

Exploited superorganisms – how life history shapes the reproductive strategies of honeybees

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Not all honeybees stick to the likable picture that is often drawn of them: In South Africa, cape honeybees (*A. mellifera capensis*) can make a pseudoclone of themselves the next queen of a colony. They are, from a genetic point of view, a real threat to their own queen. They will pass their own genome on to the next generation instead of providing support for the whole colony. Scientists have called these individuals “reproductive cheats“. In this essay I will use this example to show how changes in the mode of reproduction of organisms are linked to social parasitism within species and that several reproductive strategies can emerge and exist in parallel. This depends on the life circumstances of the individual.

Generally, honeybees are characterized by a *reproductional hierarchy*, where only one individual – the queen – reproduces. Other females of the group “selflessly sacrifice their reproductive opportunities [and thus] their own selfish interests” (Holmes and Beekman, 2016). Important features of this “monopolized reproduction” (Jarosch, 2012) are:

- 1) haplo-diploidy: fertilized eggs are diploid and develop into females, non-fertilized eggs are haploid and develop into males, which are clones of the queen.
- 2) the ability of workers to reproduce parthenogenetically, and
- 3) the tendency of queens to mate with multiple males (polyandry). (Holmes et al., 2015)

Beekeepers therefore do not only see the queen as the mother of the colony, they also know that different subfamilies in a hive have different *father-queens*, whose clones fertilized the young queen. Mating flights occur only in the beginning of a queen's life. These flights are dangerous not only for the queen, but also for the whole colony. If the young queen does not return, the workers will have a big problem to solve: They can only make new queens, as long as they have female larvae to raise with royal jelly – this means, that these bees will be the royal phenotype. In the absence of a queen and her pheromones, a colony can become *hopelessly queenless*¹. Some of the worker bees develop

¹There are several reasons, which could account for a lost queen: (1) She simply died for reasons like old age, attack by other colonies, (2) she is taken away by a beekeeper in order to artificially replicate managed colonies. In these cases, there are still young female larvae left in the hive. A third way (3) for a queen to leave her colony is the swarming process, the annual natural reproduction process of the colony as a super-organism. In this case, there are queen cells left behind within the colony, which have already reached the state of the first metamorphosis. These

functional ovaries and parthenogenetically produce male eggs². This is a way for the colony to spread much of its gene-pool, even if the colony itself is doomed because males do not take part in reproductive tasks such as foraging and rearing brood.

South African honeybees (*Apis mellifera capensis*) have long been known to behave differently. The first scientific report of this dates back to 1912: In queenless hives, queen cells with fully-developing larvae were reported. Initially this was explained as the bees “stealing eggs from other hives” (Onions, 1912). But soon it became evident, that in this species the asexual production of females (= thelytoky)³ was rather the rule than the exception⁴. Thelytoky is an important advantage in case of queen losses (Neumann et al., 2011). Holmes et al. (2010) found that replacement queens are often of worker origin. But also in a queenright, non-emergency situation around 10% of all the workers produced during one season seemed to be the daughters of worker bees (Beekman 2009). During the swarming period, as much as 40-60% of queens are of worker origin (Allsopp et al., 2010). Capensis workers seem to prefer to lay their eggs into queen cells and use a “window of opportunity” around the swarming time to specifically place their eggs and increase the possibility for them to become queens. Some of these “cheating“ workers do not even originate from that colony. Thelytokously produced queens generally reproduce sexually again (Beekman et al., 2011). From a Weismann-Darwinistic point of view, a worker whose genetic clone is the next queen of a colony, has an enormous fitness benefit over a worker that is helping the colony to raise her 0.25-0.75% related siblings (Holmes and Beekman, 2016). This applies even more, if they can take over a foreign colony.⁵ Thelytoky in honey bees can therefore also be an incentive for competition between reproducing workers.

This behavior is not exclusively found in *A. m. capensis*, even though it seems to be very rare in other honeybee species: When *A. mellifera* virgin queens are exposed to carbon dioxide they can be induced to lay eggs. Mackensen (1947) used this technique and found that sometimes they produced female eggs. Recently, in *A. cerana*, an Asian honeybee species, indications of thelytoky have also been reported (Holmes et al., 2015).

But coming back to the peculiar case of *A. m. capensis*: One lineage of laying workers has apparently spread

cells are already capped and only depend on the right temperature to develop into queens within 12 days.

