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Maternity of emergency queens in the Cape honey bee, *Apis mellifera capensis*

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Abstract

During reproductive swarming, some workers of the Cape honey bee, *Apis mellifera capensis*, lay eggs in queen cells, many of which are reared to maturity. However, it is unknown if workers are able to lay in queen cells immediately after queen loss during an episode of emergency queen rearing. In this study we experimentally de-queened colonies and determined the maternity of larvae and pupae that were reared as queens. This allowed us to determine how soon after queen loss workers contribute to the production of new queens. We were further interested to see if workers would preferentially raise new queens from queen-laid brood if this was introduced later. We performed our manipulations in two different settings: an apiary setting where colonies were situated close together and a more natural situation in which the colonies were well separated. This allowed us to determine how the vicinity of other colonies affects the presence of parasites. We found that workers do indeed contribute to queen cell production immediately after the loss of their queen, thus demonstrating that some workers either have activated ovaries even when their colony has a queen or are able to activate their ovaries extremely rapidly. Queen-laid brood introduced days after queen loss was ignored, showing that workers do not prefer to raise new queens from queen brood when given a choice. We also detected non-natal parasitism of queen cells in both settings. We therefore conclude that some *A. m. capensis* genotypes specialize in parasitizing queen cells.

Keywords: apiary, *Apis mellifera capensis*, non-natal parasitism, queen cell

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Introduction

Insect societies are characterized by reproductive division of labour and workers do not normally reproduce in the presence of a queen. The absence of worker reproduction is intriguing because in most species workers are capable of laying eggs that develop into viable males and will do so if queenless (Bourke 1988). Such 'altruistic' worker behaviour is best explained by inclusive fitness theory, which posits that a worker's total reproductive output is enhanced by personal sterility (Hamilton 1964a,b). However, kin selection theory also predicts the potential for conflicts over reproduction. Because insect societies are rarely comprised of clones, the reproductive optima of colony members do not completely overlap because of relatedness asymmetries within colonies (Beekman & Ratnieks 2003). As a result, insect colonies require mechanisms that control the expression of selfish interests by individuals.

In polyandrous species such as honey bees (*Apis*) an important mechanism for controlling selfish behaviour by individual workers is worker policing, the removal of worker-laid eggs (Ratnieks 1988). In arrhenotokous populations, in which workers lay haploid eggs that develop into males, workers are more related to the

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sons produced by the queen (relatedness, $r = 0.25$) than to the average worker-produced son ($r \approx 0.125$) (Ratnieks 1988). As a result, workers can, in theory, increase their inclusive fitness (Hamilton 1964a,b) by refraining from individual reproduction (Wenseleers *et al.* 2004) and by removing any eggs laid by workers (Ratnieks & Visscher 1989).

In contrast to all other subspecies of honey bee, workers of the Cape honey bee from South Africa, *A. mellifera capensis*, regularly produce diploid female offspring without mating via thelytokous parthenogenesis (Onions 1914; Verma & Ruttner 1983). The shift to thelytokous worker reproduction changes the reproductive options for workers and reduces the selective pressures that favour policing (Greeff 1996; Beekman & Oldroyd 2008). This is because workers are related to their own female offspring by unity ($r = 1$) and are therefore equally related to the average female progeny of their (half and full) sister workers ($r \approx 0.25$) as they are to the progeny of their queen (Greeff 1996). Thus, in colonies of *A. m. capensis*, worker reproduction has been predicted to be higher and worker policing lower than in arrhenotokous subspecies (Greeff 1996; Moritz *et al.* 1999). Thelytoky also means that *A. m. capensis* workers can contribute to the production of new queens (Beekman & Oldroyd 2008; Boot *et al.* 2008; Jordan *et al.* 2008). Because caste in honeybees is solely determined by larval feeding, any diploid egg can be raised as a queen provided the larva is fed appropriately (de Wilde & Beetsma 1982). If a worker successfully becomes the mother of the new queen she is genetically reincarnated as that new queen, resulting in an enormous fitness benefit for the individual worker. As the resident queen is herself equally related ($r = 0.5$) to her own sexually produced offspring and the thelytokously produced offspring of her daughters, she is predicted to be largely indifferent to queen production by workers (Greeff 1996). However, competition among workers for the production of new queens is expected to be intense. This is because of the significant differences in relatedness between workers to potential queen-destined brood. If a worker is the mother of the new queen she is related to the new queen by unity. If the worker's super-sister (a sister sharing the same father) is the mother of the new queen, the relatedness between the worker and the new queen is 0.75. But if a half sister is the successful mother, the relatedness is only 0.25.

