Cryptic sexual populations account for genetic diversity and ecological success in a widely distributed, asexual fungus-growing ant

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Sex and recombination are central processes in life generating genetic diversity. Organisms that rely on asexual propagation risk extinction due to the loss of genetic diversity and the inability to adapt to changing environmental conditions. The fungus-growing ant species *Mycocepurus smithii* was thought to be obligately asexual because only parthenogenetic populations have been collected from widely separated geographic localities. Nonetheless, *M. smithii* is ecologically successful, with the most extensive distribution and the highest population densities of any fungus-growing ant. Here we report that *M. smithii* actually consists of a mosaic of asexual and sexual populations that are nonrandomly distributed geographically. The sexual populations cluster along the Rio Amazonas and the Rio Negro and appear to be the source of independently evolved and widely distributed asexual lineages, or clones. Either apomixis or automixis with central fusion and low recombination rates is inferred to be the cytogenetic mechanism underlying parthenogenesis in *M. smithii*. Males appear to be entirely absent from asexual populations, but their existence in sexual populations is indicated by the presence of sperm in the reproductive tracts of queens. A phylogenetic analysis of the genus suggests that *M. smithii* is monophyletic, rendering a hybrid origin of asexuality unlikely. Instead, a mitochondrial phylogeny of sexual and asexual populations suggests multiple independent origins of asexual reproduction, and a divergence-dating analysis indicates that *M. smithii* evolved 0.5–1.65 million years ago. Understanding the evolutionary origin and maintenance of asexual reproduction in this species contributes to a general understanding of the adaptive significance of sex.

Attini | clonality | Formicidae | thelytoky | mutualism

The vast majority of metazoans reproduces sexually, enjoying the benefits of genetic recombination (1–3) such as rapid adaptability to novel ecological conditions (4, 5) and the purging of deleterious mutations from their genomes (6, 7). However, relative to sexually reproducing organisms, an asexual female doubles its fitness by transmitting its entire genetic material to the next generation (8). Despite such obvious short-term fitness advantages, asexual organisms occur only sporadically throughout the tree of life and are predicted to be evolutionarily short-lived and doomed to early extinction (9–11). In contrast to the short-term advantages of asexuality, the adaptive value of sexuality, that is, genetic recombination, is expected to be of long-term benefit (2, 12–14). There remain in evolutionary biology significant unexplored questions about whether sexual reproduction is favored by natural selection over short evolutionary time spans and, if not, why sexual reproduction persists as the prevalent mode of reproduction, given that the selective benefits are deferred. Studying the origin and evolution of parthenogenetic lineages, and understanding how genetic diversity is generated and preserved in such lineages, is essential to answering these questions.

Asexual reproduction by females, or thelytokous parthenogenesis, has recently been reported in queens of the fungus-growing ant *Mycocepurus smithii* in three geographically distant populations in Latin America: Puerto Rico (15), Panama (16), and Brazil (17). The widespread geographic distribution of asexuality and the complete absence of males from field collections and laboratory colonies suggested that *M. smithii* might be obligately asexual (16, 17), and one study proposed that asexuality in this species might be ancient (16). Among bees, wasps, and ants, thelytokous parthenogenesis has so far been observed in the Cape honey bee (18, 19) and in 12 distantly related species of ants (17, 20–23). Population-genetic studies of some species revealed a diversity of highly complex genetic systems, including different cytogenetic mechanisms used to produce workers and queens, facultative sexual reproduction, and clonal male lineages (23–27). Asexual eusocial Hymenoptera produce diploid offspring via meiotic parthenogenesis, or automixis, in which a limited amount of genetic variability is generated through fusion of sister nuclei (28–31). In contrast, mitotic parthenogenesis, or apomixis, in which offspring are genetic clones of their mothers, has not been demonstrated unambiguously in social insects.

