

UNEXPLAINED SPLIT SEX RATIOS IN THE NEOTROPICAL PLANT-ANT, *ALLOMERUS OCTOARTICULATUS* VAR. *DEMERARAE* (MYRMICINAE): A TEST OF HYPOTHESES

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We investigated sex allocation in the Neotropical ant *Allomerus octoarticulatus* var. *demerarae*. Because *Allomerus* is a plant symbiont, we could make geographically extensive collections of complete colonies and of foundresses in saplings, allowing us to estimate not only population- and colony-level sex allocation but also colony resource levels and the relatednesses of competing ant foundresses. This species exhibits a strongly split sex ratio, with 80% of mature colonies producing $\geq 90\%$ of one sex or the other. Our genetic analyses (DNA microsatellites) reveal that *Allomerus* has a breeding system characterized by almost complete monogyny and a low frequency of polyandry. Contrary to theoretical explanations, we find no difference in worker relatedness asymmetries between female- and male-specialist colonies. Furthermore, no clear link was found between colony sex allocation and life history traits such as the number of mates per queen, or colony size, resource level, or fecundity. We also failed to find significant support for male production by workers, infection by *Wolbachia*, local resource competition, or local mate competition. We are left with the possibility that *Allomerus* exhibits split sex ratios because of the evolution of alternative biasing strategies in queens or workers, as recently proposed in the literature.

KEY WORDS: Ant-plant symbiosis, local mate competition, local resource competition, mating system, relatedness asymmetry, resource abundance hypothesis.

In recent years, it has become clear that there is great deal of variation in sex biasing strategies in ant societies (reviewed in

Chapuisat and Keller 1999). The breeding system—the number, relatednesses, and relative reproductive outputs of queens within a colony—constitutes a key feature of social life (Ross 2001) that directly affects the relatednesses among colony members (especially workers), and thus, governs the indirect benefits that workers gain by helping to raise brood. Most importantly, because

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the Hymenoptera are haplodiploid organisms, such that diploid females are produced from fertilized eggs and haploid males from unfertilized ones, there are relatedness asymmetries across workers (females engaged in other tasks than reproduction) and sexual individuals (queens and males). For instance, when colonies are headed by one singly mated queen, workers are three times more related to their sisters than to their brothers, whereas queens are equally related to their daughters and their sons (Trivers and Hare 1976). Kin selection thus predicts a conflict between queens and workers, with queens favoring a balanced sex allocation and full-sibling workers a three times larger investment in females than in males. As a consequence, the breeding system imposes selection pressures on sex bias strategies in queen(s) and/or workers (Trivers and Hare 1976; Pamilo 1991; Chapuisat and Keller 1999). One of the most interesting and well-studied of such phenomena in ants is the existence of so-called “split” or bimodal sex ratios, where some colonies produce mainly female sexuals and others produce mainly male sexuals (Boomsma and Grafen 1990, 1991; Kümmerli and Keller 2009).

Several sets of hypotheses have been proposed to account for the occurrence of split sex ratios in ants and other social Hymenoptera (see also Kümmerli & Keller 2009). Firstly, differences in sex allocation among colonies could be caused by variation in the degree of relatedness asymmetry across colonies, where the variation is caused by cross-colony variation in queen number, in relatedness between con-colonial queens, or in the number of mates per queen, or by male production by workers (Yu 1987; Boomsma and Grafen 1990; Ratnieks 1991; Boomsma 1993; Bourke and Chan 1994; Ratnieks and Boomsma 1997). Workers can bias sex ratios adaptively by rearing mostly females in colonies with high relatedness asymmetry and by rearing mostly males in colonies with lower relatedness asymmetry (Beekman and Ratnieks 2003). Sex allocation biasing may stem, for example, from destruction of male brood (Aron et al. 1994, 1995; Passera and Aron 1996; Sundström et al. 1996), from channeling a larger proportion of the female brood into the queen caste rather than the worker caste through differential feeding (Hammond et al. 2002), and/or from the production of males by workers. Worker production can generate extreme male bias if populations exhibit both queenright and queenless colonies; if the latter produce only males, queenright colonies will be selected to specialize in female production.

Secondly, it has been suggested that colonies can vary in the nature of sex ratio manipulation strategies and in the identity (queen or worker) of the individuals that employ these manipulations (Helms et al. 2005). For example, queens may exert control over sex allocation by regulating the caste fate of diploid eggs they produce (i.e., by laying worker-destined eggs rather than queen-destined eggs) (de Menten et al. 2005) or by biasing the primary sex ratio (the proportion of haploid-male eggs laid) (Passera et al.

2001). These biasing strategies may be exhibited, theoretically, either by the queens or by the workers. They are known collectively as “alternative biasing strategies,” and models have argued that they can result in split sex ratios (Roisin and Aron 2003; Reuter et al. 2004; Helms et al. 2005). The term “alternative biasing strategies” refers to the existence of two distinct pure strategies across colonies, whereby, for instance considering queens, some queens put a strict limit on the number of diploid eggs available and others provide diploid eggs *ad lib.*, or some queens produce only male eggs whereas others produce both sexes at equal frequency; or considering workers, some eliminate all males in some colonies while others eliminate none, or workers in only some colonies manipulate the brood sex ratio and determine the caste of the female brood. Polymorphism in queen/worker strategies is maintained by frequency-dependent selection. Although ant queens in some species have long been known to be able to exert control over the developmental fate of diploid eggs (total queen control) or over sex-allocation by laying more or fewer male eggs (partial queen control) (Nonacs 1986b; Pamilo 1990), “alternative biasing strategies” have not yet been demonstrated empirically.

Thirdly, colony resource level has been suggested as a proximate cause of sex ratio variation, either because environmental heterogeneity in the supply of resources will cause some colonies to have easier access to resources that are needed for the production of one sex only (Grafen 1986), or because increasing colony resources allows a greater proportion of diploid brood to be reared as female alates, whose development is usually costlier and thus the diploid brood would have otherwise been channeled into becoming workers rather than reproductives (Nonacs 1986a; Crozier and Pamilo 1993; Rosenheim et al. 1996). Fourthly, differences in dispersal strategies between the sexes could affect sex allocation through mechanisms such as local mate competition (LMC; Hamilton 1967) or local resource competition (LRC; Clark 1978; Frank 1987). This is because the fitness return from investing into the less dispersing sex is a diminishing function of the total investment in sexuals (Frank 1987). Under LMC, competition among sons for mates lowers the reproductive value of males with increased investment in sexuals. Thus, colonies are predicted to produce males up to some threshold, and then invest all remaining resources into female reproductives. Under LRC, dispersal of females is limited and related queens compete for access to resources (nest sites, food), so that the fitness return through females decreases with increased investment relative to the fitness return through males. More productive colonies are therefore expected to invest proportionally more in males.

Fifthly, the queen replenishment hypothesis holds that in highly polygynous ants, the production of new queens occurs preferentially, or only, in colonies that contain relatively few queens, because these are the colonies that benefit most from recruiting new queens (Brown and Keller 2000, 2002). This mode of

reproduction may also lead to intense LRC between queens within colonies and is often associated with dramatically male-biased sex ratios (Bourke and Franks 1995). Lastly, it has been suggested that maternally inherited parasites, by manipulating host reproduction in ways that enhance their transmission to future generations, may influence sex allocation in social Hymenoptera (Bourke & Franks 1995). One candidate for such sex ratio manipulation is *Wolbachia*, a genus of cytoplasmically transmitted bacteria known to widely infect Hymenoptera, and particularly ants. *Wolbachia* can bias the sex ratio of brood toward females, via either cytoplasmic incompatibility or the induction of parthenogenesis (Wenseleers et al. 1998).

