

- C. elegans* larval development by neuronal expression of a TGF- $\beta$  homolog. *Science* 274, 1389–1391.
15. Fujiwara, M., Sengupta, P., and McIntire, S.L. (2002). Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron* 36, 1091–1102.
16. Greenspan, R.J., Tononi, G., Cirelli, C., and Shaw, P.J. (2001). Sleep and the fruit fly. *Trends Neurosci.* 24, 142–145.
17. Cirelli, C., and Tononi, G. (1999). Differences in brain gene expression between sleep and waking as revealed by mRNA differential display and cDNA microarray technology. *J. Sleep Res.* 8 (Suppl. 1), 44–52.

<sup>1</sup>Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK. <sup>2</sup>MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK. E-mail: [debono@mrc-lmb.cam.ac.uk](mailto:debono@mrc-lmb.cam.ac.uk)

DOI: 10.1016/j.cub.2008.01.002

## Social Evolution: Reincarnation, Free-Riding and Inexplicable Modes of Reproduction

We like to believe that human societies are the most complex in the animal kingdom, with intricate family structures and a unique repertoire of sophisticated social interactions. A new study reveals an insect society so complex that we are forced to reconsider our role as conquerors of complexity.

Seirian Sumner<sup>1</sup> and Laurent Keller<sup>2</sup>

Social Hymenoptera — ants, some bees and wasps — are renowned for their vast, complex societies. Their simplest societies comprise a single reproductive queen mated to a single male, but most are more complex, consisting of multiple queens and even multiple, multiply mated queens. The ultimate eusocial outcome is the evolution of a specialized caste of workers who forage, rear brood and defend the colony. Workers cannot mate, but the hymenopteran haplodiploid system of sex determination means they retain the ability to lay male (haploid) eggs which, if not ‘policed’ (eaten) by other workers, add extra complexity to the emerging mosaic of family life.

Cape honeybee (*Apis mellifera capensis*) colonies have a single queen which, like all honeybee queens, mates with many males. Uniquely, workers of this species can reproduce asexually, resulting in diploid female offspring. They therefore have the potential to reincarnate themselves genetically as queens, and compete with their mother queen and fellow sisters over queen production. To maximize the transmission of genes between generations, workers should want to lay their own queen eggs (genetic relatedness  $r \sim 1$ ), but they have no preference over raising sisters or nieces ( $r = 0.3$  in both instances, assuming effective paternity of 10). By contrast, the queen is equally related to her daughters and worker-laid granddaughters and so should be

indifferent as to who lays the eggs. On the basis of these unusual relatedness values, Greeff [1] predicted that worker policing should be absent or reduced in the Cape honeybee, such that workers contribute substantially to the production of new queens [2].

To test this prediction, Jordan *et al.* [3] swapped queens or brood between colonies in order to distinguish queen-produced from worker-produced offspring by microsatellite genotyping. They found that almost 60% of the new queens were offspring of workers. At face value, this appears to be unequivocal evidence of an absence of worker policing, as predicted by kin-selection theory if worker reproduction does not entail too high a cost at the colony level [1,4]. However, the genetic analyses revealed unanticipated complexity, with over 65% of worker-produced queens being the offspring of workers originating from foreign colonies. This has two important consequences. Firstly, because the eggs of such ‘drifting’ workers might not be policed as efficiently as those of natal workers, we cannot conclude that the high rate of worker-produced queens stems from a low rate of worker policing. Secondly, the genetically based prediction that worker policing should be reduced becomes largely invalid given that unrelated workers contribute most to queen production. Instead, we would expect strong selection for policing by resident workers, as previously reported by Pirk *et al.* [5],

although several other studies have reported an absence of policing [6–8].

Social parasitism by drifting workers has been reported in other (arrhenotokous) honeybees. In the Cape honeybee, the added incentive of producing parthenogenetic queens ( $r \sim 1$ ) makes social parasitism a lucrative strategy. Drifting behaviour might therefore be more common in species where parthenogenetic production of queens by workers is possible. Although scarce, the available data are in line with this prediction, with the average proportion of drifting workers in Cape honeybees (6.9%) being 2.5 times higher than in other honeybee species [9]. (It remains to be tested whether the swapping of queens and brood may have altered the recognition mechanisms and consequently the rate of worker drifting and policing of worker-laid eggs. For example, exposure to eggs from several queens may increase worker acceptance of non-nestmate brood [10].) Jordan *et al.* [3] suggest that the propensity to drift may be genetic. If this is true, then social parasitism may be a behavioural polymorphism for an alternative reproductive strategy, maintained in the population by balancing selection. Colonies with worker policing will exclude reproductive free-riders, thus avoiding the costs of parasitism but also missing out on producing parthenogenetic queens. Colonies that lack worker policing allow worker queen production but run the risk of being parasitized. Thus, the frequency of a drifting (or policing) genotype may be maintained in the population by frequency-dependent balancing selection, in a similar way to classical host–parasite systems.

An analysis of the microsatellite genotypes revealed unanticipated complexity in the reproductive genetic system. Three of the brood were homozygous and shared alleles with the resident queen at all six microsatellite loci. These brood were

unlikely to be haploid males, or arise because the queen's mate or the foreign worker(s) share one allele with the queen at each locus. The most parsimonious explanation is that these brood were parthenogenetic offspring of queens. Previous studies revealed that Cape honeybee workers reproduce by automictic parthenogenesis with central fusion, restoring diploidy by fusion of the two central meiotic products. Consequently, offspring share the same genotype as their mother at loci that did not cross-over. Under this mode of reproduction, the probability that a heterozygous locus becomes homozygous is at most one-third per generation [11]. Jordan *et al.* [3] found much higher rates, with three of the 16 queen-produced daughters and seven of the 23 worker-produced daughters being homozygous at all loci. Other modes of parthenogenesis that might explain the high rate of homozygosity — such as apomictic parthenogenesis or automictic parthenogenesis with terminal fusion — are incompatible with the sex determination mechanism in honeybees, where only individuals heterozygous at the sex-determining locus become female; eight of the 10 homozygous brood were shown to be female through morphology or microsatellite analysis at the U\_351B microsatellite, which is located near the sex locus.

