Social Hybridogenesis in the Clonal Ant *Cataglyphis hispanica*

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Summary

With a few rare exceptions, the vast majority of animals reproduce sexually [1–3]. Some species have, however, evolved alternative modes of reproduction by shifting from classical bisexuality to unorthodox reproductive systems, like parthenogenesis, gynogenesis, or hybridogenesis [4, 5]. Under hybridogenesis, both the maternal and paternal genomes are expressed in somatic tissues, whereas the germline is purely maternal. Recently, a form of hybridogenesis at the level of the society has been reported in some ants, where purebred females develop into reproductive queens and interlineage hybrids into sterile workers [6]. Here, we report a unique case of social hybridogenesis in the desert ant *Cataglyphis hispanica*. Workers are produced exclusively from interbreeding between two distinct genetic lineages, whereas male and female sexuals are produced by asexual reproduction through parthenogenesis. As a consequence, all workers are pure hybridogens, and only maternal genes are perpetuated from one generation to the next. Thus, queens of *C. hispanica* use sexual reproduction for colony growth, whereas they reproduce asexually through parthenogenesis for germline production.

Results

In hybridogenetic species, females of hybrid origin discard the paternal genome prior to meiosis and produce gametes carrying no paternally derived genes [7–9]. The eggs are then fertilized with sperm of the paternal species resulting in a hybridogen, which consists of a clonally inherited maternal part and a sexually inherited paternal part (Figure 1). Hybridogenesis, therefore, results in a situation in which both the maternal and paternal genomes are expressed in somatic tissues, whereas the germline is purely maternal.

A form of social hybridogenesis was recently reported in two ant genera: *Pogonomyrmex* harvester ants and *Solenopsis* fire ants. Hybrid mating between individuals originating from different genetic lineages results in the production of sterile workers (analogous to the "soma" of solitary organisms), whereas mating between individuals from the same genetic lineage produces new reproductive queens (the "germline") (Figure 1). In the *Pogonomyrmex barbatus*/*rugosus* species complex, colonies are headed by a single queen that mates multiple times with males of their own as well as with males of the alternate lineage. Purebred females become queens, and interlineage hybrids become workers [6, 10, 11]. A similar reproductive system evolved in a hybrid zone between two fire ants: *Solenopsis geminata* and *S. xyloni* [12]. Colonies of *S. xyloni* contain multiple queens each mated with a single male. Queens mated with a male of their own species produce only reproductive females, whereas queens mated with a *S. geminata* male produce only workers. Because males of Hymenoptera arise from unfertilized, haploid eggs by arrhenotokous parthenogenesis, they belong to the queen lineage.

While conducting a population genetic study, we discovered a unique case of social hybridogenesis in which paternal genes are not transmitted, but only maternal genes are perpetuated across generations, in the desert ant *Cataglyphis hispanica*. We found the existence of two genetic lineages across four populations sampled. Queens mate with a single male originating from the alternative lineage than their own. Remarkably, workers are all interlineage hybrids, whereas the sexual line is purely maternal.

Colonies of *C. hispanica* Are Monogynous and Monandrous

A single queen was found in 34 out of the 38 colonies excavated from the two populations of Bonares (B) and Lucena del Puerto (L). The 555 workers sampled from queen-right colonies and genotyped at the five polymorphic microsatellite markers were unambiguously assigned to the queen present in their nest. Moreover, in the four colonies in which no queen was collected, worker genotypes (n = 74) were compatible with single maternity. Mother-offspring genetic analyses from 369 lab-raised worker progenies of 30 queens indicated that 28 queens were singly mated, and two were doubly mated, resulting in an average number of patrilines per colony of 1.07 ± 0.26 ($P_{nondetection}$ = 0.04). Genetic analysis of the sperm content of queens' spermatheca (n = 20) did not reveal alleles that were not detected in parent-offspring analyses.

