Establishment of Cape honey bees as social parasites in African honeybee colonies

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Since Cape honey bees cause huge losses of African bee colonies by taking over reproduction, they are expected to establish easily after invading a colony. However, by artificial introduction of marked bees, we found that African bees are by no means defenceless. Cape bees started to reproduce in only one out of four colonies; in two other colonies all Cape bees were removed within three days. In the last African colony some of the Cape bees were removed, and the remaining Cape bees had not developed into egg-layers after 12 days. Apparently African bees can readily detect and remove potential Cape parasites after invasion into their colonies. Conditions inside the colony may be critical in defining vigilance of the bees. This suggests that alternative beekeeping management systems can reduce damage due to Cape parasites.

Keywords: Apis mellifera capensis, Apis mellifera scutellata, worker reproduction, social parasitism, ovary activation

Social parasitism of African honey bees, *Apis mellifera scutellata*, by the Cape honey bee, *A. m. capensis*, has caused huge losses amounting to tens of thousands of African honeybee colonies (Allsopp 1993) in the central and northern parts of South Africa. These losses, the 'Capensis Problem', are due to the 1990 migration by beekeepers of some Cape honeybee colonies beyond their natural range (Allsopp 1993), and their behaviour in African honeybee colonies (Neumann & Hepburn 2002, Neumann & Moritz 2002).

Once inside African colonies, Cape workers are able to activate their ovaries and to start reproducing (Hepburn & Allsopp 1994). Unlike workers of other *A. mellifera* races which lay haploid eggs, workers of the Cape honey bee produce diploid eggs by thelytoky: parthenogenetic reproduction giving rise to females (Ruttner 1977). Their brood is necessarily nursed by African workers, which provide Cape larvae with more food than their own larvae (Calis *et al.* 2002, Allsopp *et al.* 2003). This results in the production of intermediates between workers and queens that are physically more adapted to reproductive behaviour (Beekman *et al.* 2000, Calis *et al.* 2002, Allsopp *et al.* 2003). Due to the increasing number of laying Cape bees the social organisation in the colony disappears and the colony usually dies, but some Cape bees may spread to other colonies (Hepburn & Allsopp 1994). Since the parasitic bees replicate thelytokously, this has led to pseudo-

clones of social parasites spreading in the African population (Kryger 2001).

Worker ovary activation is normally rare in queen-right honeybee colonies (Ratnieks 1993), and this also holds for Cape bees (Hepburn *et al.* 1991, Hepburn 1992). Workers that do activate their ovaries are usually recognised by their nestmates (Sakagami 1954, Velthuis 1976, Blom 1991, Visscher & Dukas 1995), which may react by harassing the particular workers in order to stop the activation. Since Cape bees are successful as parasites in African colonies (Martin *et al.* 2002b) it is clear some do activate their ovaries. However, parasitic Cape bees have been observed to be attacked by African workers (Allsopp 1995), indicating that at least in some circumstances African colonies may resist potential Cape parasites. We can confirm these observations as, in preliminary experiments to introduce Cape bees into queen-right African colonies, the Cape bees failed to establish in some cases. Therefore we studied survival and ovary activation of Cape bees introduced into African colonies in detail.

MATERIALS AND METHODS

Source of honey bees used

Two Cape honeybee colonies, *A. m. capensis*, were obtained from a population at Cape Point (Fig. 1). This population is considered to be the least hybridised with African bees, because it is far south from the hybrid zone and undisturbed by beekeeping activities (Allsopp & Hepburn 1997, Hepburn & Radloff 2002; Fig. 1). Another two colonies were obtained from Nieuwoudtsville (Fig. 1), representing colonies from the natural hybrid zone between *A. m. capensis* and *A. m. scutellata* (Hepburn & Crewe 1991). Four African honeybee colonies, *A. m. scutellata*, were obtained from Kenhardt (Fig. 1), which is deep inside the distribution area of the African bee (Hepburn & Radloff 2002). All colonies were kept in one-storey Langstroth-hives and occupied about 10 frames. Parasitic Cape bees were obtained from infested African colonies around Pretoria (Martin *et al.* 2002b; Fig. 1). Combs with capped brood were transported over-night to Stellenbosch (Fig. 1) and the brood was allowed to emerge in an incubator.

