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A critique of "Asexually Produced Cape Honeybee Queens (*Apis mellifera capensis*) Reproduce Sexually," authors: Madeleine Beekman, Michael H. Allsopp, Julianne Lim, Frances Goudie, and Benjamin P. Oldroyd. Journal of Heredity. 2011:102(5):562–566

Reproductive Biology of the Cape Honeybee: A Critique of Beekman et al.

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Abstract

Laying workers of the Cape honeybee parthenogenetically produce female offspring, whereas queens typically produce males. Beekman et al. confirm this observation, which has repeatedly been reported over the last 100 years including the notion that natural selection should favor asexual reproduction in *Apis mellifera capensis*. They attempt to support their arguments with an exceptionally surprising finding that *A. m. capensis* queens can parthenogenetically produce diploid homozygous queen offspring (homozygous diploid individuals develop into diploid males in the honeybee). Beekman et al. suggest that these homozygous queens are not viable because they did not find any homozygous individuals beyond the third larval instar. Even if this were true, such a lethal trait should be quickly eliminated by natural selection. The identification of sex (both with molecular and morphological markers) is possible but notoriously difficult in honeybees at the early larval stages. Ploidy is however a reliable indicator, and we therefore suggest that these "homozygous" larvae found in queen cells are actually drones reared from unfertilized eggs, a phenomenon well known by honeybee queen breeders.

The Cape honeybee, *Apis mellifera capensis*, shows several unique features in relation to reproduction and pheromonal dominance, which makes it an excellent model system for understanding the evolution of reproductive dominance in social hymenoptera. This is partly due to the fact that workers of the Cape honeybee are able to produce diploid female offspring from unfertilized eggs (Onions 1912; Anderson 1963), a process called thelytoky (Crozier 1975). These female offspring can then develop either into workers or queens (Anderson 1963; Ruttner 1977). The underlying cytological mechanism is well understood and is based on automictic parthenogenesis with a central fusion (Verma and Ruttner 1983) that prevents meiotic recombination (Moritz and Haberl 1994). Even recombination due to crossing over is heavily reduced (Baudry et al. 2004).

Recently, Beekman et al. (2011) reported in this journal that asexually produced Cape honeybee queens reproduce sexually. This is correct, but far from being a novel finding. Over the past 100 years, it has been repeatedly reported that new queens produced in *A. m. capensis* colonies were laying worker offspring (Onions 1912; Anderson 1963; Ruttner 1977; Moritz et al. 1996; Allsopp and Hepburn 1997; Moritz et al. 2011), and these queens behave no differently from queens produced from fertilized eggs (Jack 1917; Hepburn and Guye 1993; Tribe and Allsopp 2001).

Thelytokous parthenongenesis in the Cape honeybee results from the automictic fusion of the 2 central of the 4 meiotic products to restore diploidy (Tucker 1958; Verma and Ruttner 1983). Therefore, the question in Beekman et al. (2011) should not be whether mated queens produce diploid worker offspring from fertilized eggs or reproduce asexually; but rather whether a mated Cape honeybee queen of laying worker origin can produce male offspring. To address this question, the authors should have sampled larvae from drone cells rather than worker cells (Beekman et al. 2011). However, even such a finding would have had only rather limited novelty since again it has been shown repeatedly over the past 100 years that impaternate female progeny of virgin queens can occur (Onions 1912; Mackensen 1943; Tryasko 1969; Tryasko 1975; Velthuis et al. 1990; Oldroyd et al. 2008). However, the conditional use of sex when producing workers or queens reported from 2 ant species (Pearcy et al. 2004; Fournier et al. 2005) might also occur in Cape honeybees but has not been addressed experimentally by Beekman et al. (2011). For this purpose, they should have sampled both larvae from queen and worker cells. Nevertheless, recently it has been shown that queens fertilize eggs when producing queens and workers (Moritz et al. 2011).