Parthenogenetic Production of Male and Female Sexuals

A total of 24 new queens (gynes) were produced in 7 of the 38 colonies (mean number of gynes per colony ± SE = 3.43 ± 0.95). All showed strictly identical multilocus genotypes to their mother queen. None carried alleles of their mothers' mate at any of the five markers studied, indicating that they had been produced by thelytokous parthenogenesis (see Table S1 available online). The probability of these gynes arising from mating with males having no diagnostic allele at the five loci was low, ranging from 0.049 to 4.74×10^{-7} across colonies (Supplemental Experimental Procedures). In line with parthenogenetic production of gynes, the coefficient of relatedness (r) between a queen and her reproductive daughters was 1.0 ($SE_{Jackknife}$ = 0; n = 7 colonies). Moreover, 51 males were raised in 14 colonies (mean number of males per colony ± SE = 3.64 ± 0.61). All were haploid and carried alleles of the colony queen (Table S1), consistent with their production by arrhenotokous parthenogenesis.

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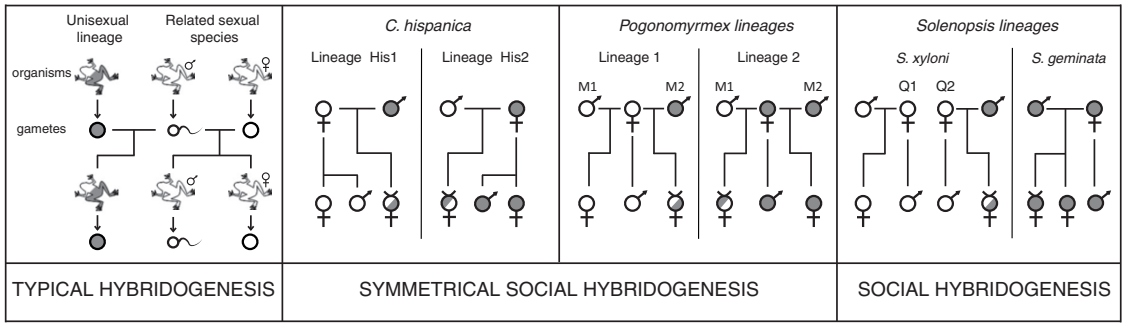

Figure 1. Hybridogenesis and Its Social Counterparts in Three Ant Taxa

First panel describes a typical hybridogenesis pattern. Females of hybrid origin produce gametes bearing only the maternal genome, after discarding the paternal genome; the eggs are then fertilized with sperm of the paternal species, resulting in a hybridogen that consists of a clonally inherited maternal part and a sexually inherited paternal part (modified from [5]). Gray and white colors represent nuclear genomes from two distinct parental species. Panels 2–4 show the three different forms of social hybridogenesis reported in ants. Lineage label corresponds to the queens' lineage origin. Gray and white colors refer to the different interacting lineages or species. Offspring obtained from mating between individuals originating from the same or different genetic lineages/species are shown (new queens —, males _, and workers –). The multiple mates in the *Pogonomyrmex* lineages are labeled M1 and M2. The coexistence of several nest mate queens in *S. xyloni* is indicated by Q1 and Q2 (panel for *Solenopsis* modified from [14]). Because reproductive offspring are produced by asexual reproduction in *C. hispanica*, only maternal genes are perpetuated across generations, as is the case under typical hybridogenesis. Both the maternal and the paternal intralineage genomes are transmitted to the next generation of queens in *Pogonomyrmex* and *Solenopsis* ants.

Workers Arise Exclusively from Sexual Reproduction

Across the four studied populations, all 820 genotyped workers (n = 58 colonies) harbored alleles from both the queen and her mate(s), indicating that they were produced by sexual reproduction. Notably, 776 workers (94.6%) were heterozygous at the five loci surveyed; the remaining 44 workers were heterozygous at four loci but homozygous at one locus (Cc 26) (Figure 2). Observed worker heterozygosity by loci was significantly higher than expected under random mating (G total = 51.74; df = 4; $p < 0.0001$). The mean observed heterozygosity ranged from 0.94 to 1 according to the population sampled (expected heterozygosity, 0.54–0.57). Thus, alleles carried by the queens and their mates were almost never identical.

