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Dedicated to the memory of William Donald Hamilton

Polyandry in social Hymenoptera — disunity in diversity?

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Multiple mating by queens occurs in many species of social Hymenoptera despite its likely costs. Hypotheses to explain multiple mating include a need for more sperm than provided by a single male, the convergence of queen and worker sex-allocation optima and various genetic diversity hypotheses. For some species the sperm need hypothesis fails since queens retain only a single male's worth of sperm. In other cases, sperm store does increase with the number of matings. Similarly for the sex-allocation and genetic diversity hypotheses, data from some species are in support, those from others are not. Comparative analysis reveals a negative correlation between level of genetic diversity (of which queen mate number is an important determinant) and parasite load; findings in the best-studied case are complex: monandry and higher levels of polyandry are each selectively favored over moderate polyandry. Out of 14 identifiable hypotheses five are judged most useful for future work. Unfortunately, the search for a simple unitary model to explain all cases seems futile. A model encompassing all of these factors is desirable for studies on single species, but would be complex. Comparative analyses remain desirable, but should encompass the likelihood that different factors predominate in different groups.

Introduction

Multiple mating by female social insects has been of wide general interest, because it is an apparently maladaptive behavior. Likely costs of polyandry can be readily identified and include increased predation risk, energetic costs (probably most important in species with independent colony founding), increased risks of acquiring sexually transmitted diseases, and a reduction of relatedness between nestmates (at first sight a threat to the very foundations of eusociality). Despite these likely costs, the phenomenon is widespread among eusocial ants, bees and wasps (Page 1986, Boomsma & Ratnieks 1996, Crozier & Pamilo 1996, Fjerdingstad *et al*. 1998, Oldroyd *et al*. 1998, Bekkevold *et al*. 1999, Boomsma *et al*. 1999, Foster *et al*. 1999, Paxton *et al*. 1999, Pedersen & Boomsma 1999, Villesen *et al*. 1999, Murakami *et al*. 2000, Schmid-Hempel & Schmid-Hempel 2000, Tay & Crozier 2001), although in most species most queens mate singly (Boomsma & Ratnieks 1996, Strassmann 2001).

Intensive and extensive theoretical work has examined multiple mating by social insect queens in an effort to find adaptive explanations. A large number of sometimes very complex hypotheses have been proposed, most of these concerning potential benefits to queens arising from the increased intracolonial genetic diversity that results when multiple males co-father a queen's offspring. These hypotheses (Table 1) have often been explored both empirically and theoretically (as reviewed below), but often only one possible hypothesis has been examined, the approach being to find a single explanation for polyandry.

Empirical tests of plausible hypotheses for polyandry have yielded support in some species but not in others. For example, evidence that high intracolonial genetic diversity resulting from polyandry increases fitness correlates was obtained for the bumble bee *Bombus terrestris* (Shykoff & Schmid-Hempel 1991, Liersch & Schmid-Hempel 1998, Baer & Schmid-Hempel 1999, 2001) but no such effects were found for the ant *Lasius niger* (P. J. Gerstch, E. J. Fjerdingstad & L. Keller unpubl.). These differences in results need not reflect differences in power of the tests involved (although the sample sizes are often necessarily small), but rather reflect real differences in the biology of the species in the relative strengths of various factors affecting the queen mating frequency. The striking differences between species in queen mating frequencies may further reflect such differences. In most species queens mate once or with just one or two males, but in other species queen mating frequency is extreme (sometimes exceeding 100 mates per queen). The phylogenetic scale of this variation can be quite fine, for example the ant tribe Attini includes both habitually monandrous and highly polyandrous genera (Villesen *et al*. 1999, Murakami *et al*. 2000), and in the bee genus *Bombus* most species appear strictly monandrous but a significant fraction of *B. hypnorum* queens are polyandrous (Schmid-Hempel & Schmid-Hempel 2000).

Due to the apparent inability of any one hypothesis to explain polyandry convincingly so far, a pluralistic approach to the problem is required. For that reason we here discuss core aspects of our current knowledge on adaptive explanations for multiple mating by social insect queens, suggesting approaches likely to be of value for future studies. We start by considering the many hypotheses that have been proposed.

Assessing the hypotheses: general remarks

Hypotheses 1–7 in Table 1 do not pertain just to social insects but potentially to all insects, although a need to increase effective population size (hypothesis 2) is more likely to be important in social insects than in others (Pamilo & Crozier 1997). However, no such relationship between polyandry and population size has been observed (Wilson 1963), nor is it expected if the sole benefit is to the population or species. All the other hypotheses except 3 and 14 were assessed by Crozier and Page (1985) on the basis of the belief that multiple mating is more frequent in species with larger colonies (Cole 1983, Boomsma & Ratnieks 1996), leading them to reject any hypotheses which did not accord with this relationship.

Unfortunately, neither Cole (1983) nor Boomsma and Ratnieks (1996) included a phylogenetic dimension in their studies. Boomsma and Ratnieks (1996) found a significant positive association between paternity rate and colony size for monogynous ants, which persisted when results were averaged per genus. Subjecting these data to comparative analysis using the phylogeny of Schmid-Hempel and Crozier (1999) via the CAIC

Table 1. Hypotheses for the evolution of polyandry in social Hymenoptera.