²Workers physically hinder their sisters from reproducing and remove worker-laid eggs. These mechanisms have been called “policing”. They could explain the evolutionary process to suppress workers ovaries development in the first place, because there would be an energetic loss for the individuals who are trying to reproduce without an outcome (aquiescence theory) Wenseleers et al. (2004).

There are hardly found any worker eggs in queenright honeybee colonies of any subspecies. However, recently it has been shown that hopelessly queenless colonies attract worker bees from other hives. They use the willingness of natal workers to stop policing and breed worker-layed eggs, thus reproducing successfully in a non-natal colony. Strangely but in accordance with commercial beekeeper’s experiences, in some colonies no worker-laid eggs can be found, ever after being hopelessly queenless for a prolonged period of time. Maybe, these colonies have never turned off their policing mechanisms (Chapman et al. 2010).

³Thelytoky involves fusion of the meiotic products, two nonhomologous pronuclei as if one of the nuclei acted as a sperm. The precise mechanisms and the risk of them are explained in Oldroyd et al. (2008).

⁴Thelytoky is rare among animal taxa, where sexual production dominates. In eusocial haplo-diploid species, however, it seems to be quite common, especially in ants (Rabeling and Kronauer (2013), for a review see Goudie and Oldroyd (2014)).

⁵How could reduced self- reproduction have evolved in the first place? Hamilton’s inclusive fitness theory gives an explanation: Assuming that worker reproduction reduces the rearing capacity of the colony, it would be beneficial for workers to support their colony rather than focusing on their own reproduction (Hamilton (1964) as cited in Wenseleers et al. (2004)). A colony is made up of several subfamilies with different father-queens, workers are mostly half-sisters. Thus, workers share more genes with the drones laid by their mother, than by drones laid by their sisters. This would lead to inclusive fitness benefits (Ratnieks (1988) as cited in Chapman et al. (2010)).

to hives of another subspecies, *A. m. scutellata*.⁶ Workers invade *scutellata* colonies and become “pseudoqueens, an intermediate caste with [ovaries and] queenlike pheromone[s]”. This entirely asexual lineage is commonly called “the clone”. This clone has caused much damage and is held responsible for the loss of hundreds of *scutellata* colonies (Moritz, 2002), because its pseudoqueens and workers do not contribute to the hives’ foraging and rearing activities. Thus, the colonies inevitably collapse. Neumann et al. (2011) discovered that all the invasive *capensis* individuals could be traced back to one ancestral worker genome. As an intraspecific social parasite, it is sometimes compared to a form of cancer in eusocial superorganisms (Goudie and Oldroyd, 2014)⁷. Looking at the honeybee genome, thelytoky brings along a lot of costs, which are still being discussed. Overall, thelytokous reproduction seems to imply a reduced viability in each succeeding generation.⁸

All in all, in *A. m. capensis* three reproductive strategies can be observed:

- 1) *The queen* shares the sexual reproductive approach of other honeybee subspecies.
- 2) Asexually reproducing *workers* use a window of opportunity to make their daughters the next queen of the hive, but otherwise rarely reproduce.
- 3) Only a *parasitic lineage* can endure the cost of asexuality: With high rates of reproduction and low maternal investment it is enough that only a few of the eggs survive. They restart the parasitism circle and „it hardly matters if many of [the eggs] are inviable“.

The example of *Apis mellifera capensis* shows how costs and benefits of sexual reproduction very much depend on the life history of the individual and its environment, rather than on its genome.

⁶The emergence of the clone was a human-influenced process and very much depends on beekeeping practices. It would not have occurred without the transference of many hives from one region to another (Hepburn and Allsopp (1994)). It seems, that *A. capensis* does not become parasitic within its natural range, which is separated from the *A. scutellata* range by a hybridization zone (Beekman et al. (2008)). Do we witness an example of human-induced processes of allopatric species evolution (cp. Neumann and Moritz (2002), Neumann et al. (2011))? Researchers also warn of the genetic consequences of migratory beekeeping (Byatt et al. (2015)).

⁷ Given that Goudie et al. (2012) have reported that rarely also drones of the clone appear, maybe this cancer-like disease could even be sexually transmitted. Later they showed, that the clone and thelytokous workers of sexual *A. capensis* populations can both produce arrhenotokous eggs (=male parthenogenetic). Clone alleles might therefore find their way into the genome of other colonies and subspecies.

⁸e.g. the loss of heterozygosity, which has to be controlled in every generation. For a review on the genetic implications of thelytoky and on the corresponding costs and benefits see Goudie and Oldroyd (2014).

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Outline

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