Populations Consist of Two Genetic Lineages

Within each population, maximum-likelihood analyses indicated that reproductives clustered into two genetically distinct groups. Allele frequencies were significantly different between the two groups for each of the five loci (Fisher's exact test, $p < 0.0001$), with four loci (Cc11, Cc54, Cc76, and Cc93) being diagnostic of the genetic group in each population (Figure 3; Figure S1). In populations L, B, and Los Ranchos de Guadiamar (R), the two genetic groups were formed by queen and male pools, respectively. In population Aznalcóllar (A), reproductives of both sexes belonged to each of the two groups. Remarkably, in all colonies, the queen and her mate(s) belonged systematically to alternative genetic groups, and all workers were the progeny of these hybrid matings.

Across the four populations, neighbor-joining analyses of the four genetic group pairs identified within populations indicated the presence of two clusters, hereafter called His1 and His2 lineages (Figure 4). Queens from populations B and R belonged to the His1 lineage, whereas those from population L belonged to the His2 lineage; queens of both lineages were collected in population A. The average genetic distance estimated according to Kimura's two-parameter method in our data (628 bp long sequence) ranged from 0.013 (His1) to 0.021 (His2) within the lineages and was 0.019 between the two lineages.

Discussion

Our results show an original form of social hybridogenesis in the ant *C. hispanica*. Queens mate with males originating from a different genetic lineage to produce hybrid workers, but they produce new reproductives asexually through parthenogenesis. Thus, both the maternal and paternal genomes are expressed in the worker force, whereas the "germline" is purely maternal. From an inclusive fitness perspective, males of *C. hispanica* are an evolutionary dead end because they consume resources but cannot transmit their genes to the next generation unless hybrid reproductives are produced. In a reproductive system where males are evolutionarily redundant, we would expect this sex to disappear entirely, resulting in a system in which reproduction by queens is exclusively asexual (as is the case in asexual populations of the ant *Mycocepurus smithii* [13]). Nevertheless, we found no parthenogenetically produced workers in *C. hispanica*, indicating that males are still required for the production of workers. Therefore, should one lineage cease producing males, the entire system will be driven to extinction. The reproductive system found in *C. hispanica* differs from that reported in the sister species *C. cursor*. In both species, queens use sexual reproduction and parthenogenesis for the production of the worker and queen castes, respectively [14]. However, no genetic lineages were found in *C. cursor*. Moreover, the occasional production of gynes by the queens though sexual reproduction and by the workers through thelytokous parthenogenesis contributes to the transmission of the male genes over generations in this species. In addition, about 2.5% of the workers are parthenogenetically produced in *C. cursor*.

Social hybridogenesis in the monogynous and monandrous *C. hispanica* appears strongly different from that observed in *Pogonomyrmex* and *Solenopsis*, for at least two reasons (Figure 1). First, in these species, both the maternal and the paternal intralineage genomes are transmitted to the next generation of queens. By contrast, as is the case in other hybridogenetic species [5], only maternal genes are perpetuated across generations in *C. hispanica*. Second, in *Pogonomyrmex* and *Solenopsis* ants, each colony needs both intralineage

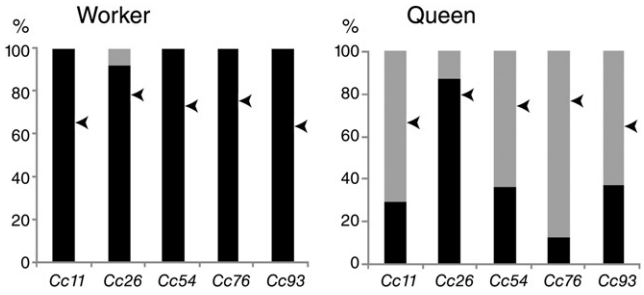

Figure 2. Proportion of Heterozygous and Homozygous Loci in Workers and Queens across the Four Populations Sampled

The mean expected heterozygosity by locus, assuming that female offspring are produced by sexual reproduction with random mating within each population, is indicated by arrowheads. Heterozygous (black) and homozygous (gray) loci are shown for workers (n = 820) and queens (n = 58).

and interlineage mating partners to complete a full reproductive cycle. Colonies either contain multiple queens, as in *Solenopsis*, or they are headed by a single, multiply mated queen, as is the case in *Pogonomyrmex*.