As expected, *A. m. capensis* workers have recently been shown to successfully compete with the resident queen for the production of new queens during reproductive swarming (Jordan *et al.* 2008; Allsopp *et al.* 2010). Interestingly, *A. m. capensis* workers mainly become reproductively active when their colony is producing new queens; outside of this period, rates of worker reproduction are not much higher than in normal arrhenotokous populations (Beekman *et al.* 2009).

When a queen is lost suddenly, worker honey bees select young female brood (eggs or larvae less than 3 days old) and build 'emergency' queen cells around this brood in order to rear queens (Winston 1987). If, as suggested by Beekman *et al.* (2009) workers do not lay eggs outside periods in which the colony is preparing to rear new queens (i.e. during reproductive swarming and queen supersedure where a new queen is raised before a failing queen dies), we would not expect any queen cells to contain brood produced by workers immediately after the queen is suddenly lost. We tested this hypothesis by moving queens to a new hive and collecting queen cells reared in the now queenless original colony. In the following week we provided each queenless section with young brood from their queen, thus offering the queenless colonies the choice of their own queen-laid or worker-laid brood from which to rear new queens. We performed this unnatural manipulation because we were interested to determine if, given a choice between queen-laid and worker-laid brood, workers would refrain from laying eggs in queen cells and raise queens from queen-laid larvae instead.

As a previous study found a large contribution of new queens to be the offspring of non-natal and potentially parasitic individuals (Jordan *et al.* 2008), we performed our experiment under two different layouts, one mimicking an apiary situation where workers could easily move from one colony to another and a more natural situation where colonies were more widely spaced (more than 100 m apart). If some individual workers specialize on entering non-natal colonies to parasitize the queen cells with their eggs (Jordan *et al.* 2008), we would expect to find offspring of non-natal workers in both situations. Alternatively, if non-natal workers mainly move from one colony to another due to apicultural practises and drift of foragers between colonies (Allsopp *et al.* 2010), we would expect to find fewer non-natal offspring when the colonies are widely spaced than when they were closely spaced.

Materials and methods

Experimental manipulations

Experiments were conducted from December 2008 to February 2009 using *A. m. capensis* colonies originating from the Stellenbosch area (33°56′ S, 18°51′ E), Western Cape, South Africa. The experiment was repeated twice. For each trial we chose eight colonies, two each from four separate apiaries. The colonies each contained at least eight frames of bees, a marked and laying queen, at least four frames of brood and a honey super.

Colonies were placed at two separate sites in such a way that all the colonies at each site had different apiary origins. At the first site, 'Le Verger', the colonies (LV1-4 in trial 1 and LV5-8 in trial 2) were kept on a single pallet similarly to common beekeeping practice. At the second site, 'Asara', the colonies (A1–4 in the first trial and A5–8 in the second trial) were isolated from each other by a distance of at least 100 m in a highly heterogeneous landscape.

We simulated sudden queen-loss by removing each queen from her colony along with a frame of brood and ≈2000 workers. The queens and accompanying brood and workers were transferred to nucleus hives and moved to a new site some 20 km away. Twenty pupae from worker-cells were sampled from each colony at this time in order to construct consensus genotypes for each queen. We also collected wingtips from all queens for genetic analysis.

Five days after queen-removal, we inspected the queenless colonies and removed all queen cell contents (larvae and pre-pupae, hereafter QCCs) for genotyping, exhaustively destroying any cell that contained an egg or larva. At this time, worker-laid eggs were observed in all queenless colonies and the original queens were laying at their new location. We then transferred one frame containing queen-laid eggs (from the queenright nucleus hives containing the original queens) to their respective queenless colony. The queenless colonies thus had a second opportunity to choose between rearing a new queen from worker- or from queen-laid brood. Five days later, we inspected the colonies again and harvested QCCs. No further queen-laid brood was added, so the queenless workers could only rear queens from their own (or a parasite's) worker-laid brood. We then inspected each colony a further two times (7–10 days after the previous check), harvesting all QCCs found at each inspection. At the final inspection, one queen cell was left in each colony to allow them to re-queen. Once the new queen was laying, we collected her wingtips for genetic analysis.