The aim of this study is to characterize the genetic structure of colonies and the breeding system of the ant *Allomerus* cf. *octoarticulatus* var. *demerarae* Wheeler (Myrmicinae, named *A. demerarae* in some previous papers [Yu and Pierce 1998; Yu et al. 2001, 2004], hereafter *Allomerus*) and to use these data to explain patterns of sex allocation in this species. This species exhibits highly skewed sex ratios, which motivates our study, and some useful characteristics for sex ratio studies. Firstly, *Allomerus* is a symbiotic plant-ant, i.e., an ant that is strictly associated with a host-plant (Yu and Pierce 1998). This life history allows us to collect whole colonies and to quantify colony resource availability, both with high confidence. Secondly, *Allomerus* appears to have a simple breeding structure, with previous dissections revealing that colonies appear to be monogynous (Yu and Pierce 1998; Frederickson 2006). Nonetheless, colony sex ratios are highly split, with colonies specializing in the production of male or female sexuals (Yu and Pierce 1998; Frederickson 2006). We assess the evidence for or against each of the above models that account for sex ratio specialization in social Hymenoptera, with the exception of the queen replenishment hypothesis because *Allomerus* is not highly polygynous.

Materials and Methods

STUDY SYSTEM

The host-plant of *Allomerus octoarticulatus* var. *demerarae* is the Neotropical ant-plant *Cordia nodosa* Lam. (Boraginaceae). *Cordia nodosa* is an understory treelet found across Amazonia (Wheeler 1942), and in Peru it is principally inhabited by *Allomerus* and at least three species of *Azteca* Forel (Dolichoderinae). Each plant hosts one ant colony, with rare exceptions ($\approx 1\%$) in which double-trunked plants can host two different colonies and, thus, two different ant species (Yu and Pierce 1998; Yu et al. 2001, 2004). The plant provides its resident ant colony with housing in the form of hollow swellings (domatia) at branch internodes (Appendix S1) and with a small volume of lipid and carbohydrate-rich food bodies (Solano et al. 2005), although *Allomerus*' diet appears to consist mostly of insects that are captured on the host-plant surface (Davidson et al. 2003; Dejean et al. 2005).

In return for plant-derived resources, *Allomerus* and *Azteca* workers actively patrol and protect new shoots (Yu and Pierce 1998; Frederickson 2005; Edwards et al. 2006a, 2007). Domatia are a useful index of plant size (Yu and Pierce 1998). We focused on the more abundant *Allomerus*, which is a castration parasite of *C. nodosa*. Workers destroy flowers and thereby prevent most fruiting, thus diverting resources from plant reproduction to growth, benefiting the ant (Yu and Pierce 1998; Edwards and Yu 2008; Frederickson 2009). *Azteca* spp. do not castrate. The system relies thus proximately on the persistence of *Azteca*, which compete with *Allomerus* for host-plants (Yu and Pierce 1998; Yu et al. 2001, 2004).

STUDY SITES

We analyzed data collected from three different sites in Amazonian Peru. Two sites, TPL (Tambopata Jungle Lodge, bordering the Tambopata National Reserve; 12.83°S, 69.40°W), and EBCC (Estación Biológica Cocha-Cashu, in the Manu National Park; 11.89°S, 71.40°W), are located in southern Peru, in the department (province) of Madre de Dios, while the third site RNAM (Reserva Nacional Allpahuayo-Mishana; 3°58'S, 73°25'W) is in northern Peru, in the department of Loreto. TPL and EBCC are ca. 240 km apart, while RNAM is around 1000 km distant from the two others. Madre de Dios (ca. 2100 mm rain/year [Yu et al. 2001]) and Loreto (2400–3200 mm rain/year [Sombroek 2001]) are both characterized by extensive, mesic to seasonal lowland tropical rain forest, with rainfall seasonality being much greater in Madre de Dios than in Loreto.

DATASETS

We took advantage of several datasets (see Fig. 1 for a synoptic diagram). At TPL, we collected a subset of workers and the queen (but no alates) from 20 mature colonies inhabiting adult *C. nodosa* trees. At RNAM, we collected workers, all alates (pupae and adults), and the queen(s) from 181 mature colonies, revisiting a dataset from Frederickson (2006). Additionally, we used previously published colony fecundity counts from TPL (54 colonies, Yu et al. 2004) and EBCC (31 colonies, Yu and Pierce 1998). We referred to the 54-colony nongenotyped TPL dataset as TPL₅₄, distinguishing it from the 20-colony genotyped TPL dataset, which we designated as TPL_{G20}.

In short, we had two colony collections for genetic analysis, RNAM and TPL_{G20}, which were both used for worker genotyping and for describing the mating system. The RNAM collection was also used for studying the link between sex allocation bias and worker relatedness. And we had three colony collections for estimating colony and population sex ratios: TPL₅₄, EBCC, and RNAM (again) (Fig. 1).

For these colonies we also had an estimate of host-plant size in terms of domatia number. We used domatia number because

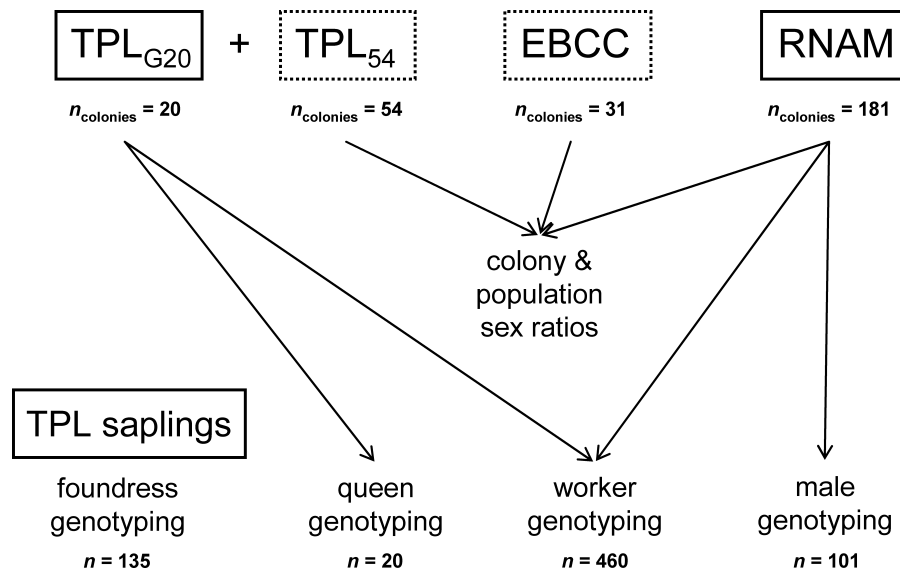


Figure 1. Synoptic diagram of the three different datasets and their use in this study. Solid boxes indicate collections that were used for genotyping, and dashed lines indicate previously published datasets that were used only for estimating colony and population sex ratios. In the TPL_{G20} dataset, only workers and queens were collected. In the TPL₅₄ and EBCC datasets, only alate counts were available, and in the RNAM dataset, alates, workers, and queens were collected and counted.

C. nodosa exhibits dichasial, sympodial branching (one domatium per internode, Appendix S1). We approximated colony size by the number of domatia because domatia are a limiting resource for plant-ants, providing a convenient measure of total resource availability (space, housing and food, Appendix S1) (Yu and Pierce 1998; Edwards et al. 2006a; Frederickson 2006). *Allomerus* ants convert their host-plants into passive traps for insect prey (Dejean et al. 2005), so the bigger the plant, the more the trapping surface. Additionally, our use of domatia number as a proxy for colony size is supported by a previously published study of eight Neotropical ant-plant species, including two ant-plant species that host *Allomerus* spp., which found that worker number and domatia number (or estimated volume) are tightly and positively correlated (Fonseca 1999).