Our data reveal that reproductives from the four sampled populations share the same two gene pools, forming a single pair of genetic lineages. This contrasts with the *Pogonomyrmex barbatus/rugosus* species complex, where several pairs of dependent genetic lineages do occur. However, because both lineages of a pair are completely dependent on one another in an obligate mutualism to produce workers, reproduction in *C. hispanica* and in *Pogonomyrmex* corresponds to a reciprocal or "symmetrical social hybridogenesis" (SSH) [15]. The occurrence of two distinct interbreeding genetic lineages in *C. hispanica* does not correspond to differences between the male and female genomes (Figure 4) due, for example, to sons being clones of the colony father and new queen clones of the colony mother [16–18] or to segregation distortion [19]. It results from the conjunction of two reproductive patterns: the parthenogenetic production of gynes, whereby mothers transfer all their genes to the reproductive females, and strict reproduction between partners with different genotypes. This systematic outbreeding may be explained by prezygotic reproductive isolation (males and females of the same lineage may avoid mating together) and/or postzygotic reproductive isolation (reduced fertility or inability to produce workers from same-lineage mating). Colonies of *C. hispanica* are headed by a single queen mated once. Few colonies produce gynes, and, for those who do, the number of gynes produced per colony is small. One should therefore expect strong selection for prezygotic isolation with gynes discriminating among potential males to mate only with the alternate lineage, otherwise a huge proportion of gynes mating inappropriately would die every generation. Discrimination between genetic lineages could be based on chemical cues. Hydrocarbon profiles of the wax layer on the cuticle have indeed been shown to mediate a wide range of behaviors in social insects, including kin recognition, fertility signaling, or mating choice [20].

The maintenance of the dependent lineage system reported here requires that reproductives are pure-breeding lineages and that F1 interlineage worker hybrids are viable. Thus, a viable intergenome must be maintained, whereas the two genetic lineages may diverge over time. Mitochondrial deoxyribonucleic acid (DNA) analyses indicate similar levels of haplotype divergence both between and within lineages, suggesting that the lineages are not reciprocally monophyletic and that the hybridogenetic system reported here may be of very recent origin. We found that all pure-breeding reproductives resulted from asexual reproduction. However, it remains unknown whether viable hybrid reproductives could occasionally be produced and compromise the persistence of the system. Such interlineage reproductives might arise from occasional production of gynes by sexual reproduction or from production of daughter queens and males by worker reproduction through parthenogenesis. Orphaned workers of *C. hispanica* lay both haploid and diploid eggs that may have interlineage genomes. However, whether these eggs can develop into fully grown and reproductively capable adults is uncertain because all the 199 sexuals (genotyped and inferred) of our sample were of pure-breeding lineages. Hybrid reproductives may also carry genetic information from the queen's mate and, then, represent the only possible way for males to have a nonzero fitness because males seem to transmit their genes only to the worker caste.

We found a single lineage in three out of four populations studied. This strongly suggests that colonies from different lineages structure in patches within populations. This may result from a combination of two processes: the parthenogenetic production of new queens and colony foundation by budding, whereby young mated queens establish a new colony at a walking distance of their natal nest with the help of a worker force. Reduced dispersal of genetically highly similar females is expected to generate strong microgeographical genetic structure. Interestingly, asexual production of new queens and budding are also closely associated in four ant species [14, 16–18] and termites [21]. In this context, males must be the dispersing sex to ensure interlineage mating.

Hybridogenesis in *C. hispanica* combines the genetic benefits of asexual reproduction, the genetic diversity from sexual reproduction, and the high level of heterozygosity of the hybrid worker force. By using alternative modes of reproduction for the queen and the worker castes, queens of *C. hispanica* increase the transmission rate of their genes to their reproductive female offspring, while maintaining some level of genetic

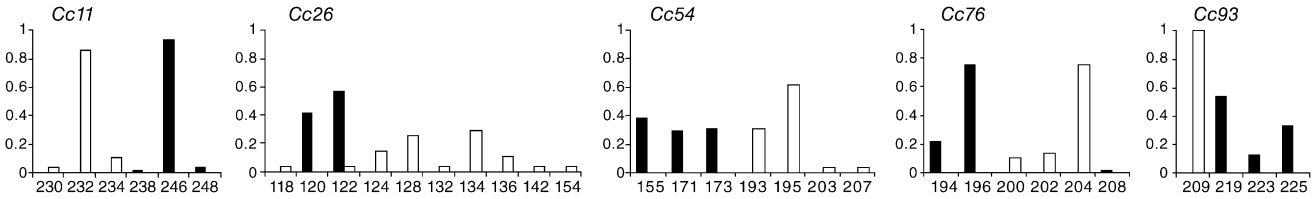

Figure 3. Allele Frequencies for Queens and Their Inferred Mates at the Five Microsatellite Loci Surveyed, in Population B

Data for queens (n = 27) are represented with black bars and for their inferred mates (n = 29) with white bars. See also Figure S1 for all populations.