Genetic analyses

DNA was obtained from the queens' wingtips, adult workers and QCC using a high salt extraction method (Aljanabi & Martinez 1997). For workers we used two to three legs, for QCCs we used an amount of tissue approximately the size of a match head. Tissue was added to 500 μL reaction buffer [0.1 mg/mL Proteinase K (Promega), 10 mM NaCl, 10 mM TRIS (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% sodium dodecyl sulphate in a 1 mL centrifuge plate]. A stainless steel bead was added to each well and tissue was homogenized for 5 min on each side at 25 Hz using a TissueLyser (Qiagen). Tissue from wingtips (queens) was frozen with liquid nitrogen and crushed using a mortar in a 1.5 mL microcentrifuge tube before adding 500 μL reaction buffer [0.2 mg/mL Proteinase K, 10 mM NaCl, 10 mM TRIS (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% sodium dodecyl sulphate]. Samples were incubated overnight at 55 °C. After incubation 1/25 volume of 5 M NaCl was added to each sample before incubation for 30–45 min at −20 °C followed by centrifugation for one hour at 4300 rpm at 4 °C. Following centrifugation, 200 μL of the supernatant was added to 400 μL 99.7% ethanol and samples were incubated at −20°C for 1 h. Samples were again centrifuged at 3830 *g* and 4 °C for 2 h before discarding the supernatant and rinsing twice with 70% ethanol. Samples were air-dried and then resuspended in 50 μL 1 × Tris-EDTA buffer.

All worker samples and QCCs were examined at five to seven microsatellite loci: Am006, Am008, Am014, Am046, Am052, Am059 and Am061 (Solignac *et al.* 2003). These microsatellite markers were amplified in two duplex and one triplex polymerase chain reactions (duplex 1: Am014/Am061, duplex 2: Am006/Am008 and triplex 1: Am046/Am052/Am059). Duplex 1 consisted of 0.1 μL of forward and reverse primer for Am014 and 0.133 μL of forward and reverse primer for Am061, along with 0.1 μL each of dATP, dTTP, dCTP and dGTP; 0.4 μL $MgCl_2$; 0.5 μL 10 × PCR Enhancer Solution (Invitrogen); 0.8 μL 10 × TAQ-Ti Polymerase reaction buffer (Fisher Biotec); 0.03 μL TAQ-Ti DNA Polymerase (Fisher Biotec); 0.27 μL H_2O and 2 μL genomic DNA. Reagents in triplex 1 were as for duplex 1, except for primer volumes (0.033 μL of forward and reverse primer for Am046, 0.1 μL of forward and reverse primer for Am052 and Am059) and H_2O volume (0.404 μL). Reagents in duplex 2 were as for triplex 1 and duplex 1 except for the primer volumes (0.1 μL of the forward and reverse primer for each) and that no water was added. Total reaction volumes were 5 μL. Three hundred and eighty four amplifications were performed simultaneously on an Eppendorf 384 Thermal Cycler. PCR conditions were: initial denaturation period of 94 °C for 7 min; 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 45 s and a final extension period of 15 min.

PCR products from each reaction were diluted 1:10 and 1 μL of each diluted product was added to 5 μL formamide and 50 μL LIZ DNA size standard (Applied Biosystems). Samples were run on a 3130*xl* Genetic Analyser (Applied Biosystems) with capillary length 36 cm and injection time of 15 s at 1200 V for 41 min. Resulting data files were analysed using GeneMapper 3.7 (Applied Biosystems). Microsatellite allele sizes were distinguishable due to a unique combination of dye colour and amplicon size range.

Determining maternity of QCCs

We constructed consensus queen genotypes from either the queens' wingtips or if the wing tips' DNA did not successfully amplify, the queen-laid worker samples collected from each colony in week 1. We compared these queen genotypes with the QCC genotypes to determine if QCCs were the offspring of queens or workers. Queen-produced QCCs must share at least one allele with the queen at all loci whereas the offspring of workers may not.