Although many theoretical studies predict the costs and benefits of sex, little is known about the evolution of asexuality at the organism level (2). To study the origin and maintenance of parthenogenesis and to elucidate the mechanisms generating genetic diversity in parthenogenetic lineages, we investigated the evolutionary history of the asexual fungus-growing ant *M. smithii*. To test for obligate asexuality in *M. smithii*, we developed highly variable short tandem repeat (or microsatellite) markers and analyzed colonies from multiple populations across the species's broad range, extending from Mexico to Argentina and including some Caribbean islands (32, 33). To identify the genetic structure within and between populations of *M. smithii* and to infer the cytogenetic mechanism underlying parthenogenetic reproduction, we genotyped sterile workers and reproductive queens from 234 colonies. Clonality was inferred by genetic identity between nest mates. Controlled laboratory breeding experiments complemented our field observations. To test for a potential hybrid origin of parthenogenesis in *M. smithii*, we reconstructed a molecular phylogeny of the genus *Mycocepurus*. An additional fine-scaled mitochondrial phylogeny of asexual and sexual *M. smithii* populations was used to investigate whether asexuality arose

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once or multiple times independently from sexually reproducing ancestors. Lastly, we performed a divergence-dating analysis to estimate the time span over which parthenogenesis has persisted in *M. smithii*, because asexuality was previously proposed to be of ancient origin.

Results

Population-Genetic Analyses. A total of 1,930 *M. smithii* individuals from 234 colonies collected at 39 different localities in Latin America (Fig. 1 and Table S1) was genotyped at 12 variable microsatellite loci yielding 106 alleles (range: 2–15 alleles per locus). The number of alleles per locus per individual never exceeded two, indicating diploidy of females. Of the genotyped populations, 89.7% ($n = 35$) showed population-genetic signatures of clonality, whereas 10.3% ($n = 4$) showed an increase of unique multilocus genotypes, indicative of genetic recombination caused by sexual reproduction.

Asexual populations. A total of 1,647 individuals from 218 colonies in 35 populations exhibited genetic signatures of clonal reproduction. Asexual reproduction was characterized by sharing of repeated multilocus genotypes among individuals (Table S1), maximum deviation from random mating ($F_{IS} = -1$; Table S2), and a low genotype-to-individual ratio (i.e., G:N approaching 0, whereas a G:N of 1 indicates that each individual is genetically distinct from another) (Table S1). To determine the number of independently evolved asexual lineages that arose at different localities from the sexual population, we estimated the probability that slightly different multilocus genotypes originated from separate sexual events ($p_{sex} > 0.01$) instead of arising from accumulated mutations or scoring errors ($p_{sex} < 0.01$). In addition, clonal diversity (R) was calculated.

Among all *M. smithii* populations, 66 asexual genotypes were identified, 57 of them representing unique multilocus genotypes ($R = 0.86$; Tables S2 and S3). Five repeated multilocus genotypes were shared between 10 geographically proximate populations (~10–40 km distance), and three unique genotypes were identified in seven geographically distant populations (~700–2,600 km distance; Tables S2 and S3). Calculating the probability that repeated multilocus genotypes from different populations originated from distinct sexual events revealed that identical multilocus genotypes belong to the same clonal lineage ($p_{sex} < 0.01$), indicating long-distance dispersal events of individuals from the same asexual lineage. No genetic variation was present within repeated multilocus genotypes ($F_{IS} = -1$), but significant genetic variance was structured among them [analysis of molecular variance (AMOVA); $F_{ST} = 0.624$, $P = 0.01$].

A comparison of the 57 unique multilocus genotypes revealed high frequencies of low genetic distances between genotypes, resulting in a bimodal frequency distribution of genetic distances and indicating the potential existence of mutations or scoring errors in clones (34). Eleven multilocus genotype pairs differed from one other genotype only by a single allele, reducing the number of asexual lineages that potentially originated from distinct sexual events to 46 ($p_{sex} < 0.01$, $R = 0.69$). Further lowering the threshold and allowing two to six alleles to be shared among multilocus genotypes within an independently evolved clonal lineage, we identified 43 ($R = 0.65$) to minimally 38 ($R = 0.57$) independently evolved clonal lineages.

