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Anniversary of a beekeeper's discovery of thelytoky in Cape honey bees

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Significance:

- The laying workers of the Cape honey bee continue to negatively affect the South African beekeeping industry, with more losses suffered in the northern regions of the country.
- The reproductive parasites enter susceptible host colonies, activate their ovaries, and lay diploid eggs, leading to colony dwindling and collapse.
- Diploidy in eggs produced by unmated laying workers arises from thelytokous parthenogenesis, first discovered in honey bees by a hobbyist beekeeper.
- We examine the consequences of thelytokous parthenogenesis and outline what is being done to understand and limit the spread of the laying workers of the Cape honey bee.

Introduction

The existence of thelytoky in Cape honey bees was first documented in 1912 – making 2022 the 110th anniversary of this discovery. The significance of this discovery took a number of decades to be fully appreciated. The biology of sex determination in honey bees – where males are produced from unfertilised eggs while females arise from fertilised eggs – allows the reproductive female to control the sex ratio of her offspring. Hence, queens in honey bee colonies bias the sex ratio in favour of producing large numbers of females (that will be workers) and only producing males seasonally when needed. This gives rise to an exclusively female social group characterised by a single reproductive female (queen) and large numbers of non-reproductive females (workers). As workers are unable to mate, any offspring they produce should be male. Finding that Cape honey bee workers can produce female offspring was an anomaly that required extensive investigation to unravel its biological significance.

South Africa is home to two subspecies of honey bees. One is *Apis mellifera scutellata,* also known as the Savannah honey bee predominantly found in the northern parts of South Africa, extending northwards into various parts of East Africa. The second subspecies – *A. m. capensis* also known as the Cape honey bee – is native to the Western Cape region of South Africa in the Fynbos Biome. Separating these two subspecies is a natural and stable zone of introgression, restricting the naturogenic spread of the Cape honey bee outside of its native region (Figure 1A). *A. m. capensis* workers are usually darker than the typically yellow and black *A. m. scutellata*. Although colour alone is an unreliable feature to separate the subspecies, it has some utility. Unlike honey bee populations in the northern hemisphere, the majority of honey bees in South Africa are wild and not managed.

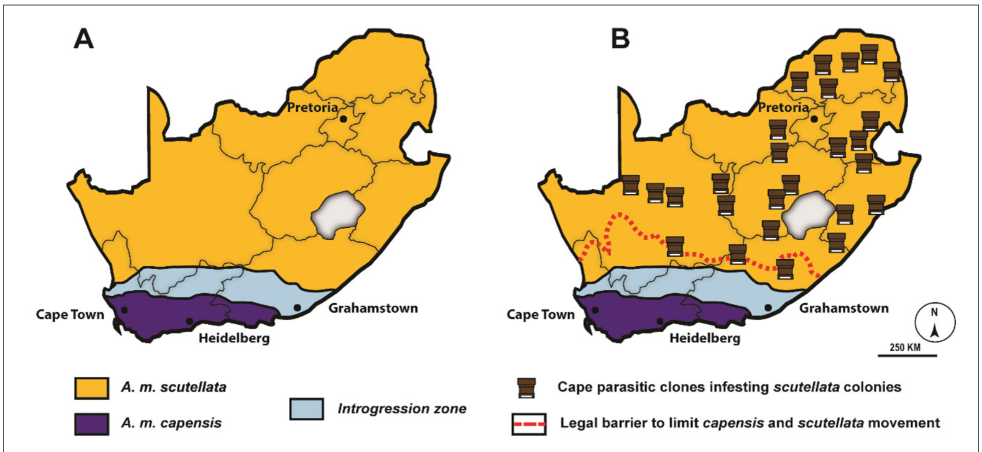

Figure 1: Map of South Africa showing the distribution of the two subspecies of honey bee – *Apis mellifera scutellata* (yellow) and *A. m. capensis* (purple) – (A) prior to 1990 and (B) after the anthropogenic establishment of parasitic laying workers (brown hives) after 1990. The red dotted line shows the legal barrier (Reg. 159/5 February 1993) enacted to restrict the movement of colonies of *A. m. capensis* and *A. m. scutellata* beyond the stable zone of introgression (light blue).