To distinguish thelytokous offspring of a natal worker from the offspring of the queen, we exploited a quirk of thelytokous meiosis. If during thelytokous meiosis with central fusion a crossover event occurs between the locus and the centromere there is a one-third chance that an allele present in the worker mother will become homozygous in her offspring (Baudry *et al.* 2004; Pearcy *et al.* 2006; Oldroyd *et al.* 2008). When homozygozity occurs, there is a 50% probability that the homozygous allele will be from the male parent (Allsopp *et al.* 2010). Therefore, the probability that a worker's offspring will be homozygous for a paternally-derived allele is $1/2 \times 1/3 = 1/6$ per locus (if the queen that produced the reproductive worker was heterozygous and did not share an allele with the worker's father) (Allsopp *et al.* 2010). Hence, if a QCC shares a single allele with the queen at most loci, but is homozygous at one locus or more and does not share this allele with the queen, the QCC was produced thelytokously by a worker (Allsopp *et al.* 2010). Given the high recombination rates found in *A. mellifera* (Beye *et al.* 2006), we can assume that recombination occurs at all loci located 50 cm or more from the centromere. When the locus is located less than 50 cm from the centromere, recombination rates are reduced by centromeric interference. Apart from Am061 and Am059, all loci used were more than 50 cm from the centromere based on the Solignac-4 genetic map of the honey bee (Weinstock *et al.* 2006). Loci Am061 and Am059 are less than 50 cm from their centromere (7.3 and 29.6 respectively), but can still indicate worker maternity when they become homozygous for a paternal allele.

The above interpretation could be in error if a non-natal worker shares an allele with the queen at all loci except the homozygous locus or loci by chance. This would result in classifying a QCC as worker-laid when in fact it was the offspring of a non-natal worker. We therefore calculated the probability that a random worker in the population could share an allele with the queen at the i loci at which the QCC shared an allele with the queen: $\prod_i p_j$, where p_j is the frequency of the allele j shared by the resident queen and the QCC at the i^{th} locus. When the QCC carried two different queen alleles at a locus, we used the average of both allele frequencies.

A QCC heterozygous at all loci and carrying a queen allele at all loci can be the offspring of a worker if crossing over has not led to homozygozity at one or more loci. The probability of not detecting such a worker-derived offspring and thus erroneously declaring it queen-laid is $(1 - 1/6)^j$ where j is the number of loci examined (Allsopp *et al.* 2010). QCCs were generally examined at between five and seven loci, so the approximate probability of erroneously declaring any one natal-worker-laid QCC as being queen-laid ranged from 0.28 to 0.40. We therefore underestimate the contribution of natal workers to QCC.

A worker-produced offspring also has a $1/2 \times 1/3 = 1/6$ chance of becoming homozygous for a maternally-derived allele when recombination has occurred. Hence, if a QCC shares a single allele with the queen at most loci, but is homozygous for a queen allele at one locus or more, the QCC may have been produced thelytokously by a worker. However, this kind of QCC could also arise as the result of the queen having mated to a male carrying the same allele as the queen at the locus or loci homozygous in the QCC. We calculated the probability of such matings in the following way. First, for all loci examined we calculated the average frequency of the two alleles carried by the queen as $(p_{1i} + p_{2i})/2$, where p_{1i} is the population frequency of the first queen allele at the ith locus, and p_{2i} is the population frequency of the second queen allele at the i^{th} locus. We then calculated α, the average of these averages over the i loci. For each QCC we then determined n, the number of loci homozygous for a queen allele. The probability that a QCC would be homozygous for a queen allele at any of n loci due to a male mating with the queen sharing an allele with her at any of n loci was then estimated as α^n. To obtain population allele frequencies we genotyped one worker per colony from a total of 158 colonies collected from within the Western Cape (sampling years: 1984, 1993, 2006 and 2009). When an allele was not present in the population but was found in our study, we used a frequency of 0.05. The population allele frequencies are given in Table S2 (Supporting information).

