Mating for variety increases foraging activity in the harvester ant, *Pogonomyrmex occidentalis*

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Abstract

Multiple mating by females characterizes most insect species, but is relatively uncommon in social insects. Females may mate with multiple mates because they experience the direct benefits of increased survival or fecundity, to acquire high quality mates, or to lower the risk of reduced fecundity by mating with incompatible males. We used the extensive natural variation in mating frequency in the western harvester ant, *Pogonomyrmex occidentalis*, to test the hypothesis that increased mating by the queen leads to an increase in colony performance. Colonies with greater genetic diversity began to forage earlier in the day and foraged for longer time periods. The workers which initiated foraging were a nonrandom subset of the genotypes present in the colony. We used a statistical approach to correctly predict the direction and magnitude of the correlation between genetic diversity and colony foraging activity.

Keywords: activity, ants, behaviour, multiple mating, polyandry, social insects

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Multiple mating by females, once thought to be rare, is now recognized as very common (Jennions & Petrie 2000). The realization that taxa traditionally considered monogamous, such as birds, have high levels of female multiple mating has led to an exploration of the possible benefits gained by females that are polyandrous. Females may mate with males to produce high quality offspring — either solely male offspring (i.e. sexy sons: Wedell & Tregenza 1999; Sakaluk *et al*. 2002) or all offspring (Petrie 1994; Welch *et al*. 1998; Wedekind *et al*. 2001; Hines *et al*. 2002). In species with immunological (Penn & Potts 1999) or genetic incompatibility (Zeh & Zeh 1996), or genomic conflict (e.g. Wilkinson *et al*. 1998), females may mate multiply if they cannot identify the best male or in order to produce a diverse set of offspring as a response to a heterogeneous environment. In a meta-analysis of nonsocial insects, Arnqvist & Nilsson (2000) found that females benefitted directly from multiple mating, through significantly increased lifetime offspring production, although female survival often decreased.

While multiple mating is common in insects (reviews of various orders in Choe & Crespi 1997; Simmons & Siva-Jothy 1998; Arnqvist & Nilsson 2000), it is relatively uncommon

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among the social insects. Although in a large number of species a fraction of queens mate twice, there are relatively few species where queens routinely mate a moderate to large number of times (Strassmann 2001). Honeybees, and other members of the genus *Apis*, are the pre-eminent example of high mating frequency; queens may mate more than 20 times (Tarpy & Nielsen 2002). For other groups, the highest mating frequencies are found in *Vespula* wasps (Foster & Ratnieks 2001), in the leaf-cutting ants (Reichardt & Wheeler 1996; Fjerdingstad & Boomsma 1998; Fjerdingstad *et al*. 1998; Boomsma *et al*. 1999), in army ants (Denny *et al*. 2004; Kronauer *et al*. 2004, 2007) and in seed harvester ants of the genus *Pogonomyrmex* (Cole & Wiernasz 1999; Gadau *et al*. 2003; Rheindt *et al*. 2004; Wiernasz *et al*. 2004).

In social insects, multiple mating by the queen potentially changes the genetic diversity in two distinct kinds of female offspring: workers and new queens. Genetic diversity in reproductive daughters may be favoured by frequency-dependent selection as a consequence of unpredictable environmental variation. Alternatively, multiple mating may permit post-copulatory paternity biasing (by either cryptic female choice or sperm competition) in order to produce higher quality queens. Biased paternity has been observed in ants (Sundström & Boomsma 2000; Fernandez *et al*. 2002), but not in honeybees (Franck *et al*. 1999, 2002). In this

way, social insects resemble any other insect species. Most of the interest in multiple mating in social insects, however, has focused on the consequences for the colony phenotype.

