The MutAnts Are Here

Daniel A. Friedman,[1,*] Deborah M. Gordon,[1] and Liqun Luo[1,2]
[1]Department of Biology
[2]Howard Hughes Medical Institute
Stanford University, Stanford, CA 94305, USA
*Correspondence: dfri@stanford.edu
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The development of CRISPR/Cas9-mediated gene knockout in two ant species opens a new window into exploring how social insects use olfactory cues to organize their collective behavior.

Ant colonies have long been one of the most accessible and fascinating examples of collective behavior in nature. Recently, genomic, epigenomic, and transcriptomic analyses have begun to unveil the molecular underpinnings of physiology and behavior in various ant species (Yan et al., 2014; Friedman and Gordon, 2016). However, without the ability to perform genetic manipulations, most hypotheses regarding ant genetics rest purely on correlative evidence and phylogenetic inference. Now, in this issue of *Cell*, Trible et al. (2017) and Yan et al. (2017) advance the field of ant genetics into a new era by successfully performing CRISPR/Cas9-mediated knockout of the gene that encodes the same key olfactory co-receptor in two different ant species.

All of the 13,000+ known ant species are eusocial (Ward 2014): they live in colonies, in which females range in reproductive capacity from being functionally sterile workers to mated and reproducing queens. Trible et al. and Yan et al. study two ant species that diverged over 100 million years ago, the clonal raider ant (*Ooceraea biroi*) and Jerdon's jumping ant (*Harpegnathos saltator*). In both studies, the authors injected freshly-laid eggs with purified Cas9 protein and synthetic guide RNAs (sgRNAs) corresponding to the targeted locus, *Orco*. Orco is a highly conserved and obligatory transmembrane co-receptor which heterodimerizes with a diversity of ligand-specific olfactory receptors in the Or gene family (Larsson et al., 2004). Among the three families of olfactory receptors in insects, the Or family has undergone a radical expansion in ants (Simola et al., 2013), hypothesized to reflect an increased ability to perceive the cuticular hydrocarbons and pheromones used in ant communication (Tsutsui 2013). However, until the genetic loss-of-function studies presented here, there was no technique that specifically disrupts the function of *Orco* (or any other gene) to investigate its function.

Ants use haplodiploid sex determination; haploid eggs develop as males and diploid eggs develop as females. In many ant species, the difference in reproductive capacity between workers and queens is entirely epigenetic, as both develop from diploid eggs. This situation is analogous to the relationship between somatic and germline cells in a multicellular organism, which differ only in epigenomic state. The clonal raider ant, *O. biroi,* is an all-female species in which diploid females asexually produce isogenic diploid daughters via parthenogenesis (Figure 1A). This facilitates transgenics because injected G0 eggs surviving till adulthood can be screened for successful *Orco* knockout (assuming the mutant is not lethal), so mutant lines can be quickly propagated. The obvious downside of clonal reproduction in *O. biroi* is that crosses are impossible. In *H. saltator,* as in many other ant species, mated diploid females lay both unfertilized haploid male eggs and fertilized diploid female eggs. However, *H. saltator* workers display a form of reproductive plasticity in which a change in social context can induce a non-reproductive worker to become a "gamergate"—an unmated female capable of producing sons by laying unfertilized haploid eggs (Figure 1B). Yan et al. take advantage of the gamergate transition of *H. saltator* to generate hemizygous mutant males, which can then be backcrossed to receptive females, producing heterozygous and homozygous mutant females.

In both *O. biroi* and *H. saltator*, homozygous *Orco* loss-of-function individuals display similar patterns of altered behavior and decreased reproductive performance, supporting the hypothesized centrality of Orco in mediating the perception of olfactory stimuli in ants. Trible et al. demonstrate that compared to heterozygous *Orco* mutant lines of *O. biroi*, homozygous *Orco* mutants are less likely to be repelled by noxious odors in a walking assay, less likely to walk in trajectories resembling their wild-type nestmates after a nest disturbance, and more likely to aimlessly wander around the nest. Additionally, homozygous mutants lay fewer eggs and have higher mortality. Yan et al. use a more complicated olfactory assay to find that *Orco* null mutants of *H. saltator* have strongly decreased sensitivity to various pure semiochemicals delivered in puffs of air, and also note increased levels of wandering outside of the nest, decreased reproductive capacity, and unique antennae-twitching behavior. These results are all consistent with the central role of olfaction for ant behavior.

