampled as a function of sample size and proportion of offspring fathered by that male. Sampling efficiency is high when males father many offspring but low when a male's representation is small. From top-left to bottom-right, the curves represent sperm bias of 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.025 and 0.01.

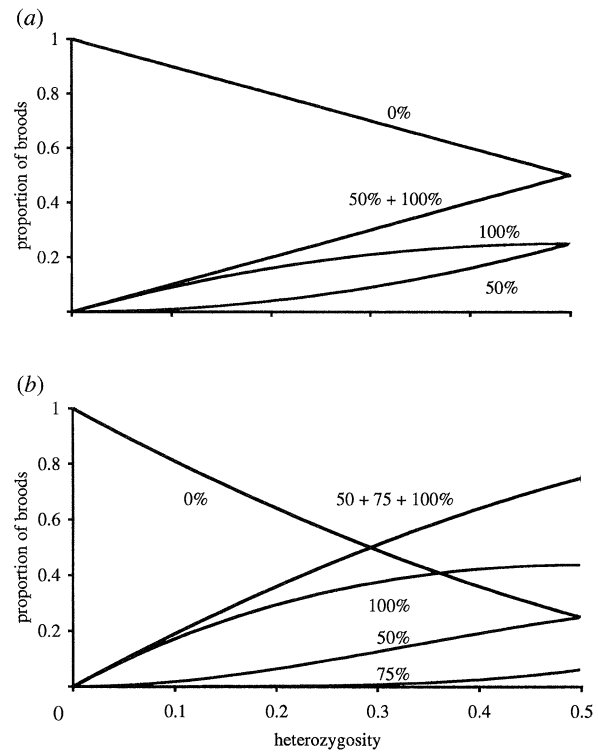

Figure 6. Detection efficiency assuming non-sampling errors to be absent. (*a*) The probabilty that the offspring of a second male can be distinguished genetically from the offspring of a first male in double-paternity broods as a function of heterozygosity at one biallelic locus. This probability is simply the probabilty that the two males are of different genotype, which equals the heterozygosity assuming Hardy-Weinberg conditions. Paternity can be assigned to 100% of the offspring when the mother queen is a homozygote and to 50%, on average, when she is a heterozygote. It is assumed that the queen genotype is known. (*b*) The probabilities of distinguishing a second male when data from two biallelic loci with equal heterozygosity are available.

allozyme variation in populations of social Hymenoptera (Crozier 1985; Graur 1985), resulting in most studies being limited to a small number of variable loci, typically 1–4 (but see Ross *et al.* 1993 for a heroic exception). Low allozyme diversity means that the mates of a single queen often carry the same marker alleles, so that offspring often inherit the same markers, but not because of identity by descent, making the offspring of different males indistinguishable (Pamilo 1982*a*). Considering two fathers and denoting the respective allele frequencies at a marker locus as q_i, the proportion of broods with undetectable double paternity (i.e. both males have the same genotype) is Σq_i^2. A second unlinked marker locus with allele frequencies r_i reduces non-detection to $(\Sigma q_i^2)(\Sigma r_i^2)$ and so on for additional loci (all assuming Hardy–Weinberg equilibrium). Detection of additional fathers can be calculated by the same principle, but becomes more tedious when non-sampling errors occur at the same time (see equation 6 below). In general, each additional father is harder to detect, and the number of alleles per locus and number of variable loci set an upper limit to the maximum number detectable. For example, detection of a third father requires, at a mimimum, one locus with three alleles or two loci each with two alleles. If only one locus with two alleles is available then the third male always has the same marker allele as one of the other two (Metcalf & Whitt 1977). Figure 6 shows detection probabilties for second matings as a function of heterozygosity at one or two biallelic loci.

Following Pamilo (1982*a*) an additional point should be mentioned. When the mating is of the form a_ia_i (queen) $\times a_i$, a_j (males) paternity can be assigned to all the offspring. However, when the mating is $a_ia_j \times a_i$, a_j only homozygous offspring can be assigned because heterozygotes, ca. 50% of the offspring, could be daughters of either male. Thus the sample size is effectively halved when queens are heterozygous at the marker locus. Where additional material is available this can be allowed for by doubling the sample size when the queen is heterozygous. When two loci are analysed an additional situation, 75% of the offspring being assignable, occurs although the probability is low (figure 6*b*). Additional loci give additional cases i.e. 62.5% and 87.5% for three males. As we write, allozyme markers are increasingly being supplemented or replaced by DNA techniques such as fingerprints, RAPD's and microsatellites (for a technical review and characterization of the various DNA techniques, see Westneat & Webster 1994). The increased genetic variability that DNA techniques can uncover means that detection efficiency will rise. However, DNA techniques have no effect on sampling efficiency. When the effort and cost of using DNA are relatively high, their application may result in smaller samples and larger non-sampling errors.

Pamilo (1982*a*, 1993) has presented calculation procedures that can alleviate some of these non-detection and non-sampling problems. In an early paper (Pamilo 1982*a*), detection probabilities for double mating are given for both homo- and heterozygous queens. The effect of male allele frequencies (non-detection error) and offspring sample size (non-sampling error) are simultaneously taken into account for genetic information from up to two biallelic marker loci. This method of estimating detection efficiency for

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double mating can be extended to include information from three loci with 2–3 alleles, but rapidly becomes difficult with more genetic variation. Recently, Pamilo (1993) has shown that the non-sampling detection error can be quantified in a more general way for any number of mates i.e. Σp_i^2 can be corrected for sampling error for queens where 2, 3, 4, or more matings have been detected, by:

$$\Sigma p_i^2 = (N\Sigma y_i^2 - 1)/(N-1) \quad (6)$$

where y_i are the observed proportional contributions of the queen-mates and N is the number of offspring analysed. However, as stated by Pamilo (1993), this method only allows an unbiased estimation of the effective number of mating types, which is lower than the effective number of fathers when non-detection errors occur. With data based on allozymes, both methods by Pamilo (1982*a*, 1993) allow accurate estimations of effective paternity in populations with only single and double mating. In populations where triple and higher matings occur in appreciable frequency Σp_i^2 will tend to be overestimated, unless data from highly variable microsatellites with very small non-detection errors (e.g. Estoup *et al.* 1993, 1994, 1995) are available.

The above shows why genetic data from mothers and female offspring can best be used to estimate effective paternity frequency when the raw data on genotypes per nest (to estimate the non-detection error by calculation of population-wide allele frequencies among males) and sample size (to estimate the non-sampling error) are also published. Unfortunately, these data are often not published. Reasonable approximations of m_e are still possible with data on overall allele frequencies (the assumption must be made that frequencies are equal in both sexes, which is not always the case; e.g. Boomsma *et al.* 1993; Pamilo 1993), mean sample size, and mean sperm bias. Otherwise, effective queen mating frequencies can only be estimated indirectly from the population-wide estimate of relatedness (b_{ff}) among the female offspring of a single queen using equation (1), provided such overall relatedness estimate is given. In the latter situation useful information at the level of individual colonies is lost. Detailed information on paternity in individual colonies according to the direct estimation procedure by Pamilo (1982*a*, 1993) is important in some questions, such as for making accurate tests of split sex-ratio theory (Sundström 1994, 1995).