Finally, we also used a collection of 135 queens founding colonies on saplings at TPL (Edwards et al. 2006b), revisiting collections from Yu et al. (2004). The TPL queen dataset was used for measuring the relatedness between queens colonizing the same saplings. We also compiled local host-plant-size distributions: $n = 951$ trees at TPL (Yu et al. 2004), $n = 1006$ trees at EBCC (Yu and Pierce 1998), and $n = 181$ trees at RNAM (Frederickson 2006).

POPULATION AND COLONY GENETIC STRUCTURE

Genotypes of 10 workers per colony were determined for the five most polymorphic DNA microsatellite loci (Ad040, Ad045, Ad109, Ad127, and Ad166; Debout et al. 2006) for the TPL_{G20} dataset and for 26 colonies from the RNAM dataset. The sole queen from each of the TPL_{G20} colonies was also genotyped.

For both the RNAM and the TPL_{G20} datasets, we used the program RELATEDNESS 5.0.8 (Queller and Goodnight 1989) to estimate mean regression relatedness r_{w-w} among colony members, giving each colony equal weight. Standard errors were obtained by jack-knifing over colonies. The expected (and asymptotic) relatedness of two workers in the same monogynous colony is 0.75 if they belong to the same patriline and 0.25 if they belong to different patrilines (Trivers and Hare 1976).

For the TPL_{G20} dataset, because we had the queen genotypes, we could deduce the paternal alleles of each worker by subtracting the maternal genotype. We then used the maternal and inferred paternal genotypes to confirm that between-father and between-parent relatednesses were not significantly different from zero. The absence of inbreeding allowed us to test for polyandry with the formula $m = 2/(4r_{w-w} - 1)$, where m is the genetic effective number of sexual partners per queen (Chevalet and Cornuet 1982) and to test for (undetected) polygyny with the formula $n = 3/(4r_{w-w})$, where n is the genetic effective number of queens per colony (Ross 1993). We used the same formulae to estimate m and n in the RNAM dataset, but with the caveat that we had to assume monogyny when we reconstructed paternal genotypes to test for inbreeding.

We therefore also used the software package MATESOFT v1.0 (Moilanen et al. 2004) to provide independent estimates of effective mate number and of the proportion of multiply mated queens. MATESOFT is a software package for the analysis of mating systems in male-haplodiploid organisms that takes into account both allelic frequencies and the Mendelian segregation of alleles. Estimates are based on the assumption that (1) marker loci are codominant,

neutral, and unlinked; (2) population allele frequencies are the same for males and females; (3) there is no inbreeding; and (4) multiple mates of the same female are not related. For all procedures, the population-wide allele frequencies are entered so that gene similarities owing to a common genetic background are taken into account. Following advice given in the documentation, when the Mendelian probability was very low ($P \leq 0.05$) that a group of workers was derived from a single monogynous colony, thus indicating polygyny, we either purged the data from the worker that caused the probability to be low (if only one worker was concerned out of the 10 workers genotyped), or we omitted the group from further analysis (if ≥ 2 workers appeared to be of a possible alien mother).

Lastly, for both the RNAM and the TPL_{G20} datasets, we calculated the frequency α of sib-mating from $F = \alpha/(4 - 3\alpha)$ (Pamilo 1985), where F stands for the inbreeding coefficient (i.e., F_{it}). The estimate for F (the correlation between pairs of genes within individuals) was done through a two-level (individual and colony), hierarchical F -analysis of variance using the software package GDA v1.1 (Lewis and Zaykin 2001) because individuals within colonies are related and so do not represent independent samples. Confidence intervals were obtained by bootstrapping over loci 10,000 times. We thus calculated the inbreeding corrected coefficient of relatedness r^* according to the equation of Pamilo (1985): $r^* = (r - 2F/(1 + F))/(1 - 2F/(1 + F))$.

LOCAL RESOURCE COMPETITION

To test for local resource competition (LRC), we used the 135 queens from the TPL sapling dataset to estimate the relatednesses of foundresses collected from the same sapling. We used the software package COLONY v1.2 (Wang 2004) to determine the number of genetic families to which the foundresses belong, under the hypothesis of a single diploid queen. We analyzed the dataset first under the assumption of strict monoandry and then relaxed the assumption. Under the first assumption, the sampled sexual females can be either full-sibs from the same colony or nonsibs from different colonies; under the second assumption, females can also be half-sibs with the same mother but different fathers.

SEX RATIO ANALYSIS

Estimating dry weights of sexuals was done by weighing ants previously dried 24 h in an oven at 70–75°C, following Keller and Passera (1989) and Sundström (1995). To prevent the reabsorption of ambient humidity by dried ants, ants were kept inside the tightly closed oven and removed immediately before weighing. Average adult dry weights (\pm SE) were 1.305 ± 0.027 mg ($n = 8$) and 1.920 ± 0.160 mg ($n = 6$) for males and females respectively in EBCC (these were used for sex ratio calculations in TPL and EBCC), and 1.021 ± 0.029 mg ($n = 7$) and 1.955 ± 0.073 mg ($n = 12$) for males and females in RNAM.

Only adult and pupal alates could be identified to sex. Therefore, our measures are snapshots, not lifetime estimates. We have three independent sets of sex ratio data: TPL₅₄, 30 colonies from EBCC, and 181 colonies from RNAM. Sex ratios are computed as female proportion, female/(female + male), and we calculated three different ratios: (1) numerical sex ratio (nSR), the numerical proportion of females among all sexuals; (2) investment sex ratio (iSR), the proportional investment in females among all sexuals estimated from dry weight, i.e., the number of pupal and eclosed alates multiplied by the average adult dry weights (to get an estimate of the investment at the time that alates leave the nest); and (3) corrected investment sex ratio ($ciSR$), the proportional investment in females among all sexuals (iSR) corrected by the difference in the female to male energetic cost ratio (Boomsma 1989; Boomsma et al. 1995).

Statistical analyses were performed using SPSS v13.0 (SPSS Inc., Chicago IL) or GLMSTAT v6.0.0 (Beath 1997). Means \pm standard errors are given. In the text, “female-only” and “male-only producing colonies” indicate colonies producing only one sex (i.e., 100%). They are denoted as F and M colonies. B colonies produce both sexes. In all figures, sex ratios are corrected investment sex ratios ($ciSR$).

MALE PRODUCTION

One reason for extreme male bias could be the production of males by workers. To assess this possibility, we compared the genotypes of the males with the reconstructed queen’s genotype in nine colonies from the RNAM dataset. Seven of these colonies were producing only male alates, while the last two were producing both sexes. One hundred and one males were collected (6–20 males per colony) and genotyped for the same five most polymorphic loci that were used for the workers (Ad040, Ad045, Ad109, Ad127, and Ad166). Sons of queens must carry a queen-derived allele at all loci and, as a group, they should not display more than two alleles at a single locus. Sons of workers may carry with equal probability either an allele derived from the mother or the father of the worker. Any male that carries a nonqueen allele is a worker’s son. However, because sons of workers may carry queen alleles at all loci by chance, the probability of nondetection was estimated following Foster et al. (2001), as $P_{non-detect} = \sum_i^n p_i (0.5^{l_i})$, where n is the number of patriline in the nest, p_i is the proportional contribution of the i th father to the brood, and l_i is the number of informative loci analyzed at the i th patriline. An informative locus is one where the queen and her mate have different alleles so that the workers carry an allele that the queen does not.

TESTING FOR WOLBACHIA INFECTION

Colony-level variation in *Wolbachia* infection could also be an explanation for extreme split sex ratios. To assess this hypothesis, 90 workers were tested for the presence of *Wolbachia* by

amplifying *Wolbachia* DNA using the primers Wf81F and Wf691R. We tested 10 workers per colony for five female-only colonies and four male-only colonies from the RNAM dataset. Workers were the same as those used in the genetic studies. We used three individual mosquitoes from French lines, known to be totally infected, as controls.