In 1993, beekeepers in South Africa were estimated to have lost 50 000–100 000 honey bee colonies, but not due to pests, diseases or pesticides, which are the usual causes of decline in honey bee populations in many parts of the world.[1] These beekeepers were facing a new challenge, from dark worker bees that were invading healthy but susceptible host colonies, laying unfertilised diploid eggs and producing other dark workers that were also reproductively active. The newly emerged workers would in turn seek out and invade other colonies, eventually leading to dwindling and collapse of the infested hosts, called the 'Capensis calamity'.[2] These laying workers have spread widely into the *A. m. scutellata* native range, with the spread attributed to their presence in managed honey bee colonies (brown hives, Figure 1B). Subsequently, Pirk et al.[3] showed that beekeepers in South Africa were losing about 41% of their colonies annually, with a significant cause of this loss attributed to the presence of *A. m. capensis* parasitic workers.

This ability of Cape honey bee workers to lay eggs that develop into diploid (females) individuals had been described by a little-known hobbyist beekeeper George William Onions (Figure 2), in a landmark paper describing reproductive parasitism, published in the *Agricultural Journal of the Union of South Africa*.[4]

Figure 2: George William Onions (1866–1941), the hobbyist beekeeper who was among the first to make critical and detailed observations of reproductive parasitism by *Apis mellifera capensis* laying workers.

G. W. Onions

George William Onions was born in 1866 to William Onions and Miriam Lockhart in Cape Town, South Africa. He was the second of three children, though unfortunately when he was still young his mother and both siblings died of diphtheria. His father would soon pass away too, leaving the young Onions in the care of his aunt. At the age of 28, he married Jessie Elizabeth Massey and together they had seven children.

Not much is known about his formal education, though from accounts given by his daughters, Onions is said to have been mostly self-taught. By profession, Onions was an electrician, operating a business that installed electrical wiring in buildings. He was also a keen inventor and is said to have invented an X-ray machine and even made a small telescope, which he mounted on a stone and used to observe stars.

His biggest passion, however, was beekeeping and this was evident in his landmark paper 'South African "Fertile-Worker Bees"' published in 1912[4], in which he described himself as '…*a practical beekeeper and queen breeder who has always endeavored to verify in his own experience, the facts and theories of bee culture…*'. From his understanding of scientific bee literature, it seems that he was also a keen reader of this literature and curious about basic honey bee biology.

Indeed, Onions was a beekeeper who purely through observation and basic experimentation came to important discoveries, later verified by researchers using modern analytical techniques. Starting his observations in 1901[4] to 1913[5], he described how in 1901 he clipped the wings of a virgin queen, mistaking it for a mated queen due to the presence of hundreds of freshly laid eggs in the colony. He later found out that the eggs had been laid by workers of African origin that had infested his Italian honey bee colonies (Mr Onions daughter, Mrs E.J. Walton, confirmed that her father imported bees from the USA and Europe, in an effort to breed honey bee races more docile than *A. m. scutellata*). Onions described that the 'dark coloured' bees had the ability to lay eggs, even in the presence of a queen. In describing the life cycle of a colony infested by laying workers, he showed that these workers would seek out susceptible colonies that were small or queenless and activate their ovaries (verified through dissections) to lay eggs. The eggs laid would emerge as workers which – Onions observed – were not performing the typical colony maintenance tasks such as brood rearing, comb building and foraging and would instead become reproductively active. This would eventually lead to the death of the host queen, and dwindling and eventual collapse of the host colony.

Further, Onions recorded that the laying workers had a well-developed 'sperm sac', which was smaller but structurally similar to that of the queen. In 1913, only queens were known to have spermathecae, making this a very controversial claim. He went on to describe that the spermatheca of the Cape bees was empty (unlike that of a mated queen), and thus, these bees had produced workers from unfertilised eggs. This observation was in clear disagreement with the prevailing understanding of the time as described by the Polish theologian and bee scientist Johann Dzierżoń who in 1845 discovered that unfertilised eggs produced males and thus drones while fertilised eggs produced females and thus workers or queens (Figure 3A). By 1906, Dzierżoń's observations were accepted