Name	Notes and example citations
1. Convenience polyandry	Females are indifferent to who fathers their offspring, and are prepared to mate multiply to avoid confrontations with males. (Thornhill & Alcock 1983).
2. N_E increase	Species with very small population sizes mate more often in order to increase the effective population size (Wilson 1963).
3. Genetic compatibility	By mating more than once females avoid mating only with a male with whose genes theirs make a specific low-fitness combination; refers chiefly to avoidance of inbreeding and outbreeding depression and of selfish genetic elements. (Tregenza & Wedell 2000).
4. Transfer of benefits	Males supply nutrients as through nuptial feeding, or substances with the sperm that assist oviposition (Vahed 1998).
5. Sperm competition (adult fitness)	The competitive abilities of sperm correlated with the fitness of the adults they yield, so that fostering sperm competition by polyandry yields offspring with higher reproductive output (Starr 1985).
6. Sperm competition (winning sperm)	Polyandry leads to more effective sperm in fertilizing more eggs than do competitors, leading to grandsons of the polyandrous female being more effective when they mate with polyandrous females (Keller & Reeve 1995). Polyandrous species should tend to show sperm competition.
7. Sperm limitation	Queens are able to yield larger or longer-lived colonies if they obtain more sperm than is delivered by a single male (Cole 1983).
8. Queen-worker competition	Queens mate multiply because this increases their reproductive output compared to that of the workers (Moritz 1982).
9. Sex-allocation manipulation	Queens mate multiply in order to bring the optimum sex-ratio for their worker progeny closer to their own (Moritz 1985).
10. Rapid fission	Queens mate multiply to increase reproductive competition between worker subgroups, favoring more rapid fission (Getz *et al*. 1982).
11. Daughter queens more variable	A parallel argument to one of those for the origin and persistence of sexual reproduction: in a variable environment polyandry (through increased intrabrood genetic diversity) increases the chance that some of a queen's offspring will survive and lowers the risk that they will compete with each other (Crozier & Page 1985).
12. Genetic polyethism	Workers differ genetically in their aptitudes and proclivities for various tasks (purely in behavior, or in morphology as well), and polyandry maximises the probability of obtaining an optimal mix of genotypes (Page *et al*. 1989).
13. Sex-locus load	Polyandry reduces the variance between colonies of the production of diploid males due to matched matings at the sex locus; under some demographic conditions this favors monandry, under others polyandry (Crozier & Page 1985).
14. Herd immunity	Polyandry increases the diversity of worker genotypes in the colony with respect to disease resistance, increasing the overall colony resistance to epidemics (Schmid-Hempel 1994).

Other than hypotheses 3, 4, and 6, all these hypotheses have been proposed to explain polyandry in social Hymenoptera. Hypotheses 8 through 14 are applicable only to social insects in that they reflect group effects. All these factors either uniformly favor polyandry, or favor polyandry for some parameter values, disfavor it for others. Mating risk (eg., from predation pressure, disease transmission, energy costs) uniformly acts to favor monandry.

package (Purvis & Rambaut 1995) confirms the findings of Boomsma and Ratnieks (1996), but with a weak trend indicated (the *t*-value for the association just exceeds the threshold for a one-tailed test; analysis not shown). Thus, while this association should be tested further, a reasonable working hypothesis remains to give particular weight to predictions of an association of level of polyandry with colony size in examining the hypotheses.

The reasons advanced by Crozier and Page (1985) for discounting hypotheses 1, 2, 5, 8, and 10 still appear cogent and, we suggest, these hypotheses are therefore not likely to be useful for guiding future research (Crozier & Page 1985).

The remaining nine hypotheses all have theoretical or empirical support, or both, for their occurrence as real phenomena affecting various fitness components. Especially in monogynous species, it is likely that they are all operational at the same time (Fjerdingstad & Pedersen 1998). Most of the hypothesized factors favor increased polyandry, being balanced against mating risk, and many may operate simultaneously in any one species (Fjerdingstad & Keller 1998). The fact that there is evidence for some factors strongly favoring polyandry (Baer & Schmid-Hempel 1999) in at least one species which is monandrous underlines the point that the final result stems from a balance of favoring and disfavoring selective forces (Baer & Schmid-Hempel 2001).

Assessing the hypotheses: analysis of nine hypothesis

Of the nine remaining hypotheses three involve selection favoring genetic variation within colonies (12: genetic polyethism; 13: sex-locus load; 14: herd immunity), and are usually called the ‘genetic diversity’ hypotheses. Hypothesis 12 (daughter queen variability) also rests on considerable levels of variation. Of the remainder, for only one (7: sperm limitation) is the presence of any genetic variation irrelevant.

Hypothesis 3: genetic compatibility

The genetic compatibility hypothesis suggests that females mate with multiple males to reduce the chance of mating with a male whose genes, when combined with the female’s, produces a low-fitness genotype. Under this hypothesis, (Zeh & Zeh 1997) are subsumed many hypothesized genetical factors, including inbreeding and outbreeding depression (Jennions & Petrie 2000). The risk of outbreeding depression is likely to be transitory in evolutionary terms, reflecting selection for reinforcement (Coyne & Orr 1989). Within species of solitary insects, inbreeding avoidance appears to be frequent as affecting mate choice, but the risk appears to be very small of a female mating with an unrelated male possessing genes that, in combination with hers leads to combinations detrimental to her offspring (Tregenza & Wedell 2000).

Given the very broad and heterogeneous definition of ‘genetic incompatibility’, it can also refer to any of those separately listed hypotheses concerning genetic variation. We have, however, thought it more useful to consider it separately from these other components, in part because of their long history in discussions of polyandry in social Hymenoptera. Once we remove the hypotheses on genetic variation traditional in social insect discussions, inbreeding remains as the chief ‘genetic incompatibility’ factor.

It is worth noting that genetic incompatibility of itself does not favor polyandry, unless further conditions are met. Thus, from the point of view of a particular female genotype, let p be the frequency of males leading to maximum offspring survival and let q equal the frequency of males who would father offspring with a survival rate of $(1 - s)$ of that of the superior males for this female. If she cannot choose her mate in terms of genetic compatibility, the mean fitness of a female who mates once is:

$$1 - sq \tag{1a}$$

If the female mates twice, again without mate choice, the fitness becomes

$$1 - 2hspq - sq^2, \tag{1b}$$

where h denotes the tendency of cases where the female has mated with one superior and one inferior male to resemble either the case of mating with a superior male (h = 0) or an inferior male (h = 1) alone. If the males have the same probability of fertilizing eggs, then $h = 0.5$ and the fitness of a twice-mating female is the same as that of a once-mating female. Unless the life history leads to $h < 0.5$ there is no selection for polyandry.

