

DISPATCH

Evolution: A social parasite was born from a virgin

Michel Chapuisat

Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland.

E-mail: michel.chapuisat@unil.ch

The sudden appearance of small winged queens within a lineage of asexually reproducing ant workers reveals that such social parasites can appear abruptly. The parasitic queens differ in a large genomic region, suggesting that a supergene instantly equipped the social parasite with a suite of co-adapted traits.

Most ant species have a morphologically differentiated caste of workers that care for the brood of queens. This permanent reproductive division of labor is the hallmark of an extreme form of integrated social organization, called 'eusociality'¹. But the ant family is rich in exceptions, unusual life-histories and surprising phenotypes. These exceptions to the rule are great systems to study the evolutionary pressures and molecular mechanisms of division of labor. In this issue of *Current Biology*, Waring Tribble, Daniel Kronauer and colleagues² document that clonal queenless workers of a raider ant suddenly produced miniaturized queens, following loss of heterozygosity in a large cluster of genes. These fascinating results suggest that an ancient supergene underlies the abrupt appearance of a pre-adapted socially parasitic lineage.

On multiple occasions, ant, bee and wasp species have become social parasites of other species, a process that has long fascinated evolutionary biologists. In *The Origin of Species*, Darwin³ discussed at length the intriguing observation that some ant species are hopeless without care-giving workers from other species, which they steal as brood.

Darwin proposes that live pupae originally stored as food by predatory ants subsequently became their care-givers, a coherent explanation that has pretty well survived the test of time⁴. But brood robbing is only one form of social parasitism⁵. Some species, like the European red wood ants, practice temporary social parasitism, whereby queens settle in nests of other species, before producing their own workers. Other species, called 'inquilines', are permanent social parasites. In inquiline species of Hymenoptera, the worker caste has been lost and the queens entirely depend on care provided by host workers of another species.

The evolutionary origin of inquiline, workerless social parasites remains hotly debated⁴⁻⁶. A prevailing model is that parasitic lineages evolved gradually from within their hosts, through intermediate forms that were facultatively parasitic. Host and parasites are generally phylogenetically close, but not necessarily the closest lineages. Moreover, we do not know how parasitic lineages achieved reproductive isolation from host lineages, and facultative intermediates showing both parasitic and host behaviors are rare, which questions whether, and how, inquiline social parasites gradually diverged from their hosts in sympatry^{4,5}. Finally, we know nothing about the genomic underpinnings of social parasitism. Finding which genetic changes led to parasitic lineages can reveal the proximate mechanisms of caste differentiation, and how such parasitic lineages evolved.

Novel insights into the genomic underpinnings of inquiline social parasitism unexpectedly came from a serendipitous discovery Tribble and colleagues² made within a colony of clonal raider ants (*Ooceraea biroi*). This small, inconspicuous ant from Asia has been spread by human trade to many tropical and subtropical islands⁷. *O. biroi* has a most unusual reproductive system for ants: it normally lacks queens while its workers reproduce asexually. Because of this asexual reproduction, nestmate workers are almost clones⁸. Yet, they show division of labor, with cycles in which periods of sedentary, synchronized reproduction alternate with periods of raids, during which workers hunt for brood of other ant species. This system has unique advantages to study division of labor, as researchers can control the genotype and age of each individual in a social group^{8,9}. They can also be bred in captivity for many generations.

While screening colonies of *O. biroi* in the laboratory, Tribble and colleagues² noticed winged individuals that looked different from the normal workers (Figure 1). This was surprising, as winged queens were unknown in this species. Moreover, these queen-like individuals were worker-sized and much smaller than the queens of related species. After measuring various body parts and checking their allometric relationships, the authors concluded that these tiny queens did not correspond to normal queens of ancestors of the genus *Ooceraea*, but resembled miniaturized workerless queens that are inquiline social parasites of other ant species¹⁰.

In a series of experiments, Tribble and colleagues² explored the hypothesis that queen-like individuals behave as inquiline social parasites. They found that, compared to workers, queen-like individuals had a reduced tendency to forage, laid twice as many eggs and produced brood that had a competitive disadvantage when reared together with worker offspring. This is fully consistent with inquilinism, but the parasitic behavior of the queen-like individuals deserves

further testing. Do they try to infiltrate foreign colonies? Are they better dispersers than workers? For example, can they fly, or do they succeed at rearing brood independently, without the help of workers? Do they occur in the field, and if so, at which frequency? More analyses will be needed to fully elucidate the function of this peculiar queen-like phenotype.