Results

MATING SYSTEM AND GENETIC STRUCTURE OF *ALLOMERUS OCTOARTICULATUS* CF. *DEMERARAE*

The five tested microsatellite loci revealed between 9 and 29 alleles per locus, with allele frequencies ranging from 0.002 to 0.521. Polymorphism was high in both populations, RNAM and TPL, with respectively 80 and 92% of alleles having a frequency of more than 0.01. Overall population relatednesses for both sites were not different from zero (TPL: 0.0007 [$SE_{\text{jackknife}} = 0.0008$]; RNAM: -0.0004 [0.0013]), and within both sites, we obtained high among-colony F_{st} estimates (TPL: 0.37, $CI_{95\%}$: [0.36, 0.39]; RNAM: 0.38 [0.37, 0.40]) indicating a high genetic divergence among nests. These indicate the absence of genetic structure at the scale of each studied population.

The fixation index F was significantly different from zero (TPL: 0.13, $CI_{95\%}$: [0.07, 0.19]; RNAM: 0.06 [0.01, 0.12]) indicating that the frequency of sib-mating may reach a maximum value of α between 0.21 (RNAM) and 0.37 (TPL). However, from genotyped queens and inferred fathers (whose genotypes were deduced through pedigree analysis) in the TPL_{G20} dataset, estimates of overall population relatednesses among queens, among the queen's mates (i.e., among fathers within queen), and between queens and their mates, were, respectively, -0.0475 ($SE_{\text{jackknife}} = 0.0077$), -0.0081 (0.0208), and 0.0009 (0.0122), all values either below or not significantly greater than zero. (Values below zero suggest that two individuals share fewer genes than average.)

Dissections of whole colonies indicate that *Allomerus* is almost always monogynous: 20/20 colonies from TPL_{G20}, 34/34 colonies from EBCC (Yu and Pierce 1998), and 162/181 (89.5%) colonies from RNAM (Frederickson 2006) were found to contain only one queen. In RNAM, it is likely that most or all of these apparently polygynous colonies were in fact different, incipient colonies of *Allomerus* that had cocolonized the same plant, since the host-plants in most cases were small (seven or fewer domatia) and at most, only half of the domatia were occupied (Frederickson 2006).

Consistent with these observations, the average genetic relatedness among nestmate workers r_{w-w} was 0.687 ($SE_{\text{jackknife}} = 0.029$; $CI_{95\%}$: [0.630, 0.744]) in TPL_{G20} and 0.7134 (0.0241; [0.666, 0.761]) in RNAM. These values were either not significantly (RNAM) or only marginally significantly different (TPL_{G20}) from 0.75, as expected under the hypotheses of monogyny (one reproductive queen per colony) and monoandry (the

queen has mated only once). However, average worker relatedness did range from 0.40 to 0.86 across colonies, and the inbreeding-corrected relatedness estimates r_{w-w}^* were 0.596 in TPL_{G20} and 0.674 in RNAM suggesting that some polygyny and/or polyandry does occur in this species.

Following Ross' equation (1993), the mean genetic effective number of queens per colony was found to be marginally significantly greater than one in one of the populations: 1.232 ± 0.112 in TPL_{G20}, and not different from one in the other: 1.087 ± 0.052 in RNAM. We further confirmed the strong tendency toward monogyny by genotyping the sole queen from each of the TPL_{G20} colonies, and we found that the genotypes of workers and queens were in all cases consistent with the hypothesis of a single queen. However, out of the 46 colonies analyzed, the MATESOFT package did detect two colonies, one each in TPL_{G20} and RNAM for which the Mendelian probability of being monogynous was low, and in these colonies more than two workers appeared to have been derived from an alien mother. Pedigree analyses showed that two queens were sufficient to explain all 10 genotypes in each case. From these estimates and our dissection results above, we conclude that monogyny is the rule in *Allomerus*, although the species may exhibit a very low frequency of polygyny.

The average number of males mated with the colony queen, estimated from the equation of Chevalet and Cornuet (1982), was not significantly greater than 1: 1.353 ± 0.211 in TPL_{G20}, and 1.121 ± 0.095 in RNAM. However, facultative polyandry in *Allomerus* was detected by the MATESOFT analysis, which estimated effective mate number to be 1.093 ± 0.011 ($CI_{95\%}$: [1.018–1.199]) in TPL_{G20} and 1.183 ± 0.013 ($CI_{95\%}$: [1.074–1.335]) in RNAM. These two values were not significantly different from each other. In both sites, the maximum estimated number of mates was 3. The estimated proportions in MATESOFT of multiply mated queens were 0.201 ± 0.021 ($CI_{95\%}$: [0.049–0.393]) in TPL_{G20} and 0.309 ± 0.019 ($CI_{95\%}$: [0.154–0.502]) in RNAM. We conclude that *Allomerus* exhibits facultative polyandry and does so at a higher frequency than it does polygyny.

Both TPL_{G20} and RNAM thus provide very similar genetic estimates for all mating system parameters, suggesting that the mating system of *Allomerus* does not vary over the censused range, which spans Amazonian Peru.

SPLIT COLONY SEX RATIOS AND POPULATION SEX RATIOS

The numbers of colonies containing adult sexuals were respectively 35 in TPL₅₄, 24 in EBCC, and 94 (107 if colonies with only alate pupae are also included) in RNAM. Thus, from the 265 colonies sampled, 153 (58%) produced adult sexuals, and of these, 144 produced more than five sexuals. We reconfirmed with the TPL₅₄ dataset the phenomenon previously documented in EBCC (Yu and Pierce 1998) and in RNAM (Frederickson 2006) that

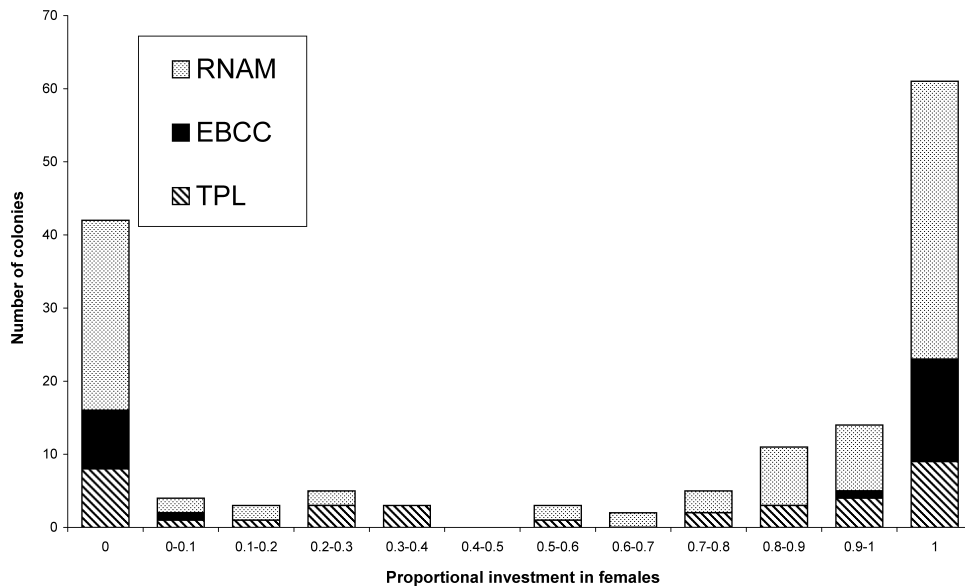


Figure 2. Bimodal frequency distribution of corrected investment sex ratios (proportion of females; *ciSR*) at the three study sites. Data are from mature colonies producing adult sexuals.

the colonies exhibit strongly split sex ratios (Fig. 2). In TPL₅₄, 17 (48.5%) colonies produced alates of only one sex, and more colonies produced highly female-biased ($nSR \geq 0.9$; 13 colonies) than highly male-biased sex ratios ($nSR \leq 0.1$; 9 colonies). Pooled over all three sites (TPL₅₄, EBCC, RNAM), highly female-biased colonies were about 1.5 times more frequent than male-biased colonies (Fig. 2).