Several hypotheses have been proposed to explain how increased genetic variance among workers improves colony performance. Increased genetic diversity may reduce pathogen load by producing a worker force that is more resistant to diseases and less likely to transmit them to colony mates than one that is genetically uniform (Hamilton 1987; Sherman *et al*. 1988, 1998; Schmid-Hempel 1994; reviewed in Schmid-Hempel 1998). The strongest support for this hypothesis comes from experimental work on multiple mating and pathogen resistance in bumblebees (Baer & Schmid-Hempel 1999, 2001), honeybees (Tarpy 2003; Seeley & Tarpy 2007) and *Acromyrmex* ants (Hughes & Boomsma 2004).

Colonies in which the queen mates a large number of times may have higher fitness because workers differ genetically in their propensity to perform certain behaviours. As a consequence, a genetically diverse colony will contain a large array of specialist workers (Fuchs & Moritz 1999). This hypothesis has received support from the finding that the thresholds for performing tasks has a genetic component in honeybees (Page & Robinson 1991; Page *et al*. 1992, 1995; Robinson 1992; Toma *et al*. 1999; Ben-Shahar *et al*. 2002) and can affect colony characteristics. A genetic basis for the propensity of individuals to perform specific tasks has also been documented in wasps (O'Donnell 1998), ants (Stuart & Page 1991; Snyder 1992, 1993; Julian 1998; Julian & Cahan 1999; Fraser *et al*. 2000; Julian & Fewell 2004) and social caterpillars (Costa & Ross 2003).

Some studies have found only a weak or no correlation between genetic diversity and colony performance. Neumann & Moritz (2000) found no effect of natural variation in mating frequency on colony size, honey production or mite resistance in domestic honeybee colonies. In artificial colonies of Argentine ants (*Linepithema humile*), genetic diversity did not affect growth under laboratory conditions (Rosset *et al*. 2005). In leaf-cutter ants (*Acromyrmex octospinosus*), high and low genetic diversity groups survived equally poorly when exposed to high concentrations of a fungal pathogen, although high diversity groups survived significantly better at low doses (Hughes & Boomsma 2004). In the ant *Lasius niger*, queen size was correlated with both mating frequency and colony survival (Fjerdingstad & Keller 2004).

The effect that natural variation in mating frequency has on colony performance in the field has received little attention. In *Pogonomyrmex occidentalis*, genetic diversity is correlated with the rate of colony growth, an important correlate of colony fitness (Cole & Wiernasz 1999; Wiernasz *et al*. 2004). One causal explanation is that increased growth results from increased foraging success due to differences in colony activity patterns. For some ant species, the time costs incurred during foraging are much more important than the energetic costs of foraging (Fewell *et al*. 1996; Nielsen

2001). For *Pogonomyrmex* ants, the value of food recovered is 100–1000 times greater than the energetic cost of retrieval so that the only significant costs in foraging are time costs (Fewell 1988; Weier & Feener 1995; Morehead & Feener 1998). With more time to forage, a colony may increase the effectiveness of food discovery or processing, or the number of foraging trips. In *P. occidentalis*, earlier onset of activity led to a longer duration of foraging and greater food retrieval by the colony (Cole *et al*. submitted).

In this study, we use the large natural variation in mating frequency in the western harvester ant, *P. occidentalis*, to examine the relationship between genetic diversity and colony performance. We measure the relationship between mating frequency and three parameters of colony activity — onset, duration and cessation of foraging — in order to test the hypothesis that increased mating by the queen leads to an increase in the duration of foraging. If the offspring of different males (i.e. patrilines) become active at different times, the onset of activity in the colony is determined by the earliest starting patriline. When the queen mates with multiple males, she samples this population of onset times: colonies whose queens have mated with more males are expected to have an earlier onset time than colonies with fewer patrilines, because of the relationship between the minimum of a sample (the patriline with the earliest onset) and the sample size. Thus, we expect a negative correlation between onset time and patriline number. The analogous prediction for the cessation of activity is that colonies with greater diversity will stay active longer than those with low diversity.