In 20 clonal populations, only a single multilocus genotype was encountered across different colonies. In 15 populations, two to maximally six multilocus genotypes coexisted at a single site (Table S1). In five populations, all or a subset of multilocus genotypes differed by one to six alleles, suggesting a single colonization event followed by diversification within clonal lineages due to the accumulation of mutations or scoring errors (Table S1). In contrast, 12 populations harbored multilocus genotypes differing by 7–15 alleles, indicating independent colonization events of these sites by distantly related clonal foundress queens. The highest diversity of clonal lineages ($n = 5$) was discovered at a Peruvian lowland rainforest site (Los Amigos).

Genetic uniformity across all loci within colonies suggests either mitotic parthenogenesis (apomixis) as the cytogenetic mechanism underlying thelytokous parthenogenesis in *M. smithii* or, alternatively, automixis with central fusion and low recombination rates. To trace the genotypes of reproductive individuals over multiple generations, we propagated *M. smithii* colonies in the laboratory for six consecutive generations and genotyped all 93 queens at the end of the experiment. All queens were genetically identical across generations, and transitions from a heterozygous locus in the mother to a homozygous locus in the offspring was not observed, as would be expected under automixis with central fusion. Interestingly, in field-collected populations in which 7 of the 11 multilocus genotype pairs differ by only a single allele and are identical at all other loci, we observed that one genotype was heterozygous at a given locus whereas the other was homozygous at the same locus. These transitions could indicate a switch from heterozygosity to homozygosity, as expected under automixis. Without knowing which one of these two is the maternal or the offspring genotype, however, it is not possible to distinguish between a transition from a heterozygous to a homozygous state caused by infrequent recombination or an accumulation of "somatic" mutations.

Recombining populations. Four Amazonian populations, distributed along the Rio Amazonas and the Rio Negro (Fig. 1), exhibited population-genetic signatures of genetic recombination, indicative of sexual reproduction (Tables S1 and S2). Among 283 genotyped individuals, 210 multilocus genotypes were identified, resulting in high genotype-to-individual (G:N) ratios, ranging from 0.71 to 1 (Table S1). Recombinant populations were characterized by inbreeding indices diverging from genetic fixation ($F_{IS} = -1$), ranging from 0.03 to −0.77, and observed and expected heterozygosities were similar, as expected for populations under Hardy–Weinberg conditions (Table S2).

Because multiple colonies were collected from the Caldeirão population in Amazonas, Brazil (Fig. 1), we investigated this

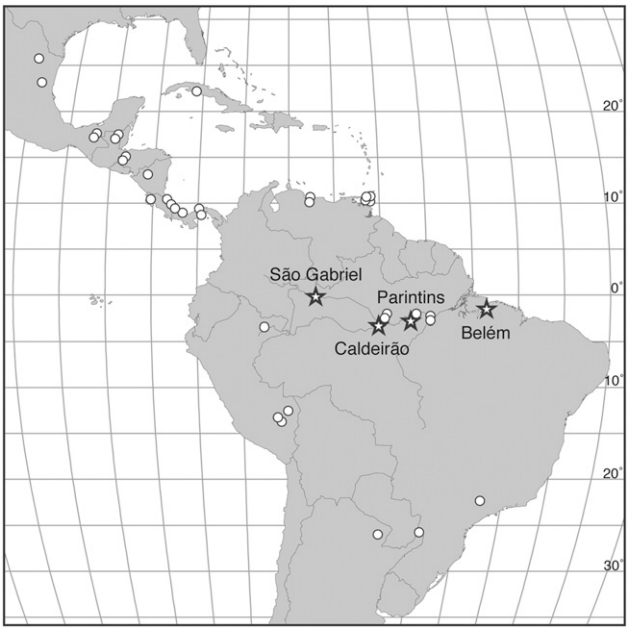

Fig. 1. Geographic distribution of sexual (stars) and asexual (circles) *M. smithii* populations. Localities refer to the sexual populations, distributed along the Rio Amazonas and the Rio Negro. Asexual populations are widely distributed in Latin America, ranging from northern Mexico to northern Argentina. Lines of longitude and latitude are separated by units of 5°.