One way that h could be lowered below 0.5 is if females are able to select which sperm fertilize their eggs in order to maximize the fitness of their offspring (Jennions & Petrie 2000). A large study using six isofemale lines and a tester stock of *Drosophila melanogaster* found numerous specific strain-by-strain interactions in sperm displacement, but no tendency of the sperm of males of the same strain as the female to be displaced by other-strain sperm, although same-strain sperm did have a disadvantage in displacing other-strain sperm (Clark *et al*. 1999). Given the complexity of the results and the unlikelihood of females encountering isogenic males in the field, these findings do not support a role for inbreeding avoidance in *Drosophila* polyandry. In social Hymenoptera, which spend a brief period mating and then use the sperm for often many years afterwards, the ability of females to select sperm from males of the better phenotype is probably vanishingly small. Also, the spermathecal capsule of social Hymenoptera consists of a single often rather spherical sack, with no easy opportunity for storing sperm from different males in different places — in contrast, *Scathophaga stercoraria* females have three or four spermathecae, which increases the potential for post-mating mate choice (Ward 2000).

With respect to inbreeding, such effects have been shown over and above those of the sex locus for honeybees (Kerr 1976), and it is reasonable to believe that there is a genetic load of deleterious recessive alleles in outbreeding Hymenoptera (Kerr 1976, Crozier 1985, Werren 1993). The strong tendency of social insects to have a dispersive mating flight is best understood as an adaptation to reduce the level of inbreeding (Crozier 1980, Crozier & Pamilo 1996), although inbreeding sufficient to raise F to about 0.09 is known from *Pogonomyrmex occidentalis* (Cole & Wiernasz 1997) and to 0.14 in *Formica exsecta* (L. Sundström, L. Keller & M. Chapuisat unpubl.), still relatively low values (by comparison, an offspring of two human first cousins has F = 0.0625). Major exceptions occur for those inbreeding social parasites where there is 100% mating between nestmates (e.g., Buschinger 1989).

Give the considerations above, genetic compatibility effects seem unlikely to be consistent in affecting mating biology of social insects, other than behavioral adaptations to avoid heterospecific matings and mating with close relatives. Selection imposed by matched matings at the sex locus will vary in strength and direction depending on ecological and demographic factors; it remains a good project to see if honeybee queens mate less often than expected with drones bearing a matching allele, and if despite expectation they use sperm with a matching allele less often than expected.

Hypothesis 4: transfer of benefits

The transfer of benefits hypothesis suggests that females mate with multiple males to accumulate fitness-enhancing resources provided by males. Nuptial gifts (Vahed 1998) have not been reported for Hymenoptera and they should be easily visible to investigators; furthermore the importance of nutritional gifts from male mates would likely be limited in many species that found colonies assisted by workers or that enter other colonies as parasites. This possibility is therefore very unlikely to be important for social Hymenoptera.

There is a distinction between nuptial gifts, being nutritious offerings intended as incentives to mate, and accessory substances transferred with the sperm, by definition following a successful mating. Accessory substances have been best studied in *Drosophila* and *Scathophaga*, where they have various deleterious effects on female fitness (Snook 2001). Analysis of the sperm plug in *Bombus* reveals materials thought to reduce female receptivity to further mating (Baer *et al*. 2000, Baer *et al*. 2001). Hence, although as always further information would be useful, the information so far mitigates strongly against accessory secretions selecting for polyandry in social Hymenoptera.

Furthermore, if nutritional accessory secretions are transferred from males to queens during mating, one would expect multiply-mated queens to have a higher survival until the time the first workers emerge or to produce more offspring during that stage. No empirical data is available for testing the former hypothesis and data on 100 incipient colonies of the ant *Lasius niger* shows no evidence for the latter (E. J. Fjerdingstad & L. Keller unpubl.).

Hypothesis 6: winning sperm

The winning sperm hypothesis suggests that females mate multiply because sperm competitve at fertilizing eggs yield sons (or for Hymenoptera, only grandsons) producing similarly competitive sperm. Under this view the only characteristic being favored is the transmission of inherited sperm competitive ability, without any necessary correlation between competitive ability and characteristics of the resulting zygotes. Provided that polyandry occurs occasionally, eggs within polyandrous females will be fertilized by highly competitive sperm more often than expected from the number of males involved. Competitive ability may increase through males producing more sperm or, as suggested by Keller and Reeve (1995), by specific interactions between particular sperm genotypes. In this regard, it has been suggested that ejaculates compete as entities, with specialized sperm types, such as apyrene sperm in some lepidopterans suggested to function to flush out or physically destroy the sperm of previous males (Silberglied *et al*. 1984). In Hymenoptera, sperm types of the polyandrous parasitoid *Dahlbominus fuscippenis* include some incapable of fertilizing the egg, but it seems unlikely that these are soldier sperm because they block the micropile to all sperm, not just those of competing males (Wilkes & Lee 1965).

Evidence for the significance of this factor (selection for competitive sperm) in the evolution of polyandry in eusocial insects is lacking; differences in the abilities of honeybee males to fertilize eggs has been found once (Harbo 1990) but not otherwise (Sasaki *et al*. 1995, Haberl & Tautz 1998, Franck *et al*. 1999) indicating that in a particularly favorable system for this effect it is weak or absent.

Although it cannot be completely dismissed, the winning-sperm hypothesis poses difficulty in terms of guiding research. Many species of ants are known with occasional polyandry (Boomsma & Ratnieks 1996), indicating that selection for winning sperm has not yet been effective in these cases in overcoming the effects of mating risk. As seen for other hypotheses, the existence of the phenomenon is insufficient to demonstrate its evolutionary effect, and life-pattern predictors of its strength remain to be determined, hindering the application of comparative methods.

