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Multiple Queens versus Multiple Mates: A Test of the
Polygyny/Polyandry Tradeoff Hypothesis in the Ant
Veromessor pergandei

Rachel Mellen

In partial fulfillment of College Honors, May 2016
Advisor: Dr. Sara Helms Cahan

Abstract

According to evolutionary theory, cooperation should evolve most easily in closely related groups. In colonies of ants, bees, and wasps, within-colony relatedness is maximized when all offspring are descended from a single queen mated with a single male. However, in many species colonies are not composed of strictly nuclear families, suggesting there may also be benefits to greater genetic diversity. To increase genetic diversity, a queen can mate with multiple males, termed polyandry, or join with other queens to cohabitate and raise offspring together, termed polygyny. Both options have associated costs, leading to an expected tradeoff in whether queens invest in polygyny or polyandry.

In this project, I tested the polygyny/polyandry tradeoff hypothesis in the desert seed harvester ant *Veromessor pergandei*. This species forms three different types of colonies across its geographic range: single queen, polygynous, and a temporary form of polygyny where colonies begin with multiple queens but they aggressively compete until only one queen is left alive. If the polygyny/polyandry tradeoff hypothesis explains their mating behaviors, I predict that solo-founding queens should mate with more males than polygynous queens, and polyandry would be higher in temporary groups than in permanent groups. Queens and their offspring from each geographic area were genotyped at six highly variable microsatellite loci to infer the number of males with which the queen had mated. Results showed tentative support for a tradeoff between polygyny and polyandry, but implicated a more complex array of factors than genetic variation only influencing mating frequency in *V. pergandei*.

Introduction

Eusocial insects form colonies consisting of at least one reproductive queen and her sterile offspring, a system made possible through the sizable indirect benefits these sterile offspring receive from kin selection (Crozier 2008). Due to the haplodiploid genetic structure of insects in Hymenoptera, workers share 75% of their genetic material, making intra-colonial relatedness very high (Nonacs 1988). Such high relatedness is only possible through monogamy, suggesting eusociality could only evolve under monogamous conditions (Hughes et al. 2008a). However, both polygyny, multiple reproductive queens in one colony, and polyandry, one queen mating with multiple males, are common in Hymenoptera, suggesting an evolutionary advantage to deviating from the ancestral monogamy (Hughes et al. 2008b). Polyandry reduces relatedness within a colony, with half-sisters sharing only 25% of their genetic material (Sundström and Ratnieks 1998). Polygyny reduces average intra-colonial relatedness even more sharply, particularly when groups are formed of unrelated queens, leading to workers within a colony that may be completely unrelated (Kellner et al. 2007). Though eusociality must evolve through monogamy, greater genetic variation than allowed by monogamy can be beneficial, and will not cause eusociality to break down once it has evolved (Keller and Reeve 1994, Hughes et al. 2008b). Genetic variation can reduce the spread of infection within a colony, and increase foraging success by widening the range of environmental conditions the colony can successfully function in, increasing the resilience of the colony and by extension the fitness of the queen (Schmid-Hempel 1994, Hughes and Boomsma 2004, Wiernasz et al. 2008). Moreover, founding a colony in a group may give queens a better chance of surviving long enough to establish a lasting colony (Rissing and Pollock 1987). A group of queens has a greater chance of producing more workers and producing them sooner than a single queen, giving the new colony a better chance of surviving both conflicts with other colonies and greater ability to forage (Offenberg et al. 2012, Sasaki et al. 2005). Additionally, in

harsh conditions, cooperation can promote survival of all. Each queen consumes less of her own resources when the burdens of excavating a nest and producing workers are shared, increasing her chance of survival despite stresses such as starvation or desiccation risk (Cahan 2001). Because of the benefits to queen survival of forming colonies in a group, polygyny is likely to develop based on environmental conditions, suggesting it may be more likely to drive patterns of polyandry.

Both polygyny and polyandry are effective at creating a genetically diverse colony, but each has a unique set of individual-level costs. This is expected to create a tradeoff between polygyny and polyandry (the Polygyny/Polyandry Tradeoff Hypothesis, Keller and Reeve 1994); once one had evolved, the second would not provide sufficient benefits relating to its costs, and should be selected against. Predation risk is high for queens during their mating flight, and prolonging that mating flight in order to mate multiply increases that risk (Yasui 1998). Mating with multiple males also increases the likelihood of disease transmission (Crozier and Fjerdingstad 2001). Flying, mating, excavating a nest, and producing offspring also all require energy, which is a limited resource for a queen who must rely on fat reserves until her first workers mature (Keller and Reeve 1994). Queens mate while in flight, and as such mating multiply requires a longer and more energetically expensive flight, potentially lowering an individual's ability to produce enough initial offspring to create a viable colony (Crozier and Fjerdingstad 2001).

Polygyny is also an inherently costly strategy. In many species, queen groups break down once the first crop of workers has been raised, resulting in inter-queen aggression, and the death of all but a single foundress (Aron et al. 2009). Even when polygyny is long-lasting, cohabitation necessitates the sharing of resources and, as such, any one polygynous queen has a smaller share of resources to devote to producing offspring than a solitary queen (Brent et al. 2008, Hughes et al. 2008). Additionally, many polygynous groups form dominance hierarchies, where dominant

queens are able to reproduce more and control a larger proportion of available resources. Lower-ranked queens may produce many fewer offspring, if they are able to reproduce at all (Kellner et al. 2007). These costs can be reduced through forming polygynous colonies with sisters, or through forming a polygynous colony through the adoption of daughter queens into their natal colony (Boulay et al. 2014). In these cases, queens gain fitness benefits from both their own offspring, and indirectly from offspring produced by their kin, a benefit that is the bases for theories of kin selection (Haapaniemi and Pamilo 2012). However, not all polygynous colonies are of closely related queens, indicating that in some cases the costs of sharing resources are not great enough to discourage grouping with other queens even when those queens are unrelated (Helms Cahan and Helms 2012).