Establishment of freshly emerged bees in queen-right colonies

For introductions we used 0-24 hours old bees that had emerged in an incubator. Bees of five different origins were introduced into two African colonies: African bees, hybrid bees, parasitic Cape bees, normal Cape bees and normal Cape bees that had been raised in an African colony. The latter resulted in worker-queen intermediates similar to the parasitic bees (Beekman *et al.* 2000, Calis *et al.* 2002, Allsopp *et al.* 2003). As a control, parasitic Cape bees, normal Cape bees and hybrid bees were also introduced into a hybrid and a Cape colony. Introduced bees were always unrelated to the host colonies.

The bees were marked using coloured plastic tags glued to the thorax. For introduction, a cage containing the marked bees was connected to a hole in the

top-cover of the hives. Subsequently, marked bees were counted every 3 days for up to 12 days after introduction to estimate survival. In the African colonies ovary activation of the three groups of Cape bees was monitored over time by examining 10 bees of each group collected directly after the bee counts.

Establishment of bees in African colonies kept in observation hives

Two African colonies were housed in observation hives with two brood combs and one honey comb on top. Marked bees were collected and introduced as before. Presence was recorded twice a day. Separate introductions were performed sequentially. Both observation hives received the same groups of bees and each group consisted of 20 individuals.

Four separate introductions were performed. At first we introduced the same groups of bees (0-24h) as in experiment 1: parasitic Cape bees, normal Cape bees raised in Cape colonies, normal Cape bees raised in African colonies, hybrid bees and African bees (Introduction 1). Ninety-seven hours later we introduced

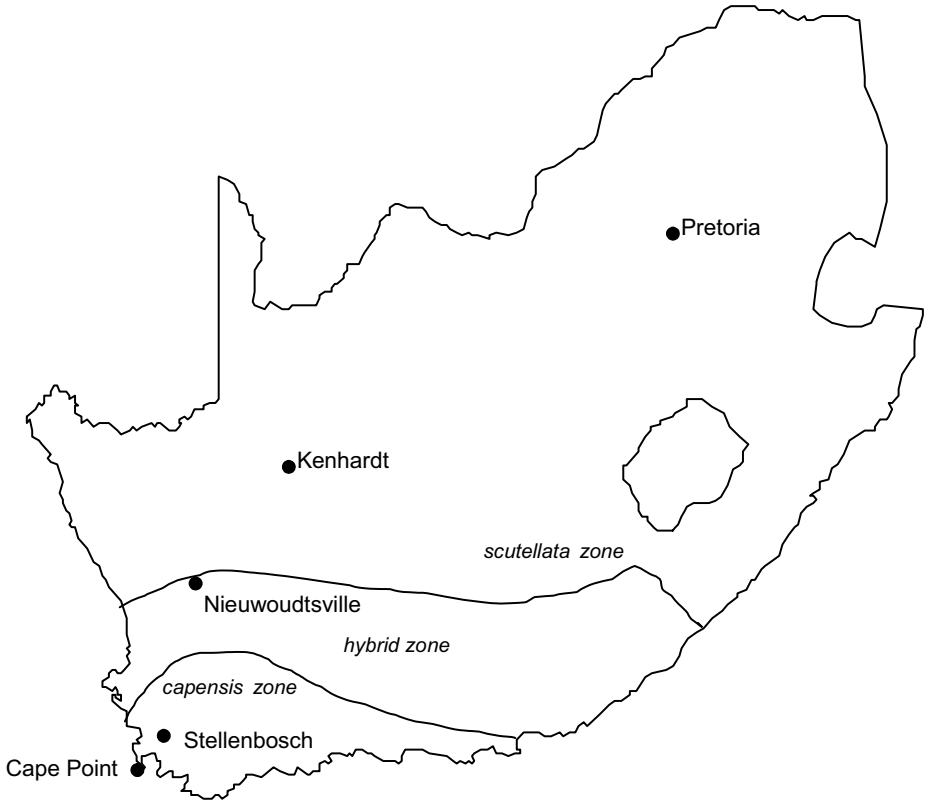

Figure 1. Map of South Africa showing the sites from which the bees were collected. Zones with *A. m. capensis*, with *A. m. scutellata*, and with their hybrids are indicated following Hepburn & Radloff (2002).

Table 1. Percentage of fully active ovaries (n=20) in Cape bees in African colony 1.

Age	Parasitic bees	Cape bees raised in African colony	Normal Cape bees
3	0	0	0
6	35	10	5
9	80	0	10
12	60	0	0

another two groups: Cape foragers collected from the flight entrance of a Cape Point colony and just emerged normal Cape workers (0-24h) (Introduction 2). After 150 hours we introduced Cape foragers and African foragers (Introduction 3). Finally, after 217 hours, we introduced groups of 10 normal Cape workers and 10 parasitic Cape bees that had been kept in two different queenless African colonies for a week, together with 20 freshly emerged African workers (0-24h) (Introduction 4).