Hypothesis 7: sperm-limitation

The sperm-limitation idea is a simple one — increased sperm stores enable increased reproductive life-span for queens and hence increased long-term fecundity (Hamilton 1964, West-Eberhard 1975, Cole 1983, Starr 1985). As noted above, there is an association between colony size and paternity level, although it is weak. Crozier and Page (1985) doubted that sperm need is a plausible evolutionary force, noting that selection on male size to match that of the females is expected. They noted that the effect could be temporarily valid [in evolutionary time] if there was a lag between the evolution of increased queen size and that of females. Other authors (Hölldobler & Wilson 1990) suggested that in some cases optimal male size in terms of inter-male competition could be smaller than required to yield a sperm load sufficient to fill a queen's spermatheca.

Honeybees are well-known to present very high levels of polyandry with, at least in *Apis mellifera*, no more sperm stored than could be supplied by a single male, and no increase in sperm stored with additional matings (e.g., Tarpy & Page 2000). However, for at least some congeners, the picture is not as clearcut as for *A. mellifera*. Thus, in *A. koschevnikovi* a drone-contains 1.7 ± 0.16 million sperm, queen oviducts contain 12–21 million sperm and the spermatheca 2–3 million sperm (Koeniger *et al.* 1994). From genetic data, queens mate 10–20 times, with the effective mate number being 10.5 ± 8.4 (Rinderer *et al.* 1998). These data indicate that a queen mates many more times than required to obtain the sperm seen in the spermatheca; the preponderance of sperm that do not arrive at the spermatheca has suggested that the queen's anatomy is adapted to excluding most sperm so as to maximise genetic diversity (R. E. Page pers. comm.). In *A. andreniformis*, sperm transfer is much more efficient than for *A. koschevnikovi*, with sperm numbers in drones and queens indicating mating frequencies between 4 and 14 (Koeniger *et al.* 2000). Microsatellites revealed somewhat higher numbers of matings with a range of 10–20 (Oldroyd *et al.* 1997), suggesting that queens mate in excess of the requirement to gain the sperm stored. The picture is pronounced for *A. dorsata*, whose queen spermathecae contain sperm deliverable by less than two males (Koeniger *et al.* 1990) yet which mate on average 26 times (Moritz *et al.* 1995, Oldroyd *et al.* 1996) and occasionally over 100 times (B. P. Oldroyd pers. comm.). Because queens of these species do require more sperm than delivered by a single male, they clearly need to mate with several males, but the very high numbers in excess of this requirement may result from other selective factors.

Vespine wasps vary between species in queen mating frequency (Foster *et al.* 1999) but too few counts of sperm from spermathecae (Stein & Fell 1994) are yet known to make a contribution to this area.

In ants, the genera with the highest reported mate numbers are *Pogonomyrmex* (Cole & Wiernasz 1999) and the higher attines *Atta* and *Acromyrmex* (Kerr 1961, Reichardt & Wheeler 1996, Fjerdingstad *et al.* 1998, Bekkevold *et al.* 1999, Boomsma *et al.* 1999, Villesen *et al.* 1999, Murakami *et al.* 2000). A possible exception is *Atta texana*, reported by Moser (1967) to store no more sperm than produced by one male; but the situation may resemble that of *A. colombica*, where there is a high variance in sperm load between males and queens store no more sperm than the mean male load (Fjerdingstad & Boomsma 1997), yet there is a significant regression of sperm stored on mate number (Fjerdingstad & Boomsma 1998).

The sperm-limitation hypothesis is difficult to test directly, because of the great longevity (as long as several grant lifetimes) of social insect queens (*see* Keller & Genoud 1997). The payoff is not that more multiply-mated queens have more offspring at a given age than less multiply-mated queens still active at the same age, but rather that queens with more sperm have a high probability of continuing for more seasons than those with less. Indirect arguments are possible, such as the numbers of sperm needed to last a lifetime (Fjerdingstad & Boomsma 1998), and should it become possible to determine queen ages with sufficient reliability — such as through lipofuscin accumulation (Vila *et al.* 2000) — it may be possible to check if increasing mate number increases longevity. However, such an increase could also result from benefits from genetic diversity, so that comparative analyses of the association with multiple mating with predictors not applicable to other factors might yield a better chance of success.

Queens of the imported fire ant, *Solenopsis invicta*, have been estimated to store sperm sufficient for between six and seven years and their sperm supply has been shown to decline dramatically with time (Tschinkel 1987). We do not know if queens ever outlive their sperm supply, but if they do then it is likely that infertility is death, either through the extinction of the colony or possibly through the killing of the queen in favor of a fertile one (Wilson 1966, Tschinkel & Howard 1978). If queen longevity could be improved by larger sperm stores, then selection for either or both of polyandry and more efficient sperm transfer would be expected.

Comparative analyses may be profitably directed towards hypothesized reasons for sperm-need rather than just the factor itself, given the paradox it presents in evolutionary terms (Crozier & Page 1985). These sub-hypotheses are phyletic lag (males are increasing in size to match queen needs, but they lag behind) and that male-male competition favors males with smaller sperm loads than can be accommodated by a female's spermatheca. Multiple mating by males (Boomsma 1996, Reichardt & Wheeler 1996) may result if males can father more offspring by spreading their sperm load across several females (either if females differ in quality, or as a bet-hedging strategy), if the benefit to female fecundity follows a curve of diminishing returns, or if mating with a non-virgin female [to forestall the future workers opting for an all-male sexual brood, carrying no paternal genes (Boomsma 1996)]. However, this hypothesis does not differ fundamentally from the other two — males would then be selected to have more sperm so as to fill the spermathecae of multiple females if nothing constrained maximum male size.

In three *Pogonomyrmex* species (Davidson 1982, Abell *et al*. 1999, Wiernasz *et al*. 2001) larger males are more successful at mating than are smaller ones, but in *Atta colombica* Fjerdingstad and Boomsma (1997) noted that males with larger thoraces had smaller sperm contents and suggested that this may indicate that these two fitness traits are negatively correlated and that the most aerially proficient males can deliver fewer sperm. We may expect male size (and hence male sperm complement size) to be constrained more often in species that mate on the wing (such as *Atta colombica*), where agile (or simply small) males may experience a higher mating success, than in ground-mating species (such as *Pogonomyrmex*) where large male size may be favored. Although our suggestion that the males with the most sperm (or largest overall size) may sometimes be less successful at mating is conjectural for social insects, a case of just such stabilizing selection is known from an aerially mating midge, *Chironomus plumosus* (Neems *et al*. 1998).