If the Polygyny/Polyandry tradeoff drives the evolution of mating behavior, the evolution of polygyny should reduce the likelihood that polyandry will evolve. Surveys of mating frequency across species seem to support the presence of this tradeoff, as there is a negative correlation between polygyny and polyandry in Hymenoptera (Keller and Reeve 1994, Hughes et al. 2008). However, at the within-species level, tests of the tradeoff hypothesis have been equivocal. In support of the hypothesis, some species studied have shown the expected negative correlation between polyandry and polygyny in individual species (Qian et al. 2011, Haapaniemi and Pamilo 2012) However, this has not been the case for every study, other species show polyandry and polygyny to coexist, or even to be positively associated, indicating the need for further study to understand what affects the relationship between the two strategies (Schmid-Hempel and Crozier 1999, Pendersen and Boomsma 1999, Rubin et al. 2013). Moreover, there are other factors that could potentially affect mating behavior. Queen body mass may also affect selective pressures on mating behavior, as higher body mass could lower a queen's energetic cost to mate, potentially

leading to more matings (De Souza et al. 2013), Colony density may also influence mating behavior, as greater colony density could increase availability of mates, thus reducing the cost of finding additional mates.

Veromessor pergandei is an ideal species for testing for a tradeoff between polygyny and polyandry. Polyandry, as well as both solitary and polygynous colony founding behavior are present within the species (Ode and Rissing 2002, Cahan et al. 1998). Polygynous colonies are made up of unrelated queens and, once founded, either remain cooperative permanently, or break down into fighting between queens and the deaths of all but one (Helms Cahan and Helms 2012, Helms et al. 2013). Solitary, permanently polygynous, and temporarily polygynous colony types are geographically distinct, with polygyny having evolved in response to differing environmental factors across *V. pergandei*'s range (Helms and Helms Cahan 2012). Permanently polygynous colonies form in the harshest parts of the range, such as Death Valley, most likely due to the increased chance of survival from founding a colony with a group of other queens (Cahan 2001, Rissing and Pollock 1987). Solitary colonies form in the most optimal areas of the range, and temporarily polygynous colonies form in intermediate areas (Cahan 2001). This allows comparison of how levels of polyandry are different between colony founding types within the same species. If the polygyny/polyandry tradeoff has been important in shaping mating behavior in *V. pergandei*, queens from solitary-founding regions should show the highest levels of polyandry, and those from regions where queens form temporary polygynous groups should show higher levels of polyandry than those from areas where groups are permanent, due to the temporary nature of genetic variation from polyandry in a group that will not last. Within permanently polygynous groups, polyandry should also be negatively correlated with the magnitude of polygyny.

To test for a tradeoff between polyandry and polygyny within this species, mating frequencies of queens from the three behavioral regions were compared by allowing individual queens to produce offspring in the lab, and then genotyping those offspring at six highly variable microsatellites to infer the number and relative contributions of their male mates. Pedigree-effective mating frequency was calculated to determine the representation of the males each queen mated with in her offspring; this statistic is a function of the absolute mating frequency as well as paternity skew, which measures the extent to which paternity is shared equally or biased. The polygyny/polyandry tradeoff hypothesis predicts a negative relationship between the two strategies, so within permanently polygynous regions number of foundress queens was compared to absolute and effective mating frequency, to determine whether polyandry showed a negative relationship with magnitude of polygyny. Absolute mating frequency and pedigree-effective mating frequency were both compared to queen body size and colony density to control for the potential effects of these factors on mating frequency.

Methods

Newly mated queens were collected 1-5 days after their mating flights at 9 sites in *V. pergandei*'s range, and shipped overnight to the University of Vermont. Each queen was shipped

Table 1

Collection sites and samples sizes. Means from personal communication with Helms and Helms-Cahan.

in a ventilated tube with a moist cotton ball, and weighed on arrival. Of the 73 total queens used in this experiment, 39 were from permanently polygynous sites, 19 queens were from solitary sites, and 15 queens were collected from temporarily polygynous sites (Table 1). All collections occurred in 2009 and 2010.

Site	Colony Type	Colonies sampled	Mean Queen Number per nest	Mean Queen Body Mass	Mean Colony Density
Anza Borrego, CA (ANZA)	Solitary	16	1.005	35.945	8.828
Whitewater Canyon, CA (WW)	Solitary	3	1.034	32.759	16.404
McCartney Rd, AZ (MC)	Temporary Polygyny	9	3.625	35.436	10.378
Robbins Butte, AZ (RB)	Temporary Polygyny	4	1.333	31.537	3.409
Gila Bend, AZ (GB)	Temporary Polygyny	2	1.455	32.485	8.865
Boulder City, CA (BC)	Polygyny	9	16.57	25.773	13.995
Bakersfield, CA (BK)	Polygyny	10	1.776	29.963	no data
Mitchell Caverns, CA (MI)	Polygyny	10	3.129	27.545	0.985
Palmdale, CA (PA)	Polygyny	10	2.143	28.987	no data

Once at the University of Vermont, queens were individually weighed to the nearest 0.01mg on a Mettler Toledo microbalance and placed in glass 16x150mm tubes with water held in place at one end with a cotton ball to provide humidity and allowed to produce workers and brood. While producing brood, queens were kept in complete darkness at 28°C. After the first workers eclosed as adults, the lighting schedule was changed to 12 hours light, 12 hours darkness, and a diet of Kentucky bluegrass seeds was provided. Colonies were sacrificed after producing 20 or more workers and brood, or at the end of three months if they did not produce additional offspring. Sacrificed colonies were preserved in 95% ethanol. A subset of the queens were used in a separate experiment and were not available for further analysis; a total of 39 queens (53%) remained and were genotyped along with their offspring.