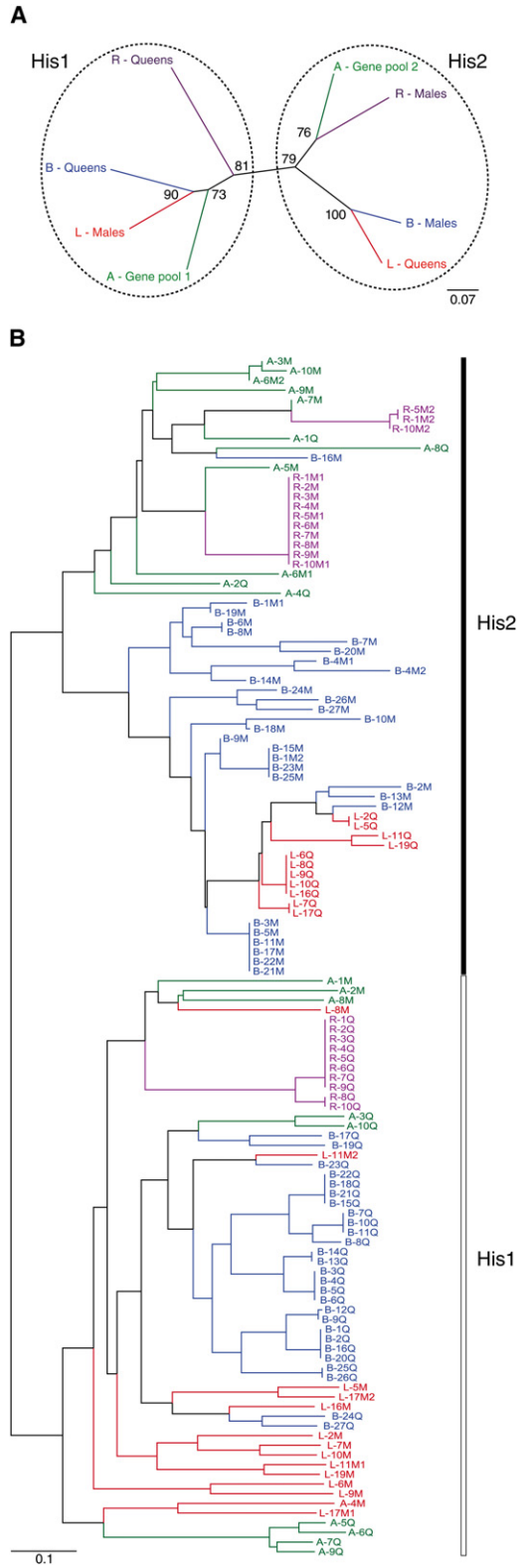

Figure 4. Number of Distinct Lineages across All Sites Sampled

(A) Neighbor-joining dendrogram based on Cavalli-Sforza genetic distance between within-site groups. Numbers on nodes indicate bootstrap percentages (1,000 replicates on loci).

(B) Neighbor-joining dendrogram based on allele sharing genetic distances of 58 queens (labeled Q) and their 66 inferred mates (M) collected over four populations: A (green), B (blue), L (red), R (pink). M1/M2 labels differentiate multiple mates. The collection number of each nest is given with the letter of the site.

diversity in the worker force [14]. Hybridization has long been shown to provide fitness benefits due to heterosis in both plants and animals, e.g., by enhancing growth rate of hybrids and/or by improving resistance to stressful conditions and ability to survive in restricting habitats [22–26]. In ants, the high level of heterozygosity among workers could impact colony fitness by causing a form of social heterosis, giving colonies a competitive advantage in some environments. For instance, worker heterosis could allow exploitation of a wider range of resources more effectively [27] or enhanced resistance to parasites and pathogens [28–30]. However, hybridization may also result in outbreeding depression due to genetic incompatibilities, with hybrids experiencing reduced fertility or sterility [31–33]. Interestingly, in *C. hispanica*, the use of asexual and sexual reproduction by queens for the worker and reproductive castes, respectively, allows the expression of the positive effects of hybridization without its negative impact on reproduction. Because hybrid individuals exclusively become nonreproductive workers, the potentially negative consequences of hybrid sterility are null, whereas workers may even benefit from nonreproductive heterosis effects [12, 34].