Hypothesis 9: sex-allocation manipulation

The sex-allocation manipulation hypothesis suggests that queens mate multiply because selection then leads to a worker-controlled sex allocation closer to their own optimum under selection. In male-haploid social insects selection favors workers changing the colony's pattern of sex-allocation in response to its relatedness structure (Boomsma & Grafen 1991, Crozier & Pamilo 1996). Within a monogynous population, for example, colonies with a once-mated queen will then adopt a highly female-biased sex allocation and ones with a twice-mated queen a more male-biased one (Ratnieks & Boomsma 1997), an example of sex-ratio compensation. With increasing mate number, the optimum colony sex allocation pattern under worker control approaches that of the queen, and this avenue for manipulation of the workers has been suggested as yielding selection for polyandry (Moritz 1985, Ratnieks & Boomsma 1997); *see* review by Crozier and Pamilo (1996).

There is evidence for the occurrence of sex-ratio compensation in many studies (Crozier & Pamilo 1996: chapter 5) but support for the sex-ratio manipulation hypothesis has been found in only two cases, those of the ants *Formica exsecta* (Sundström *et al*. 1996) and *F. truncorum* (Sundström & Ratnieks 1998), with the only other study so far (E. J. Fjerdingstad, P. J. Gertsch & L. Keller unpubl.) finding no differences in observed sex allocation between single and double-paternity cases in 113 *Lasius niger* colonies. Productivity differences were also sought for in the same 113 *Lasius niger* colonies, but not found (P. J. Gertsch, E. J. Fjerdingstad & L. Keller unpubl.). A first requirement for continued consideration of the hypothesis for any case would be that the change in worker mediation of the sex ratio postulated to drive polyandry is observable. For cases where a convergence of queen optima and the worker-controlled sex ratio is observed, it is unclear (as for most of the hypotheses) whether this effect is causal or not, with tentative acceptance contingent on disproof of all other hypotheses. Given that the effect becomes increasingly weaker as higher levels of polyandry are achieved, it does not seem likely for example that this hypothesis is plausible for the various highly-polyandrous honeybee species (*see* Oldroyd *et al.* 1997).

Comparative analysis potentially offers the best way forward, but predictors for when this effect predominates are not apparent.

Hypothesis 11: daughter queens more variable

The daughter queens more variable hypothesis suggests that polyandry would be more likely in an environment in which daughter-queens experience a greater variety of microhabitats in which to settle (Crozier & Page 1985). This could be tested via comparative analyses or by comparing different populations of the same species that differ in queen mating frequency. Characterization of the actual colony foundation sites and subsequent colony conditions would be required to test the hypothesis. The hypothesis, however, may not provide a strong selective factor because in at least some cases ant queens tend to select their preferred microhabitat (at least to some degree) rather than accepting potential sites at random (Wilson & Hunt 1966). The fact that the *Pogonomyrmex* species and the attines have unusually homogenous diets and superficially homogenous habitats and yet show very high levels of multiple mating, reduces the likelihood of this hypothesis further although, especially for *Pogonomyrmex*, temporal variation (Hedrick 2000) remains to be considered. Also, the very high levels of polyandry found in honeybees [e.g., averaging 26 males in *Apis dorsata* (Moritz *et al.* 1995, Oldroyd *et al.* 1996) but occasionally exceeding 100 (B. P. Oldroyd pers. comm.)] are not readily explicable under this hypothesis, because genotypic diversity in broods of male-haploid species increases negligibly after about 7 matings (Crozier & Page 1985, Crozier & Pamilo 1996).

Hypothesis 12: genetic polyethism

The genetic polyethism hypothesis suggests that polyandry yields a more efficient worker force. Genetic polyethism has been abundantly demonstrated in honeybee species (e.g., Bhagavan *et al.* 1994, Breed 1988, Calderone & Page 1988, Frumhoff & Baker 1988, Oldroyd *et al.* 1994, Page *et al.* 1989, Robinson & Page 1988, 1995) and has been found for ants in artificially constructed (Stuart & Page 1991, Julian 1998) and natural (Snyder 1992, 1993, Julian 1998) colonies. However, in only two of the ant studies (Stuart & Page 1991, Julian 1998) could maternal effects be eliminated. Improved colony efficiency has been demonstrated in more as against less genetically diverse honeybee colonies in one study (Fuchs & Schade 1994) and highly suggestive indications obtained in others (Calderone & Page 1992, Oldroyd *et al.* 1993, Page *et al.* 1995, Fewell & Bertram 1999). For ants, in which some species have morphological task-associated variation between worker castes (or 'subcastes' according to terminological preference), genetical variation in the proportion of these subcastes has been demonstrated between colonies in *Pheidole dentata* (Johnston & Wilson 1985) and strongly indicated within colonies for *Camponotus consobrinus* (Fraser *et al.* 2000).

For genetic polyethism to select for polyandry requires appropriate behavioral dominance, defined as occurring when the overall colony behavior tends more to that of one genotype than expected on the basis of its proportion of the workforce (Charnov 1978, Craig 1980, Pamilo 1982, Bulmer 1983, Crozier & Pamilo 1996). As an hypothetical example, behavioral dominance occurs if 10% of the colony are specialists for a task which no other members can do, but they enable the colony to perform the task more than 10% of the times required. No model has yet appeared explicitly including this effect, but that of Fuchs and Moritz (1998) does so implicitly. If behavioral dominance is strong enough for benefits of specialism, then there need be no upper limit to the level of polyandry selected for.

Various studies on honeybees indicate the operation of behavioral dominance at least for low levels of genetic diversity. For ants, behavioral dominance has been repeatedly demonstrated in the sense that a colony benefits disproportionately from the presence of different (morphological) castes (Oster & Wilson 1978, Wilson 1980, Hölldobler & Wilson 1990), but if there is no genetic variation in the population for these characteristics then polyandry would not be selected for, making the indications cited above for genetic variation for caste proportions quite important. In terms of genetic polyethism, however, there is no evidence yet that polymorphism affects any fitness-related traits.