population in detail to test for sexual reproduction. Genotyping of 243 individuals (234 workers, 5 queens, 4 spermatheca contents) revealed the existence of 173 unique multilocus genotypes, of which 132 multilocus genotypes were represented by single individuals whereas the remaining 41 multilocus genotypes were shared by 111 individuals. Among the shared multilocus genotypes, two or at most six nestmates carried identical genotypes. After removing identical genotypes from the dataset, we tested whether genotypes that differ by only a single allele are derived from distinct sexual events or from somatic mutations or scoring errors. Among those unique genotypes (n = 173), 55 multilocus genotypes likely belonged to the same clonal lineage ($p_{sex} < 0.01$), whereas 118 multilocus genotypes probably originated from distinct sexual events ($p_{sex} > 0.01$). This result indicates that 48.6% (118 multilocus genotypes out of 243 individuals) of the genotyped individuals result from sexual reproduction. Such a mixture of recombinant and clonal offspring within a single population suggests that sexual *M. smithii* queens either occasionally reproduce parthenogenetically or, alternatively, that a larger number of clonally reproducing queens coexists with sexual queens in the same colony. Facultative asexual reproduction by otherwise sexual queens seems more likely, however, given the high number of shared genotypes in the Caldeirão population (n = 41), contrasting with the low number of individuals sharing a multilocus genotype (n = 2–6). After excluding repeated genotypes, observed and expected heterozygosities were almost identical (H_o = 0.372, H_e = 0.369) and the inbreeding index was indicative of random mating (F_{IS} = −0.009) (Table S2).

To directly test whether queens were fertilized, the abdomens of four (out of five) queens were dissected, revealing sperm-filled spermathecae and reproductively active ovaries. The spermatheca contents (n = 4) were identified as sperm under 200× magnification and subsequently genotyped. The sperm from each spermatheca were haploid at all loci, as expected from hymenopteran males developing from unfertilized, haploid eggs. In addition, haploidy at all loci indicates that the queens were singly mated. Furthermore, a subset of paternal alleles matched alleles found in workers which were not present in queens (Table S4). Hence, workers exhibited recombinant genotypes representing both maternal and paternal alleles. The combined evidence demonstrates that the *M. smithii* population from Caldeirão reproduces sexually and, although males have as far as we know never been collected, sperm content clearly reveals their existence.

The genetically recombinant population from São Gabriel da Cachoeira showed that all nestmates (n = 8) were genetically distinct (G:N = 1, H_o = 0.365, H_e = 0.315, F_{IS} = −0.172), consistent with strict sexual reproduction (Tables S1 and S2). However, in the Belém colony (G:N = 0.96, H_o = 0.451, H_e = 0.466, F_{IS} = 0.034) and the Parintins colony (G:N = 0.71, H_o = 0.650, H_e = 0.398, F_{IS} = −0.773), few individuals shared a multilocus genotype, suggesting mixed sexual and parthenogenetic reproduction in these populations.

Only a single clonal lineage (from Trinidad) shared a multilocus genotype with the sexual population from São Gabriel da Cachoeira, suggesting that sexual lineages may continuously spawn asexual lineages. To further explore whether sexual populations give rise to asexual lineages, we analyzed the genetic structure of unique multilocus genotypes of asexual and sexual populations. Genotypes of sexual populations group as distinct genetic clusters in the 3D plot generated by a nonmetric multidimensional scaling (NMDS) analysis (Fig. 2). In the discriminate analysis of principal components (DAPC) analysis, the asexual genotypes as a whole and the four clusters of sexual genotypes are significantly different from each other [Wilks's lambda = 0.098, approximate F ratio = 80.247, df (12, 685), P < 0.0001]. Only a few asexual genotypes grouped inside clusters of sexual genotypes, indicating genetic proximity. Greater genetic distances between clones and sexual clusters most likely indicate that the clones originated from sexual source populations other than the four that were sampled, or perhaps that they are of older evolutionary origin and thus highly diverged. Limited overlap between sexual clusters further indicates that the genetic variability of sexual populations was not exhaustively sampled for *M. smithii* as a species.