For the second QCC harvest, we further noted from which brood frame (queen- or worker-laid) the QCCs were collected. When a QCC on the frame that originated from the colony that contained the original queen possessed a queen allele at all loci, the QCC could not be distinguished as being either queen-laid or worker-laid unless it was homozygous at one or more loci (see above).

Queen cell contents (QCCs) produced by reproductive parasites are easily distinguished from QCCs produced either by the queen or natal workers when they carry two non-identical alleles not carried by the resident queen at any locus (Jordan *et al.* 2008; Allsopp *et al.* 2010).

Because one of our aims was to compare the contribution of non-natals to QCC between the two colony placements, we performed a 2×2 contingency table analysis of the total number of QCCs produced by queens and natal workers combined vs. non-natal parasites in the colonies set out in an apiary setting (Le Verger) vs. the more natural setting (Asara).

Results

All colonies except colony A4 produced the most queen cells immediately after de-queening (Table 1). Colony A4 was excluded from the analyses as this colony failed to produce any queen cells until very late in the experiment when no queen-laid eggs were present. Subsequent harvests were characterized by a steep drop in queen cell production in all colonies (Table 1).

Workers contributed to QCCs immediately after the queens were removed (Table 1 and Table S1, Supporting information). The contribution of non-natals to queen cell maternity did not differ between the colonies in the two settings (first trial: $\chi^2_1 = 2.804$, $P = 0.08$; second trial: $\chi^2_1 = 0.106$, $P = 0.47$) (Table 1). Only four out of a total of 57 non-natal-laid QCC could be traced back to one of the other colonies in the trial (see Table S1, Supporting information). Interestingly, this was the case even in the Le Verger setting where the four experimental colonies were placed very close together. After we left the last queen cells in the colonies, six successfully reared a laying queen. Four of these queens were offspring of non-natal workers (colonies A3, A5, A7 and LV3) (see Table S1, Supporting information). Thus, despite the theoretical prediction that workers from different patrilines should compete fiercely over which individual becomes the mother of the new queen (Beekman & Oldroyd 2008), most colonies manage to produce a viable queen and this queen was often the offspring of a non-natal worker.

Workers constructed queen cells on the introduced frame containing queen-laid brood in only five colonies. Only two of these QCC were compatible with being queen-laid (one in colony A2 and one in LV7; see Table S1, Supporting information). Two QCC collected from frames containing queen brood were offspring of a non-natal worker (colony A7 and A8; see Table S1, Supporting information). Hence, workers did not show

Table 1 Number and percentage of QCC produced by the queen or natal workers (queen- or worker- laid: QL or WL) thus including QCC that could have been produced by natal workers but were not homozygous for a paternal allele (see 'Materials and methods'), natal worker-laid (WL) and foreign worker-laid (FL) in both trials pooling colonies per setting. 'Totals' are the sum of all four dates of all colonies. Colonies in the Le Verger setting were placed close together on a single pallet. Colonies in the Asara setting were spaced apart. On December 10 (first trial) and January 28 (second trial) a frame containing queen brood was introduced into the colonies. Hence on December 15 (first trial) and February 3 (second trial) QCC were collected from either frames containing worker brood or queen brood. Genotypes of all individuals used to construct this table are given in Table S1 (Supporting information). The genotype of only one QCC collected from the queen frame could have been attributed to the queen (colony LV7; see Table S1, Supplementary information). Combining the number of QCC produced by the queen and natal workers and comparing those with the number produced by foreign workers, shows that the number of foreign-laid QCC did not differ between the different apiary settings (first trial: $\chi^2_1 = 2.804$, $P = 0.08$; second trial: $\chi^2_1 = 0.106$, $P = 0.47$)