To determine the number of patrilineages present in each colony, the offspring and queen, if available, were individually genotyped at 6 microsatellite loci: L18, L5, LxAGT1, Myrt3, Po3, and Ppal12, which have been identified as both highly variable and passed down through Mendelian inheritance in this species (Helms and Helms Cahan 2012). DNA was extracted by drying each sample for 20 minutes at 50°C in a drying oven, crushing the dried sample with a sterilized homogenizer, and adding 250µl of 5% Chelex-100 resin solution, followed by 20

minutes incubating at 90°C on a hotplate (Helms and Helms Cahan 2012). Microsatellite loci were amplified in multiple reactions, in a total of 3 PCR reactions per sample: set 1 containing 5µM L5, LxAGT1, and PPal12, set 2 containing 5µM L18, Myrt3, and Po3, and finally a single-locus amplification of 5µM L18 only. PCRs were conducted using a 10µl reaction of: 2µl DNA sample, 2.8µl H₂O, 2µl Qiagen Q solution, 1µl 10x Qiagen buffer, 0.75µl 2mM dNTPs, 0.4µl 25mM Qiagen MgCl₂, and 1µl of primer mix containing forward and reverse primers for each set of microsatellite amplifications. To check for successful replication of DNA, 5µl was removed from the control well and two randomly selected wells in each plate, and run on a 1.5% agarose gel. Prior to genotyping, each of the three PCRs were combined in a ratio of 10µl set 1:10µl set 2:5µl L18. Genotyping was performed using the UVM Cancer Center's Genescan service with LIZ600 as an internal size standard.

Microsatellite data was analyzed using Genemapper (Applied Biosystems, Foster City, CA, USA), allele calls were made by the program and confirmed by eye. Male genotypes, unknown queen genotypes, absolute mating frequency, and paternity skew were calculated using MATESOFT v. 1.0 (Moilanen et al. 2004). In cases of multiple possible genotypes calculated for males and queens, selections of genotypes were made based on probabilities assigned by MATESOFT and genotypes assigned by eye based on the fewest possible patriline. Pedigree-effective mating frequency was calculated based on the sum of squared paternal contributions given by MATESOFT, corrected for sample size by the program using Pamilo's (1993) correction. JMP v. 12.1.0 (SAS Institute Inc., Cary, NC, USA) was used to calculate statistics. Both absolute mating frequency and pedigree-effective mating frequency were analyzed using a nested ANOVA, with site nested within colony type to account for sampling effects based on the sites used, and sample size as a covariate for absolute mating frequency. Both measures of mating frequency were

analyzed again with a second ANOVA, with mean queen body mass, mean colony density, and mean number of foundresses as covariates. Sample size was added as a covariate to absolute mating frequency, but was not used in analyses of effective mating frequency as the value already corrects for sample size. Site was not included in the second ANOVA, as there were not enough sites sampled to support such a complex analysis. The effect of colony type on average paternity skew was analyzed using an ANOVA. Individual queen body mass and mean foundress number were individually regressed against absolute mating frequency and pedigree-effective mating frequency within each colony type, with the exception of mean foundress number and solitary colonies, as they only have one queen. Colony density was not analyzed within colony types due to small sample size.

Results

Colony Type

The number of males queens mated with was significantly associated with colony type, when analyzed with sample size and site nested within type as covariates (ANOVA, $F_{2,72}=15.60$, $p<0.001$)(Fig. 1A). Site nested within type had a significant effect as well (ANOVA, $F_{6,72}=p<0.02$), but sample size was nonsignificant (ANOVA, $F_{1,72}=0.500$ $p=0.48$). Solitary and temporarily polygynous colony types were not significantly different ($p>0.05$), while permanently polygynous colonies showed significantly fewer patriline lines than either ($p<0.05$ in both pairwise comparisons)(Fig. 1A). However, when analyzed with average queen body mass, colony density, sample size, and foundress number per site, there was no independent effect of colony type on absolute mating frequency (ANOVA, $F_{2,46}=1.99$ $p=0.15$).

Pedigree-effective mating frequency was, on average, lower than absolute mating frequency for all three colony founding types, with means dropping from 3.89 to 3.75 in solitary areas, 3.07 to 2.55 in temporarily polygynous areas, and 1.97 to 1.80 in permanently polygynous areas (Fig. 1A,1B). Effective mating frequency also was significantly associated with colony type