In all three sites, the population investment sex ratio (*iSR*) was biased toward females (Table 1, significantly so in RNAM and EBCC), but only in the larger RNAM dataset (181 colonies) was the *ciSR* significantly different from 1:1 (or 0.5 as computed here). Statistical significance in RNAM is due in part to the larger sample size, but the estimated sex ratio is also more female biased. We did not correct for tree-size frequencies because the colony sex ratio did not change with number of domatia (see paragraph *Colony size and colony resource availability* within next section of results).

Table 1. Estimates of population sex ratios (% of females). *n*: number of colonies, *nSR*: numerical sex ratio, *iSR*: investment sex ratio, *ciSR*: *iSR* with Boomsma's 0.7 power conversion. See Sex Ratio Analysis within Materials and Methods for details. Asterisks indicate significant differences from 0.5, equal production of each sex.

Site	<i>n</i>	<i>nSR</i>		<i>iSR</i>		<i>ciSR</i>	
		Mean	SE	Mean	SE	Mean	SE
TPL	35	0.543	0.074	0.636	0.071	0.609	0.072
EBCC	24	0.617	0.101	0.703*	0.095	0.678	0.097
RNAM	94	0.688*	0.044	0.808*	0.037	0.776*	0.039

TESTS OF HYPOTHESES ACCOUNTING FOR BIASED SEX ALLOCATION

Variation in intracolony relatedness

In the RNAM dataset, we found no link between intracolony relatedness and the degree of sex ratio bias (Fig. 3). The mean intracolony relatednesses were respectively 0.7307 (± 0.0264 SE) for the female-only producing colonies, 0.7458 (± 0.0090 SE) for the male-only producing colonies, and 0.7103 (± 0.0297 SE) for colonies producing both sexes (0.6557 \pm 0.0327 SE, if the single polygynous colony was included). None of the pairwise comparisons was significantly different (*t*-tests: all two-tailed *P*-values ≥ 0.137 [Fig. 4]). Similarly, across-colony variation in numerical sex ratio, *nSR*, is not associated with variation in r_{w-w} , the worker mean relatedness (Spearman rank correlations, $r_s = 0.074$, $n = 26$, $P = 0.718$; the result remains unchanged if the polygynous colony is removed and/or if we use *ciSR*). Finally, we found no evidence that polyandrous colonies (regardless of the software used to estimate the number of fathers per colony, RELATEDNESS or MATESOFT) produce more male-biased sex ratios (Fig. 4), and there was no difference in the mean number of patriline between the three types of colonies (*t*-tests: all pairwise comparisons, $P \geq 0.12$). In short, we cannot explain variation in the colony sex ratio with variation in worker relatedness levels.

Colony size and colony resource availability

As expected from previous studies (Yu and Pierce 1998; Yu et al. 2004; Frederickson 2006), there was a positive relationship between colony size (domatia number) and the number of adult sexuals produced (males + females, Spearman rank correlations: $r_s = 0.694$, $n = 265$, $P < 0.001$ [Fig. 5]). This relationship was

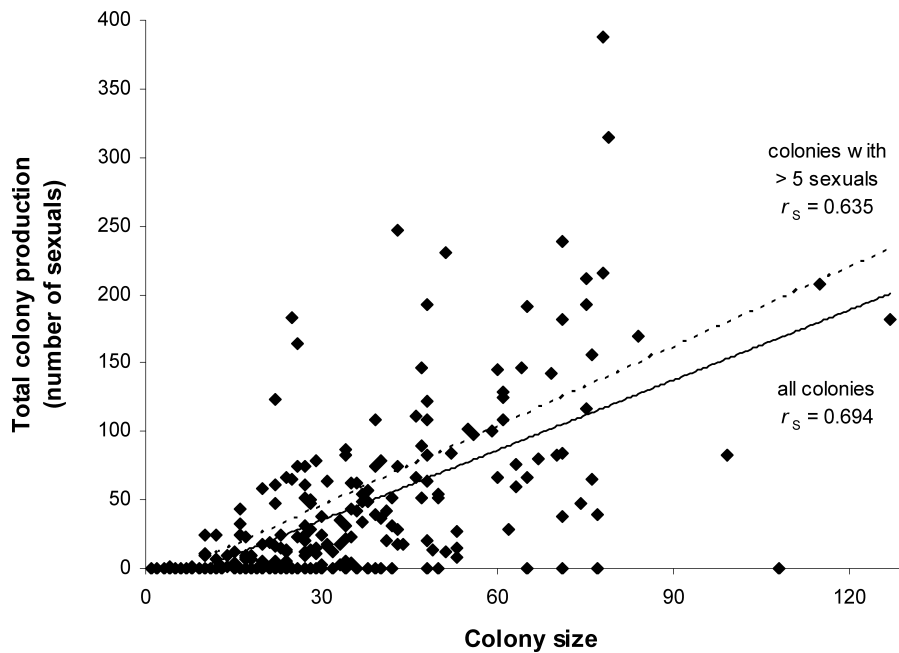


Figure 5. Total colony fecundities (number of sexuals) as a function of colony size (domatia number) for all colonies from the three study sites. Correlation is significant at $P < 0.001$ (Spearman rank correlations: $n = 265$, $r_s = 0.694$ [plain line]). Significance remains unchanged when considering only colonies producing at least five sexuals ($n = 144$, Spearman's $r_s = 0.635$ [dotted line]).

$P_{\text{TDMS}} = 0.17$ with their removal (the plants have 78 and 79 domatia).

Redoing the analysis with the method of Nonacs (1986b) also gave a nonsignificant result: the partial correlation of sex ratio iSR on colony size (number of domatia), with total sexual productivity (TDMS) held constant, was not significant ($r = -0.018$, d.f. = 150, $P = 0.17$). Thus, at equivalent productivity, decreasing colony sizes, i.e., numbers of workers (those who are controlling for productivity), did not result in lower female bias.

With nSR as the response variable, we used logistic regression (generalized linear model, binomial errors, scale estimated for overdispersion, $n_{\text{tot}} = 153$) and found a significantly positive main effect of plant size (DOM, $F_{1,151} = 6.06$, $P < 0.015$, Appendix S3, Fig. S3a). However, the effect of domatia was minor (variance explained = 3.86%) and attributable to the influence of only three large plants (≥ 100 domatia in TPL₅₄ and EBCC) that had strongly female-biased sex ratios. Removal of these three plants rendered the interaction and main terms nonsignificant ($P_{\text{interaction}} = 0.289$, $P_{\text{SITE}} = 0.112$, $P_{\text{DOM}} = 0.081$). Host-plants with fewer than 100 domatia account for more than 99.9% of all trees censused in EBCC and RNAM (Appendix S2; the full size structure at TPL is not available, but large plants are also rare there, D. Yu personal observation). Large, *Allomerus*-inhabited plants are killed by the cerambycid beetle, *Trachysomus* sp. (Yu and Pierce 1998).

Substituting total dry mass of sexuals (TDMS) for domatia (DOM) as a predictor produced more complicated results. The interaction effect of TDMS with SITE was not significant ($P =$

0.08), but both are highly significant as main effects, such that the female sex ratio increases with TDMS and is generally highest in RNAM ($P_{\text{TDMS}} < 0.0001$, $P_{\text{SITE}} = 0.002$, $F_{3,149} = 7.46$, $P_{\text{total}} = 0.001$, variance explained = 13.1%, Fig. S3b). However, again, there are two influential points with high TDMS and 100% female sex ratio (Fig. S3b). Removal of these points and reanalysis reveal a significant interaction effect ($F_{2,145} = 3.54$, $P = 0.032$), and the female sex ratio increases with TDMS in two sites, RNAM and EBCC.