Beekman et al. (2011) finally argue that natural selection should favor asexual reproduction in *A. m. capensis*. Indeed, this is true for a wide region of the fitness parameter space as had been noted several decades ago (Moritz 1989; Greeff 1996a, 1996b). However, Beekman et al. (2011) attempt to support that contention with the results of Jordan et al. (2008) who claimed to have found parthenogenetically produced homozygous female offspring. The evidence for this exceptionally surprising finding (homozygous diploid individuals develop into diploid males in the honeybee that are cannibalized by workers, Mackensen 1951) was, however, weak. Given the fundamental discrepancy of the basic knowledge on both sex determination and the cytogenetics of thelytokous parthenogenetics in honeybees, the data should have been supported by a cytogenetic confirmation of previous studies on the thelytokous parthenogenetic mechanism in the Cape honeybee. Because central fusion prevents any recombination of chromosomes, the identical genotype of the mother is restored apart from rare crossing over events (Moritz and Haberl 1994; Neumann and Moritz 2002; Baudry et al. 2004). Unless a robust analysis confirms that the sampled larvae were female, it seems more parsimonious to explain the observed homozygosity by Jordan et al. (2008) as male eggs being laid accidentally in queen cells, which is a common phenomenon in apicultural queen rearing (Fell and Morse 1984).

We feel Beekman et al. (2011) do not produce an evolutionary plausible explanation by stating that they could not find any homozygous individuals beyond the third larval instar because they are not viable. Clearly, any lethal trait is exposed to strong selection and if this were the result of the thelytoky trait in *A. m. capensis*, we would simply not be able to use this honeybee as a model for studying the evolution of social behavior today.

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Response to "Reproductive Biology of the Cape Honeybee: A Critique of Beekman et al." by Pirk et al.

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In our paper "Asexually produced Cape honeybee queens (*Apis mellifera capensis*) reproduce sexually" (Beekman et al. 2011), we report the simple finding that queens known to be the asexual offspring of workers mate and produce new workers sexually. Our rationale for the study was 2-fold. First, we knew that unmated *Apis mellifera capensis* queens can lay eggs both thelytokously and arrhenotokously (Oldroyd et al. 2008), and we thus wanted to see if our queens had mated or were laying workers thelytokously. Second, asexually produced queens would not be able to produce offspring sexually if thelytoky is strictly genetically determined (Lattorff et al. 2005, 2007) and transferable across castes.

Pirk et al's critique appears to revolve around 2 main points: 1) the fact that it has been known for more than a century that *A. m. capensis* queens are produced by laying *A. m. capensis* workers and that these queens reproduce sexually; and 2) the contention that completely homozygous larvae found in queen cells are more likely to be diploid drones than females thelytokously produced by the resident queen. The latter point is not the topic of the paper Pirk et al. seek to critique and is a reference to an earlier publication (Jordan et al. 2008). Nevertheless, we shall respond to both issues.

We fully appreciate that it has been "known" for a century that queenless *A. m. capensis* colonies are capable of requeening themselves from laying worker offspring and that these new queens "behaved no differently from queens produced from fertilized eggs." To the best of our knowledge, however, this has never been genetically confirmed. As "normal" as these colonies appear, it remained possible that worker offspring in these colonies were thelytokously produced. Beekman et al. (2011) confirm what was always believed but previously never known: that the thelytokously produced queens of Cape honeybees reproduce sexually and are indeed normal.

We are at a loss with respect to the suggestion made by Pirk et al. that we should have investigated if these queens could produce haploid drones. As we have shown that all the queens are able to reduce the genetic complement allowing for fertilization resulting in diploid females, their ability to produce haploid drones is surely trivial.

We acknowledge that the origin of the completely homozygous larvae found by Jordan et al. (2008) remains elusive. We have attempted to use the technique described in Oldroyd et al. (2011) to determine if the homozygous individuals were heterozygous at the region flanking the complementary sex-determining locus, *csd*. Our unpublished result showed that at