Activation of ovaries in a queenless colony

Ovary activation of Cape bees was also studied in a queenless African colony. In this experiment we introduced two groups of freshly emerged bees (0-24h) from a Cape colony from Stellenbosch (Fig. 1) in an African colony from Kenhardt (Fig. 1). One group of Cape bees was raised in their own colony and the other was raised in an African colony. Hundred and fifty bees of each group were introduced into the host colony that consisted of five frames full with bees, four frames of brood of all stages, but without a queen. Samples of about 20 bees per group were taken after 4, 7, 10 and 13 days. Ovary activation of the bees sampled was determined.

RESULTS

Establishment of freshly emerged bees in queen-right colonies

In African colony 1, the number of counted bees remained constant over time (Fig. 2). Cape bees of all origins exhibited some ovary activation, although parasitic bees more often showed fully active ovaries (Table 1). Interestingly, the gut of bees without active ovaries was packed with pollen, whereas guts of bees with active ovaries were strikingly less full and uncoloured. Since mature eggs were present in Cape bees, this African colony was considered to be successfully parasitized.

In contrast, the number of Cape bees observed in African colony 2 decreased over time, whereas the number of hybrid and African bees remained constant (Fig. 2). In addition, 38% of the parasitic Cape bees, 13% of the normal Cape bees raised in an African colony and 5% of normal Cape bees raised in their own were found dead

outside the hive in the first three days. No hybrid and African bees were found, indicating that Cape bees were selectively killed inside the colony. Five out of 116 Cape bees that were sampled from this colony during twelve days showed some ovary activation, but no mature eggs were found. Apparently, this colony was not parasitized as yet, although some Cape bees remained in the colony.

In the Cape Point colony, the number of Cape bees observed remained constant over time (Fig. 2). After twelve days all recovered Cape bees were dissected and showed no ovary activation. In the hybrid colony from Nieuwoudtsville, no Cape bees were recovered after twelve days except for one normal Cape bee (Fig. 2). The number of introduced hybrid workers remained high.

Establishment of bees in African colonies kept in observation hives

The most striking result from the successive introductions was that no Cape bees were allowed to remain inside the colonies. If freshly emerged Cape bees were introduced, they were no longer observed after two to three days, whereas