Finally, we also compared the mean sexual productivity (TDMS) from colonies producing only males, only females, and both sexes and found no significant differences (t -tests: all pairwise comparisons, $P > 0.07$) (Table 2). P -values only increased when colonies producing less than 10 mg of dry weight (approximately five sexuals) were omitted and/or when correcting dry masses with Boomsma's factor (t -tests with equal variances: all pairwise comparisons, $P \geq 0.16$). The same results were obtained within each of the three sites (Table 2), except in RNAM, where productivity in M colonies was significantly smaller than that in B colonies ($t_{34,205} = 2.967$; $P = 0.005$ / with Boomsma's correction: $t_{36,583} = 2.788$; $P = 0.008$). Correcting for the nine multiple tests, however, renders this one difference marginally or nonsignificant.

In summary, we find no statistical evidence for an effect of colony resource level or size on colony investment sex ratio (iSR). With the numerical sex ratio (nSR) as the response, the samples suggest that colonies in very large trees

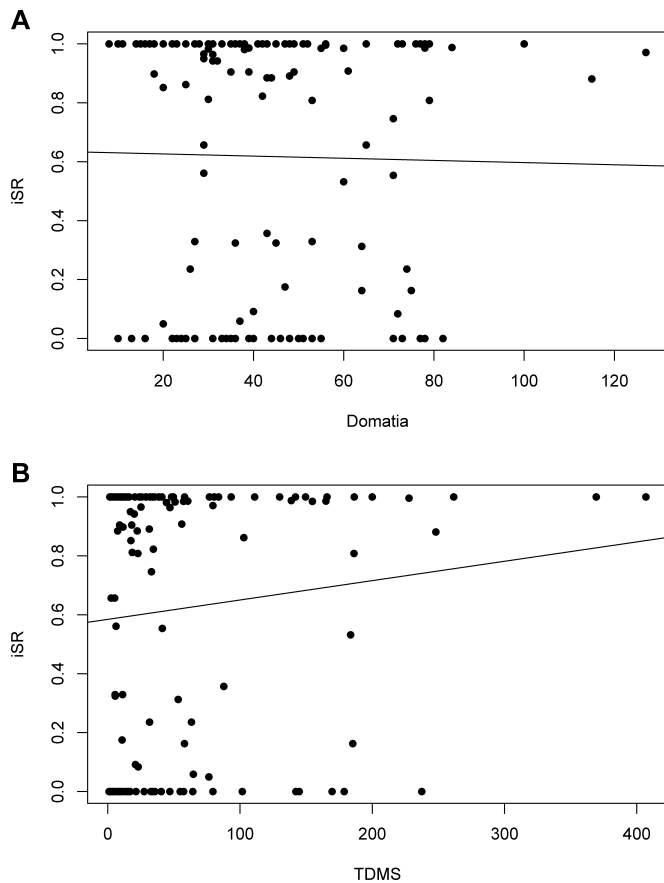


Figure 6. Regressions of the colony sex ratio (iSR) on colony resource level. Colony female sex ratio is estimated by the investment ratio (iSR , Table 1). Colony resource level can be estimated by host-plant size (number of domatia, DOM) or by the total dry mass of sexuals (TDMS, i.e., male + female mass). Two models were analyzed: DOM + SITE + DOM \times SITE and TDMS + SITE + TDMS \times SITE, where SITE is a categorical variable for the three collection sites. Colony investment sex ratio (iSR) is not explained by either A. colony size (DOM) or B. colony productivity (TDMS). Statistical details in the text.

are female-biased, but such trees are exceedingly rare. There is also a statistical suggestion that the numerical sex ratio in two sites becomes more female-biased as total productivity (TDMS) increases.

Table 2. Estimates of mean sexual productivity (TDMS) for colonies producing both sexes (B), or only males (M), or only females (F). n : number of colonies. 10+ colonies: colonies with a sexual dry mass greater than 10 mg, i.e., with more than about five sexuals. Standard errors are given inside brackets.

	n	M	n_M	F	n_F	B	n_B
All colonies	153	40.19 [± 8.73]	42	61.96 [± 12.11]	61	64.48 [± 9.93]	50
All 10+ colonies	110	64.76 [± 12.50]	25	87.89 [± 16.09]	42	73.98 [± 10.88]	43
TPL	35	88.90 [± 32.36]	8	121.17 [± 51.65]	9	101.84 [± 19.29]	18
EBCC	24	82.05 [± 15.44]	8	119.86 [± 30.13]	14	43.80 [21.38]	2
RNAM	94	12.33 [± 3.03]	26	26.59 [± 6.16]	38	43.44 [± 10.03]	30

Local resource competition (LRC) and local mate competition (LMC)

To test for the presence of LRC, we collected 135 live foundresses from 88 saplings at TPL (mean per sapling: 1.53; median: 1.00) and used COLONY software to group the foundresses into full-sib genetic families. Under the assumption of monoandry we obtained 87 pure full-sib genetic families. Of the 25 saplings containing more than one *Allomerus* queen, not one contained multiple queens from the same family (i.e., born in the same mother colony). When the assumption of monoandry was relaxed, we obtained 35 families (with a reconstructed mate number per queen ranging from 1 to 6, to obtain high posterior probabilities). Still, only two out of the 25 saplings contained two members of the same family (out of three and five foundresses present, respectively).

The female numerical sex ratio nSR did not increase significantly with total numbers of sexuals (male + female alates), either as a main effect or in an interaction with SITE (logistic regression, scale estimated because of overdispersion, $F_{2,147} = 1.45$, $P_{\text{interaction}} = 0.237$; $F_{1,151} = 1.55$, $P_{\text{total alates}} = 0.216$). Qualitatively similar results were obtained using iSR and TDMS (or $ciSR$ and corrected TDMS) as the response variables (data not shown).

Finally, within the TPL study site, we failed to detect any significant isolation-by-distance among the foundresses ($n = 135$; slope = -0.0034 ; $P = 0.095$), and only 4% of the foundresses could be assigned to one of the adult colonies in the 16-ha quadrat.

Worker reproduction

None of the 101 males ($n = 9$ colonies) examined carried non-queen alleles, consistent with no worker reproduction. In line with this, the within-colony mean genetic relatedness between workers and males r_{w-w} was 0.237 ($SE_{\text{jackknife}} = 0.016$; $CI_{95\%}$: [0.081, 0.378]; min: 0.143; max: 0.319). This value is not significantly different from 0.25, as expected when all males are produced by the queen. The mean estimated probability of nondetection of worker-produced males was low, 0.176, further suggesting that worker reproduction is rare, or even absent, in *Allomerus*.

Table 3. Tested hypotheses for split sex ratios in *Allomerus octoarticulatus* var. *demerarae*.

Hypothesis	Main prediction	Dataset	Support for hypothesis?
Relatedness asymmetry	Among-worker relatedness is higher in female-producing than in male-producing colonies	RNAM	None
Queen replenishment hypothesis	A mode of reproduction in highly polygynous ants, often associated with male-biased, population sex ratios	TPL ₅₄ , EBCC, RNAM	Not applicable
Worker reproduction	In male-producing colonies, some (or all) males are produced by workers and not by the queen	RNAM	None
Parasite infection	Colony female-bias may be due to presence of <i>Wolbachia</i> bacteria	RNAM	None
Resource abundance hypothesis	Increased colony resource level is correlated with a more female-biased sex ratio	TPL ₅₄ , EBCC, RNAM	None, except possibly the largest colonies
Local mate competition	Female bias increases with colony fecundity	TPL ₅₄ , EBCC, RNAM	None
Local resource competition	More productive colonies are expected to invest proportionally more in males, and foundresses competing for the same saplings should be related	TPL ₅₄ , EBCC, RNAM, TPL saplings	None
Queen control over sex allocation	Population sex ratio should be 0.5 and/or greater male investment in small colonies	TPL ₅₄ , EBCC, RNAM	None
Resource variation hypothesis	Colonies differ in their access to protein and/or carbohydrates	TPL ₅₄ , EBCC, RNAM	None

Wolbachia infection

Wolbachia have sometimes been found present in preliminary tests (Y. Milton, J. Russell, and M. Frederickson, unpublished data), but our survey of 90 workers found no signs of *Wolbachia* amplification, in contrast to the three control mosquitoes that showed clear amplification bands. We thus did not find any support for an effect of *Wolbachia* infection on the sex ratio.