Cross-fostering experiments revealed that the phenotypic differences between queen-like individuals and workers were due to genetic variation, not to phenotypic plasticity². In short, queen-like individuals laid eggs that always developed into queen-like individuals, and workers laid eggs that developed into workers, independently of the care-givers and rearing conditions. The most likely explanation for this strict transmission is that the queen-like phenotype is the result of genetic mutations. Experimental breeding data further indicate that queen-like mutants can persist at low frequency within colonies predominantly composed of workers. Overall, it seems likely that queen-like mutants and wild-type workers are controlled by a balanced genetic polymorphism that persists because of frequency-dependent selection, such that the parasitic genotypes being favored when rare and disfavored when common.

Because both the parasitic queens and their worker ancestors are almost clonal, sequencing each lineage provided immediate access to the underlying genomic changes. The comparison of queen-like mutants and workers revealed that a 2.25 million base pair long stretch of DNA had completely lost heterozygosity in the queen-like lineage (Figure 1). This part of chromosome 13, amounting to about 1% of the total genome, was homozygous in the queen-like ants and corresponded to one of the two haplotypes found in wild-type workers. This genetic architecture suggests that a group of linked genes — a supergene — underlies the differentiation between the queen-like mutants and wild-type workers.

A supergene is a cluster of loci segregating as a single Mendelian unit. In sexually reproducing species, recombination between alternative supergene haplotypes is suppressed following structural changes in the DNA sequence, like chromosomal inversions, or through epigenetic mechanisms. Supergenes control coordinated shifts in multiple morphological, behavioral and physiological traits, and are associated with various intra-specific polymorphisms^{11,12}. A supergene, therefore, provides a powerful molecular mechanism to generate a balanced equilibrium between a 'cheater' parasitic lineage and a host lineage.

In *O. biroi*, the queen-like mutant exhibits multiple traits adapted to social parasitism: small body size, wings, high fecundity, reduced foraging and exclusive production of queen-like individuals, without any worker offspring. Yet, the queen-like mutant appeared from the wild-type ancestor in at most a few generations (Figure 1). Indeed, apart from the loss-of-heterozygosity stretch on chromosome 13, the queen-like lineage differed from wild-type workers by very few changes in the DNA sequence, corresponding to the number of mutations that would be expected if sequences diverged five or six generations ago. This raises an interesting question. How could such a complex adapted phenotype appear suddenly, likely in a single-step mutation, without a long period of natural selection?

The best answer to this question is that the tract of linked genes underlying the parasitic phenotype evolved long ago, in some sexual ancestor. Supergenes spread because the absence of recombination prevents maladaptive combinations of alleles, or because they captured selfish genetic elements distorting transmission ratio¹¹⁻¹³. Supergenes persist as balanced polymorphism due to various forms of frequency-dependent selection, including disassortative mating, overdominance (heterozygote advantage), or selection varying through space and time¹³⁻¹⁵. But in clonal lineages, there is no selection

pressure for recombination suppression and no reason for a supergene to evolve, as all alleles are co-transmitted. Moreover, Tribble and colleagues² found that in *O. biroi*, the low-heterozygosity region on chromosome 13 has accumulated transposable elements and structural variants, indicative of an ancient origin and long absence of recombination¹⁶. Together, these theoretical and empirical arguments indicate that the supergene triggering queen-like development did not evolve recently in clonal workers, but was inherited from sexual ancestors of *O. biroi*, or acquired by introgression from some related sexual lineage. The supergene then remained silent in clonal heterozygous workers, for at least several generations, until a loss of heterozygosity mutation, likely due to a rare recombination event during asexual reproduction, suddenly produced individuals homozygous for the supergene, which developed into queen-like mutants (Figure 1). In short, a rare mutation re-activated a 'fossil' supergene, that likely evolved long ago in a sexual lineage. If this scenario is correct, comparative search in related species will shed light on the origin of this 'supergene for social parasitism'.