Our hypothesis assumes that the differences in worker foraging behaviour have a genetic basis, and that genotypes differ in the time interval over which they will actively forage. We assume a trade-off at the level of the individual worker, i.e. a genotype which begins foraging early in the morning ceases foraging before the colony as a whole becomes inactive, while an individual which begins foraging later in the day stays active longer. Colonies with more genotypes are more likely to have longer time to forage relative to colonies with fewer genotypes. If this trade-off does not exist, then the best genotype (the one with the greatest temporal range in foraging activity) would be expected to sweep to fixation. Several possible mechanisms could produce differences among genotypes (e.g. thermal tolerance, Gilchrist *et al*. 1997 and David *et al*. 2003; tempo of activity, Burkhardt 1998); however, we do not test for a specific mechanism here.

Materials and methods

We quantified activity in 76 harvester ant colonies by observing the onset and cessation of foraging activity. The colonies used in this study were part of (Block 1, $N = 21$ colonies) or peripheral to (Blocks 2 and 3, $N = 29, 26$ colonies, respectively) our long-term study site in western Colorado.

The blocks were distributed in a rough semicircle: blocks 1 and 2 were separated by ~450 m; blocks 2 and 3 by ~650 m. Within a block, the average distance between adjacent colonies was 20 m. The habitat is cold desert (altitude ~1470 m above sea level), and the vegetation is primarily desert grasses, mustards, composites and chenopods (see Wiernasz & Cole 1995 for a detailed description of the study site).

One block of colonies was monitored on a given day. Activity monitoring began before dawn. Observers approached no closer than 5 m to the nest (to minimize disturbance to the colony) observing with binoculars. Each colony was checked every 15 min until all colonies were foraging. Onset of foraging activity was scored as the first workers start to move away from the nest. Observers returned to the colonies when ambient temperature reached approximately 30 °C and checked each colony every 15 min until all had ceased foraging. Cessation of foraging was scored as two consecutive observations of no activity outside the nest. Foraging activity increases through the morning, peaks and declines until the colony shuts down when the air temperature reaches approximately 35 °C. On a few occasions, it was not possible to obtain all of the activity data for a particular colony, resulting in slightly different sample sizes for the onset and cessation of activity. Because the duration of activity required both onset and cessation measures, the sample size for duration is somewhat smaller than that of the other two parameters.

We statistically controlled for daily differences in foraging activity (for all data, $F_{9,228} = 17$, $P \ll 0.001$), nest elevation (foraging onset correlated with elevation in one block; $r = 0.4$, $P = 0.035$), nest entrance orientation (total foraging activity correlated with cos(nest entrance orientation) in one block; $r = 0.26$, $P < 0.01$) and nest size (for all nests, $r = 0.33$, $P \ll 0.001$), by using the residuals of activity measurements after removing these effects. Activity was affected by different environmental factors in different areas, because in some blocks there was little variation in an environmental variable, such as elevation, among colonies. To determine the relationship between each of the activity variables and genetic diversity we first performed an analysis of variance on each variable using block as a classification variable and the number of patrilines as a covariate. If a block did not have a significant effect, we pooled the data from all colonies and used regression to test for a relationship between the activity variables and the number of patrilines in the colony.

Microsatellite markers were used to determine mating frequency in each colony. Forty-three of the colonies had already been genotyped as part of a previous study (Wiernasz et al. 2004); the remaining 33 colonies had been sampled in the same year, so that mating frequency estimates from all colonies are comparable. We genotyped 20 randomly collected workers at four highly polymorphic microsatellite loci (with 12, 15, 19, 28 alleles; range of heterozygosities:

0.81–0.97; for details of DNA extraction and polymerase chain reaction, see Wiernasz et al. 2004). Given the high degree of polymorphism at these loci, the likelihood of two males having the identical four-locus genotype is extremely low ($< 10^{-4}$). Typically, patrilines within a colony differed in alleles for at least three loci. We used the GENOGRAPHER (version 1.6.0) software (Benham 2001) to determine each worker's multilocus genotype. Colonies of *Pogonomyrmex occidentalis* have a single queen (Cole & Wiernasz 2000; Wiernasz et al. 2004); male ants are haploid. This enables the queen's genotype and the genotypes of each male to be determined by inspection. Patriline number is thus the minimum number of males with which the queen has mated.