(*e*) *Effective paternity frequency in ants*

Given the above considerations, 19 allozyme datasets were used to estimate effective paternity frequencies directly. On average 44 broods with 14.3 offspring per brood were analysed per study. Genetic variation at 1–6 allozyme loci gave an average detection efficiency for double mating of 41 %. Detailed results are given in table 5, together with notes on methods, the occasional approximations involved, and additional biological data. Although some of the datasets only allowed upper or lower limits to be estimated, the overall picture that emerges is clear. In nine species, double paternity was never observed, and in six of these, sample size and detection efficiency were sufficiently high to conclude that effective paternity was lower than 1.05. We call this category (s), single paternity. From equation (1), the relatedness among offspring females in (s) broods is 0.75–0.725. Another important category, (s–d) single–double, has a mixture of single and double paternity, with the proportion of two-father broods being less than 50 %, to give offspring relatedness of 0.725–0.65. Because of extremely uneven paternity, *Formica transkaucasica* and *Leptothorax pergandei* have m_e values below 1.05 even though some two-father broods were detected. A third, and rarer, category, (s–m) single–to–multiple, in which three of more fathers were detected in some broods, is represented by *Formica aquilonia* and *F. truncorum*. Here effective paternity frequencies are between 1.4 and 1.5 and relatedness is as low as 0.6. In *Lasius niger* most populations belong to the (s–d) category, but the Amsterdam population listed in table 5, is (s–m) (Van der Have *et al.* 1988). This indicates that paternity can vary among populations of the same ant species (J. J. Boomsma & T. M. Van der Have, unpublished data).

The population-wide effective paternity frequency averaged across all 19 species is 1.16. This is probably a slight overestimate as upper limits of m_e were used in some cases. In the ten species in which multiple paternity was detected the mean is still only 1.23. Despite up to six fathers being detected in some broods of *Formica aquilonia* (Pamilo 1993), the variance in paternity within and among broods is so high in this species that the population-wide effective queen mating-frequency is below 1.5. Genetic studies (A, V) of 11 more species were not included in table 5, because of incomplete background data. Table 6, which is a summary of all (A, V) datasets for ants, includes these species plus four species with (D) data. The additional (A, V) data confirm the estimate of $m_e \leqslant 1.16$ given in table 5. In 9 of the 11 additional species, the number of fathers per brood was stated to be one, although some studies mention the occasional double mating indicating that all nine belonged to the (s) or (s–d) categories.

No species in table 5 had an effective paternity frequency greater than 2, which would correspond to a relatedness among female offspring below 0.5. Nor is this fourth category, (m) multiple, represented in any of the additional 11 species with genetic data (table 6). Therefore, even though multiple copulation may occur frequently, effective queen mating-frequency is low in most ants. The only good data in ants suggestive of population-wide effective paternity frequencies above two are from the leafcutter ants *Atta sexdens* (Kerr 1961) and *Atta laevigata* (Corso & Serzedello 1981) (table 6). These (D) method studies gave insemination frequencies of approximately three. Effective paternity frequencies may be lower than this if sperm clumping (which cannot be detected with D methods) occurred, or (more likely) higher, if copulating males have less than full loads of sperm stored by queens. Importantly, tables 5 and 6 give a very different picture than the one based on observations of copulations. This is shown in figure 7, where the frequencies of our paternity categories are compared with the copulation

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Table 6. *Paternity or insemination frequency, number of queens, and number of workers in 34 ant species*

(The 19 species for which effective paternity frequency (m_e) could most accurately be estimated (table 5) are marked *.)

subfamily species	paternity or insemination freq.[a]	no. queens[b]	no. workers (10^n)[c]	type of data[d]	references
Ponerinae					
Rhytidoponera chalybaea	s[e]	s[f]	2	A	Ward 1983
Rhytidoponera confusa	s[e]	s[f]	2	A	Ward 1983
Myrmicinae					
*Aphaenogaster rudis**	s	s	2	A	Crozier 1973, 1974; Talbot 1951
Atta laevigata	m	s	6	D	Corzo & Serzedello 1981; Mintzer 1990; Pereira da Silva 1975
Atta sexdens	m	s	6	D	Kerr 1961; Weber 1972
Atta texana	s–d	f	6	D	Moser 1967; Moser & Lewis 1981; Mintzer 1987
*Harpagoxenus sublaevis**	s	s	2	A	Bourke *et al.* 1988
Leptothorax acervorum	s	f	2	A	Heinze, pers. com; Stille *et al.* 1991
Leptothorax gredleri	s	f	2	A	Heinze *et al.* 1992; Heinze, pers. com.
Leptothorax longispinosus	s[g]	f	2–3	A	Herbers 1986
Leptothorax nylanderi	s–d	s	2–3	V	Plateaux 1981; Buschinger 1968
*Leptothorax pergandei**	s–d	s	2	A	Heinze *et al.* 1995
Myrmica punctiventris	s	s or f	2	A	Snyder & Herbers 1991; Banschbach & Herbers 1996
*Myrmica rubra**	s–d	f or m	2–3	A	Seppä 1994*b*; Seppä, pers. com.; Elmes & Keller 1993
*Myrmica ruginodis**	s–d	f	2–3	A	Seppä 1994*a*
*Solenopsis geminata**	s	f	5	A	Ross *et al.* 1987, 1988; Adams *et al.* 1976
*Solenopsis invicta**	s	s or m	5	A	Ross & Fletcher 1985; Ross 1993; Markin *et al.* 1973
*Solenopsis richteri**	s	f	5	A	Ross *et al.* 1987, 1988; Jouvenaz *et al.* 1989
Dolichoderinae					
*Conomyrma insana**	s or s–d	s	no data	A	Berkelhamer 1984
Iridomyrmex purpureus	s[e]	f or m	3–5	A	Halliday 1975, 1979, 1983
Iridomyrmex humilis[g]	s	m	6	D	Keller & Passera 1992; Markin 1970; Keller 1988
Tapinoma minutum	s or s–d	f	2	A	Herbers 1991
Formicinae					
*Colobopsis nipponicus**	s	s	3	A	Hasegawa 1994, Hasegawa, pers. com.
*Formica aquilonia**	s–m	m	5–6	A	Pamilo 1993; Fortelius 1994; Rosengren *et al.* 1993, pers. com.
Formica argentea	s	f	2	A	Snyder 1992
*Formica exsecta**	s–d	s or m	4	A	Pamilo & Rosengren 1984; Rosengren *et al.* 1993; Rosengren, pers. com.
Formica pratensis[h]	s–d	s (m)	4	A	Pamilo 1987; Pamilo *et al.* 1994; Rosengren *et al.* 1993; Gallé 1978
*Formica pressilabris**	s–d	m	3	A	Pamilo 1982*a*; Pamilo & Rosengren 1984; Rosengren *et al.* 1993; Czechowski 1975
*Formica sanguinea**	s–d	f	4	A	Pamilo 1982*a*; Rosengren *et al.* 1993; Rosengren, pers. com.
*Formica transkaucasica**	s–d	f	3	A	Pamilo 1982*a*; Rosengren *et al.* 1993; Rosengren, pers. com.
*Formica truncorum**	s–m	s or m	4	A	Sundström 1989, 1993 *a*,*b*, 1994
*Lasius niger**	s–d/s–m	s	4	A	Van der Have *et al.* 1988; Boomsma *et al.* 1982
*Lasius flavus**	s or s–d	f	4	A	Boomsma *et al.* 1993; Nielsen *et al.* 1976; Pontin 1978
*Lasius neglectus**	s or s–d	m	6	A	Boomsma *et al.* 1990

[a] Categorization of paternity (A, V). (s) single: double mating absent or very rare; population-wide effective mating frequency < 1.05. (s–d) single-double: double mating occurs in ca 20%–50% of queens; effective mating frequency 1.05–1.25. (s–m) single-multiple: mating frequency above two occurs regularly; effective mating frequency 1.4–2. (m) multiple: mating frequency usually greater than two; effective mating frequency > 2. (No species studied with (A, V) methods fall into this category.)

[b] Categorization of queen number per colony: s, single: single queen in > 95% nests; high relatedness (> 0.7) among female nestmates (assuming single paternity). f, few: at most a few queens per nest; intermediate relatednesses (0.25–0.7) among female nestmates. m, many: many egg-laying queens per nest; low relatedness (< 0.25) among female nestmates.