Unexpectedly, both groups also find gross neuroanatomical defects in the antennal lobe of *Orco* mutants, the initial olfactory processing center in insect brains. The insect antennal lobe consists of discrete glomeruli; each glomerulus is the target of axons of a single type of olfactory receptor neurons expressing a specific olfactory receptor, an organizational principle also conserved in mammals (Komiyama and Luo, 2006). The antennal lobe of *Drosophila melanogaster Orco* mutants has normal number of glomeruli but exhibit age-dependent axon degeneration (Chiang et al., 2009). The dramatic reduction of glomerular

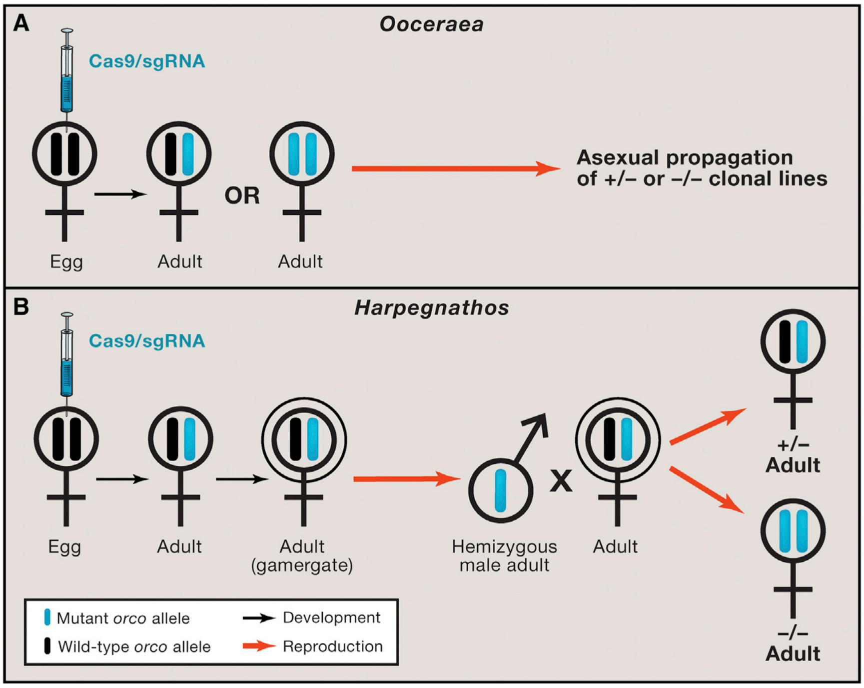

Figure 1. Reproductive diagrams for *Ooceraea biroi* and *Harpegnathos saltator*
(A) *Ooceraea biroi*.
(B) *Harpegnathos saltator*.

number in *Orco* mutants in both ant species implies a novel neurodevelopmental role for Orco, perhaps related to the drastic expansion of glomerular number (300–500 in ants compared to ~50 in *D. melanogaster*). This is a subject worthy of further investigation.

The work of Trible et al. and Yan et al. together represent a watershed moment for the budding field of eusocial insect genetics. Ants are endemic to a wide range of ecosystems, from the damp leaf litter of the tropics to the desiccating heat of the desert. Simple olfactory interactions generate the collective behavior of colonies. How has this tremendous ecological heterogeneity been facilitated by neurophysiological and epigenetic plasticity (Friedman and Gordon, 2016; Gordon, 2016)? What has been the role of genomic changes, for example the drastic expansion of the Or gene family, in accommodating adaptation to such diverse environments? Exploring these fascinating questions will elucidate the evolution of the world's most ecologically-dominant invertebrate societies.

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