Experimental Procedures

Fifty-eight nests Of *C. hispanica* were sampled between May 2009 and May 2011 from four Andalusian populations (southern Spain): 27 colonies from B, 11 colonies from L, 10 colonies from R, and 10 colonies from A. Colonies from B and L were completely excavated (n = 38) and were brought to the laboratory for queen mating-frequency estimation. New queens and males that emerged in the laboratory were genotyped to determine their mode of production.

Genotyping

Individual ant DNA was extracted using the Chelex extraction process (Bio-Rad, Hercules; [35]) following standard protocols. Ant legs were crushed and incubated for 1 hr 30 min at 85°C in 100 μl of 5% Chelex with constant agitation. After a 3 min centrifugation at 12,000 rpm, 80 μl of the supernatant was transferred into a 1.5 ml tube. To isolate sperm DNA, the queens' abdomen was dissected in a saline solution, and the content of their spermathecae was extracted following the same procedure. Seven microsatellite markers described for *C. cursor* were tested [36]; none showed null alleles, but two revealed linkage disequilibrium. Our samples were genotyped at five polymorphic microsatellite loci (Cc11, Cc26, Cc54, Cc76, and Cc93), showing 7–17 alleles across the four populations. Loci were amplified as described in [37] with PCR optimizations following the QIAGEN protocol (available upon request). PCR products were genotyped using an automated Applied Biosystems ABI 3730 Sequencer (Applied Biosystems). The size of the different alleles was determined using the Rox 350 HD internal size standard and GeneMapper 3.7 software (Applied Biosystems). Control for genotyping errors due to null alleles and allele dropouts was performed with MICRO-CHECKER [38]. Linkage disequilibrium was tested with genepop'007 [39].

Genetic and Phylogenetic Data Analysis

Colony and population genetic structure was inferred from the pedigree of 820 workers (mean number of workers genotyped per colony ± SE = 15.47 ± 1.12). Descriptive statistics (i.e., the allele frequencies, observed heterozygosity, and expected heterozygosity) were computed with FSTAT [40]. Relatedness coefficients (*r*) were estimated using the algorithm of Queller and Goodnight [41] implemented in the program relatedness 5.0.8. Colonies were weighted equally, and SEs were obtained by jackknifing over colonies.

The number of queens per colony was determined from field observations and genetic analyses for the 38 colonies excavated in B and L. Queen genotypes were determined by direct analysis (n = 34 colonies) or were inferred from worker offspring genotypes if the queen was not collected (n = 4).

Individuals were assigned as belonging to different matrilines if they did not share an allele with the queen at at least one locus. Assignment of individuals to matrilines was confirmed with the maximum-likelihood methods implemented in the program COLONY 1.2 [42].

The number of fathers contributing to the progeny of each queen was estimated from the genotype of lab-raised worker pupae from 30 queens (mean number of pupae genotyped per colony ± SE = 12.37 ± 0.22, n = 369), by reconstructing each paternal genotype from mother-offspring allele combinations. Queen mating frequency was confirmed by genotyping the sperm content of 20 queens' spermathecae. The probability of nondetection of additional patrilines due to two fathers sharing the same alleles at all loci was calculated as

$$P_{non-detection} = \prod_j \sum_i f_{ij}^2,$$

where f_{ij} is the level frequency of allele i at locus j [43].