[c] Typical number of workers in a large colony as a power of 10.

[d] A, V: genetic analysis of offspring using allozymes or visible genetic markers, respectively; D: dissections of males and queens to determine sperm numbers.

[e] Rare cases where genotype distributions within nests cannot be attributed to the offspring of a single male and single queen are probably due to multiple queens and not multiple paternity.

[f] Colonies are headed by either a single queen or several mated-workers.

[g] Renamed *Linepithema humile* (Shattuck 1992). Single paternity confirmed by allozymes (Kaufmann, Boomsma & Passera, unpubl.).

[h] The categorization of mating category (s–d) and queen number (s) is the most likely one possible, based on the limited allozyme data available (Pamilo *et al.* 1994; Pamilo, pers. com.)

frequencies from (O) category datasets in Keller & Reeve (1994).

The conclusion that multiple paternity is rare in ants is robust. Despite the low genetic variation in most allozyme studies the estimates of regression relatedness (b_{ff}) among offspring in table 5 are unbiased, because these calculations include the cumulative effects of all males that sired offspring. Although the standard

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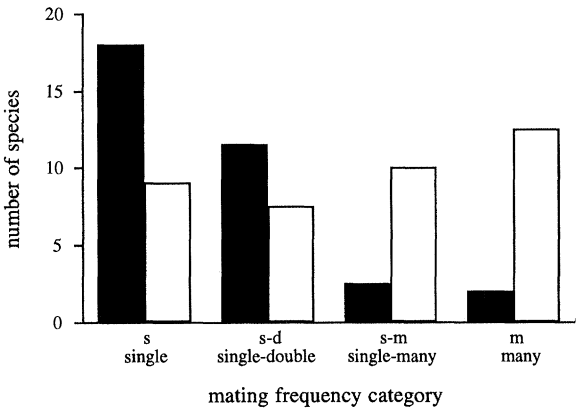

Figure 7. Frequency distribution of the (A, V, D) data from table 5 (filled areas) compared with the frequency distribution of the data on observations of copulations in the most recent review (open areas) (Keller and Reeve 1994). Observations of copulation were assigned to the (s–d) category when multiple copulation was observed but copulation frequencies above two were not reported, and to the (s–m) category when the range of observed copulations contained both single copulation and copulation frequencies above two. Observations of copulation were assigned to the (m) category when single copulation was not included in the observed range of copulation frequencies. The two distributions are significantly different ($G_{adj} = 16.24$; $P = 0.001$).

errors of the specific b_{ff} estimates are substantial, the overall mean regression relatedness across species came out at the same value (0.69) as the average based on direct comparison of mother and offspring genotypes. This result should be viewed historically. When the first reviews on queen-mating-frequency in social Hymenoptera (Page & Metcalf 1982; Cole 1983; Starr 1984; Page 1986) were made, most of the allozyme data we used (tables 5 & 6) were unavailable. In addition, given that the influential paper of Trivers & Hare (1976) had more or less dismissed multiple paternity as an important factor affecting the kin structure of colonies the main purpose of these reviews was to demonstrate that multiple copulation, insemination, and paternity occured in social Hymenoptera and to hypothesise why they might be selectively favoured. Now that more data are available it appears that Trivers & Hare were correct in assuming that multiple paternity is rare (except for derived taxa such as higher leafcutter ants), but it is likely that they were incorrect in suggesting that it was mainly due to sperm clumping.

(*f*) *Effective paternity frequency in eusocial bees and wasps*

Comparable studies of eusocial bees and wasps are fewer than in ants and most studies that are available give fewer details on the population-level allele frequencies. The data almost all refer to field-collected individuals, not to offspring reared from single queens in the laboratory. Especially in wasps such as Polistinae, this creates the problem that occasional multiple foundresses cannot always be excluded as having contributed to the colony-offspring. As in the ants, the data indicate that in bees and wasps there is also a wide range in effective paternity frequency, but with single or close to single paternity predominating.

In the social wasps, species without a morphologically distinct queen caste (Polistinae and *Microstigmus comes* (Sphecidae) have been studied) fall into either the (s) or the (s–d) category (*Polistes metricus*, *Polistes variatus*, *Polistes annularis*, and *Polistes jadwigae*; Metcalf & Whitt 1977; Metcalf 1980; Tsuchida 1994; Peters *et al.* 1995) or the (s–m) category (*Ropalidia marginata*; Muralidharan *et al.* 1986). In *Microstigmus comes* Ross & Matthews (1989) estimated average relatednesses among females in single foundress colonies at 0.63, consistent with our (s–m) category, whereas Ross & Carpenter (1991) consider this species to be effectively monandrous (s). Perhaps some of this discrepancy was due to the fact that the mother was included in the relatedness calculations. Among species with a distinct queen caste (Vespinae) two species of yellowjackets, *Vespula maculifrons* and *Vespula squamosa*, have high effective paternity frequencies (7.1 and 3.3; Ross 1986; Ross & Carpenter 1991), but at least one closely related species (*Dolichovespula arenaria*) is (s–d) (F. L. W. Ratnieks & J. J. Boomsma, unpublished data). A recent DNA microsatellite study of a colony of *Vespula rufa* by Thorén *et al.* (1995) shows that this species probably belongs to the (s–m) or (m) category.

In eusocial bees without a morphologically distinct queen caste (only Halictidae have been studied), paternity is either exclusively single (s) (*Augochlorella striata*; Mueller *et al.* 1994), or single-double (s–d) (*Lasioglossum zephyrum*; Kukuk *et al.* 1987; see also Crozier *et al.* 1987). An estimate of nestmate relatedness in *Lasioglossum laevissimum* (Packer & Owen 1994) also suggests mostly single with some double paternity. A recent study on *Halictus ligatus* (Richards *et al.* 1995) reports relatedness estimates among first brood workers of single foundress colonies of around 0.5. However, the relatively large standard errors around these estimates and the statement in the paper that direct inspection of genotypes (data for two loci, each with five alleles) showed that: 'some queens had mated more than once', suggests that this species may belong in our (s–d) or (s–m) category rather than the (m) category. It should be noted here that in species with very small colonies, such as many halictids, some males that are represented in the spermatheca may not be represented among the offspring, even when all offspring are analysed. This is a non-sampling 'error' by the queen and not by the investigator.

In bees with a distinct queen caste (these are all Apidae) paternity frequency is variable. High effective paternities (m) are well documented in *Apis mellifera* (Kerr & Bueno 1970; Woyke 1975; Adams *et al.* 1977; Page & Metcalf 1982; Koeniger 1986, 1991; Koeniger *et al.* 1989, 1994; Moritz & Southwick 1992; Lobo & Kerr 1993; Estoup *et al.* 1994). However, within the genus *Apis* there is variation. Based on sperm counts, insemination frequencies of 1–3 were thought possible in *A. florea* (Koeniger *et al.* 1989), but a recent genetic study with DNA microsatellites has shown that 5–14 patrilines were represented in samples of 23–71 offspring of this species (Oldroyd *et al.* 1995). The effective paternity in these broods was estimated to be

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5.65 ± 1.04. Another recent study with DNA microsatellites by Moritz *et al.* (1995) has estimated that 19–53 patrilines were represented in broods of queens of *A. dorsata*, giving an effective paternity of 25.56 ± 11.63. Studies (D, A) on stingless bees (Kerr *et al.* 1962; Contel & Mestriner 1974; Contel & Kerr 1976; Machado *et al.* 1984) indicate exclusively single mating in *Melipona quadrifasciata*, *Melipona subnitida* and *Plebeia droryana*. Single mating seems the rule in *Trigona postica* (Beig 1972) although multiple copulations by queens have been reported in laboratory mating boxes (Engels & Engels 1988). Such mating boxes may not represent a completely unnatural situation because *Trigona postica* males form large aggregations close to colonies with virgin queens (Engels & Engels 1988) but, as described above for *Myrmica rubra*, laboratory studies of copulation can be poor predictors of effective paternity. Data on bumblebees, *Bombus*, indicate either exclusive single insemination (Röseler 1973; Sakagami 1976) or paternity (Owen & Plowright 1980; Estoup *et al.* 1995) or insemination and paternity frequencies compatible with the (s–d) or (s–m) categories (Röseler 1973; Estoup *et al.* 1995). The data on copulation frequencies (O) in bees and wasps (Page & Metcalf 1982; Cole 1983; Starr 1984; Page 1986) were not accurate enough to allow a direct comparison with the data from the (A,V,D) categories. It thus remains to be determined whether a difference similar to that found in ants (figure 7) occurs in bees and wasps.