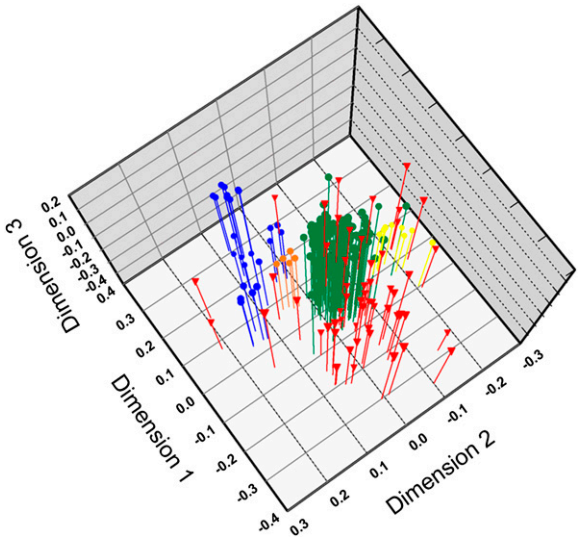

Fig. 2. Plot of 3D object coordinates resulting from an NMDS analysis derived from individual genetic distances. Colored circles represent genotypes of sexual *M. smithii* populations (blue, Belém; orange, Parintins; green, Caldeirão; yellow, São Gabriel da Cachoeira) and red triangles represent genotypes of asexual lineages.

Phylogenetic Analyses. To test the monophyly of *M. smithii* and reconstruct whether asexuality evolved once or multiple times from a sexually reproducing ancestor, we conducted a global phylogenetic analysis of the genus *Mycocepurus* and a local analysis of only *M. smithii* taxa representing a sample from each of the genotyped populations (Table S5).

In the global analyses, the monophyly of the genus *Mycocepurus* was unequivocally supported [Fig. S1; Bayesian posterior probability (BPP) = 1; maximum likelihood bootstrap proportion (MLBP) = 100], which is consistent with a previous analysis (35). Within the genus *Mycocepurus*, nine reciprocally monophyletic, highly supported groups were recognized [Fig. S1; BPP = 1, MLBP ≥ 92], supporting the existence of five new species (Fig. S1). The monophyly of *M. smithii* was well-supported [Fig. S1; BPP = 1, MLBP = 92], suggesting that extant *M. smithii* populations derive from a single most recent common ancestor (MRCA). An undescribed species from the Colombian Amazon was found to be the sister lineage of *M. smithii*, but with only weak statistical support (Fig. S1; BPP = 0.72, MLBP = 56).

For the mitochondrial gene tree of genotyped *M. smithii* populations, the statistical support for relationships between sampled individuals is generally low, as expected from the relatively weak phylogenetic informativeness of the mtDNA markers (Table S6; parsimony-informative characters = 169; 11% of mtDNA dataset). Despite this general problem, the monophyly of *M. smithii* as a species was supported by both the mitochondrial and nuclear data, suggesting that a hybrid origin of asexual reproduction is unlikely in *M. smithii*. The mitochondrial phylogeny further indicates that the sexual populations are separated into at least two distantly related groups (Fig. 3) and that relationships among asexual populations are in some cases correlated with geography. Three sexual populations form a reasonably well supported clade (BPP = 0.96, MLBP = 59) that also includes two clonal pop-

ulations (Fig. 3). The sexual population from Belém forms the sister lineage to a clade consisting of asexual populations from the Amazon and Trinidad. This relationship, however, is only weakly supported (BPP = 0.51). Neither the asexual nor the sexual populations are reconstructed as monophyletic under any possible rooting (Fig. 3), consistent with the hypothesis of independent evolutionary origins of asexuality. Based on Bayes factors (BF), the likelihoods of phylogenies resulting from analyses in which the asexual populations are constrained to be monophyletic are significantly worse fitting to the data than those resulting from unconstrained analyses [ML: 2ln(BF) = 137.82; Bayesian: 2ln (BF) = 124.1], further indicating multiple independent origins of asexuality.