Studies that measure the genetic characteristics of social insect colonies typically use the effective mating frequency or intracolony relatedness. These measures are well-suited to questions that concern the average worker phenotype of the colony. Our study examines the extremes of colony behaviour (onset of foraging). In this case, the number of patrilines is more appropriate, because of the statistical relationship between the minimum of a sample and the sample size (see below). A sample size of 20 workers is likely to underestimate the number of times the queen has actually mated in colonies where the mating frequency is high (more than eight males). The only method available for estimating the extent of the underestimate is that of Pamilo (1993). This correction estimates the effective mating frequency corrected for sample size. Pamilo's correction number and the minimum patriline number are highly related [regression of corrected number on minimum number, $P < 0.001$; y-intercept of –0.4 (SE = 0.7) and slope = 1.0 (SE = 0.1)]. While we cannot determine the exact number of times a queen has mated, our use of the minimum number is not biased with regard to the questions we are asking.

We assumed that phenotypic differences among colonies were due to genotypic differences between workers. We addressed this assumption by asking whether workers foraging at different times belong to different patrilines. We selected nine colonies which varied in both the duration and onset of activity, but which were spatially proximate, and collected the first 10 workers leaving each colony. These workers were genotyped using standard methods (Wiernasz et al. 2004; see above), and the distribution of patrilines compared to that of workers taken from the entire colony to test the null hypothesis that early workers represent a random sample of patrilines from each colony. We used {Σ[(observed number – expected number in each patriline)^2]/ expected number} as our test statistic. The expected number of workers was obtained from the original sample of workers ($n = 20$) previously collected from each colony. To establish significance levels, we compared this statistic to a distribution of randomly generated values which were produced as follows. We generated a random sample of 10 workers by sampling with replacement from the original worker

distribution, and calculated the statistic. We repeated this procedure 10 000 times. To compare colonies, we used the ratio of the observed statistic to the mean of the randomized distribution. Values of the ratio greater than one indicate colonies where there is a nonrandom concentration of patrilines among the first 10 workers. Values less than one indicate colonies where the first 10 workers are more uniformly distributed among patrilines than expected. We used the fraction of the distribution that is more extreme than the observed statistic to establish significance levels (e.g. if 5% of random samples had larger values for the statistic, then $P = 0.05$). We combined the results from multiple tests using Fisher's method for combining probabilities (Sokal & Rohlf 1999).

Colonies differ in the number of patrilines, which represents a sample of size N, and in onset time, which is equivalent to the minimum of the sample. If patrilines differ in onset time, we expect that the onset of colony activity and the number of patrilines in the colony will be negatively correlated; the minimum of a sample declines with increasing sample size. We can predict the expected magnitude, as well as the sign, of the correlation coefficient if we assume that patriline onset times are normally distributed. This prediction is based on the statistical relationship between the expected mean and variance of the minimum as a function of sample size (Mosteller & Rourke 1973). From the known mean and variance of colony onset times, we derived the mean and variance of the underlying (but unknown) distribution of patriline onset times. The mean sample size (mean number of patrilines) = 6.3. Using standard tables (Table A-16, Mosteller & Rourke 1973), we find that the expected value of the minimum for a sample of this size is 1.30 standard deviates below the mean of the underlying distribution. Similarly, the expected variance of the minimum is 0.412 of the variance of the underlying distribution (Table A-17, Mosteller & Rourke 1973). The observed variance in colony onset times is 395.7 (with a mean of 0, because the data are residuals). The estimated standard deviation (SD) of

the underlying distribution of patriline activity times is thus $(395.7/0.412)^{1/2} = 31$ min, and the estimated mean is $(1.3 \times 31) = 40.3$ min. We used the estimated mean and variance to calculate the expected onset time for each mating frequency category (e.g. for a colony with two patrilines, the expected onset time is 17.4 min less that the mean of the patriline distribution [= 0.56 standard deviates $\times$ 31]). To compare our data with expected values, we regressed the observed onset time for a colony on the expected onset time (the minimum) based on its mating frequency (the sample size). We tested the hypothesis that the slope of the regression should not be different from one.