Two possibilities may explain the apparent preservation of heterozygosity at the sex-determining region in highly homozygous individuals. First, there may have been genomic duplication in the sex-determining region, such that the U\_351B primers amplify two different microsatellites and homozygous individuals might appear heterozygous at U\_351B (if the duplicated regions are different sizes) and develop as females (if the two copies of the sex-determining locus are functional). The second possibility is that a peculiar system of reproduction maintains heterozygosity in the sex chromosome region, via automictic parthenogenesis with terminal fusion (in the absence of recombination). But this would require the sex locus to be located near the centromere where there is little recombination, which is not the case in the honeybee [11]. We propose a novel mode of reproduction to explain these data, involving ameiotic

parthenogenesis with preferential elimination of chromosomes that do not bear the sex-determining locus. This would lead to female offspring bearing the same genotype as their mother at the chromosome with the sex-determining locus, and haploid genotypes at all other chromosomes. This could be tested by determining the ploidy of homozygous females for each chromosome individually.

The Jordan *et al.* [3] study adds an unanticipated level of complexity to the already intricate life of the Cape honeybee. A colony comprises an eclectic cocktail of relatives and non-relatives, with the mother queen, her aberrant daughters workers and their asexual royal offspring, the queen's own royal offspring (both sexual and asexual!), and the asexual offspring of numerous unrelated free-riders. This family structure, the most baroque reported in any living organism, is yet more evidence that the consort of haplodiploidy and caste-determination systems sets the evolutionary stage for the emergence of extraordinary reproductive and genetic systems in social insects [12–16].

#### References

1. Greeff, J.M. (1996). Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Phil. Trans. R. Soc. Lond. B* 351, 617–625.
2. Beekman, M., and Oldroyd, B.P. (2008). When workers disunite: intraspecific parasitism in eusocial bees. *Annu. Rev. Entomol.* 53, 19–37.
3. Jordan, L.A., Allsopp, M.H., Oldroyd, B.P., Wossler, T.C., and Beekman, M. (2007). Cheating honeybee workers produce royal offspring. *Proc. R. Soc. Lond. B* 275, 345–351.
4. Hammond, R.L., and Keller, L. (2004). Conflict over male parentage in social insects. *PLoS Biol.* 2, 1472–1482.
5. Pirk, C.W.W., Neumann, P., and Ratnieks, F.L.W. (2003). Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits. *Behav. Ecol.* 14, 347–352.
6. Calis, J.N.M., Boot, W.J., and Allsopp, M.H. (2003). *Capensis* honeybees: crucial steps leading to social parasitism. *Proc. Exper. Appl. Entomol. NEV* 14, 39–43.
7. Beekman, M., Good, G., Allsopp, M.H., Radloff, S.E., Pirk, C.W.W., and Ratnieks, F.L.W. (2002). A non-policing honey bee colony (*Apis mellifera capensis*). *Naturwiss.* 89, 216–220.
8. Moritz, R.F.A., Kryger, P., and Allsopp, M.H. (1999). Lack of worker policing in the Cape honeybee (*Apis mellifera capensis*). *Behav.* 136, 1079–1092.
9. Sumner, S., Lucas, E., Barker, J., and Isaac, N. (2007). Radio-tagging technology reveals extreme nest drifting behaviour in a eusocial insect. *Curr. Biol.* 17, 140–145.
10. Helantera, H., Martin, S.J., and Ratnieks, F.L.W. (2007). Prior experience with eggs laid by non-nestmate queens induces egg acceptance errors in ant workers. *Behav. Ecol. Sociobiol.* 62, 223–228.
11. Baudry, E., Kryger, P., Allsopp, M.H., Koeniger, N., Vautrin, D., Mougel, F., Comuet, J.M., and Solignac, M. (2004). Whole genome scan in thelytokous-laying workers of the Cape honey bee (*Apis mellifera capensis*): central fusion, reduced recombination rates and centromere mapping using half-tetrad analysis. *Genetics* 167, 243–252.
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. Helms-Cahan, S., and Keller, L. (2003). Complex hybrid origin of genetic caste determination in harvester ants. *Nature* 424, 306–309.
14. Pearcy, M., Aron, S., Doums, C., and Keller, L. (2004). Conditional use of sex and parthenogenesis for workers and queen production in ants. *Science* 306, 1780–1783.
15. 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.
16. 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.

<sup>1</sup>Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK. <sup>2</sup>Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland.  
E-mail: Seirian.Sumner@ioz.ac.uk, Laurent.Keller@unil.ch

DOI: 10.1016/j.cub.2008.01.004

## Evolutionary Biology: Genomic Clues to Original Sex in Fungi

Sexual identity in fungi is conferred by a diverse group of transcription factors. Analysis of a Zygomycota genome, representing a basal branch within the fungi, indicates that HMG-domain proteins were present as ancestral sex determinants and suggests a mechanism for the evolution of eukaryotic sex chromosomes.

Paul S. Dyer

Fungi have some of the most fascinating and diverse sex lives in

nature. They include species with two defined sexes of opposite 'mating type', which undergo typical mating between complementary partners