The sudden appearance of a queen-like parasitic phenotype in a colony of clonal workers documented by Tribble and colleagues² is fascinating, but becomes slightly less miraculous when one considers that the underlying genetic module likely evolved gradually in sexually reproducing ancestors. Large non-recombining regions, or supergenes, can persist as balanced polymorphisms for millions of years, jump species boundaries, and play major roles in maintaining intra-specific alternative social phenotypes, dispersal phenotypes, sexes, or ecotypes¹¹⁻¹⁴. Such supergenes usually have ancient origins and complex evolutionary trajectories¹³⁻¹⁵. The *O. biroi* 'supergene for social parasitism' seems no exception. While the large group of linked genes controls an exquisite combination of co-adapted traits for social parasitism, much remains to be learned on when, where, and

how this genetic module evolved, which role it might play in speciation, and how it ended up in a clonal ant lineage.

References

1. Boomsma, J.J., and Gawne, R. (2018). Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. *Biol. Rev.* *93*, 28-54. 10.1111/brv.12330.
2. Tribble, W., Chandra, V., Lacy, K.D., Limón, G., McKenzie, S.K., Olivos-Cisneros, L., Arsenault, S.V., and Kronauer, D.J.C. (2023). A caste differentiation mutant elucidates the evolution of ant social parasites. *Curr. Biol.* *33*, 1047-1058.
3. Darwin, C. (1859). *On the origin of species* (John Murray, London).
4. Borowiec, M.L., Cover, S.P., and Rabeling, C. (2021). The evolution of social parasitism in *Formica* ants revealed by a global phylogeny. *Proc. Natl. Acad. Sci. U.S.A.* *118*, e2026029118. 10.1073/pnas.2026029118.
5. Buschinger, A. (2009). Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecol. News* *12*, 219-235.
6. Cini, A., Sumner, S., and Cervo, R. (2019). Inquiline social parasites as tools to unlock the secrets of insect sociality. *Philos. Trans. R. Soc. B* *374*, 20180193. 10.1098/rstb.2018.0193.
7. Wetterer, J.K., Kronauer, D.J.C., and Borowiec, M.L. (2012). Worldwide spread of *Cerapachys biroi* (Hymenoptera: Formicidae: *Cerapachyinae*). *Myrmecol. News* *17*, 1-4.
8. Oxley, P.R., Ji, L., Fetter-Pruneda, I., McKenzie, S.K., Li, C., Hu, H.F., Zhang, G.J., and Kronauer, D.J.C. (2014). The genome of the clonal raider ant *Cerapachys biroi*. *Curr. Biol.* *24*, 451-458. 10.1016/J.Cub.2014.01.018.
9. Ulrich, Y., Saragosti, J., Tokita, C.K., Tarnita, C.E., and Kronauer, D.J.C. (2018). Fitness benefits and emergent division of labour at the onset of group living. *Nature* *560*, 635-638. 10.1038/s41586-018-0422-6.
10. Aron, S., Passera, L., and Keller, L. (1999). Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination. *Proc. R. Soc. B* *266*, 173-177. 10.1098/rspb.1999.0618.
11. Thompson, M.J., and Jiggins, C.D. (2014). Supergenes and their role in evolution. *Heredity* *113*, 1-8. 10.1038/hdy.2014.20.
12. Schwander, T., Libbrecht, R., and Keller, L. (2014). Supergenes and complex phenotypes. *Curr. Biol.* *24*, R288-R294. 10.1016/J.Cub.2014.01.056.
13. Chapuisat, M. (2023). Supergenes as drivers of ant evolution. *Myrmecol. News* *33*, 1-18. 10.25849/myrmecol.news_033:001.
14. Llaurens, V., Whibley, A., and Joron, M. (2017). Genetic architecture and balancing selection: the life and death of differentiated variants. *Mol. Ecol.* *26*, 2430-2448. 10.1111/mec.14051.
15. Wellenreuther, M., and Bernatchez, L. (2018). Eco-evolutionary genomics of chromosomal inversions. *Trends Ecol. Evol.* *33*, 427-440. 10.1016/j.tree.2018.04.002.
16. Kim, K.W., De-Kayne, R., Gordon, I.J.J., Omufwoko, K.S., Martins, D.J.J., French-Constant, R., and Martin, S.H.H. (2022). Stepwise evolution of a butterfly supergene via duplication and inversion. *Phil. Trans. R. Soc. B* *377*, 20210207. 10.1098/rstb.2021.0207.