(*g*) *Some implications of low paternity frequency*

We conclude that multiple paternity is well-documented in only one genus each of eusocial bees, wasps, and ants (*Apis*, *Vespula*, *Atta*). All three genera have morphologically distinct queen and worker castes and workers which cannot mate or found colonies, so that multiple paternity is not expected to cause the loss of eusociality. Multiple paternity is thus likely to have evolved after eusociality in each.

The rarity of high effective paternity frequencies in the eusocial Hymenoptera has several general implications. This can be seen by evaluating the basic differences between our (m) mating category and the three other mating categories (s, s–d, s–m). First, it is only in the (m) category that multiple mating has a major effect on intranest genetic diversity in most colonies. Second, it is only in the (m) category that the average relatedness between sisters (0.25–0.5) is equal to to or lower than the relatedness between mothers and daughters (0.5). Third, only such a relatedness structure would provide a general argument for selection against eusociality in haplodiploid lineages where all females are mated and retain their complete reproductive potential. The currently available data do not support the idea that multiple mating has been a general constraint in the early evolution of eusociality in Hymenoptera. More data on mating systems in solitary and primitively eusocial bees and wasps are needed, however, to settle this issue. Fourth, only an (m) mating system provides, all other things being equal, a general argument in favour of reduced worker fertility via mutual worker policing in those lineages where eusociality is established and where workers can only lay unfertilized eggs (Woyciechowski & Łomnicki 1987; Ratnieks 1988; Ratnieks & Visscher 1989).

4. CORRELATIONS BETWEEN PATERNITY, COLONY SIZE AND QUEEN NUMBER

In ants, two correlations with queen mating-frequency have been examined. Cole (1983) showed that queen mating-frequency is positively correlated with colony size (number of workers), and hypothesised that queens heading large colonies are under selection to copulate with multiple males to avoid running out of sperm (sperm limitation hypothesis; see also Crozier & Page 1985). Keller & Reeve (1994) showed that queen mating-frequency is negatively correlated with queen number, supporting the hypothesis that genetic variability generally has a positive effect on colony fitness. They tested the specific hypothesis that multiple mating functions primarily to increase genetic variation, but has a mortality cost to queens during mating. This hypothesis predicts that multiple mating will be less frequent when intracolony genetic variability is already guaranteed by polygyny (figure 2).

We tested both correlations using the data in table 6. Paternity frequencies were categorized as before: (s), (s–d), (s–m), (m). If a species fell into two categories it was scored half in both. Colony populations were categorized following Cole (1983) as the power of 10 of the number of workers in a typical large colony, with $\leqslant 10^3$ being small, and $\geqslant 10^4$ large. Categorizing queen number per nest was more difficult. In some species (*Solenopsis*, *Iridomyrmex* and *Formica*) populations can be either monogynous or polygynous. This situation seems to represent a distinct category (Elmes & Keller 1993; Rosengren *et al.* 1993) and was scored separately (table 7). The categories of queen number per nest were: (s) single, single queen in more than 95% of the nests, giving relatedness among offspring female nestmates close to 0.75, assuming single paternity; (f) few, effective number of queens per nest < 3, giving relatednesses > 0.25 if queens are unrelated and paternity per queen is 1; (m) many, effective number of queens per nest > 3, giving relatedness < 0.25 when queens are unrelated. When nest queens are related the threshold between our (f) and (m) category can increase to at most 9 queens (when all queens are full sisters) instead of 3. Table 7 shows how species fall into the various categories.

(*a*) *The sperm-limitation hypothesis*

The correlation found by Cole (1983) is supported when the analysis is restricted to exclusively monogynous ant species (s). The correlation between effective paternity or insemination frequency and colony size is positive and significant (Spearman's rank coefficient, corrected for ties (Zar 1984) gives $r_s = 0.8011$; $P_{\text{one-tailed}} < 0.0025$, $n = 11$). This correlation remains significant when the data are averaged per genus ($r_s = 0.8428$; $P_{\text{one-tailed}} < 0.01$; $n = 8$). When there are several queens per nest (f) the correlation is

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Table 7. *Maximum worker population of colonies (power of 10), queen number, and effective paternity frequency for 34 ant species (table 5)*

(Some species have populations in both the s or f/m categories for queen number. Data for these species are given in the 'variable' subcolumns, to distinguish them from species with zero or minor variation in queen number among populations: 'fixed' subcolumns. In these cases, and also when queen mating-frequency could not be unambiguously categorized (see table 5), species may appear in more than one cell.)

	effective paternity frequency of queens											
effective no. queens per colony	s single; $m_e < 1.05$				s–d single-double; m_e 1.05–1.25				s–m single-multiple; m_e 1.4–2				m multiple; $m_e > 2$	
	fixed		variable		fixed		variable		fixed		variable		fixed	
s	*A. rudis*	2	*S. invicta*	5	*L. pergandei*	2	*F. exsecta*	4	*L. niger*	4	*F. truncorum*	4	*A. laevigata* [a]	6
single	*H. sublaevis*	2			*L. nylanderi*	2–3	*F. pratensis*	4					*A. sexdens* [a]	6
	M. punctiventris	2			*L. niger*	4								
	R. chalybaea	2			*C. insana*	—								
	R. confusa	2												
	C. nipponicus	3												
	C. insana	—												
f	*F. argentea*	2	*I. purpureus*	3	*T. minutum*	2	*M. ruginodis*	2–3						
few	*L. acervorum*	2			*M. rubra*	2–3								
	L. gredleri	2			*F. transkaucasica*	3								
	M. punctiventris	2			*F. sanguinea*	4								
	T. minutum	2			*L. flavus*	4								
	L. longispinosus	2–3			*A. texana* [a]	6								
	L. flavus	4												
	S. richteri	5												
	S. geminata	5												
m	*I. humilis*[a]	6	*I. purpureus*	5	*F. pressilabris*	3	*M. ruginodis*	2–3	*F. aquilonia*	5–6	*F. truncorum*	4		
many	*L. neglectus*	6	*S. invicta*	5	*L. neglectus*	6	*F. exsecta*	4						
							F. pratensis	4						

[a] In four species where sperm data are used the estimate is for insemination frequency, not paternity frequency

positive but non-significant (corrected for ties: $r_s = 0.3818$; $P_{\text{one-tailed}} = 0.1$; $n = 13$). For multiple-queen species, (m), colony sizes are larger but vary little across paternity/insemination categories to give a non-significant rank correlation coefficient close to zero. For the species with variable queen number colony size is also approximately constant across mating categories in each of the queen-number categories.