To test for the presence of genetically distinct interbreeding groups, the genotype of the queen and her mate(s) was inferred from an additional sample of ten workers/colony from ten colonies in population R, and ten workers from ten colonies in population A. We tested whether each of the four sites comprised reproductives of different genetic groups using the maximum-likelihood method implemented in the population assignment program STRUCTURE 2.3.3 [44] with a burn-in of 50,000 and run lengths of 50,000 under an admixture ancestry model. To determine the number of distinct lineages across all sites, we performed two neighbor-joining clustering analyses based on Cavalli-Sforza and Edwards chord distance between the genetic groups detected in the previous within-site analyses and on the shared-allele distance (D_{AS}; [45]) between pairs of reproductives (i.e., 58 queens and their 66 mates). Calculation of distances and construction of neighbor-joining dendrograms were performed with the software POPULATIONS 1.2.32.

Genetic divergence within and between lineages was estimated based on the sequencing of a portion of 628 bp of the COI gene (see Supplemental Experimental Procedures).

Supplemental Information

Supplemental Information includes one figure, one table, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2012.04.060.

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References

1. Williams, G.C. (1975). Sex and Evolution (Princeton, NJ: Princeton University Press).
2. Bell, G. (1982). The Masterpiece of Nature: the Evolution and Genetics of Sexuality, CUP Archive Edition (London: Croom Helm).
3. Barton, N.H., and Charlesworth, B. (1998). Why sex and recombination? Science *281*, 1986–1990.
4. Suomalainen, E., Saura, A., and Lokki, J. (1987). Cytology and Evolution in Parthenogenesis (Boca Raton, FL: CRC Press Edition).
5. Avise, J.C. (2008). Clonality: the Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals (New York: Oxford University Press).
6. Helms Cahan, S., Parker, J.D., Rissing, S.W., Johnson, R.A., Polony, T.S., Weiser, M.D., and Smith, D.R. (2002). Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. Proc. Biol. Sci. *269*, 1871–1877.
7. Schultz, R.J. (1969). Hybridization, unisexuallity and polyploidy in the teleost *Poeciliopsis (Poecilidae)* and other vertebrates. Am. Nat. *103*, 605–619.
8. Vrijenhoek, R.C. (1993). The origin and evolution of clones versus the maintenance of sex in *Poeciliopsis*. J. Hered. *84*, 388–395.
9. Som, C., Bagheri, H.C., and Reyer, H.U. (2007). Mutation accumulation and fitness effects in hybridogenetic populations: a comparison to sexual and asexual systems. BMC Evol. Biol. *7*, 80.
10. Julian, G.E., Fewell, J.H., Gadau, J., Johnson, R.A., and Larrabee, D. (2002). Genetic determination of the queen caste in an ant hybrid zone. Proc. Natl. Acad. Sci. USA *99*, 8157–8160.
11. Sirviö, A., Pamilo, P., Johnson, R.A., Page, R.E., Jr., and Gadau, J. (2011). Origin and evolution of the dependent lineages in the genetic caste determination system of *Pogonomyrmex* ants. Evolution *65*, 869–884.
12. Helms Cahan, S., and Vinson, S.B. (2003). Reproductive division of labor between hybrid and nonhybrid offspring in a fire ant hybrid zone. Evolution *57*, 1562–1570.
13. Rabeling, C., Gonzales, O., Schultz, T.R., Bacci, M., Jr., Garcia, M.V.B., Verhaagh, M., Ishak, H.D., and Mueller, U.G. (2011). Cryptic sexual populations account for genetic diversity and ecological success in a widely distributed, asexual fungus-growing ant. Proc. Natl. Acad. Sci. USA *108*, 12366–12371.
14. Pearcy, M., Aron, S., Doums, C., and Keller, L. (2004). Conditional use of sex and parthenogenesis for worker and queen production in ants. Science *306*, 1780–1783.
15. Parker, J.D. (2004). A major evolutionary transition to more than two sexes? Trends Ecol. Evol. (Amst.) *19*, 83–86.
16. Fournier, D., Estoup, A., Orivel, J., Foucaud, J., Jourdan, H., Le Breton, J., and Keller, L. (2005). Clonal reproduction by males and females in the little fire ant. Nature *435*, 1230–1234.
17. Pearcy, M., Goodisman, M.A.D., and Keller, L. (2011). Sib mating without inbreeding in the longhorn crazy ant. Proc. Biol. Sci. *278*, 2677–2681.
18. Ohkawara, K., Nakayama, M., Satoh, A., Trindl, A., and Heinze, J. (2006). Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Vollenhovia emeryi*. Biol. Lett. *2*, 359–363.
19. Kulmuni, J., Seifert, B., and Pamilo, P. (2010). Segregation distortion causes large-scale differences between male and female genomes in hybrid ants. Proc. Natl. Acad. Sci. USA *107*, 7371–7376.
20. Howard, R.W., and Blomquist, G.J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu. Rev. Entomol. *50*, 371–393.
21. Matsuura, K., Vargo, E.L., Kawatsu, K., Labadie, P.E., Nakano, H., Yashiro, T., and Tsuji, K. (2009). Queen succession through asexual reproduction in termites. Science *323*, 1687.
22. Fry, J.D., Heinsohn, S.L., and Mackay, T.F. (1998). Heterosis for viability, fecundity, and male fertility in *Drosophila melanogaster*: comparison of mutational and standing variation. Genetics *148*, 1171–1188.
23. Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L., and Paige, K.N. (1998). Tracking the long-term decline and recovery of an isolated population. Science *282*, 1695–1698.
24. Burke, J.M., and Arnold, M.L. (2001). Genetics and the fitness of hybrids. Annu. Rev. Genet. *35*, 31–52.
25. Keller, L.F., and Waller, D.M. (2002). Inbreeding effects in wild populations. Trends Ecol. Evol. *17*, 230–241.
26. Charlesworth, D., and Willis, J.H. (2009). The genetics of inbreeding depression. Nat. Rev. Genet. *10*, 783–796.
27. Nonacs, P., and Kapheim, K.M. (2007). Social heterosis and the maintenance of genetic diversity. J. Evol. Biol. *20*, 2253–2265.
28. Baer, B., and Schmid-Hempel, P. (1999). Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature *397*, 151–154.
29. Hamilton, W.D. (1987). Kinship, recognition, disease, and intelligence: constraints of social evolution. In Animal Societies: Theories and Facts, Y. Ito, J.L. Brown, and J. Kikkawa, eds. (Tokyo: Japan Science Society Press), pp. 81–102.
30. Schmid-Hempel, P. (1998). Parasites in Social Insects (Princeton, NJ: Princeton University Press).
31. Barton, N.H., and Hewitt, G.M. (1989). Adaptation, speciation and hybrid zones. Nature *341*, 497–503.
32. Orr, H.A. (1995). The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics *139*, 1805–1813.