Polyethism is more likely to be important for larger than for smaller colonies (Boomsma & Ratnieks 1996), but since this prediction also holds for others of the nine hypotheses other predictors should also be sought for comparative studies.

Hypothesis 13: sex-locus load

The sex-locus load hypothesis suggests that under some ecological and social conditions a reduction of the variance for the production of diploid males is selected for, and under others an increase. Selection under this hypothesis results from the production of homozygotes for the sex locus, which become diploid males either dying during development or yielding largely or wholly sterile adults (Crozier 1975, Cook & Crozier 1995). These diploid males constitute a fitness load on colonies of queens that made a matched mating (have mated with a male carrying one of their sex locus alleles). Explicit to the model for this hypothesis, and implicit in all genetic-variation hypotheses, is behavioral dominance, namely that, for polyandry to be selected for, genetic variation assists colonies with more genetic variation sufficiently more on average to compensate a polyandrous strain for the reduction in frequency of those less-variable colonies with optimum genotypes. In this case, the optimum phenotype is to have no diploid males at all. Multiple mating reduces the frequency of this optimum phenotype but increases the proportion of the colonies with some diploid males. Demographic models suggest that, in many cases, colony reproduction late in the colony growth curve leads to selection for polyandry, but reproduction early in the colony growth curve favors monandry (Crozier & Page 1985, Pamilo *et al.* 1994, Crozier & Pamilo 1996). Fire ants (*Solenopsis invicta*) face severe competition from other colonies after emergence, and the production of any diploid males is fatal (Ross & Fletcher 1986). Honeybee colonies lack this early competition, and modelling indicates that colonies with some diploid males tend to approach the mature colony size of colonies with none, but that colonies with high proportions of diploid males are severely disadvantaged (Page 1980). An alternative model is that polyandry is favored if the diploid males can be removed early enough and replaced with fresh diploid eggs (Ratnieks 1990). Such removal may occur more efficiently in colonies with intermediate numbers of diploid males (as in a twice-mated queen with one matched mating) than in colonies with high numbers (as in a once-mated queen with a matched mating). This model involving the timing of diploid male removal yields behavioral dominance, and explains the case of meliponines better than does the demographic model — meliponines produce new colonies as swarms, yet mate singly because the diploid males grow to adulthood causing a severe burden to the colony (Ratnieks 1990).

The nature of the behavioral dominance determines the directionality of selection, towards monandry or polyandry. The strength of selection may also vary between species, mediated for example by the amount of resource required to produce a single male relative to individuals of other castes. Thus, if in one species males are very large relative to workers (as in army ants) then the loss of resources through diploids becoming males would generally be greater than in a species in which males are smaller than workers (as in many other ants).

We can now derive an heuristic model for sex-locus selection driving the evolution of double mating. Let the fitness of a once-mating strain be:

$$W_1 = a + (1 - a)(1 - d) \qquad (2)$$

where d = the loss of fitness due to the colony presenting 50% of its diploids as male, and $a = (n - 2)/n$ where n is the effective number of sex

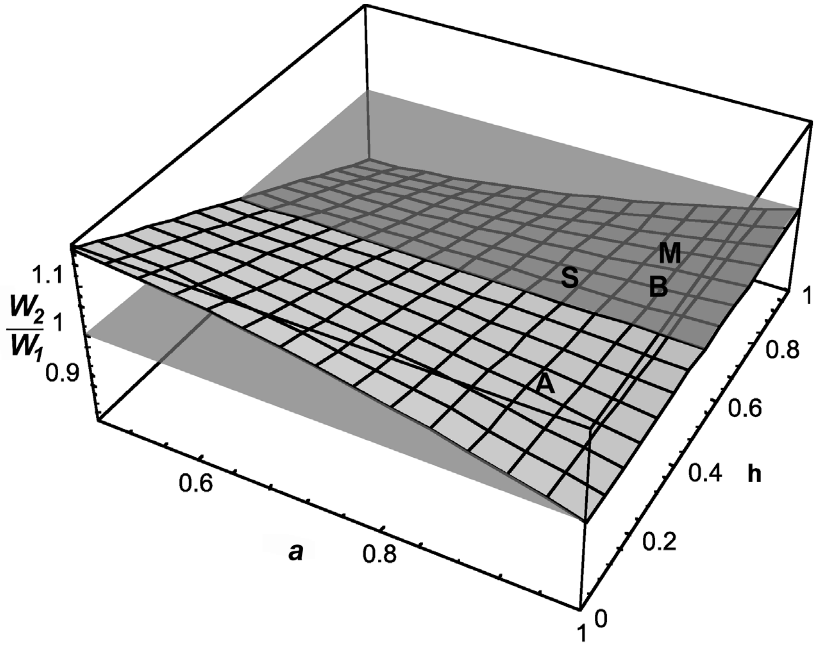

Fig. 1. Selection for queens mating twice or once. Selection for mating twice is favored when the fitness of a twice-mating strain (W_2) exceeds that of a monandrous strain (W_1), i.e., $W_2/W_1 > 1$. The squared curved surface indicates the value of W_2/W_1 for different values of $a = (n - 2)/n$, where *n* is the effective number of sex alleles} and *h* (the degree to which a colony in which one of the queen's alleles matches that of a mate resembles a colony in which no alleles are matched, $h = 0$, or resembles a colony in which both queen alleles are matched, $h = 1$). The fitness decrement in a colony with both queen alleles matched compared to one with no alleles matched (*d* in Eq. 3) is here assumed to be a constant 0.5. Selection favors mating twice for all cases with $h < 0.5$, but the strength of selection depends on d and the effective number of sex alleles. Based on the reported numbers of sex alleles, the placements are shown for parameter *a* for *Apis mellifera* (A: Yokoyama & Nei 1979), *Bombus terrestris* (B: Duchateau *et al.* 1994), *Melipona compressipes* (M: Kerr 1987), and *Solenopsis invicta* (S: Ross *et al.* 1988). The *h* placement for *Bombus terrestris* is based on the observed monandry of this and most congeners and the results of Baer and Schmid-Hempel (2001).

alleles. The parameter *a* is the probability of a unmatched mating.