Cole's (1983) result is supported because he excluded highly polygynous ants from his analysis because of difficulties in estimating worker number. Our impression, however, is that estimates of colony size are about equally accurate for all categories of queen number and that inaccuracies are at most one order of magnitude. Zero correlation between paternity/insemination and colony size in obligate and facultatively polygynous ants is important because queens in polygynous societies are not under the same selection for high fecundity as monogynous queens, and suggests a modified version of the Cole-hypothesis: not colony size but the lifetime fecundity of queens is predicted to correlate positively with mating-frequency. Increase in queen number above 1 per colony relaxes selection on individual queens to store a lot of sperm (and possibly a male's need to transfer a lot of sperm) to ensure sufficient fecundity and longevity.

A major conceptual problem with the sperm limitation hypothesis is the question of why natural selection has not caused male size or sperm content to increase in those species with highly fecund queens, such that a single copulation would be sufficient for queens (Starr 1984; Crozier & Page 1985). Empirical evidence shows that evolution in this direction has occurred because queens of more fecund species, measured as number of ovarioles, store more sperm (Tschinkel 1987), and the number of sperm produced by males and stored by queens covary positively (Page 1986). In bumblebees multiple paternity is likely to result in higher sperm storage (Röseler 1973; Estoup *et al.* 1995), but this is not the case in *Apis* (see above). This puzzle is significant given that many monogynous ants have mid-air mating flights where the predation cost of additional matings is unlikely to be trivial for either sex. In addition, the observed correlation between paternity frequency and colony size does not in itself prove the Cole (1983) hypothesis. The data show a correlation between queen mating-frequency and colony size (Cole 1983) or between paternity frequency and colony size (this study), but the causal mechanism may be either selection for fertility or greater selection for genetically diverse offspring in large colony species (see next section), or both.

(*b*) *The genetic diversity hypothesis*

The conclusions of Keller & Reeve (1994) are, in general, not supported. There is no tendency for the species in table 7 to be in the line of cells along the

diagonal from the bottom-left to top-right (corrected for ties: $r_s = -0.014$; $n = 28$; $P > 0.5$). However, this may be partly due to variation in colony size. Only small colony species are found in the top-left cells (s, s) whereas large colony species, 10^5–10^6, occur towards the bottom and right. In other words, colony size increases with both increasing queen number and paternity/insemination frequency, but species with many multiple-mated queens per nest do not seem to have larger colonies than species with either many single-mated queens or a single multiple-mated queen. Restricting the analysis to species with both large (10^4–10^6) colonies and non-variable queen number does give a negative rank-correlation between paternity/insemination and queen number (corrected for ties: $r_s = -0.534$; $P_{\text{one-tailed}} \approx 0.05$; $n = 11$), which is marginally significant. Unsurprisingly, this trend is no longer significant when species of the same genus occurring in one cell of table 7 are considered to be a single datum (corrected for ties: $r_s = -0.451$; $P_{\text{one-tailed}} > 0.1$; $n = 9$).

The support above is at best suggestive and requires *post hoc* modification of the original hypothesis. Keller & Reeve suggest that the few comparative data on *Atta* are consistent with their hypothesis. In this genus colony size is always large (table 6). Two monogynous species belong to the highest (m) paternity/insemination category (Kerr 1961; Corso & Serzedello 1981), whereas a polygynous species, *Atta texana*, is in category (s–d) (Moser 1967; Mintzer 1987). Within-genus comparisons of this kind will likely provide more powerful tests of Keller & Reeve's prediction when enough data on effective paternity and effective queen number become available.

(*c*) *Separating predictions from the two hypotheses*

Predictions that are exclusive to one or the other hypothesis would be valuable. One such prediction concerns the pattern of sperm use. In the sperm limitation hypothesis the selective factor is the number of sperm, and multiple insemination could lead to any pattern of paternity from random to highly clumped. In contrast, the genetic diversity hypothesis predicts that sperm should be mixed. The discovery of many species with multiple insemination and extreme sperm clumping would considerably weaken the genetic diversity hypothesis. However, non-clumped sperm does not automatically support the genetic diversity hypothesis because this may arise as an unselected consequence of the sperm storage mechanism. On the other hand, the elaborate sperm mixing mechanism found in *Apis mellifera* (Koeniger & Ruttner 1989), combined with the storage of only one male's worth of sperm (Page 1986), is inexplicable by the sperm limitation hypothesis and suggests some definite advantage from increased colony genetic diversity. Data suggest that one advantage is a reduced average effect of diploid males on colony performance (see below).

Because our analysis suggests that queen fecundity may be a more important factor than colony size, controlling for queen fecundity (number of ovarioles) in a comparative study may provide a way of testing the genetic diversity hypothesis. The genetic diversity hypothesis would be supported if effective paternity and effective maternity frequencies were negatively correlated after controlling for queen fecundity. A useful test of the sperm limitation hypothesis could be made by comparing sperm storage and copulation frequency for queens from a single population. A positive correlation between the two, if found, would support the sperm-limitation hypothesis. However, a decisive test of the sperm limitation hypothesis would also necessitate showing that, among queens with broods of a given paternity frequency (to eliminate genetic diversity factors), those with more sperm stored have higher fitness.

5. SELECTIVE REASONS FOR SINGLE OR LOW PATERNITY

More emphasis has been placed on determining the selective causes of multiple mating (e.g. Sherman *et al.* 1987) than on the causes of single or low mating-frequencies. This is understandable given the difficulty posed by multiple paternity to the evolution of eusociality. However, as shown above, species with high effective paternity frequency are obligately eusocial, so there is little or no chance of reversion to non-eusociality.

Here we briefly discuss some factors which may selectively favour queens producing single or low paternity broods, with the aim of showing that this is frequently predicted by the relevant theory, assuming that additional copulations have some cost to queens. The abundance of species with single or low paternity frequencies implies that there are many conditions under which queens cannot enhance their fitness by multiple copulation. Note that copulation outside the natal nest is an act in which queens can make decisions unencumbered by manipulation or control by other females (queens or workers), although in some cases, e.g. *Melipona*, queens may not be able to mate more than once because of male control, specifically the plug formed by the torn-off male genitalia (Kerr *et al.* 1962). However, in *Apis mellifera*, subsequent males can remove the mating plug (Koeniger 1991), suggesting that plugs are not an absolute barrier to multiple copulation.

(*a*) *Diploid males*

Data from *Apis mellifera* suggest that multiple paternity enhances colony performance by reducing the deleterious effect of diploid males (Woyke 1980), in agreement with Page & Metcalf's (1982) variance reduction hypothesis, but with a different biological explanation of the fitness curves involved (Ratnieks 1990*a*; for data see Woyke 1980, 1981, 1984). Colonies producing 25% diploid males (as would result from double paternity with equal sperm use, with one male carrying the same sex-determination allele as one of the queen's two alleles) performed better, in terms of population, honey storage, and the efficiency with which the brood chamber was used, than the mean of

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colonies producing 50% or 0% diploid males (as would result, respectively, from single paternity with or without a matched mating).

In *A. mellifera* multiple paternity is effective in reducing the mean cost imposed by diploid males because diploid males are destroyed as young larvae, before much time or energy is invested in them (Ratnieks 1990*a*). However, when diploid males are reared to adulthood this will result in selection for single copulation (Pamilo 1991*b*; Ratnieks 1990*a*; Pamilo *et al.* 1994) when the cost of 25% diploid males is closer to the cost of 50% than 0% diploid males. Two plausible examples of this, both in genera with single paternity, are known. In *Melipona quadrifasciata* queens which produce diploid male offspring are executed by the workers, who recruit a replacement sister queen (Camargo 1982). Increased mating frequency would increase the probability of producing diploid males, and likely the probability of being executed. In monogyne fire ants incipient colonies headed by queens which have made a matched mating die, because the diploid males consume most of the limited nutritional resources available (Ross & Fletcher 1986). It is likely that 25% diploid males would be lethal given that males are much larger than workers and would consume more than 25% of the resources (Ross & Fletcher 1986), and that incipient colonies are under intense intraspecific competition (Tschinkel & Howard 1983). Both these scenarios could be tested by appropriate experiments.