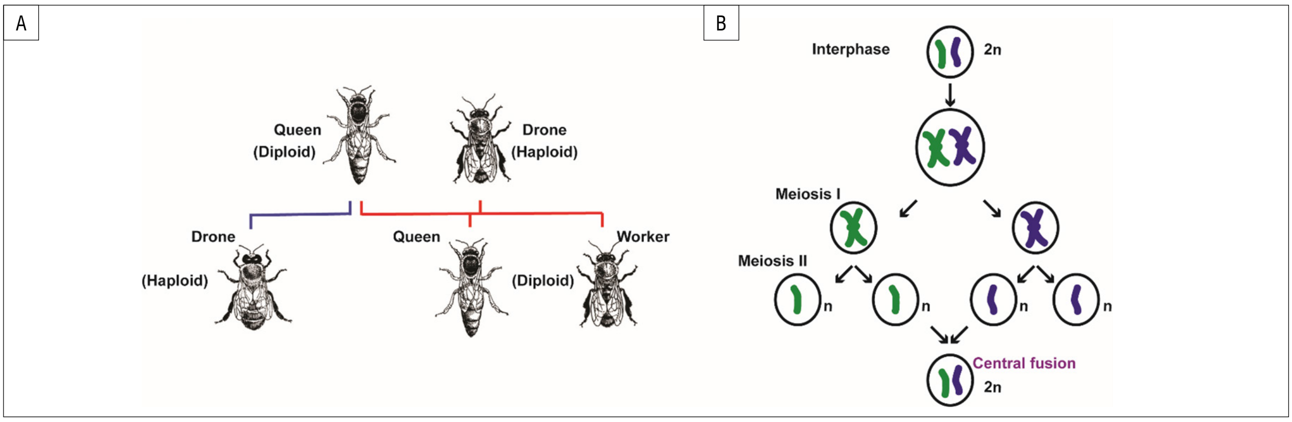

Figure 3: Sex determination by haplodiploidy as described by Johann Dzierżoń in 1845 where (A) unfertilised (haploid) eggs become drones while fertilised eggs become workers or queens. However, the diploid state can be restored in thelytokous parthenogenesis (B), through the fusion of the two central pronuclei in meiosis II, as happens in the laying workers of *Apis mellifera capensis*, leading to emergence of females from unfertilised eggs, thus being an exception to Dzierżoń's rule.

as the established mode of sex determination in honey bees, therefore requiring Onions to defend his observations that the reproductively active Cape honey bee workers were laying unfertilised eggs that led to the emergence of female bees. It would be more than 75 years before Savitri Verma and Friedrich Ruttner in 1983 explained how diploidy is restored in these bees, experimentally confirming Onion's observations.[6]

For the time being though, Onions had to contend with working with Rupert Jack, a Rhodesian government entomologist who took it upon himself to validate the veracity of Onions' incredible claims[7] given the fact that Onions was not an entomologist. One of the fiercest critics of the observations by Onions came from van Warmelo who refuted Onions' conclusions based on the findings of Dzierżoń and claimed that if the 'fertile workers' were producing workers and not drones, these fertile workers must have mated with drones.[8]

Prior to 1910

A previous attempt had been made to explain the curious behaviour of the laying workers of *A. m. capensis* by Sir Henry de Villiers who, as well as serving as the first Chief Justice of South Africa, was an avid beekeeper. In a study published in 1883, Lord de Villiers described his discovery of eggs laid in cells in a colony that had just recently moved into his hive, and that had caged its queen to prevent her from absconding. In further observations, he noticed that the adult bees emerging from these eggs were workers. Possibly based on Dzierżoń's work and given that there was no other queen in the colony, Lord de Villiers concluded that the eggs must have been laid by the caged host queen and passed through the holes of the cage to workers who then deposited them in the brood cells.[9]

1920–1960

A large portion of the bee research in South Africa at this time focused on attempts to breed a more docile race of the South African bees, through the importation of European bees. Much of this work was championed by A. E. Lundie (Bee Culture Laboratory of the Bureau of Entomology). Although not much research into the laying workers of *A. m. capensis* took place during this time, Lundie published an article confirming the ability of Cape honey bees to lay worker-destined eggs.[10]

It was R. H. Anderson (Agricultural Department, Plant Protection Research Institute of the Agricultural Research Council) who first reexplored the findings of Onions. Anderson reported that laying workers were present in *A. m. capensis* colonies, even in the presence of the queens.[11] Further, he showed that, following the removal of a queen, infighting would take place, after which some workers would quickly activate their ovaries and begin laying eggs. In validating Onions' findings, Anderson further showed that the laying *A. m. capensis* workers had activated ovaries with many ovarioles per ovary, a large spermatheca that contained no sperm and, very importantly, that the laying workers were producing female offspring parthenogenetically. This was 50 years after Onions' groundbreaking paper.

After 1970

Prior to 1970, beekeeping in South Africa was mainly done for the purpose of producing hive products such as honey, wax, and propolis. However, the industry underwent a major transition after 1970 with the realisation that there was a demand for pollination services that could be more lucrative than simply selling hive products. To achieve this, the beekeepers began moving their colonies from place to place – a process that greatly facilitated the spread of the *A. m. capensis* laying workers, leading to dwindling and collapse of the infested host colonies.