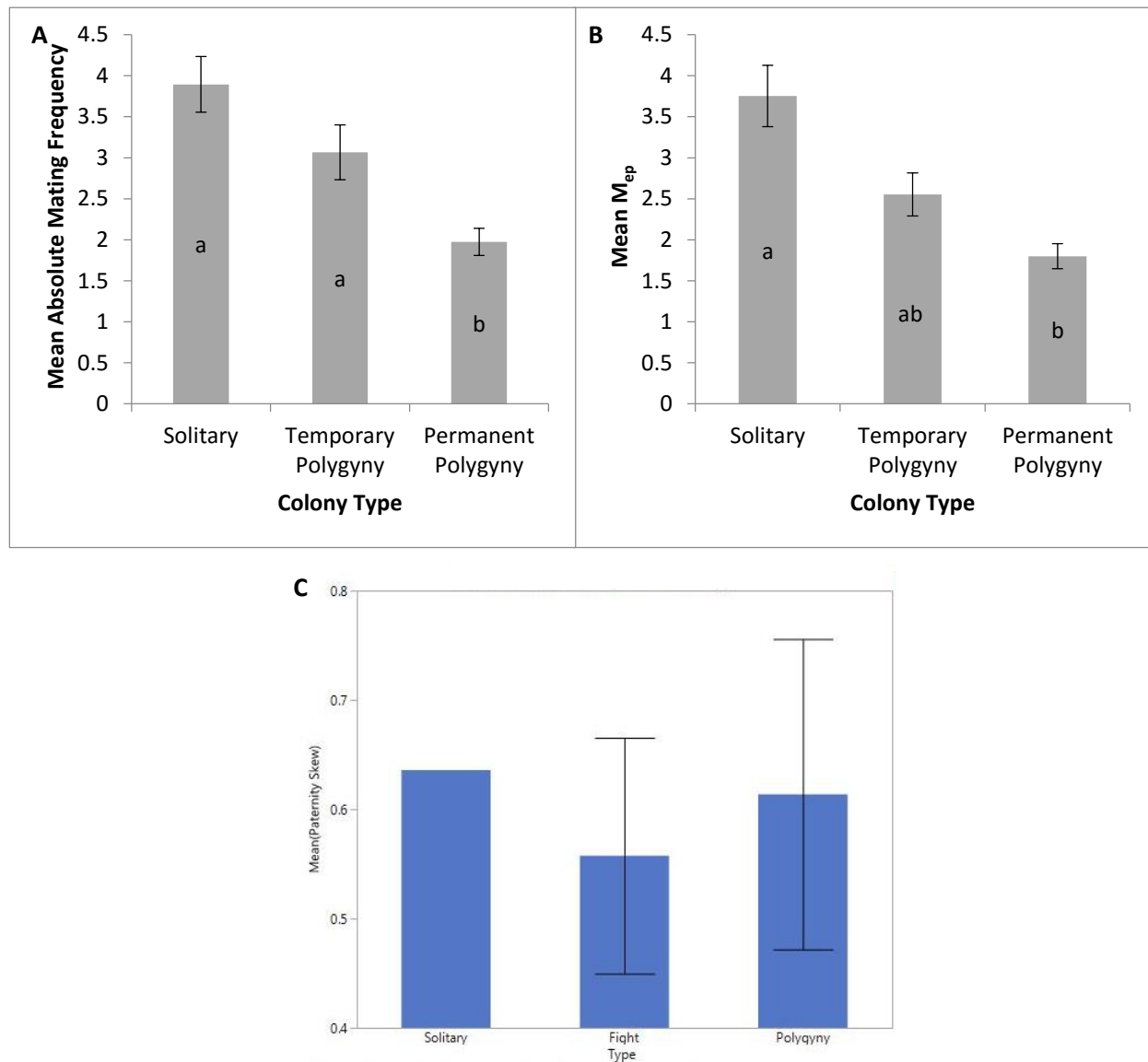


Figure 1

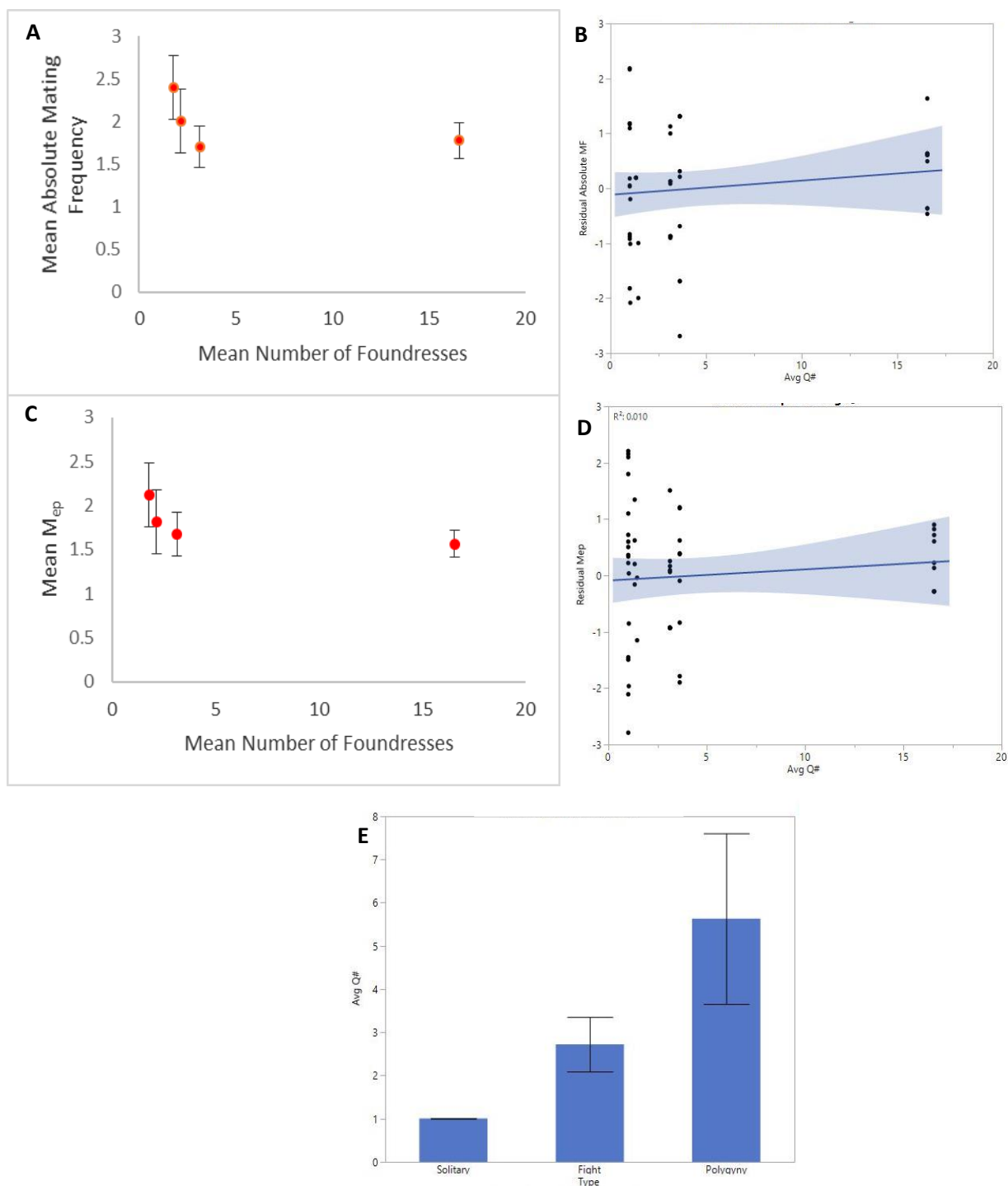
Bar graphs of (A) mean absolute mating frequency \pm SE and (B) mean pedigree-effective mating frequency (M_{ep}) \pm SE, by colony type. b represents a significantly different result from a, ab represents an intermediate result significantly different from neither. (C) Bar graph of mean paternity skew \pm 95% confidence interval, by colony type. “Fight” corresponds to temporary polygyny, “polygyny” to permanent polygyny.

(ANOVA, $F_{2,72}=14.92$ $p<0.001$). Site did not show a significant relationship with effective mating frequency (ANOVA, $F_{6,72}=1.81$ $p=0.11$). Though effective mating frequency was similar to absolute mating frequency in that it was significantly lower in permanently polygynous colonies than in solitary ($p<0.05$), unlike absolute mating frequency temporarily polygynous colonies showed an intermediate value of effective mating frequency that was not significantly different from solitary or permanently polygynous colonies (Fig. 1B).