(*b*) *Disease*

Multiple paternity is hypothesised to enhance colony performance (e.g. survival) in an environment where microparasites detrimental to the colony occur. This hypothesis depends, among other things, upon the existence of genetic diversity in host resistance and parasite virulence.

Concerning host resistance, some data from *Apis mellifera* suggest that different host genotypes may vary in susceptibility to *Bacillus larvae*, the cause of the disease American foulbrood (Rothenbuhler & Thompson 1956; Laidlaw & Page 1984). However, the first study showed only small differences in resistance between different genetic stocks and the second study fortuitously noted the effect in a single colony. In *A. mellifera* most of the disease-fighting ability of both individuals and the colony apparently comes from generalized defensive mechanisms, such as hygienic behaviour, the proventricular valve, high brood nest temperature, etc (reviewed Seeley 1985) that are effective in combatting different strains and even species of disease-causing organism. Coevolution between disease-causing organisms and hosts can generate the necessary genetic diversity in virulence and resistance (Seger & Hamilton 1988), but a recent theoretical study has suggested that this is a relatively unlikely outcome in plants (Parker 1994). Perhaps more likely is the maintenance of moderate or low diversity, with occasional 'revolutions' as a new strain of microorganism becomes established, leading to a new resistance genotype in the host species.

Empirical data to test the hypothesis are few. One study of *A. mellifera* showed no difference in the amounts of four diseases in colonies with low or high genetic diversiy (Ratnieks 1989). Data from the bumble bee *Bombus terrestris* suggest that a protozoan parasite can spread more easily among low diversity versus high diversity experimental groups of workers (Shykoff & Schmid-Hempel 1991), but this result was not repeatable subsequently (Schmid-Hempel & Schmid-Hempel 1993), and is in any case problematic given the strong evidence for single mating in *B. terrestris* (Estoup *et al.* 1995). More generally, even if it could be shown that a disease can spread more easily among a low diversity group of workers than among a higher diversity group, this does not necessarily indicate that all high diversity groups will suffer less disease, as the shape of the function relating reproductive fitness to parasite pressure and paternity determines whether selection works in favour or against multiple paternity (Schmid-Hempel 1994). The spread of disease-causing organisms in social animals has two major components: spread among colonies, and spread within individual colonies. Shykoff & Schmid-Hempel's (1991) study is relevant to the second component because all their experimental groups included one infected individual. Another recent study on bumblebees has addressed the first component (Durrer & Schmid-Hempel 1994). The disease hypothesis can favour low diversity and hence single mating. In particular, groups of higher genetic diversity could be more susceptible to contracting novel infections, because their higher diversity means that they contain individuals susceptible to a wider range of parasite genotypes. The susceptible individuals, once infected, may function as the proverbial 'one bad apple', raising the level of disease propagules within the colony to levels high enough to infect less susceptible individuals.

(*c*) *Division of labour*

High worker genetic diversity is clearly not an absolute requirement for effective division of labour. Fire ants, for example, have single paternity and single-queen colonies yet have elaborate division of labour (Porter & Tschinkel 1985; Ross 1993). However, there can be genetic differences in the threshold at which workers undertake different tasks or switch between tasks, as shown by numerous studies of *Apis mellifera* (reviewed by Page & Robinson 1991). Nevertheless, there is no convincing evidence that honey bee colonies with greater genetic diversity have higher survival or reproduction than low diversity colonies because of more efficient or flexible division of labour (Oldroyd *et al.* 1991, 1993, 1994; Fuchs & Schade 1994). The differences found in these studies relate to foraging behaviour or apply to unnaturally small colonies, and do not directly address colony performance. Woyciechowski & Warakomska (1994) showed that worker genetic diversity had no effect on the diversity of pollen collected. Evidence on this issue may also suffer from complications due to non-additive genetic effects. That is, certain combinations of

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subfamilies perform better than others (Moritz & Hillesheim 1989; Oldroyd *et al.* 1992).

(*d*) *Sex allocation*

When workers facultatively alter colony sex allocation in response to variation in the genetic diversity of the brood, as is predicted theoretically (Ratnieks 1990*b*; Boomsma & Grafen 1990, 1991) and found in nature (Queller *et al.* 1993; Sundström 1994; Evans 1995), queens may enhance their inclusive fitness by copulating with a greater number of males, thereby being more likely to head a colony rearing male reproductives. (Population-wide female-biased sex allocation ratios (Trivers & Hare 1976) mean that male offspring are more valuable to queens than female offspring.) The gain in inclusive fitness to queens diminishes as effective paternity frequency increases, so that a third copulation is less likely to be favoured than a second copulation (Ratnieks & Boomsma 1995). Of greatest relevance to the ranges of paternity frequency shown to occur in this paper is the possibility that facultative sex allocation by workers can result in both single and double-mated queens having the same fitness, thereby maintaining a mixed mating strategy among queens with the effective paternity frequency low. Equality of fitness occurs because the gain from the second copulation reduces as the population-wide effective paternity frequency increases, whereas the cost of the second copulation remains the same (Ratnieks & Boomsma 1995).

Although genetic diversity of colonies is important in this hypothesis, because workers are assumed to assess queen mating-frequency based on some phenotypic expression of the genetic diversity of their sisters (Boomsma & Grafen 1990, 1991; Ratnieks 1990*b*), its importance is subtly different than in the disease or diploid male hypotheses. For example, it is not necessary that the colony as a whole has a higher survival or productivity, which is an essential component of the other hypotheses. Furthermore, moderate sperm bias may not be important if workers assess diversity as the total number of odors in the colony (Ratnieks 1990*b*), but would be important if the accuracy of paternity frequency assessment by workers depended on the relative paternities of multiple fathers (i.e. if multiple paternity is harder to assess when sperm use is unequal) (e.g. Boosma 1996).

Another facet of this mechanism is that it cannot cause increases in paternity frequency unless workers already facultatively alter colony sex allocation in response to variation in the diversity of nestmates. Facultative sex allocation is very unlikely to occur in species which have exclusively single-paternity broods, because there will be no selection on workers for facultative allocation (Hasegawa (1994) provides the first empirical evidence for non-split sex ratios in an ant species lacking variation in paternity and maternity). Thus, facultative sex allocation can only increase paternity frequency from an initial state of mixed single and double paternity. Double paternity would not need to be extremely frequent initially, and could be due to any factor, selected or unselected. With respect to facultative sex ratio biasing, obligate single paternity may therefore be an adaptive peak.

(*e*) *Sperm limitation*

As discussed above and by Cole (1983), the sperm limitation hypothesis predicts single copulation in species whose queens are not highly fecund.

(*f*) *Costs of copulation*

An important and little-known factor in understanding the evolution of social insect mating systems is the cost of copulation (see, for example, O'Neill 1994). Each additional copulation probably imposes an equal cost to a queen, but the benefits of each additional copulation are likely to decline (Ratnieks 1990*a*; Ratnieks & Boomsma 1995). Thus, single or low mating frequencies would be predicted to occur if the copulation cost were high, even if multiple insemination or multiple paternity resulted in fitness benefits to queens after copulation.

Our analysis of ants (the 'mating' column in table 5) showed no obvious correlation between mating behaviour or location and paternity frequency, but it is likely that some mating systems (e.g. mating on the ground) may result in additional matings being less costly than others (e.g. mating in mid-air). Probably the least costly, even approaching zero cost, is mating in the natal nest (Passera & Keller 1992). That copulations can be non-costly is shown by the honey bees *Apis mellifera* and *cerana* (reviewed in Ratnieks & Boomsma 1995). Here the mortality costs of additional matings are estimated to be very low, given that queens rarely die on nuptual flights and copulate with 10–40 males (see also Ratnieks 1990*a*).