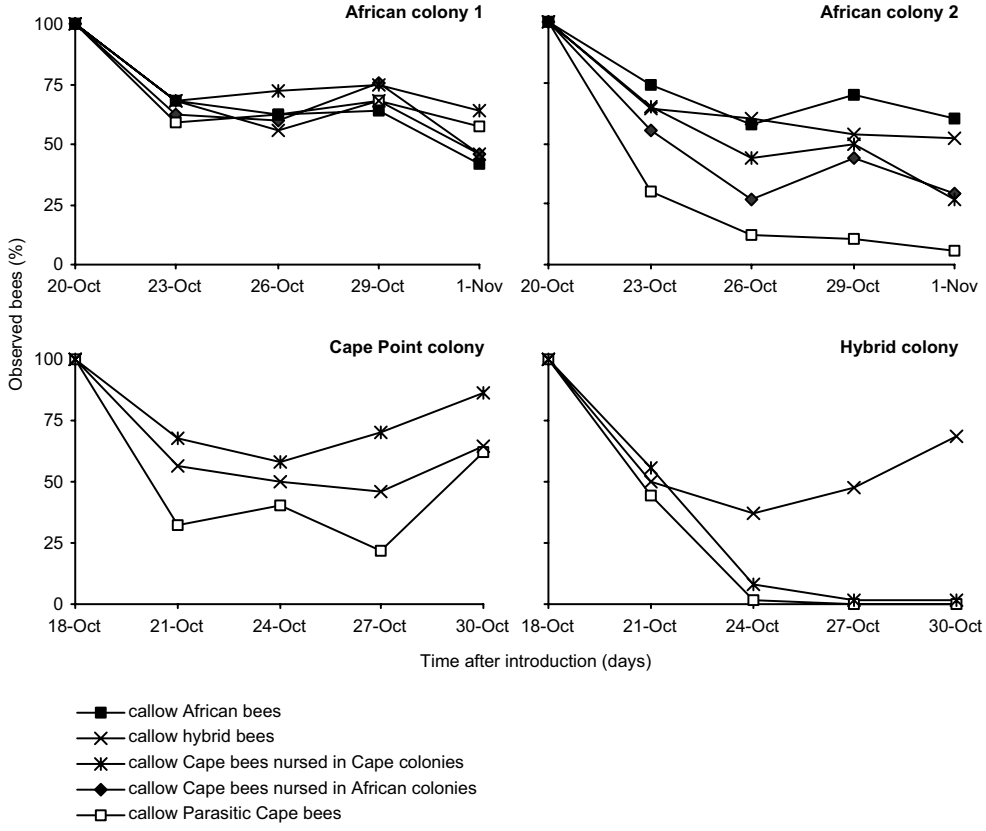

Figure 2. Percentage of marked bees observed after introduction into two African honey bee colonies, a Cape honey bee colony and a hybrid colony.

introduced hybrid and African bees remained abundantly visible throughout the experiment (Fig. 3). In Introduction 1, the three groups of Cape bees were removed at a similar rate, although in observation hive 2 they disappeared consistently faster. Marked hybrid and African bees were not removed from either observation colony. The Cape bees were seen attacked and actively removed by the African bees. Many of them were found dead outside the hives. When foragers and freshly emerged Cape bees were introduced (Introduction 2), the removal of the Cape foragers was even more rapid. Cape foragers were no longer observed after only a few hours, whereas the freshly emerged Cape bees were removed over a period of days, mirroring their removal during Introduction 1. When Cape and African foragers were introduced (Introduction 3), Cape foragers were again removed almost instantly whereas African foragers remained

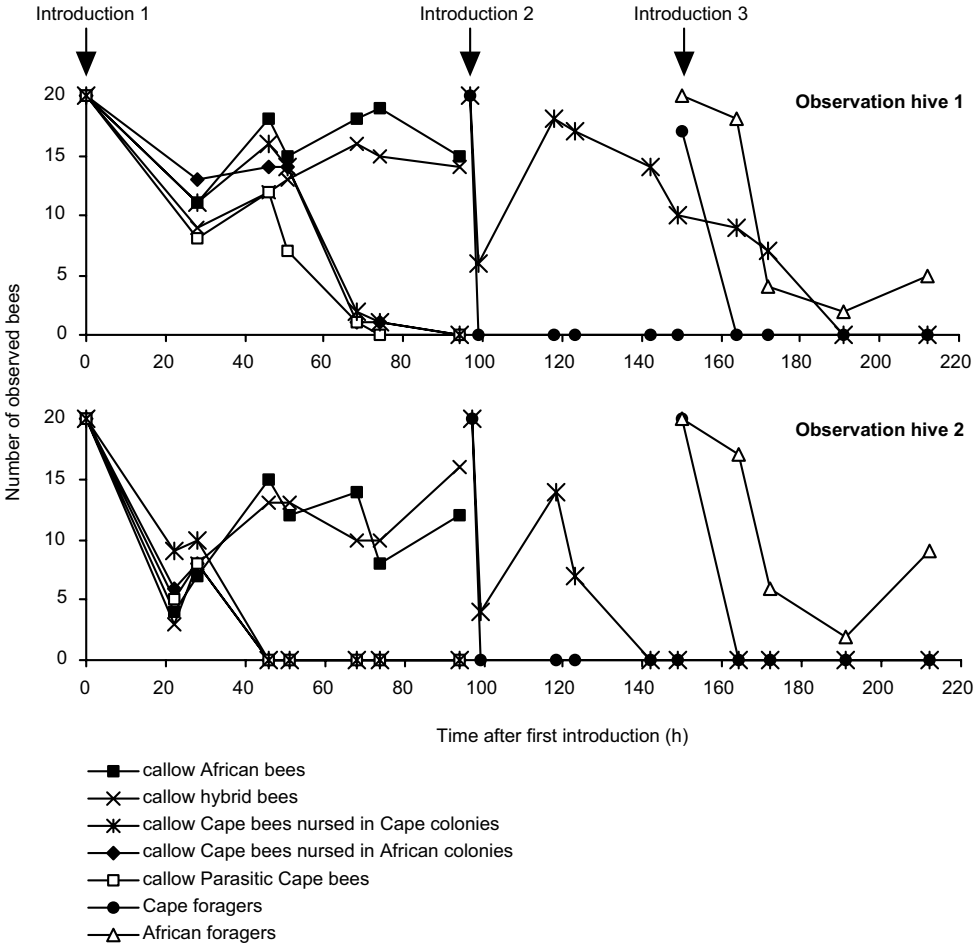

Figure 3. Number of marked bees observed after introduction into observation hives housing African honey bee colonies. During introduction in observation hive 1 three Cape foragers escaped, so only 17 Cape foragers were introduced.