Divergence-dating analysis. The stem-group age (i.e., earliest possible origin) of the fungus-gardening ants was estimated to be 52 million years (Ma) [confidence interval (CI) = 44,60] and the crown-group age was 50 Ma (CI = 43,58), consistent with estimates in Schultz and Brady (35). The estimated crown-group age of the genus *Mycocepurus* is ∼10 Ma (CI = 6,14), whereas the stem-group age is considerably older with 37 Ma (CI = 27,46), which is also indicated by a long branch leading to the MRCA shared with the sister lineage *Myrmicocrypta* (Fig. S1). The stem-group age of *Mycocepurus smithii* is ∼1.65 Ma (CI = 0.57,2.84), whereas the crown-group origin was estimated to be considerably more recent at 0.5 Ma ago (CI = 0.01,1.19). This relatively recent estimate for the evolutionary origin of *M. smithii* is consistent with the almost complete absence of genetic variability observed in the nuclear DNA sequences.

Discussion

M. smithii consists of a mosaic of sexual and parthenogenetic populations. Although separated by as much as 2,000 km, the sexual populations are located along the Rio Amazonas and the Rio Negro, suggesting the existence of a central widespread sexual (or facultatively sexual/asexual) population that has repeatedly generated asexual, clonally reproducing lineages. These asexual lineages have rapidly dispersed throughout much of Latin America, leading to the current widespread geographic distribution of the species (32, 33). The high clonal diversity in some populations indicates that independently evolved clonal lineages have colonized these habitats separately and repeatedly through time. Once an *M. smithii* lineage has lost the ability to reproduce sexually, the condition seems irreversible, resulting in our finding of genetically identical individuals in each of the 218 parthenogenetic colonies studied. The mitochondrial phylogeny of *M. smithii* (Fig. 3) identifies a statistically well-supported group that includes individuals from both asexual and sexual populations, and places the sexual populations in at least two distantly related clades. These patterns, coupled with the results of phylogenetic constraint analyses, are consistent with independent and repeated losses of sexual reproduction. Given the limitations of our sampling, it is nearly certain that additional sexual source populations, from which such closely related groups of asexual clones originated, were not sampled. The divergence-dating analysis provides a recent estimate (crown-group age: 0.5 Ma; CI = 0.01,1.19) for the origin of the presumably sexual most recent common ancestor of extant *M. smithii* populations, indicating that secondary transitions from sexual to asexual reproduction have occurred recently and possibly continue to occur in the present.

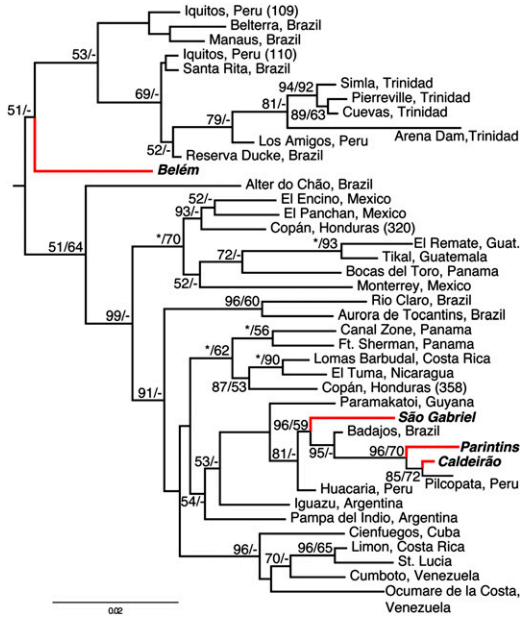

Fig. 3. Midpoint-rooted Bayesian phylogram of *M. smithii* individuals representing each of the genotyped populations based on analyses of three mitochondrial gene fragments. Bayesian posterior probabilities (×100) and ML bootstrap proportions are indicated as BPP/MLBP. Red branches and bold font indicate taxa from sexually reproducing populations. All other taxa represent asexual populations. (Scale bar, number of substitutions per site.)