Results

Both the onset and duration of foraging activity were affected by genetic diversity. In the analysis of variance using block as a classification variable, and the number of patrilines as a covariate, there was no significant block effect on any variable (onset: $F_{2,73} = 1.2$, $P > 0.3$; cessation: $F_{2,67} = 0.08$, $P > 0.8$; duration: $F_{2,67} = 0.59$, $P > 0.5$). For onset time, patriline number was a significant covariate ($F_{1,72} = 9.09$, $P = 0.004$, Fig. 1A) in the analysis of variance. When data from blocks are combined, there is a significant effect of patriline number on onset time. Colonies with high levels of multiple mating became active significantly earlier (Fig. 1A, standardized regression coefficient, $b = -0.32$, $N = 76$, $P = 0.005$) than those with low levels. One colony, with the largest number of patrilines, also had the earliest onset time. The negative relationship between onset time and patriline number is robust to deletion of this point ($F_{1,71} = 4.87$, $P = 0.03$). However, it is statistically more valid to delete pairs of points — the two points with the greatest positive and negative influence on the regression. In this case, the effect of patriline number is also significant ($F_{1,70} = 7.185$, $P = 0.009$). The cessation of activity was unrelated to genetic diversity (Fig. 1B; $b = 0.04$, $N = 70$, $P > 0.5$). The onset of
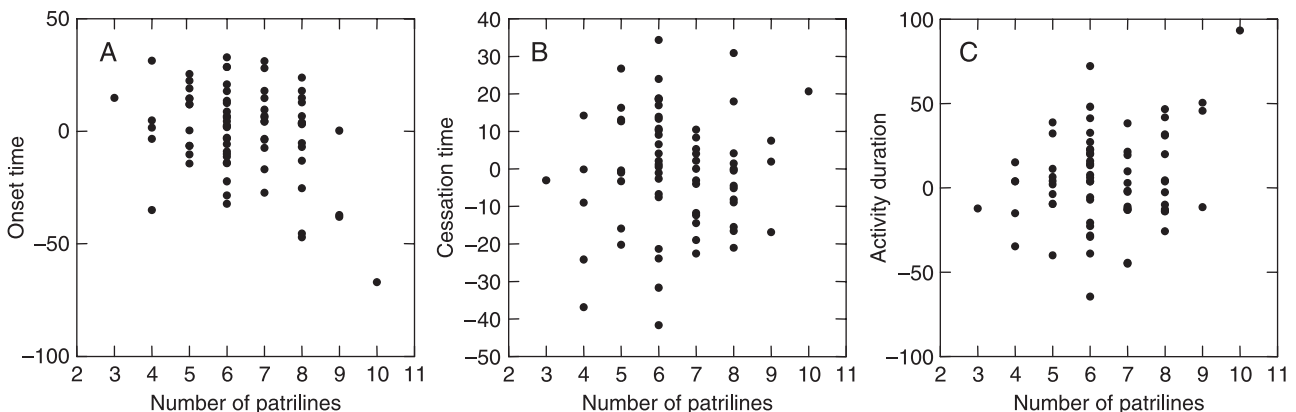