Discussion

Our genetic data suggest that *Allomerus* colonies are almost always the product of a single, sometimes multiply mated, queen. We found genetic evidence for polygyny in only two colonies and all but a few genotyped workers could be assigned to the resident queen. Therefore, the weak genetic diversity that is observed within colonies of *Allomerus* appears to be due mainly to polyandry. Most queens (70–80%) mate once, and the rest mate with two or three males, resulting in a mean effective mating frequency below 1.3. This estimate of polyandry is an upper bound, because within any given colony, generally only one or two workers out of the ten genotyped differed in their genotypes, and we cannot exclude the possibility that some small, incipient colonies in a side branch were collected while collecting the whole tree.

Mating and sex allocation strategies are similar at both ends of the censused range, which are separated by around 1000 km. Indeed, other than the fixation index F , the TPL_{G20} and RNAM

datasets provide statistically indistinguishable genetic estimates for all mating system parameters, suggesting that the mating system of *Allomerus* does not vary over the Amazonian part of Peru. Colony split sex ratios are encountered in all studied sites, and population sex ratios are biased toward females, significantly so at EBCC (*iSR* only) and at RNAM (*iSR* and *ciSR*).

WHAT DOES NOT EXPLAIN SPLIT SEX RATIOS IN ALLOMERUS?

Our results allow us to discard several explanations with confidence (Table 3). Firstly, variation in worker relatedness asymmetry (e.g., Boomsma and Grafen 1991; Fournier et al. 2003) does not explain split sex ratios: polygyny is very rare, and the detected polyandrous and polygynous colonies are not more likely to produce male-only or even more male-biased sex ratios (Fig. 4). The rarity of polygyny is also inconsistent with the queen replenishment hypothesis for split sex ratios (Brown and Keller 2000, 2002). Secondly, sex ratio bias due to worker reproduction (i.e., parthenogenetic production of males by workers) or to heterogeneous *Wolbachia* infection among colonies fails to receive support.

Thirdly, our analyses largely fail to support the resource abundance hypothesis (Nonacs 1986a; Bourke and Franks 1995): mean productivity (TDMS) in female-only colonies is not significantly greater than in male-only colonies (Table 2), and investment in females (*iSR*) is not higher in larger colonies (domatia) or in colonies

with more resources invested in sexuals (TDMS [Fig. 6]—same results with *ciSR* and corrected TDMS). The female numerical sex ratio (*nSR*) is significantly higher in larger or more resource-rich colonies, but mainly because of two or three very large colonies. In the latter set of tests, the use of logistic regression weights more fecund colonies more heavily in the dataset. In summary, there is a hint that the most resource-rich colonies specialize in females, but this effect does not explain female-specialization in the vast majority of colonies (Table 2, Appendices S2, S3).

The same analyses applied to the resource abundance hypothesis also argue against local mate competition (Hamilton 1967) and the more stringent constant male hypothesis [CMH] (Frank 1987), since LMC/CMH also predict that female bias should increase with colony fecundity, but, as we outline above, this prediction is weakly upheld at best. CMH also predicts that every colony should produce at least a few males and that small colonies should be male-specialists, neither of which occurred in our dataset. Finally, in those cases where we detected polyandry, among-father relatednesses were not different from zero, but LMC is based on competition amongst related males for mates.

Local resource competition (Frank 1987; Pamilo 1991) also is not supported: all (or almost all) foundresses collected from the same sapling are unrelated. Moreover, postarrival survivorship of *Allomerus* foundresses (i.e., once they have arrived at a host-plant) is estimated to be low (28%, Yu et al. 2001; 13%, Frederickson 2006), so even when related foundresses do colonize the same sapling, it is likely that parasites and competitive displacement by *Azteca* queens (Yu et al. 2004) will further reduce the probability of nestmate competition. Also, the population sex ratio is not male-biased (Table 1), nor is there any indication that the female numerical sex ratio decreases with colony size or total fecundity, nor does the production of males (in mg) increase with total sexual productivity (TDMS), as is expected after Pamilo (1991). In sum, these results do not suggest that related *Allomerus* foundresses regularly compete for saplings. We note that our foundress collections cover the full temporal range of establishment, from recent arrivals with eggs only to queens with nanitic workers on the verge of establishing a colony (Yu et al. 2004). Although it is theoretically possible that failure to find related foundresses on the same sapling could be explained if sibling foundresses preferentially displace each other right after arrival, rather than fighting nonkin, this would not be consistent with expectations from kin selection theory.

Total queen control over the fate of diploid eggs (Nonacs 1986b; Pamilo 1990) also is not supported since the queen's optimal sex ratio is parity, but we observed female-biased population sex ratios in all three collection sites, significantly so at two sites (*iSR*, Table 1). On the other hand, it remains possible that queens exert partial control over the colony sex ratio (Chan & Bourke 1994), for example, by increasing the fraction of unfertilized eggs

laid, which must become male sexuals (Passera et al. 2001). However, we did not find that male investment is greater in host-plants with few domatia, i.e., in small colonies (the number of domatia is correlated with the number of workers). This result suggests that, at a higher queen-to-worker ratio, queens do not have greater control of sex allocation, as expected under this hypothesis (Nonacs 1986b), or queen-restriction of diploid eggs is too costly in small colonies, due to its effect on worker numbers and colony growth rate.

Finally, the natural history of *Allomerus* is inconsistent with the resource variation hypothesis (Grafen 1986). The nitrogen-isotope ratio ($\delta^{15}\text{N}$) of *Allomerus* workers is more similar to predatory arthropods (Acari, Araneida, Hemiptera, Pseudoscorpionida) than to plant-chewing insects (Coleoptera, Orthoptera, and Diptera) or sap-feeding trophobionts (Homoptera) (Davidson et al. 2003), which suggests that *Allomerus* colonies derive a non-trivial portion of their nitrogen from insect prey (captured using specialized traps constructed on the plant surface, Dejean et al. 2005). In short, all *Allomerus* colonies in an area appear to be sampling from the same, diverse community of plant-feeding insects, and this, combined with the facts that host-plant densities are high ($\approx 30\text{--}80$ plants ha^{-1}) and the failure to detect a host-plant size effect (with the possible exception of the largest host-plants), makes it unlikely that colonies will differ greatly in their access to dietary nitrogen and/or carbohydrates. We have observed that male- and female-biased colonies can be separated by only a few meters (D. Yu, personal observation).

WHAT MIGHT EXPLAIN SPLIT SEX RATIOS IN *ALLOMERUS*?

By process of elimination, we are left with the explanation that split sex ratios in *Allomerus* are the result of the coevolution of queen and/or worker biasing strategies, as recently modeled by Roisin and Aron (2003), Reuter et al. (2004), and Helms et al. (2005). The latter model is the most general, so we focus on it.