33. Greig, D., Louis, E.J., Borts, R.H., and Travisano, M. (2002). Hybrid speciation in experimental populations of yeast. Science *298*, 1773–1775.
34. Umphrey, G.J. (2006). Sperm parasitism in ants: selection for interspecific mating and hybridization. Ecology *87*, 2148–2159.
35. Walsh, P.S., Metzger, D.A., and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques *10*, 506–513.
36. Pearcy, M., Clemencet, J., Chameron, S., Aron, S., and Doums, C. (2004). Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. Mol. Ecol. Notes *4*, 642–644.
37. Timmermans, I., Hefetz, A., Fournier, D., and Aron, S. (2008). Population genetic structure, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis sabulosa*. Heredity (Edinb) *101*, 490–498.
38. Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes *4*, 535–538.
39. Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. *8*, 103–106.
40. Goudet, J. (1995). FSTAT (vers 1.2): A computer program to calculate *F*-statistics. J. Hered. *86*, 485–486.
41. Queller, D.C., and Goodnight, K.F. (1989). Estimating relatedness using genetic markers. Evolution *43*, 258–275.
42. Wang, J.L. (2004). Sibship reconstruction from genetic data with typing errors. Genetics *166*, 1963–1979.
43. Boomsma, J.J., and Ratnieks, F.L.W. (1996). Paternity in eusocial Hymenoptera. Philos. Trans. R. Soc. Lond. B Biol. Sci. *351*, 947–975.
44. Pritchard, J.K., Stephens, M., Rosenberg, N.A., and Donnelly, P. (2000). Association mapping in structured populations. Am. J. Hum. Genet. *67*, 170–181.
45. Chakraborty, R., and Jin, L. (1993). A unified approach to study hypervariable polymorphisms: statistical considerations of determining relatedness and population distances. EXS *67*, 153–175.