When analyzed with average queen body mass, colony density, and number of foundress queens as covariates, the effect of colony type on effective mating frequency remained significant (ANOVA, $F_{2,72}=4.16$ $p<0.03$) (Fig 1B). Independent of these other factors, pedigree-effective mating frequency was significantly higher in solitary than in temporarily polygynous colonies ($p<0.05$), with permanently polygynous colonies not significantly different from either, though this group had the smallest least square mean. Mean paternity skew ranged from 0.55 to 0.64 and was not significantly different among colony types (ANOVA, $F_{2,8}=0.263$ $p=0.78$) (Fig 1C).

Mating Frequency and Size of Foundress Groups

Independently of queen body mass, colony density, and colony type, queens in areas with larger foundress groups mated with an average of more males (ANOVA, $F_{2,52}=7.35$ $p<0.01$), and had more genetically variable offspring (ANOVA, $F_{1,52}=5.88$ $p<0.02$). Within permanently polygynous colonies, the number of queens in foundress groups was not significantly associated with absolute mating frequency (linear regression, $t_{38}=-0.79$ $p=0.45$) or pedigree effective mating frequency (linear regression, $t_{38}=-0.89$ $p=0.38$). Within temporarily polygynous colonies, there was a nonsignificant positive trend in absolute mating frequency (ANOVA, $F_{14}=1.34$ $p=0.20$) and

**Figure 2.**

Scatterplots of (A) mean absolute mating frequency \pm SE, and (B) mean pedigree-effective mating frequency plotted against mean number of foundress queens. Scatterplots of (B) absolute mating frequency residuals and (D) pedigree-effective mating frequency residuals plotted against mean number of foundress queens. Residuals were calculated from ANOVA's of mean body mass, mean colony density, colony type, and in the case of absolute mating frequency, sample size, to visualize the effect of foundress number independent of these other factors. (E) Bar graphs of the average number of foundresses \pm 95% confidence interval, by colony type. "Fight" corresponds to temporary polygyny, "polygyny" to permanent polygyny.

pedigree-effective mating frequency (ANOVA, $F_{14}=1.12$ $p=0.28$). Solitary colonies were not analyzed separately, as these queens do not form foundress groups.

Queen Body Mass

Larger queens mated with significantly more males than smaller queens ($F_{1,52}=6.96$ $p=0.01$) and had more genetically variable offspring ($F_{1,52}=5.25$ $p<0.03$). When both measures of mating frequency were regressed against individual queen body mass for each colony type, however, not all types showed this relationship. In primary polygynous colonies, individual queen body mass did not show a significant association with mating frequency (linear regression, $t_{37}=0.44$ $p=0.66$) or effective paternity (linear regression, $t_{37}=0.34$ $p=0.74$). In contrast, individual body mass was significantly positively related to absolute mating frequency in solitary colonies (linear regression, $t_9=3.09$ $p<0.02$), as well as to pedigree-effective mating frequency (linear regression, $t_9=2.68$ $p<0.03$). Temporarily polygynous colonies showed a nonsignificant relationship between individual body mass and both mating frequency (linear regression, $t_4=-2.34$ $p=0.10$) and effective mating frequency. (linear regression, $t_4=-2.49$ $p=0.09$). Temporarily polygynous colonies had a very low sample size for this analysis ($N=5$) due to lack of data on queen live mass for some colonies from these sites. Average body mass showed a correlation to colony type, with smaller queens in permanently polygynous colonies, and larger queens in those that are solitary and temporarily polygynous (Fig. 3C).