The Capensis calamity

In 1912, Onions reported his detailed observations describing the host-seeking behaviour of *A. m. capensis* laying workers, activation of ovaries and laying eggs and the dwindling and eventual collapse of infested colonies.[4] It would be more than 80 years before these laying workers would be recognised as a major problem for the beekeeping industry in South Africa, as described by Allsopp and Crewe[2]. The report followed a major outbreak of *A. m. capensis* laying workers that occurred in 1990–1991, in areas outside of the native range of *A. m. capensis* (Figure 1). Prior to this, there had been two other outbreaks in the 1980s as reported by Geoff Tribe and Martin Johannsmeier, although these outbreaks were on a small scale and easily contained. The 1991 outbreaks, however, involved a large number of *A. m. capensis* colonies moved outside their native region and also transport of *A. m. scutellata* colonies to the *A. m. capensis* native regions and back, greatly facilitating the spread of parasitic workers.[12]

Dietemann et al.[12] and Pirk et al.[3] showed that migratory beekeepers face higher colony losses due to the laying *A. m. capensis* parasites, and herein lies the crucial role that beekeepers can play in stopping the 'Capensis calamity'. As recommended by many researchers investigating the nature and impact of the 'Capensis calamity' on South African apiculture, beekeepers should only utilise locally caught bee colonies for beekeeping and should limit the migration of bees from place to place as migration increases the risk of exposure of colonies to infestation by parasitic workers. Further, the *A. m. capensis* honey bees should never be moved outside of their native range and into that of the *A. m. scutellata.*[13]

Huge economic losses sustained by beekeepers, the increased distribution of the parasitic laying workers and the potential threat to wild honey bee populations and to food security posed by the continuing spread of the *A. m. capensis* laying workers refocused the attention of researchers and other stakeholders of the beekeeping industry. More scientists shifted their attention to this issue, trying to understand the biology of the reproductive parasites, how these laying workers seek out susceptible colonies and evade detection by host workers, and why they are able to fully activate their ovaries and lay eggs, even in the presence of the host queen.

While most of the research prior to 1970 was based almost purely on field observations, the advent of more advanced scientific tools in gas chromatography (GC), microscopy and molecular genetics enabled researchers to address deeper questions needed to understand the biology of the *A. m. capensis* laying workers. In great collaborative and multidisciplinary efforts, scientists teamed up to work on various aspects of the biology of the laying workers, as reviewed by Mumoki et al.[14], confirming and extending the observations reported by Onions in 1912[4] and 1914[5].

Through backcross experiments, Verma and Ruttner[6] showed that the restoration of the diploid condition in *A. m. capensis* worker-laid eggs occurs through fusion of the two central pronuclei, in what is known as thelytokous parthenogenesis, thereby explaining the emergence of diploid workers from unfertilised eggs laid by unmated workers (Figure 3B). Further, using GC and GC-mass spectrometry, Crewe and Velthuis[15] and others[14] demonstrated that – in addition to the presence of a spermatheca, high ovariole number per ovary and an ability to rapidly activate their ovaries to lay eggs – the *A. m. capensis* laying workers were also producing queen-associated mandibular gland pheromones. These queen-associated signals are not just from the mandibular glands but also from the Dufour's and the tergal glands – a further indication of the involvement of multiglandular pheromone signals in establishing reproductive dominance. Various molecular studies[14] have shown that the laying Cape honey bee workers utilise queen-associated pathways in the biosynthesis of the fatty acid components of the mandibular gland pheromone. Ongoing work is focused on exploring the molecular mechanisms involved in the biosynthesis of the communication signals from the tergal and Dufour's glands.

Hepburn and Crewe[16] showed that while the principal mode of reproduction in *A. m. capensis* is thelytokous parthenogenesis, workers of this subspecies are also able to reproduce arrhenotokously (produce male offspring from unfertilised eggs) and that the contribution of worker reproduction within the South African honey bee population is significant. What governs the switch from arrhenotokous to thelytokous parthenogenesis has been the subject of ongoing research, with conflicting conclusions that will require use of functional characterisation of the genes involved using tools such as RNA*i* and CRISPR-Cas9.

Finally, as this groundbreaking discovery showed, the link between beekeepers and social insect researchers is essential and is a manifestation of 'citizen science' in action. Key aspects of the life of the reproductive parasite *A. m. capensis* were first and rather accurately described by a keen and curious beekeeper who took meticulous notes of his observations for 10 years before presenting his findings to the scientific community – findings that many scientists have built on over the years using various tools in genomics and chemical ecology, to understand the biology of the laying workers of *A. m. capensis.* Indeed, the Cape honey bee has provided us with a unique opportunity to witness the evolution of a social parasite in real time, providing us with significant insights into the evolution and regulation of the reproductive division of labour in social insect societies.

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Competing interests

We have no competing interests to declare.

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