6. PATERNITY VERSUS MATERNITY: EFFECTS ON COLONY DIVERSITY AND CAUSATION

Both multiple paternity and multiple maternity (polygyny) are important factors affecting the kin structure of colonies. Both reduce the relatedness among a colony's daughter females (workers and virgin queens). However, they are not directly comparable in either effect or causation suggesting the need for caution whenever comparisons are made.

(*a*) *Effects*

In ants, observed levels of polygyny (Rosengren *et al.* 1993) are approximately an order of magnitude more important in reducing nestmate relatedness than observed levels of multiple paternity. For example, in paternity category (s–m) the relatedness among the offspring of a single queen is 0.6–0.7 (table 5). In species such as *Apis* and *Vespula* with multiple paternity (m) relatedness is 0.3–0.5, and the lowest possible value with multiple paternity is only 0.25. However, relatednesses in polygynous ants of the (m) category can easily drop below 0.10 and may approach zero (e.g. Pearson 1983; Kaufmann *et al.* 1992; Pamilo 1993; Rosengren *et al.* 1993; Ross 1993).

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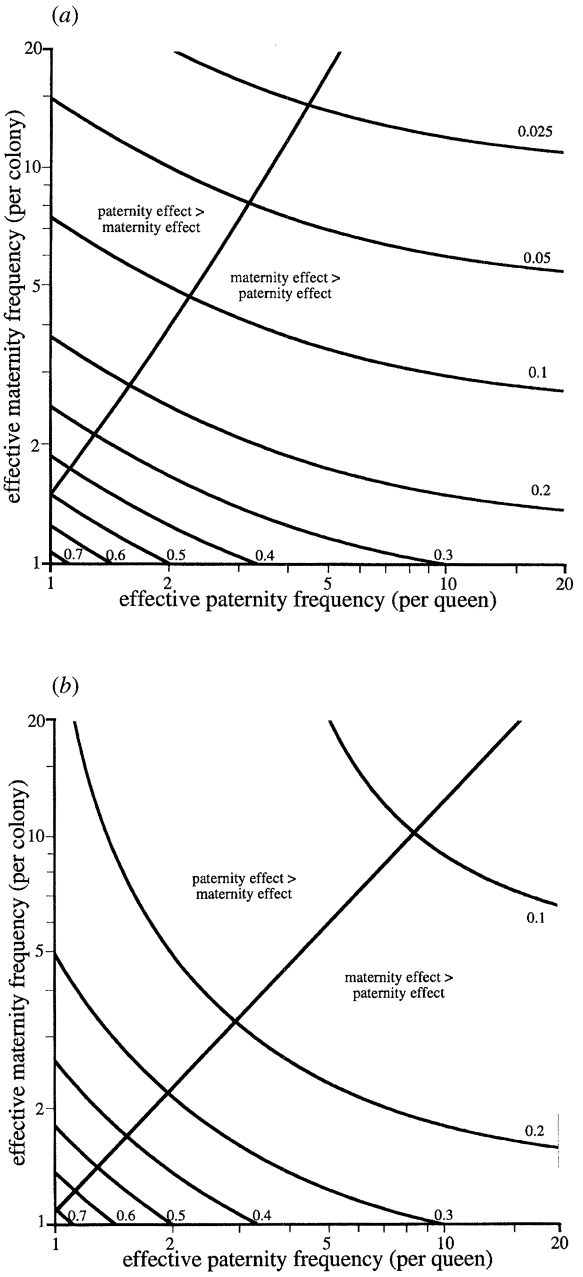

Figure 8. Lines of equal relatedness (from 0.025–0.70) as a function of effective paternity frequency per queen and effective maternity frequency per colony. The axes are logarithmic to make the distances between isolines more equal. The diagonal line connects the points where the slopes of the relatedness isolines equal minus one (because the axes are logarithmic the intersections in the figures deviate slightly from 90%). The diagonal is to the left of the $x = y$ line (not drawn) (i.e. where the diagonal would be if maternity and paternity had exactly equivalent effects), but more so when queens are unrelated (*a*) than when queens are sisters (*b*). In figure 8*b*, the isolines have been calculated assuming that colonies are headed by sister queens, all offspring of a single mother with the same paternity frequency as them. In both 8*a* and 8*b* queens are assumed to mate with unrelated males.

Some quantitative aspects of the effect of paternity and maternity frequencies are shown in figure 2. In this figure, monogyne colonies with paternity frequency in the ranges (s–d) and (s–m) would be close to the top-right corner, but highly polygynous colonies would be close to the bottom-left corner. In addition, the rates at which relatedness among nestmates reduces as a function of increasing number of queens and fathers depends on sperm bias and the relatedness among nest queens. Thus the effects of paternity and maternity frequency can take different forms.

Figure 8 shows the effects of effective paternity and maternity frequencies on relatedness for two situations: unrelated queens and sister queens. The diagonal lines connect the points on the relatedness isolines at which the slope equals −1 (because the axes are logarithmic this cannot be seen by eye). At these points equal small changes in effective paternity or maternity have equal effects on nestmate relatedness. To the left of the diagonal line changes in effective paternity have a greater effect on relatedness than changes in effective maternity, and vice versa. When queens are unrelated (figure 8*a*) the diagonal line is considerably to the left of the $y = x$ line (i.e. where the diagonal line would be if maternity and paternity had exactly equivalent effects). This indicates that, across the whole parameter space, queen number has a greater effect on relatedness than paternity. However, when queens are sisters the diagonal line comes closer to the $y = x$ line, indicating that paternity and maternity are now more equal in their effect, although maternity is still more important overall. Greater equality occurs because the sister queens are related to each other so that increases in maternity do not reduce relatedness as much as when queens are unrelated (compare also figure 2, *b* versus *c*, *d* versus *e*). In natural situations the diagonal line may be in an intermediate position as queen–queen relatedness tends to be relatively high in species or populations with few queens per colony and low (occasionally approaching zero) in species or populations with many queens per colony (Keller 1995).

Although maternity is generally more important in reducing relatedness than paternity, changes in paternity become more important than changes in maternity when maternity is already high. For example, if colonies had an effective maternity frequency of ten then a change in paternity of 1, from 1 to 2, would have a much greater effect than a change in maternity of 1, from 10 to 11. One consequence of this is that, when paternity frequency is low and queen number high, an increase in paternity will be more effective in increasing genetic diversity among colony offspring than a similar increase in queen number. Similarly, the reverse is true. For example, when a monogynous species has an effective paternity frequency of 3, adding another queen will have far more effect than adding another patriline. This complicates the 'translation' of the genetic diversity hypothesis into testable predictions. Multiple paternity and maternity may be alternative ways to increase genetic diversity from low to moderate levels, but both may be required to achieve consistently high genetic diversity (e.g. *Formica aquilonia*; Pamilo 1993).

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(*b*) *Causation*

The above (figure 8) compares the effects of paternity and maternity on nestmate relatedness. However, in reality it seems very unlikely that paternity and maternity can be traded-off directly because they are controlled by different parties, at different times in the lifecycle, and have different selective causes.