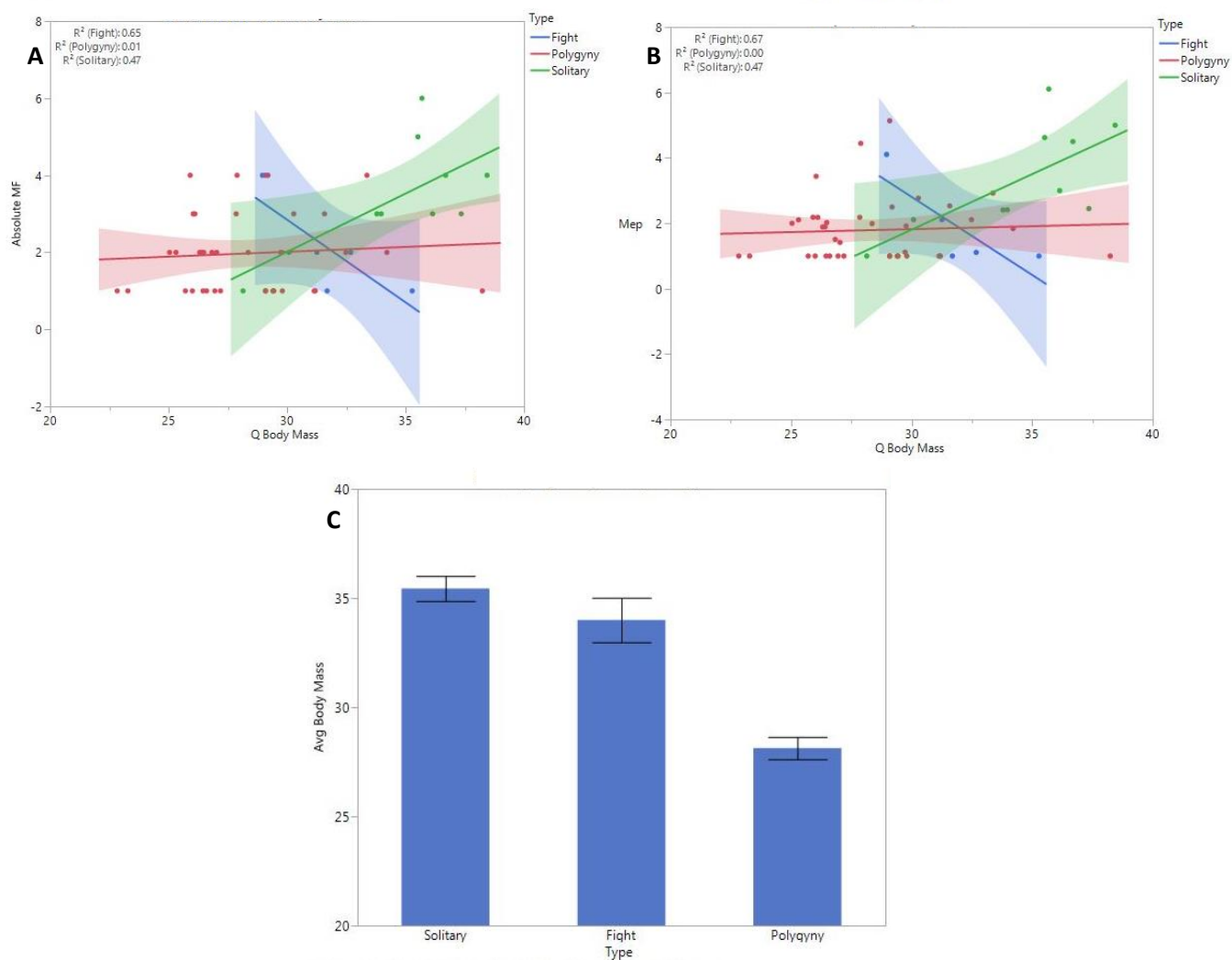


Figure 3.

Scatterplots of (A) absolute mating frequency plotted against individual queen body mass, with colony type overlaid, and (B) pedigree-effective mating frequency plotted against individual queen body mass, with colony type overlaid. (C) Average body mass $\pm 95\%$ confidence interval, by colony type. “Fight” corresponds to temporary polygyny, “polygyny” to permanent polygyny.

Colony Density

Queens from areas with a higher colony density mated with fewer males (ANOVA, $F_{1,52}=5.85$ $p<0.03$), and had less genetically variable offspring (ANOVA, $F_{1,52}=5.85$ $p<0.03$)(Fig. 4A, 4B). The solitary region had the highest mean density, followed by the fighting and permanently polygynous regions (Fig. 4C); effects of colony density could not be examined statistically with sufficient power within colony types, due to the low number of sites examined

with colony density data available (2 solitary sites, 3 temporarily polygynous sites, and permanently polygynous sites the second).

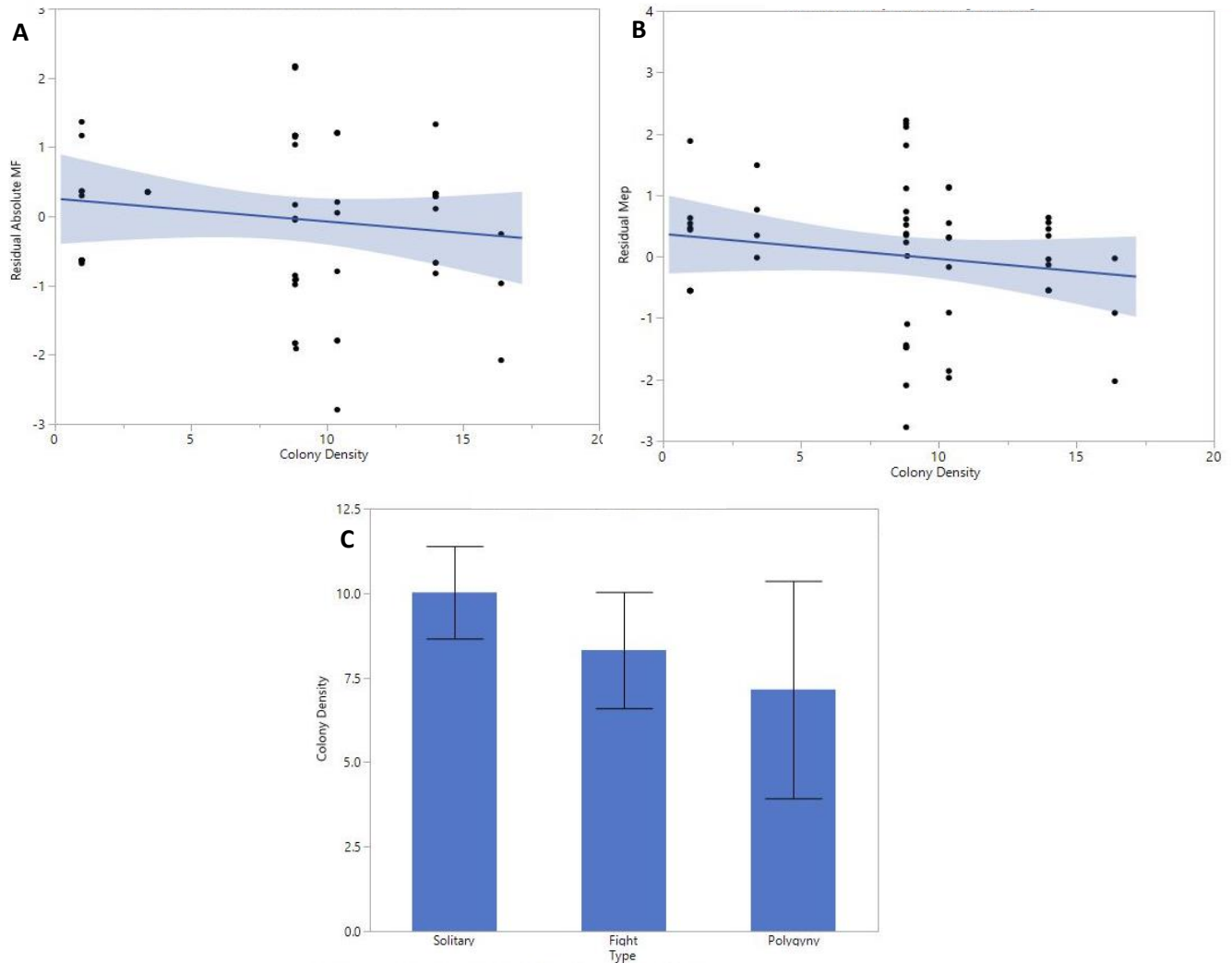


Figure 4.

Scatterplots of (A) residuals of absolute mating frequency by colony density, and (B) residuals of pedigree-effective mating frequency by colony density. Residuals were calculated from ANOVA's of mean body mass, mean colony density, mean number of foundresses, and in the case of absolute mating frequency, sample size, to visualize the effect of colony density independent of these other factors. (C) Mean colony density \pm 95% confidence interval, by colony type. "Fight" corresponds to temporary polygyny, "polygyny" to permanent polygyny.