			Likely origin of queen cell contents						
		Sampling date	QL or WL (%)	WL (%)	FL (%)		QL or WL (%)	WL (%)	FL (%)
Trial 1	Le Verger (close)	10 December 2008	8 (15.4)	37 (71.1)	7 (13.5)	Asara (distant)	6 (14.3)	33 (78.6)	3 (7.1)
		15 December 2008	0 (0)	0 (0)	0 (0)		0 (0)	6 (100)	0 (0)
		22 December 2008	0 (0)	2 (100)	0 (0)		0 (0)	3 (100)	0 (0)
		30 December 2008	0 (0)	6 (75)	2 (25)		0 (0)	6 (100)	0 (0)
		Total	8 (12.9)	45 (72.6)	9 (14.5)		6 (10.5)	48 (84.2)	3 (5.3)
		Sampling date	QL or WL (%)	WL (%)	FL (%)		QL or WL (%)	WL (%)	FL (%)
Trial 2	Le Verger (close)	28 January 2009	8 (24.2)	19 (57.6)	6 (18.2)	Asara (distant)	14 (25.0)	33 (58.9)	9 (16.1)
		3 February 2009	1 (100)	0 (0)	0 (0)		0 (0)	2 (50)	2 (50)
		9 February 2009	0 (0)	0 (0)	0 (0)		0 (0)	3 (100)	0 (0)
		19 February 2009	0 (0)	0 (0)	1 (100)		0 (0)	5 (83.3)	1 (16.7)
		Total	9 (25.7)	19 (54.3)	7 (20)		14 (20.3)	43 (62.3)	12 (17.4)

a preference for queen-laid brood when constructing queen cells.

On average, where a QCC was declared worker-laid, the probability, estimated as α^n, that a queen could have mated with a male carrying the same allele as herself at the homozygous locus was 0.03 (Table S1, Supporting information). Hence about 3% of QCC homozygous for a queen allele at one or more loci were most likely offspring of the queen and not of a natal worker.

We also used the population allele frequencies to calculate the likelihood that a QCC classified as worker-laid due to homozygozity for paternal alleles at one or more loci was in fact offspring of a non-natal worker that happened to share an allele with the queen at all other loci. On average this probability is 0.009 (Table S1, Supporting information), suggesting that QCC homozygous for non-queen alleles were classified correctly as natal worker-laid in all cases.

Sixteen QCCs were homozygous for a queen allele at all loci studied (see Table S1, Supporting information) and three were homozygous at all loci including non-queen alleles (hence these were produced by natal workers or non-natal workers).

Discussion

We found that *A. m. capensis* workers contribute to queen cell production immediately upon the loss of the queen. Previous work had suggested that during natural swarming events, *A. m. capensis* workers mainly activate their ovaries after the first queen cells have been built (Beekman *et al.* 2009). However, our present analysis shows that *A. m. capensis* workers contribute significantly to the production of queen cells immediately following queen-loss and thus either have active ovaries when their queen is present or are able to activate their ovaries within hours. Such workers may lay in areas where queen cells are normally built, along comb margins, thus increasing their chances of becoming the next queen's mother. Alternatively, reproductive workers may remove queen-laid eggs and lay in newly constructed queen cells. When queen-laid brood was introduced after queen loss almost no queen cells were constructed on the frames contained queen brood.

Interestingly we did not find higher rates of parasitism by non-natal workers in colonies within the apiary setting compared with the more natural setting (Table 1 and Table S1, Supporting information). Even more interesting is the fact that even in the apiary setting most of the parasites came from colonies that were not part of the experiment. This suggests that the majority of parasites were either a longstanding presence in the colonies, most likely originating from other colonies in the original apiaries before the experimental colonies were moved to their experimental locations or from long distance drift or active parasitism. The substantial presence of parasites in both settings favours an active parasitism explanation, however and suggests that there may be certain *A. m. capensis* genotypes that have evolved to parasitize queen cells.