The fitness of a strain mating twice is then:

$$W_2 = a^2 + 2a(1 - a)(1 - hd) + (1 - a)^2(1 - d) \quad (3)$$

where h = the tendency of colonies with one of the queen's alleles matching that of one male to resemble the case with no matched alleles ($h = 0$) or to resemble the case of both alleles matched ($h = 1$). The quantity *h* is thus a measure of behavioral dominance.

Mating twice is favored when

$$W_2/W_1 > 1 \quad (4)$$

Whether selection at the sex-locus favors mating twice as against once, and the strength of this selection, therefore depends on the values of *a*, *h* and *d*. Figure 1 shows the interplay of *a* and *h* in favoring or disfavoring multiple mating. The directionality of selection depends on *h*.

The value of *a* rests on the effective number of sex alleles. In turn, the number of sex alleles rests on the interplay between selection, mutation rate, and effective population size. The potential number of sex alleles is unknown, pending molecular characterization of the locus. Other things being equal, selection on level of polyandry via the sex locus will be more intense in smaller populations. Hence, even in cases where colonies can cope with the occurrence of 25% of the diploids being male so as to make them more similar to colonies with no diploid males as against those with 50% of the diploids being male, in large populations this selection is less likely to counter the effects of mating risk than in small populations.

Some rough placement of known species is possible within the coordinates of Fig. 1. The effective numbers of sex alleles for *Solenopsis invicta* in North America (10–13; Ross *et al.* 1988), *Apis mellifera* in Sao Paulo (18.9; Adams *et al.* 1977), *Melipona compressipes* (20; Kerr 1987) and *Bombus terrestris* (> 24; Duchateau *et al.* 1994) yield values of *a* between 0.80 and 0.92. Values of *h* are high for *Solenopsis invicta*, in that colonies not growing at a high initial rate lose in competition during the brood-raiding phase (Ross & Fletcher 1986). Values of *h* are probably low for honeybees, and Tarpy and Page (2001) have made a strong experimental attempt to elucidate the situation for the common species, *Apis mellifera*. They show that high levels of brood mortality lead to high levels of colony mortality, and with a mathematical model confirm that low mate numbers increase the risk of high brood mortality through diploid male production. While the precise relationship between mate number and actual colony fitness needs to be determined, this study is a significant achievement. However, Tarpy and Page acknowledge that selection via the sex locus would not be likely to lead to mating frequencies over 10, yet *A. mellifera* often exceeds this. They suggest that *A. mellifera* behaves to as to exceed the polyandry threshold to minimize mortality, and that queens often overshoot (i.e., they can't count very well). This explanation seems less likely for species such as *Apis dorsata* in which very much higher mating frequencies have been found (*see* above).

Hypothesis 14: herd immunity

The herd immunity hypothesis suggests that polyandry is selected for because increased genetic diversity improves the chances of a colony possessing worker genotypes resistant to any pathogen and colonies with some resistant genotypes survive better than those with none. Comparative analysis shows that parasite load is less in species with higher intracolony genetic diversity (Schmid-Hempel & Crozier 1999). This consideration is expected to lead to polyandry being commoner among monogynous than among polygynous species but this has yet to be shown (Crozier & Pamilo 1996, Schmid-Hempel & Crozier 1999). In a crucial experiment, Baer and Schmid-Hempel (1999) showed that artificially inseminated *Bombus terrestris* queens produced colonies with lower parasite loads and higher reproductive success if sperm of high as against low genetic diversity was used. However, consideration of overall fitness using the same protocol and field-maintained colonies showed that the total fitness of colonies with queens mated 2–3 times is less than those with monandrous ones, although fitness increases above monandrous levels after queens mate four times (Baer & Schmid-Hempel 2001).

Herd immunity was Bill Hamilton's favored hypothesis for the evolution of polyandry (Hamilton 1987) with the sex-locus load as his acknowledged competitor (pers. comm. to RHC). The greater resistance of *Bombus* colonies with polyandrous queens to parasite attack (Baer & Schmid-Hempel 1999) fits well with this hypothesis, but the monandry of most *Bombus* species then appeared anomalous, although the anaphrodisiac properties of the sperm plug suggested that coercion prevented females mating more than once (Baer *et al.* 2001, Sauter *et al.* 2001). The finding that *Bombus terrestris* females mating 2–3 times are worse off than monandrous ones suggests that any herd immunity benefits are overwhelmed under low levels of polyandry by some other factor or factors, such as increased intracolony conflict (Baer & Schmid-Hempel 2001).

It can be difficult to tell whether or not a higher reproductive success for more genetically diverse colonies, as reported for *Bombus terrestris* queens mated more than four times (*see* above), truly supports the herd immunity hypothesis because genetic polyethism effects could also lead to the same pattern. In species where disease defence has a strong behavioral component the two hypotheses may become intimately intertwined. The report (Cole & Wiernasz 1999) that increased colony growth rate correlates with degree of polyandry in *Pogonomyrmex occidentalis* falls into this category, but has also been criticized on technical grounds (Fjerdingstad & Keller 2000; but *see* Cole & Wiernazs 2000).

Mating risk

Occasional polyandry occurs in many species (Boomsma & Ratnieks 1996), and it is tempting to ascribe monandry and polyandry occurring within the same population to alternative equal-fitness strategies under the classic calculation that polymorphism indicates equal fitnesses (Maynard Smith 1982). However, it is also possible that such differences reflect different local conditions, and that the individuals are following a conditional strategy (Gross 1996). Thus, if changes in the operational sex ratio occur during a prolonged mating flight, then a changing mating risk may lead to overall selection for polyandry at some times, monandry at others.