Discussion

The polygyny/polyandry tradeoff hypothesis predicts that polygyny and polyandry should be negatively associated because both incur costs but achieve the same result: genetic diversity (Hughes et al. 2008b). My results tentatively show support for this hypothesis. Genetic variation in each queen's offspring derived from polyandry, measured as pedigree-effective mating frequency, was lowest in permanently polygynous colonies, where the presence of multiple queens throughout the colony cycle provides an alternative route to genetic diversity, while it was highest in queens from the solitary region where that option is not available. It is important to note, however, that social strategy may impact mating behavior both directly and indirectly, and may not be due solely to differences in genetic variation within colonies as proposed in the polygyny vs. polyandry tradeoff hypothesis (Hughes et al. 2008b, Keller and Reeve 1994). Polygyny varies across the range of this species due to environmental factors, and selective pressures acting on queens across social contexts leads to a syndrome of differences in queen morphology and behavior, all of which appear to contribute to the observed patterns. Thus, there is support in the results for a tradeoff between polygyny and polyandry, but there is also evidence that this tradeoff is linked to a more complex set of factors than genetic variation alone.

Because the number of unrelated queens contributing to the brood is different between different social strategies, the tradeoff hypothesis predicts that pedigree-effective mating frequency should be higher in solitary areas, where intra-colonial genetic variation stems from polyandry alone, and lower in polygynous areas. Moreover, due to the transient nature of the genetic variation benefit of polygyny in temporarily polygynous colonies then in permanently polygynous ones, the effect of polygyny on polyandry should be weaker when queens fight for colony ownership. The results closely match these predictions. Effective mating frequency was

higher in solitary colonies than permanently polygynous; temporarily polygynous colonies were at an intermediate level not significantly different than either. These results are in line with previous studies of polygyny and polyandry within species that have found the two to be negatively associated (Qian et al. 2011, Haapaniemi and Pamilo 2012).

There are two primary ways in which genetic variation in the offspring can be increased under polyandry: queens can mate with more males, introducing more novel alleles into the offspring, or use male sperm more uniformly, so no individual male dominates paternity. Skew was not significantly different between colony types, but absolute mating frequency, the number of males a queen mated with, was significantly lower in permanently polygynous colonies, indicating the observed difference in genetic diversity comes from mating frequency. Interestingly, when mating strategy was assessed through absolute mating frequency alone, without considering paternity skew, there was no significant difference between solitary and temporarily polygynous colonies. This suggests that genetic variation may be especially important for mature colonies, as temporarily polygynous colonies become effectively solitary after the first crop of workers is raised and only one queen remains alive. The difference among types in absolute mating frequency disappears with average queen body mass, mean colony density, and mean number of foundresses in the model, which suggests the variation may be better explained by those factors than by colony type. It should be noted, however, that it is possible an effect on mating frequency is present in *V. pergandei* populations, but could not be detected by the analysis. Within colony types, sites varied significantly from each other in absolute mating frequency, but site effects could not be included in the larger model due to sampling limitations. Sampling more sites for each colony type to support a more complex model may show an effect on absolute mating frequency that was masked by site variation.

The polygyny/polyandry tradeoff is based on a consideration of the benefits versus the costs of mating behaviors, and both of these may be directly affected by other aspects of the queen's environment and morphology. Three possibly influential factors are queen body size, which may positively affect mating frequency due to greater fitness and energy available to mate (Fjerdingstad and Keller 2004), colony density, which may positively affect availability of mates and by extension may reduce the cost of a higher mating frequency, and number of foundress queens, which is associated with but not identical to social form and by extension should be negatively associated with mating frequency. All three of these factors are correlated with colony type, both in past research and in my sample of colonies (Fig. 1C, 2E, 3C, 4E)(Cahan and Helms 2014, Cahan 2001), and all three were associated with both measures of mating frequency. This suggests that the relationship between social strategy and polyandry is likely to be more complex than the polygyny/polyandry tradeoff would suggest, with both direct, indirect, and correlated selective pressures shaping queen mating behavior. Colony density and queen body mass are expected to reinforce the increased polyandry expected in solitary areas, where both are highest. In contrast, the number of foundress queens (or magnitude of polygyny) is expected to be negatively related to polyandry predicted in permanently polygynous areas, in keeping with the negative relationship between polygyny and polyandry predicted by the polygyny/polyandry tradeoff hypothesis.. Although including these additional factors did not change the ordering of mating frequencies across types, it did shift the statistical groupings in a way that suggests that each region is impacted by these factors in a different way. Permanently polygynous colonies continued to have the lowest mean, but the residual mating frequency estimates were more variable and this region no longer differed significantly from the other two social forms, while temporarily polygynous colonies shifted to being significantly lower than solitary colonies. Thus, the effect of

colony type on effective mating frequency may not have lost its significance, but it was altered by other factors, indicating a possible mediation of effective mating frequency by body size, colony density, and foundress number, either directly through effects on the evolutionary benefits of genetic variation, or indirectly through effects on absolute mating frequency.

Queen body mass is expected to be positively associated with polyandry; in addition to the correlation with colony type, larger queens tend to have greater energy reserves, and as such are likely to suffer fewer costs from multiple matings than smaller queens (Fjerdingstad and Keller 2004). As expected, on average larger queens mated with more males than smaller queens. The positive effect on absolute mating frequency may be due to an energetic constraint of multiple matings (Keller and Reeve 1994), with larger, fitter queens more able to bear the costs of more matings, or due to factors other than queen mate choice. Body size can be an effective indicator of queen quality and fecundity (De Souza et al. 2013), potentially making larger queens more attractive as mates to males, leading to an effect of male mate choice (Bonduriansky 2001). Interestingly, however, individual queen body mass showed a significant positive effect in solitary colonies only, while polygyny did not even have a nonsignificant trend between either measure of mating frequency and body mass, and temporary polygyny showed a negative trend with both, but had a sample size too small to statistically confirm this trend. For body mass to only have an effect in solitary areas is in line with the predictions of the polygyny/polyandry tradeoff hypothesis. Because solitary queens are not founding colonies in groups, they lack the advantage in colony productivity and survival from additional foundresses (Offenberg et al. 2012). But polyandry can be associated with greater colony productivity due to the increased genetic variation, leading to increased colony survival (Cole and Wiernasz 1999). This may present another tradeoff, present only when a queen is founding a colony alone. Multiple matings are energetically expensive

(Keller and Reeve 1994), but beneficial to colony survival for those queens that can bear the cost. As larger queens are more likely to be able to bear this cost and still have the energy required to found a colony, this may lead to the observed positive relationship between queen body mass and polyandry. It would not be a factor in other regions, because group founding queens eliminate this tradeoff by founding colonies cooperatively. Repetition of this positive association between queen body mass and polyandry in solitary colonies and a test of possible causes for it could provide an interesting project for future research.