In Helms et al.'s (2005) "alternative biasing model," it is assumed that queens can bias the primary (egg) sex ratio and that workers can bias the secondary (adult) sex ratio, both at some cost to colony productivity, and antagonistic coevolution between worker and queen interests can result in colony split sex ratios. For example, if queens have the first-mover advantage (achieved by being given the faster evolutionary rate), a mixed sex ratio strategy can evolve such that some queens lay only male eggs (for a time) while others lay an unbiased (1:1) ratio. Workers in all colonies evolve to eliminate a fraction of male eggs, and the result is that some colonies produce only males and others produce mostly females. Alternatively, when workers are given the first-mover advantage, they evolve the mixed sex ratio strategy, with workers in some colonies eliminating all male eggs and others not exhibiting any bias. Queens in this scenario all evolve to lay

a male-biased egg sex ratio. In both scenarios, the evolution of mixed strategies is caused by the fact that the value of a sex is frequency dependent.

With the data we have available, we can conduct some preliminary analyses.

(1) Sexing of 1671 alate pupae and 1584 eclosed alates from the colonies in RNAM showed that the proportions of females were respectively 57% and 69% (Frederickson 2006). The difference is significant ($\chi^2_1 = 94.1$; $P < 0.01$), indicating that the eclosed cohort was more female-biased than the pupal one. This difference is consistent with both a longer development time for females compared to males and the existence of worker mechanisms biasing the sex ratio toward females. Such a late male-brood elimination, at the pupal stage, has been shown in *Formica exsecta* (Chapuisat et al. 1997). In that species it seems, however, that workers do not replace male brood with new females; instead, male culling results in less brood production.

(2) Strongly male-biased broods co-occurring with mixed-sex broods should indicate that queens exhibit the mixed sex ratio strategy. Conversely, strongly female-biased broods co-occurring with mixed-sex broods should indicate that workers exhibit the mixed sex ratio strategy (Helms et al. 2005). In our samples, more colonies were female-specialists than were male-specialists, and mixed-sex colonies also occurred (Fig. 2), which is weakly consistent with mixed worker-biasing strategies, although in Helms et al.'s model (2005), we do not expect there to be both kinds of specialists. Of course, real biological systems rarely fit any theoretical model perfectly, and the alternative biasing strategy models do not incorporate stochasticity effects (e.g., imperfect biasing mechanisms, or mistakes by queens or workers) that can weaken the fit between theory and data (e.g., Beekman et al. 2003).

(3) Mixed sex ratio strategies in queens should reduce the ability of workers to influence the sex ratio, resulting in population level sex allocation closer to equality than to 0.75. In contrast, the existence of biasing strategies in workers should result in population female allocation closer to 0.75 than to equality. In two sites, female allocations (*iSR* and *ciSR*) were closer to 0.75 than to 0.5 (Table 1), but in the third site, TPL, female allocation was closer to parity. These results are again weakly consistent with a mixed sex ratio strategy in workers. Some of the observed variation across sites is undoubtedly the result of sampling error (Table 1), but some might also be caused in part by geographical variation in the relative costs of biasing (see Figure 3A in Helms et al. 2005) or in the relative rates of queen and worker mutations at each site.

(4) Finally, because most biasing mechanisms involve the elimination (by workers) or nonproduction (by queens) of eggs, Helms et al. (2005) predict that colonies producing more extreme sex-ratio biases should be less productive than colonies producing a

greater mix of the two sexes. In our samples, we did not detect generally lower production (TDMS) in single-sex colonies, with the possible exception of the RNAM site, where we found that TDMS was lower in male-only colonies compared to colonies producing both sexes (Table 2). This is weakly consistent with a mixed biasing strategy in queens (Helms et al. 2005). Furthermore, the genus *Allomerus* is thought to be closely related to *Solenopsis* (Fernández 2007), one species of which has been shown to exhibit queen biasing of the egg sex ratio (Passera et al. 2001).

In conclusion, the process of elimination leads us to conclude that the evolution of mixed sex ratio strategies (Helms et al. 2005) is the most likely explanation for split sex ratios in this species. However, the available data are not yet sufficient to demonstrate (or to reject) its existence in *Allomerus*, much less determine whether it is workers or queens that exhibit the mixed strategy. Thus, what is now needed are direct tests of biasing mechanisms in *Allomerus*, such as measuring the egg sex ratio in male- versus female-specialist colonies (see e.g., Sundström et al. 1996; Passera et al. 2001; Aron et al. 2005; de Menten et al. 2005) and longer-term measures of the colony sex ratio. There is also the possibility that the Helms et al. (2005) model is not robust to departures from its assumptions, such as the existence of facultative polyandry in *Allomerus*, and more modeling might be required. And of course, we cannot rule out the possibility that split sex ratios in *Allomerus* have an as-yet unknown cause.

SEX RATIO EVOLUTION AND THE MAINTENANCE OF ANT-PLANT MUTUALISMS

We finish by placing this study in the context of the mutualistic symbiosis between the ant-plant *Cordia nodosa* and the ant genus *Azteca* spp. In this system, *Allomerus* is a castration parasite of the plant *C. nodosa* (Yu and Pierce 1998) and competes with *Azteca* spp. for host-plant saplings. Previous work (Yu et al. 2001, 2004) has shown that the two genera stably coexist on the single resource represented by *C. nodosa* via a trade-off between queen dispersal ability and colony fecundity. *Azteca* queens are longer-distance dispersers, able to find and colonize isolated saplings, and therefore win them by default. As a result, *Azteca* colonies have a higher population growth rate in areas with low host-plant density. In contrast, *Allomerus* queens are poor dispersers, but *Allomerus* colonies have much higher female alate fecundity, so in areas with high densities of saplings, where there is little need to disperse, *Allomerus* can colonize more saplings per unit time, allowing them to dominate. Thus, the fundamental reason that *Allomerus* can persist in this system is *Azteca*'s lower female alate fecundity, the cause of which is a mystery, since *Azteca*'s male fecundity is similar to *Allomerus*' (Yu et al. 2004) and since the evolution of higher fecundity would allow *Azteca* to capture

valuable, high-host-plant-density habitat from *Allomerus*. Thus, we hypothesize that *intraspecies* conflict over sex allocation, so that *Azteca* produces fewer female alates (via such possible mechanisms as local resource competition or queen control of egg fate) and so that *Allomerus* produces more female alates (perhaps the result of greater worker control, as suggested here), contributes to the stability of the dispersal-fecundity trade-off, which in turn contributes to the stability of *interspecies* competition, and thus, to the stability of the *Azteca*-*C. nodosa* mutualism.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. A. A domatium on a new shoot of *Cordia nodosa*. B. Interior of a domatium with *Allomerus* workers, brood, and alate larvae.

Appendix S2. Tree size (i.e., total number of domatia per tree) distribution in the three study sites TPL ($n = 951$), in EBCC ($n = 1006$), and in RNAM ($n = 181$, only AD-occupied trees).

Appendix S3. Proportional numerical investment in females (nSR) as a function of colony size (DOM domatia number; Fig. S3a), and total sexual productivity (TDMS total dry mass of sexuals; Fig. S3b), for all colonies producing sexuals ($n_{\text{tot}} = 153$). In both analyses, a generalized linear model with binomial error and logit transform (i.e., logistic regression) was used to test the following models: DOM + SITE + DOM \times SITE and TDMS + SITE + TDMS \times SITE. Preliminary analyses found that both models were highly overdispersed (residual deviance/df > 39), so scales were estimated from mean deviance rather than being set at 1 (Crawley 1993). Neither the interaction term nor the main effect of SITE was significant in either analysis ($P > 0.05$). DOM was significant at $P = 0.0156$ (Fig. S3a). However, variance explained was only 3.8% and attributable to the influence of three large plants (DOM ≥ 98) in TPL and EBCC. Removal of these three influential points rendered DOM not significant at $P = 0.086$. TDMS was not significant at $P = 0.22$. Analyses were conducted using GLMSTAT v6.0.0 (Beath 1997).

Figure S3. MFMass is TDMS.

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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