Actual values for mating risk are hard to come by, but it is widely acknowledged that it is severe (Bourke & Franks 1995: 368–369). Even in generally polyandrous groups such as the higher attines, mortality during the mating flight can be very heavy (Fjerdingstad & Boomsma 1998). *Acromyrmex* queens descend to the ground during a mating and must therefore ascend again for subsequent matings, leading to a constant risk per mating [e.g., *see* Reichardt and Wheeler (1996)]. Ground-mating species such as *Pogonomyrmex* harvester ants have lower risks (Hölldobler 1976), but it seems likely that queens are at risk as long as they remain above ground. Significant risks have been reported for *Formica* ants where females mate on the ground beneath swarms of males (O'Neill 1994). For honeybees, estimates of risk vary considerably (Palmer & Oldroyd 2000, Tarpy & Page 2001), perhaps reflecting whether or not the risks are assessed in populations subjected to coevolved predators (as in Europe) or free of them (as in the U.S.).

Consideration of mating risk is important in studies of single species in which a complete description of mating biology is attempted. At first sight variation in mating risk seems likely to be factored out in comparative analyses, but if systematic differences in mating risk occur between different groups then measures of these should be included in such analyses otherwise a major causal factor may be overlooked.

Concluding remarks

From a review of empirical studies on solitary insects, Arnquist and Nilsson (2000) concluded that multiple mating is always adaptive — multiple mated females have higher overall reproductive success than single maters and polyandry is "near ubiquitous…". As shown in our review the case for social insects is less clear, and we agree with Strassmann (2001) that, should Arnquist and Nilsson be correct, it is as reasonable to ask why so many social Hymenoptera mate once as it is to concentrate on those which are polyandrous. In fact, it is clear (Boomsma & Ratnieks 1996, Strassmann 2001) that despite technical difficulties in their study, social Hymenoptera are better known than solitary ones in terms of the incidence of polyandry, and may indeed be better known than most other major groups of animals. With respect to comparisons with other groups, such as vertebrates, social Hymenoptera present special features such as the sex locus, the extreme ability and need to store sperm for long periods, and colonies with polyethism, lack others such as repeated mating following the birth of offspring, and have a much reduced opportunity for convenience polyandry (Strassmann 2001). Comparisons between such phylogenetically divergent groups as social Hymenoptera and vertebrates are therefore not readily made.

Social insects pose severe difficulties for mating system studies because most species do not mate under captive conditions making it very difficult to test for causality. Accordingly, researchers are often restricted to correlative evidence in support or refutation of particular hypotheses. Compounding this, the great lifespan of queens, especially in many ants (Keller & Genoud 1997, Keller 1998), makes it almost impossible to measure lifetime reproductive success for queens of most species. Comparative analyses can overcome some of these problems and we encourage their application as more data accumulate on phylogenetic relationships and mating systems in the social Hymenoptera. More information on life history and ecology of individual species is needed, with explicit incorporation of this information into quantitative pre-

Table 2. Status of the nine most useful hypotheses.

Hypothesis	Model?	Demonstrated?	Fitness effects shown?	Affects andry?	Note
3. Genetic incompatibility (Inbreeding)	No	Yes	Yes		Adaptations other than polyandry appear generally to be sufficiently effective to obviate selection for polyandry, nor does inbreeding necessarily select for polyandry.
4. Benefits transfer	No	No			Unlikely in Hymenoptera generally, and especially in eusocial species.
6. Winning sperm	Yes	No			Not easily testable. Sperm competition has been little studied and the data for honeybees are equivocal.
7. Sperm limitation	?	Yes	Yes/Maybe/No		Some honeybee and ant species store more sperm than delivered by a single mating, but at least one does not and in some others the number of matings is grossly in excess of that required to deliver the amount of sperm stored.
9. Sex-ratio manipulation	Yes	Yes/No	Yes/No		Some species show facultative change of sex-allocation according to mate number, but others do not.
11. Daughter queens more variable	?				Depends on heterogeneous environments to drive polyandry but neglects habitat choice and cannot readily explain existing cases.
12. Genetic polyethism	Yes	Yes	Yes	Maybe	Genetic polyethism has been shown in honeybees and some ants and a social wasp. In honeybees genetic polyethism has been shown to improve correlates of fitness
13. Sex-locus load	Yes	Yes	Yes	Maybe	Diploid males have been shown to be a drain on colony resources in honeybees and fire-ants; andry levels in those species are consistent with theory. But whether this factor is a necessary factor in any species is uncertain.
14. Herd immunity	Maybe	Yes	Yes	Maybe/No!	Comparative analysis indicates an appropriate effect, but an effect of andry remains to be demonstrated. Bumblebees show a complex relationship between mate number and fitness.

“Model?” denotes whether or not the hypothesis has been modelled, “Demonstrated?” refers to whether the hypothesized effect has been demonstrated to occur, “Fitness?” to whether fitness effects have been shown, and “Affects andry?” refers to whether or not there is evidence that the fitness effects have mediated the current level of mating.

dictions. Finally, quantification of mating risk variation within and across species is needed.

Social insect mating system evolution has traditionally been treated separately from that of solitary insects. A large number of the hypothesised selective factors likely to be important for social insects could not apply to solitary insects, and conversely, the factors appearing most important for solitary insects seem to be of little relevance for understanding the origin and maintenance of polyandry in social insects. Our review confirms this. Based on the considerations above the status of the nine hypotheses considered in depth here is given in Table 2. From these analyses, it appears that hypotheses 7, 9, 12, 13 and 14 are likely to be the most fruitful in guiding future research. All of these hypotheses but one treat potential selective factors unique to social insects.

We propose a multifacetted approach to the search for an answer to the question of why some social insect queens mate multiply. Research programs should attempt to distinguish between the various hypotheses, not seek support for one alone. Remembering that many factors are likely to act in any one species, it may ultimately prove useful to use a path analysis approach, namely to try and assess the likely effects of the various possible factors on the population to yield the observed result, much as has been done on occasion in assessing sex-allocation (Herbers 1990).

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