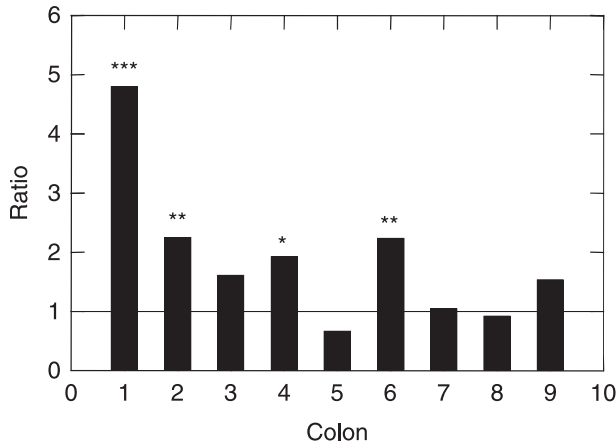**Fig. 1** The components of colony activity and the number of patrilines in colonies.

Fig. 2 Concentration of patrilines among the first 10 workers to leave the nest. Asterisks indicate significance levels: ***$P < 0.01$; **$P < 0.05$, *$P < 0.075$. Colonies had 6, 6, 9, 8, 4, 7, 5, 7, 5 patrilines.

Fig. 3 Observed and expected colony onset times (minutes), generated from the underlying distribution of patriline onset times. The solid line is the least squares regression. The dotted line is the expected slope of 1. The vertical lines are ± 2 SD based on the number of patrilines in the colony. Note that the confidence intervals are larger for smaller samples (smaller numbers of patrilines have higher observed and expected values).

foraging was five times more variable among colonies than cessation (coefficient of variation of onset = 0.53; cv for cessation = 0.11). Overall, colonies with high levels of multiple mating spent significantly more time foraging (Fig. 1C; $b = 0.25$, $N = 70$, $P = 0.036$).

The earliest workers to begin foraging did not represent a random subset of patrilines within the colony. The individual tests were significant in three of the nine colonies (Fig. 2), and the pattern was highly significant overall ($P < 0.002$; Fisher's test for combining probabilities).

Observed colony onset times were well-predicted by the expected onset times (Fig. 3; slope = 1.38 ± 0.56 SE; slope different from zero, $P = 0.016$). The slope of the relationship was not significantly different from 1 (observed values were not different from expected values, $P > 0.5$).

Discussion

As predicted, increased genetic diversity was significantly correlated with earlier onset and longer duration of foraging; however, it was uncorrelated with the cessation of foraging. Variation in the duration of foraging among colonies was thus a consequence of differences in onset time. Variation among colonies when workers stopped foraging was nearly five times less than that in foraging onset. After peaking in midmorning, foraging activity declined with increasing temperature, and colonies stopped foraging when ambient temperature reached ~35 °C. Soil surface temperatures were much higher (~55 °C). The relative lack of variation in cessation time may reflect inherent physiological limits to worker survival.

The most diverse colonies had nearly twice as much time for all activities outside the nest as did the least diverse (the average duration of activity is 4 hours). Early onset
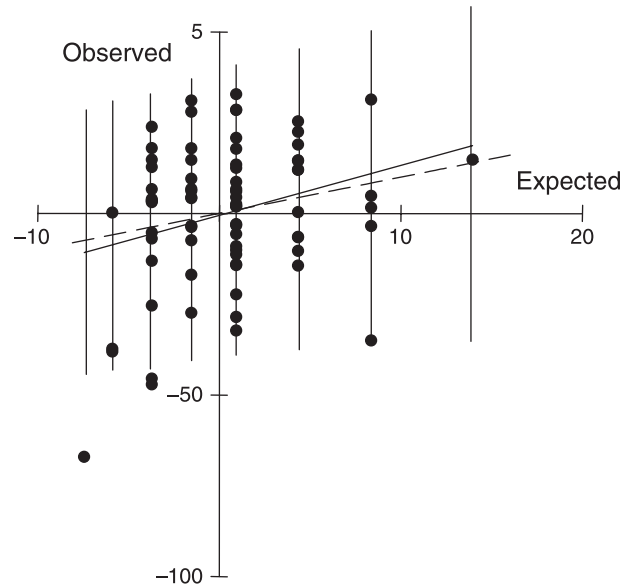
of foraging may lead to greater foraging success through a variety of mechanisms. Colonies that begin foraging early may be more likely to discover concentrations of food, be better able to recruit to and thus monopolize concentrated resources, or carry out more productive foraging trips than colonies which become active later. Elsewhere, we have shown that earlier onset of activity led to greater number of foraging trips and greater food retrieval by the colony (Cole *et al.* 2008). Increased food leads to greater reproduction in many species of ants (reviewed in Bono & Herbers 2003). Taken together, these results suggest that increasing the time period during which a colony can be active increases colony growth rates through enhanced foraging success. The selection differential for mating frequency (= the standardized regression coefficient of foraging activity on patriline number) operating through differences in foraging activity is substantial: $s^* = 0.25$ for foraging duration and $s^* = 0.32$ for onset time. Because relative growth rate is strongly correlated with colony fitness (Cole & Wiernasz 1999), foraging onset and duration should also be considered fitness proxies.