We found sixteen QCCs that were homozygous for all alleles carried by the queen at all loci examined (Table S1, Supporting information) plus three that were clearly worker-laid but homozygous at all loci. Homozygous queen-produced QCCs have been reported previously in *A. m. capensis*, but their identity remains a mystery (Jordan *et al.* 2008; Allsopp *et al.* 2010). As in our previous studies, these homozygous individuals were only found among first and second instar larvae. These larvae may have been haploid males or, potentially, diploid males produced by terminal fusion of meiotic products (Allsopp *et al.* 2010). However, Jordan *et al.* (2008) have shown that at least some of the homozygous QCC were morphologically female. The fact that such queen-produced homozygous individuals have never been found among older larvae, pupae or adults, supports the suggestion that these homozygous individuals are most likely non-viable beyond the larval stage (Allsopp *et al.* 2010). Allsopp *et al.* (2010) postulated that mated queens lose the ability to produce offspring thelytokously and that these homozygous individuals may be the results of attempts by the queen to do so, resulting in homozygosity due to some meiotic peculiarity. Thelytokous queen production by *A. m. capensis* queens would dramatically increase a queen's direct fitness. Clonal reproduction of offspring-queens has been previously reported in two species of ant, the little fire ant *Wasmannia auropunctata* (Fournier *et al.* 2005) and *Cataglyphis cursor* (Pearcy *et al.* 2006). In both ant species queens are produced predominantly asexually, while workers are always produced sexually. However, the homozygous individuals found in our study were laid prior to queen cells being produced, as the queens had been removed at least 5 days prior to the first samples being collected and workers only started to produce queen cells after the queen was lost. Therefore, the mother-queen could not have laid these homozygous eggs in queen cells in an attempt to clone herself. Moreover, given that the vast majority of QCC in our study were actually offspring of workers, most of the homozygous QCC were likely produced by workers. Thus the existence of these homozygous individuals likely arise through some unusual meiotic process that is not yet understood. It is interesting to note that the great majority (90%) of alleles that were homozygous in QCC (when the queen was heterozygous at that locus) were the queen derived allele and not a drone-derived allele (Table S1, Supporting information). During a thelytok-

ous meiosis of a worker laid egg, there should be an equal chance that the maternal (queen derived) allele or the paternal (drone derived) allele should become homozygous. The fact that it is almost always the maternal allele that is homozygous is a mystery and suggests the thelytokous meiosis of the *A. m. capensis* worker is not 'fair' and strongly favours the queen-derived genome. Elimination of the maternal genome has been suggested in the little fire ant *Wasmannia auropunctata* (Fournier *et al*. 2007) and perhaps some similar process occurs in *A. m. capensis*.

In conclusion, our study shows that workers in *A. m. capensis* colonies are always ready to lay eggs in queen cells as soon as the opportunity arises. This is interesting because the normal assumption is that colonies with a queen that contain reproductively active workers pay a cost for having these workers (Hillesheim *et al.* 1989; Montague & Oldroyd 1998). Honey bee colonies have therefore evolved a myriad of mechanisms to curtail selfish behaviour by workers as long as the queen is present (Beekman & Oldroyd 2008). We therefore predicted that *A. m. capensis* workers would only activate their ovaries and lay eggs when the colony is preparing to raise new queens (Beekman *et al*. 2009). Our current study suggests this is not the case, although we cannot exclude that *A. m. capensis* workers are able to activate their ovaries extremely rapidly. However, given that non-natal workers contribute to the production of queens immediately after queen loss, we suspect that there are certain *A. m. capensis* workers that wait for the opportunity to lay eggs in queen cells thereby increasing their chance of being reincarnated as the colony's next queen.

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This study is part of Michael Holmes' Honours project on the Cape honey bee. Madeleine Beekman and Ben Oldroyd are interested in the evolution and maintenance of sociality in the social insects. Ben's main aim is to understand the genes that are pivotal to the evolution of worker activity. Madeleine attempts to understand the evolution of sociality in general, including social vertebrates. Mike Allsopp has been working on the Cape honey bees for more than 15 years and was one of the first to describe the peculiarities of this subspecies. Julie Lim has been pivotal in finalizing the genotyping. Theresa Wossler provided logistical support that allowed Michael to stay in South Africa during this project. Madeleine, Ben, Mike and Theresa continue collaborating.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Genotypes of all queencell content (QCC). For QCC homozygous at one or more loci for a queen allele, we calculated the probability that this is due to the queen having mated with a drone carrying the same allele as the queen at that locus. When no queenbrood was present, this was not calculated. ProbabiliBes in red denote the probability of this QCC being produced by a non-natal instead of a natal worker (see text). The origin of foreign laid (FL) QCC are given when known. 'No queen eggs': no more queen eggs present in the colony at the time the QCC was produced. Colony A1-A7: colonies that were spaced apart; colonies LV1-LV8: colonies that were placed close together

Table S2 Populaton allele frequencies

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