Polygyny is not uniform, even across polygynous areas. The average size of starting nests varies across the region, and with this variation comes differences in intra-colonial genetic variation. The number of foundress queens in a nest is significantly correlated with colony type: solitary sites rarely show more than a single queen in a starting nest, while temporarily polygynous sites show significantly smaller foundress associations on average than permanently polygynous ones (Helms and Helms Cahan 2012; Fig. 2E). Contrary to expectations, queen number showed a small but positive relationship with absolute mating frequency and with pedigree-effective mating frequency across colony types, suggesting that more queens leads to an increase in both the number of mates and the genetic variation those mates provide. This effect, however, may be due to the unbalanced nature of the analysis. In eight of nine sites, foundress number averaged between 1 and 4, but in one site it averaged over 16 queens per starting nest. Data from a greater range of sites is needed to eliminate the bias this site creates in the analysis.

Within the polygynous region, the polygyny/polyandry tradeoff hypothesis predicts a negative relationship between foundress number and mating frequency; in colonies containing as many as 16 or more queens, genetic variation is already very high, and polyandry will do little to affect it. Contrary to expectation, no effect of queen number on either absolute or pedigree-

effective mating frequency was found for these colonies. Instead, I found absolute mating frequency stayed at a relatively stable mean of about two males throughout polygynous sites, an effect that was not related to number of foundresses, average or individual body mass, or colony density. This suggests something else may be maintaining a minimum of polyandry across polygynous areas. This minimum may be beneficial. Even in a highly genetically diverse polygynous colony, each individual queen only gains fitness benefits from her own offspring, so if a queen gains fitness benefits from more patriline in her own offspring, there would be selection to maintain some level of polyandry. In cases of parasite infection, even if polygyny is protective to the colony as a whole, individual queens may still suffer fitness benefits if their offspring are susceptible to the parasite, and indeed previous studies have suggested polyandry and polygyny may be selected to coexist in response to pressure from parasites (Schmid-Hempel and Crozier 1999). Alternatively, even if monandry would be optimal for polygynous queens, there may be constraints preventing that behavior. The cost of resisting additional mating attempts by males after a queen has mated once may outweigh the cost of tolerating them, leading to a higher mating frequency (Pedersen and Boomsma 1999). This retention of a stable, low level of polyandry offers an interesting avenue for further study of possible benefits and costs to queens.

Colony density at sites is expected to increase mating frequency, due to the availability of mates. Mating flights of *V. pergandei* are diffuse, rather than forming dense swarms (S. Helms Cahan, personal communication). This suggests a possible cost of finding males to mate with, as well as other costs of mating, which could be mitigated by higher colony density. Colony density is highest in solitary areas, and lowest in polygynous, so both would be expected to lead to the same overall pattern (Helms Cahan 2001; Fig. 4E). However, when both colony type and density were considered in the same analysis, the effects of density showed the opposite pattern, as queens

in less dense areas mated with significantly more males. This could occur because the benefits of genetic diversity may be density-dependent. Colony density in *V. pergandei* tends to be higher in more hospitable parts of the range (Helms Cahan 2001), which have more available resources, but also likely have more competition for these resources and for colony foundation, as a higher density of colonies means more neighbors to compete with. Brood raiding has been documented in *V. pergandei*, and brood raiding behavior can rise when colonies are more densely packed (Rissing and Pollock 1987, Adams and Tschinkel 1995). Brood raiding itself can also increase intra-colonial genetic variation (Gadau et al. 2003), and could potentially pose an alternate strategy to polyandry for increasing genetic variation within a colony. There is also the possibility that the energy investment required for more matings is more of a disadvantage in high-competition environments, where that energy could be put to use producing more or better quality offspring. A larger sample size of sites is required to better understand the effects of colony density on *V. pergandei* mating frequency.

My study was limited in its power to determine causality, as the data was only correlational. The negative correlation found between polygyny and polyandry is what would be expected if there is an existing tradeoff between the two, but determining the cause of this relationship requires further experimental study. To confirm the presence of a tradeoff, the actual benefits of genetic variation in *Veromessor pergandei* should be assessed, to determine if this species does show increased foraging success and resistance to parasites when a colony is more genetically variable, and whether these benefits increase with greater levels of genetic variation. Costs should be assessed as well, through tests of how both polygyny and polyandry alter an individual queen's ability to found a colony successfully and produce offspring. To further test the positive association found between queen body mass and mating frequency, small and large queens from solitary areas

could be allowed to mate [artificial exposure to mates], and allowed to produce offspring. If queens mating with fewer males produce fewer offspring, this would show mating presents an energetic cost, and if larger queens are less impacted by it than smaller queens, this would suggest energetic investment drives the relationship between body mass and polyandry. To study the impact of colony density on mating frequency, queen mating flights in the field could be observed and timed, to confirm whether there are differences between low and high density areas. Lab experiments artificially forcing queens to make longer or shorter mating flights could show if any change found in flight time is costly enough to be a selective pressure on queens. Results of all these experiments could shed more light on the relationship between mating frequency in *V. pergandei* and genetic variation, as well as other life history and environmental factors that may influence it.

In conclusion, my study shows a negative relationship between polygyny and polyandry is present in *Veromessor pergandei*, and tentatively supports that it is due to a tradeoff based in genetic variation. However, it also shows that polyandry is influenced by a more complex array of factors than just genetic variation, and these factors require further study to fully understand their influence on queen behavior.

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