A causal relationship of increased foraging duration, greater food intake and faster growth is logical. Experimentally generated honeybee colonies with high genetic diversity foraged more, stored more food, and grew more rapidly than colonies where the queen had mated once (Mattila & Seeley 2007). In harvester ants, increased genetic diversity also may be correlated with faster growth because

it may improve performance throughout the colony. Workers from different patrilines may differ in the rate at which they perform all tasks, including foraging. Our current results cannot discriminate between these two explanations. Regardless of the specific mechanism that translates mating frequency into activity duration, multiple mating is strongly favoured by selection.

Although the correlation between mating frequency and the onset of colony activity was significant, there was also considerable variation within a given mating frequency category. Our hypothesis assumes that patrilines differ in the onset of activity, and that the queen mates randomly with respect to male activity phenotype. As a group, early foragers were significantly different from randomly sampled patrilines, but were not distinct in all colonies. A queen that mates with more males has a greater probability, but not a certainty, of mating with males that all differ in activity phenotype. Some colonies will have multiple patrilines with similar onset times. Although the earliest workers tended to be a particular subset of possible patrilines, this need not be true in all colonies.

We have focused on the effect of genetic diversity on colony function in nature. Field studies of fitness are critical for judging the significance of a trait, because differences in features such as growth and survival may be difficult to estimate with sufficient accuracy in the laboratory. For example, Rosset *et al*. (2005) found no functional consequences of genetic diversity in artificial colonies of argentine ants (*Linepithema humile*). While laboratory studies can elucidate the mechanisms responsible for a correlation between genetic diversity and colony function, the relevance for functional effects in natural situations is problematic. The statistical mechanism that we propose requires large sample sizes to detect an effect. Such sample sizes may be difficult to achieve in the laboratory.

In the social insect species that are characterized by high mating frequencies, reproduction is virtually always restricted to the queen. Properties of the colony that increase her survival, longevity, and lifetime reproductive output function as material benefits to the queen. Material benefits are usually thought of as substances contributed by males during mating that increase female fecundity or lifespan (Arnqvist & Nilsson 2000). They have been considered relatively unimportant in social insects (Strassmann 2001), because the interval between mating and reproduction is frequently great (several years). We suggest that multiple mating provides a material benefit in the form of a genetically diverse worker force that forages for longer time periods, leading to higher colony growth rates (Cole & Wiernasz 1999; Wiernasz *et al*. 2004). Because diversity itself is the benefit, selection favours queens that mate with several males rather than with one particular type of male. However, unlike the material benefits of other species, the genetic diversity of a colony represents a benefit whose value does not decline with time.

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DCW's research ranges from the role of sexual selection in shaping phenotypic variation within and among species, to the evolution of multiple mating by females. JH is now a PhD student; her research examines how microbes mediate plant-insect interactions. DP is now the field representative for Sen. Jeff Bingaman of New Mexico. BJC's research spans many aspects of the evolutionary ecology of ants, from community structure to the evolution of social behaviour.