The combined phylogenetic and population-genetic evidence is consistent with the hypothesis that sexual reproduction was lost in ancestors of parthenogenetic *M. smithii* populations. The spontaneous loss of sexual reproduction has been proposed for the little fire ant *Wasmannia auropunctata*, in which sexual populations in the native range of this invasive species are likely the source of asexual invasive populations (36). The proximate genetic mechanisms causing the loss of sexuality are not well-understood. However, studies of Cape honey bees (37) and of parthenogenetic lineages of *Drosophila melanogaster* (38) show that a single recessive allele can cause thelytoky. These examples suggest that the high propensity for switching from sexual to asexual reproduction in *M. smithii* may be controlled by a small number of genes. Breeding experiments could test whether thelytoky is a qualitative or a quantitative trait in *M. smithii* by introgressing sexual genes into an asexual genetic background and observing the segregation pattern of the offspring.

Cyclical parthenogenesis, the alteration of asexual and sexual life stages (39, 40), is unlikely to occur in *M. smithii*. In each of the 218 parthenogenetic colonies collected in different seasons over an 8-y period (2003–2010), nestmates belonged only to one or very few clonal lineages. The nonrandom geographic distribution of sexual and asexual populations likewise suggests that the switch from sexuality to asexuality is unlikely triggered by season.

In arthropods, the evolution of asexuality is often associated with hybridization (30, 41), a mechanism so far unknown in social Hymenoptera (36). Given the monophyly of *M. smithii* and the phylogenetic congruence between nuclear and mitochondrial markers, hybridization is also unlikely to explain the origin of asexuality in *M. smithii*.

Alternatively, microorganisms such as *Wolbachia*, *Cardinium*, and *Rickettsia* have been shown to induce parthenogenesis in parasitoid wasps (42–44). Even though *Wolbachia* infections have not been detected in social Hymenoptera (45), including *M. smithii* (16), other parthenogenesis-inducing symbionts cannot be ruled out in *M. smithii*.

Although we have so far only examined a scenario in which asexual populations of *M. smithii* have repeatedly arisen from sexual populations, the nonmonophyly of the sexual and asexual populations in the mitochondrial phylogeny equally supports an alternative hypothesis: that sexual populations have repeatedly evolved from widespread asexual populations. Although evolutionary reversals from less complex to more complex ancestral traits have long been deemed unlikely (46, 47), reversals from asexual to sexual reproduction have been suggested for mites and

hawkweed (48, 49). The absence of males (17) and the lack of genetic recombination in asexual populations of *M. smithii* are consistent with the hypothesis that meiosis is dysfunctional in parthenogenetic queens. In species with haplodiploid sex determination, restoring functional meiosis would simultaneously result in recombination and the production of haploid eggs, from which males could develop (41, 50). Therefore, haplodiploid species might theoretically require only a single mutation to reevolve sexuality. However, given (*i*) that all *Mycocepurus* species for which we have biological information reproduce sexually, (*ii*) the high genetic diversity observed in the sexually reproducing *M. smithii* populations, and (*iii*) the genetic variability observed between separate clonal lineages, it seems highly unlikely that extant sexual *M. smithii* individuals descended from asexual ancestors.

Despite the large number of clonal lineages found across the broad geographic distribution of *M. smithii*, mothers and offspring from field and laboratory colonies were genetically identical across multiple generations and males were completely absent from asexual populations, suggesting apomixis as the cytogenetic mechanism underlying thelytoky. Alternatively, it is possible that *M. smithii* queens reproduce via meiotic parthenogenesis (automixis) with central fusion, a cytogenetic mechanism characterized by potentially very low recombination rates, depending on the locus's distance to the centromere, as indicated by genotype pairs that differ only at a single locus. Automixis with central fusion has been documented in social Hymenoptera (18, 19, 26, 28, 29, 51, 52), and a recent study of *W. auropunctata* reported recombination rates as low as 0–2.8% (31). Our current data, however, are insufficient to clearly distinguish between automixis with a low recombination rate and apomixis with rare gene conversion.