Table 2. Ovary activation of Cape bees (n=16-21 bees) in a queenless African colony.

	Ovaries with some activation (%)		*Fully active ovaries (%)*	
Age	Raised in African colony	Raised in their own colony	Raised in African colony	Raised in their own colony
4	0	0	0	0
7	68	69	13	19
10	38	65	7	12
13	100	100	57	33

in the colonies until the end of the experiment.

The introduction of Cape bees that had been kept in queenless African colonies for a week (Introduction 4) evoked strong balling behaviour (Pettis *et al.* 1998) from the recipient African bees, and all were removed (hence, not indicated in Fig. 3). Interestingly, all of the freshly emerged African workers introduced as a control were also balled and removed, probably because they were introduced in the same box as the Cape bees and contaminated with Cape bee pheromones.

Activation of ovaries in a queenless colony

Cape bees, regardless of whether raised in their own colony or in an African colony, readily activated their ovaries in a queenless African colony (Table 2). After 7 days substantial numbers of fully active ovaries were found and after 13 days all ovaries showed some activation.

DISCUSSION

The 'Capensis calamity', where thousands of colonies are yearly lost because Cape parasites usurp African bee colonies, suggests that parasites are easily established and start reproduction. However, our results show that African bees are by no means defenceless. In only one out of four queen-right African colonies Cape bees appeared able to lay eggs within 12 days. In the other colonies some or all Cape bees were actively removed.

Why are Cape bees removed from African colonies? Ovary activation of worker bees may evoke aggressive behaviour from nest-mates (Sakagami 1954, Velthuis 1976, Ratnieks 1988, Blom 1991, Visscher & Dukas 1995), perhaps leading to removal from the colony. In our colonies, however, removal occurs within a day after introduction, long before any activation of ovaries can be expected (Martin *et al.* 2002a; Table 2). Apparently, nest-mates are able to recognise this right from the start, probably using behavioural or pheromonal cues (Wossler 2002). Hence, the establishment of Cape bees as a social parasite and removal by defending nest-mates are two sides of the same coin; you have to activate your ovaries to become a social parasite and yet, by activating, you

announce yourself and facilitate your detection and removal. It remains unclear, however, why in some colonies the residency of Cape bees leads to social parasitism whereas in other colonies all Cape bees are removed.

There seem to be no fundamental differences between the sources of Cape bees tested in our experiments. Social parasites from Pretoria (Kryger 2001) and Cape bees that we raised in an African colony can be considered as intermediates between workers and queens which probably increases their ability to lay eggs (Beekman *et al.* 2000, Calis *et al.* 2002, Allsopp *et al.* 2003). Like normal Cape workers, these intermediates were removed from African honeybee colonies, and in the Cape Point colony they persisted without activating their ovaries. As a group, however, Cape parasites do activate their ovaries more frequent than normal Cape bees or normal Cape bees raised in an African colony (Table 1). Apparently, during the many generations in African colonies suffering from the 'Capensis Problem', there has been within-colony selection for bees that activate their ovaries (Kryger 2001, Neumann & Moritz 2002).

The considerable variation in response to Cape bees found in four African honeybee colonies argues for more research into what defines the outcome of interactions between African bees and their Cape invaders. If the outcome is defined by genetic factors, there might be a basis for selecting African honeybees that are resistant to Cape bee takeover. Alternatively, the state of the colony may be critical in defining the vigilance of the bees against nest-mates activating their ovaries. In queenless African colonies, Cape bees readily activate their ovaries and start laying (Table 2). Additionally, a queen excluder, a broodnest separated by combs without brood, or the sheer size of the colony may reduce the influence of the queen's pheromones on some of the bees leading to activation of their ovaries (Free 1987). In fact, Martin *et al.* (2002a) showed that Cape parasites activated their ovaries within 6 days above a queen excluder in an African colony, and laying by workers above queen excluders often occurs in normal Cape honeybee colonies (Moritz *et al.* 1999). Hopefully, our finding that African bees are not entirely defenceless against Cape invaders will stimulate applied research on alternative beekeeping management systems in order to reduce damage due to the 'Capensis calamity'.

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