As discussed above, copulation is under queen control (unless males can insert non-removable mating plugs or actively control sperm storage; Boomsma 1996) and the workers have no influence on paternity. In contrast, workers can have considerable influence on queen number, which is under both queen and worker control. Primary polygyny, whereby multiple newly inseminated queens cofound a nest (Hölldobler & Wilson 1990) is under the control of the queens themselves. Subsequently in the lifecycle, the workers can reduce queen number by killing or expelling queens (Keller *et al.* 1989; review Reeve & Ratnieks 1993). Workers may also affect queen number by their willingness to recruit sister queens or adopt unrelated queens. The coexistence of multiple queens in a colony is dependent on the queens being mutually tolerant of each other, being located in different subnests, or on the workers being able to prevent fighting among queens (Reeve & Ratnieks 1993). Queen number is thus a colony trait under worker control that may be modified by the workers periodically during the life of the colony, at least in those species in which replacement queens can be adopted (Keller *et al.* 1989). In contrast, paternity is a trait of individual queens and males which cannot be manipulated or changed by workers during the life of the queen. In addition, mating with more males does not reduce the relatedness of a queen to her offspring, whereas cofounding or adoption of queens by workers does have a relatedness cost for the individuals making such decisions.

A further complication is the strength of fitness costs and benefits in monogynous and polygynous colonies. When colonies are polygynous, selection on individual queens in relation to mating behaviour may be relaxed. For example, fire ant queens which have made a matched mating and produce diploid male offspring can survive in polygynous nests but not in monogynous nests (Ross & Fletcher 1986). In fact, most of the hypothesised benefits from multiple mating are weakened in a population with polygynous colonies.

7. SOME DIRECTIONS FOR FUTURE RESEARCH

Below we make a few suggestions for future research, concentrating on matters arising directly from the results and discussion presented above, and for which the current empirical data are either non-existent or would benefit from supplementation.

(*a*) *More paternity data is needed*

Current data on effective paternity in ants, bees, and wasps cover only some of the taxonomic diversity of the eusocial Hymenoptera and may not be a representative sample, because of the clear bias towards certain genera and geographic areas. For example, our table 5 represents only 25% of the 16 ant subfamilies, 3% of the 296 ant genera (Bolton 1994), and 0.2% of the ca. 9000 described ant species (Hölldobler & Wilson 1990). As a result, and given the importance of paternity frequency in social evolution, data from more species will remain valuable especially when they form components of studies on colony reproduction, disease incidence etc.

Our weak confirmation of the genetic diversity hypothesis in ants with large colonies, and our stronger support for the correlation between paternity and colony size means that data from other large-colony single-queen species would be valuable. Obvious candidate taxa are the *Oecophylla* weaver ants, the *Pogonomyrmex* harvester ants and the *Eciton* army ants. In addition, paternity frequency studies should be made in *Atta* and related genera to supplement the sperm data (see for example, Reichardt & Wheeler 1996). *Atta* and related fungus-growing ants may also prove useful in looking at the correlation between queen number and paternity (see Keller & Reeve 1994).

Sperm clumping is a little known area given the relative ease with which it can be studied using genetic markers. Where possible researchers should take multiple samples of female offspring from nests. However, these samples should be large enough so that statistical tests for temporal variation in paternity have reasonable power to detect differences. This may be easier to achieve with cheap allozyme methods than with more expensive and labour intensive DNA techniques.

(*b*) *Data for comparative studies is needed*

Data from additional species will also help greatly in making comparative studies. For example, the study of variation in effective paternity in a phylogenetic context and in relation to other variables of the mating system and colony reproduction. One candidate group for such a study are the vespid wasps (Vespidae), for which a phylogeny exists (Carpenter 1991). The Vespidae are particularly interesting because they contain species characterized by both single and multiple paternity. The social organization of the Vespidae includes non-eusocial, primitively eusocial (Stenogastrinae, Polistinae), and advanced eusocial (Vespinae) groups. Within both the Polistinae and Vespinae are socially parasitic species (e.g. *Dolichovespula arctica*, *adulterina*, *omissa*, *Vespula austriaca* ; see Greene 1991) or genera (e.g. *Sulcopolistes*, Choudhary *et al.* 1994), and taxa with swarm-founding of nests (*Provespa*, many Polistinae) can be contrasted with nest-founding by solitary queens (*Dolichovespula*, *Vespula*, many Polistinae). Another candidate group are the leafcutter ants, for which a phylogeny is currently being worked out (Chapela *et al.* 1994; Hinkle *et al.* 1994). Primitive and advanced genera of this tribe have enormous differences in the size of mature colonies, and both multiple mating and polygyny have been reported in the advanced genera

(see sections above). Ideally, such studies should cover several populations and years, so that possible intraspecific variation in effective paternity can be assessed as well.

More detailed comparative studies will also help to remove one of the shortcomings of this study. The statistical tests we have presented assume that each species, or sometimes genus, is an independent datum, whereas the data are unlikely to be fully independent (Grafen 1989; Harvey & Pagel 1991). The extent to which the number of degrees of freedom in our analyses are overestimates is unknown as the phylogeny of the ants (e.g. Baroni Urbani *et al.* 1992) in relation to the traits of interest is unknown. However, both queen number and paternity frequency can show considerable variation within a species, so that phylogenetic inertia may be low. On the other hand, some traits, such as single paternity in *Solenopsis*, are non-variable within a genus.

(*c*) *DNA techniques*

Recent advances in DNA technology have tremendously increased the diversity of genetic markers available. The application of such techniques for paternity studies in social insects (Moritz 1991; Estoup *et al.* 1993, 1994, 1995; Evans 1993; Queller *et al.* 1993; Mueller *et al.* 1994; Peters *et al.* 1995; Thorén *et al.* 1995) is likely to increase substantially in the years to come and will allow new questions to be addressed. A higher genetic diversity at marker loci will be especially useful in species where progress has been hampered by the limited diversity of allozyme markers, as in the study of societies with high paternity and maternity frequencies (Moritz 1991; Estoup *et al.* 1993, 1994; Moritz *et al.* 1995; Oldroyd *et al.* 1995), or where occasional intraspecific social parasitism occurs (Mueller *et al.* 1994).

The analysis of polymerase chain reaction-amplified (PCR-amplified) DNA will also make it possible to use small amounts of material, such as eggs, sperm, or tissue samples. This will make possible studies comparing the relative sperm contributions of males immediately after copulation, in the spermatheca, and at all stages of offspring development including the egg stage (e.g. Peters *et al.* 1995). Such studies may shed new light on currently unclear issues such as sperm competition and sperm clumping within the spermatheca, the 'black box' between copulation and paternity. Studies of stored sperm and paternity could be combined with observations of copulation, such as Hölldobler (1976) and Fortelius (1994), to investigate how copulation order affects male contributions to sperm storage and paternity, and the extent to which increased copulations lead to increased effective paternity frequency.

At present, a number of DNA techniques are still expensive and elaborate compared to allozymes, but this difference is likely to become less in the years to come (Westneat & Webster 1994). This will change technical cost-benefit considerations, but probably not at the same rate for the different DNA techniques available. For the questions addressed in this review, it seems likely that codominant Mendelian markers such as microsatellites are the most promising successors of allozyme markers, because they allow more straightforward calculation of paternity and relatedness than dominant markers such as multilocus minisatellite techniques and RAPD's.

(*d*) *Costs and benefits*

The main selective forces influencing mating frequency evolution are the costs of copulation itself, and the benefits, if any, of multiple copulation via effects on colony genetic diversity, sex allocation, queen fecundity etc. Unfortunately, these costs and benefits have received little empirical study, so that additional data would be most valuable. Mating aggregations of some species, such as ants which mate on low vegetation or the ground, could be observed to determine predation and other risks during copulation. Mortality during copulation could also be assessed by determining the loss of queens during mating flights in those species, predominantly bees and wasps, where queens return to the natal nest after mating. Quantification of the possible benefits of increased mating frequency requires data on queen survival and colony performance as a function of mating frequency, in situations where diseases, diploid males, facultative sex allocation, environmental heterogeneity etc. are important. Currently, most of the data available are for *Apis mellifera*. Further studies on this and other species are called for.

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