Conclusion

M. smithii is a recently evolved, monophyletic species consisting of a mosaic of asexual and sexually reproducing populations. Sex has been lost repeatedly in multiple lineages. Once females have lost the ability to reproduce sexually, the condition seems to be irreversible. The lack of genetic recombination and the complete absence of males in asexual populations and laboratory breeding experiments indicate that meiosis may be dysfunctional in asexual females, and thus that mitotic parthenogenesis (apomixis) is the cytogenetic mechanism underlying parthenogenesis in *M. smithii*. However, automixis with central fusion and low recombination rates cannot be ruled out as a possible alternative mechanism. Sexually reproducing populations were discovered in the center of *M. smithii*'s geographic distribution along the Rio Amazonas and the Rio Negro. *M. smithii* has high local population densities and the most extensive geographic distribution of any fungus-growing ant species, indicating its ecological success. The sympatric existence of sexual and asexual populations in the Amazon suggests that sexual populations continue to enjoy high fitness in the center of the species distribution and are not outcompeted by asexual colonies. The fitness advantage of asexual populations seems to be realized outside the range of sexual populations, where parthenogenetic queens apparently colonize vacant niches and disperse rapidly in the absence of males. Given that kin selection theory predicts that conflict over reproduction should be absent in groups of genetically identical individuals, it would be intriguing to investigate the maintenance of cooperative behavior and social conflict in *M. smithii*. Finally, given the absence of genetic variation within colonies and the presence of phenotypically distinct queen and worker castes, *M. smithii* appears to be a study organism that is well-suited for investigating the proximate mechanisms of environmentally based caste determination and for exploring the genetic basis of phenotypic plasticity.

Materials and Methods

Population-Genetic Analyses. As test statistics for asexuality, we used the existence of repeated multilocus genotypes and maximum deviation from random mating (F_{IS}) (53–55). The genotype-to-individual ratio (G:N ratio) was applied to identify multilocus genotypes (55) (Table S1). Independently evolved asexual lineages (clones) originating from separate sexual events were distinguished from slightly different multilocus genotypes that diversified through accumulation of mutations or scoring errors by calculating the probability, p_{sex}, following the methodology outlined in ref. 34 and implemented in GENCLONE 2.0 (56). The observed and expected heterozygosity for each clonal lineage (57), the proportion of clonal genotypes in a population, *F* statistics, and AMOVA were calculated in GENALEX 6 (58) and Genetic Data Analysis (59). To reveal the underlying population-genetic structure of sexual and asexual populations, we used the multivariate statistical methods (60–62) NMDS, principal component analysis, and DAPC, as implemented in PERMAP (63), GENALEX (58), and SYSTAT (Systat Software).

Phylogenetic Analyses. We conducted analyses of two distinct datasets: first, a global dataset that included 84 *M.* ingroup taxa, 32 of them *M. smithii*, and 87 outgroup taxa. The recently described social parasite *M. castrator* (64) was not included. The alignment consisted of 2,319 bp of protein-coding (exon) sequences of three single-copy nuclear genes and one mitochondrial gene and was divided into 10 partitions. Second, we conducted a local analysis of 41 *M. smithii* taxa representing one individual from each of the genotyped populations (Table S5). We obtained 1,515 bp of three mitochondrial genes and divided the alignment into two partitions (Table S6). Constrained topologies were estimated using Bayesian and ML analyses, and differences in the likelihoods of constrained versus unconstrained topologies were evaluated using Bayes factors (65–67). All ingroup sequence data were generated for this study (Table S5). Best-fit models of sequence evolution were selected for each partition under the Akaike information criterion (68) and hierarchical likelihood ratio tests as calculated in MODELTEST v3.7 (69) (Table S6). We conducted partitioned Bayesian analyses using MrBayes v3.1.2 (70). Burn-in and convergence were assessed using Tracer v1.5 (71). Partitioned ML analyses were carried out in GARLI 0.97.r737 (72).

Divergence-Dating Analysis. We used a Bayesian relaxed clock uncorrelated lognormal approach implemented in the program BEAST v1.4.8 with a Yule process as the tree prior (73–75). The root node was given a normal age prior distribution (mean = 73.5, SD = 4.5), following methodology described in ref. 76. Based on fossil data, lognormal age prior distributions were assigned to three internal nodes, as outlined in ref. 35. For more details on analyses and results, see *SI Materials and Methods* and Tables S1–S8.

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