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Parasitic Cape honeybee workers, *Apis mellifera capensis*, evade policing

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Relocation of the Cape honeybee, *Apis mellifera capensis*, by bee-keepers from southern to northern South Africa in 1990 has caused widespread death of managed African honeybee, *A. m. scutellata*, colonies[1]. *Apis mellifera capensis* worker bees are able to lay diploid, female eggs without mating by means of automictic thelytoky[2] (meiosis followed by fusion of two meiotic products to restore egg diploidy), whereas workers of other honeybee subspecies are able to lay only haploid, male eggs. The *A. m. capensis* workers, which are parasitizing and killing *A. m. scutellata* colonies in northern South Africa, are the asexual offspring of a single, original worker in which the small amount of genetic variation observed is due to crossing over during meiosis[3] (P. Kryger, personal communication). Here we elucidate two principal mechanisms underlying this parasitism. Parasitic *A. m. capensis* workers activate their ovaries in host colonies that have a queen present (queenright colonies), and they lay eggs that evade being killed by other workers (worker policing)—the normal fate of worker-laid eggs in colonies with a queen[4–8]. This unique parasitism by workers is an instance in which a society is unable to control the selfish actions of its members.

In a honeybee (*Apis mellifera*) colony, reproduction is monopolized by the single queen. In queenright colonies of European subspecies only a few workers[6] (approximately 0.01% of about 30,000 in a colony) have fully active ovaries, and most of their eggs are policed by other workers[5–8]. (Worker policing is selectively favoured because workers are more related to the sons of their mother/queen (0.25) than to the sons of their sister workers (~0.15)[4,7].) We compared ovary activation and the acceptance of eggs laid by *A. m. capensis* workers parasitizing native *A. m. scutellata* colonies in the Pretoria region of northern South Africa, with *A. m. scutellata* workers and queens. Newly emerged *A. m. capensis* workers introduced into a queenright *A. m. scutellata* colony all activated their ovaries, whereas none of the *A. m. scutellata* workers did so (Fig. 1). Furthermore, eggs laid by *A. m. capensis* workers are not effectively removed by worker policing (Fig. 2). These eggs are nearly as acceptable as eggs laid by *A. m. scutellata* queens, possibly through chemical mimicry[6,9]. In contrast, eggs laid by *A. m. scutellata* workers are policed effectively with almost none remaining after 20 h. This demonstrates that the parasitic *A. m. capensis* workers actually evade worker policing rather than exploit a situation where worker policing is lacking. An alternative hypothesis for the lack of policing, that all *A. m. capensis* worker-laid eggs evade policing, is also rejected because eggs laid by most *A. m. capensis* workers are policed in *A. m. scutellata* discriminator colonies (P. Neumann, C. W. W. Pirk and F.L.W.R., unpublished data). A second alternative hypothesis is that workers simply discriminate between diploid (queen-laid and *A. m. capensis* worker-laid) and haploid (normal worker-laid) eggs; however, studies of European honeybee subspecies have shown that this does not occur[10].

Table 1 Numbers of worker-laid eggs in *A. m. scutellata* colonies

	Colony				
Day	1	2	3	4	5
1	0	0	0	0	0
2	0	0	2	0	0
3	0	0	0	0	1
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	1	0	1	0
8	0	0	0	0	0
Total	0	1	2	1	1

Eggs were laid in drone cells to which the queen was denied access. All of the eggs laid ($n = 5$) had been removed by workers (worker policing) before inspection on the next day. (See Methods for details of the experimental design.)

Occasional worker-laid eggs were found in five *A. m. scutellata* colonies in cells above the queen excluder—a part of the hive that the queen cannot enter. All of these eggs were gone 24 h later (Table 1). This is very similar to what occurs in European subspecies of *Apis mellifera*[6,8], and further confirms that both egg laying by workers and worker policing occur at low rates in normal *A. m. scutellata* colonies.

Our results show that the parasitic *A. m. capensis* workers have at least two traits enabling them to reproduce in queenright *A. m. scutellata* colonies. The first is the ability to activate their ovaries in a queenright colony, and the second is the ability to lay eggs that evade worker policing. In this respect they resemble the anarchistic worker honeybees that occasionally occur in European subspecies[7,10,11]; however, anarchistic workers lay haploid eggs that develop into males. By laying thelytokous diploid eggs the *A. m. capensis* workers do not merely reproduce, they replicate;

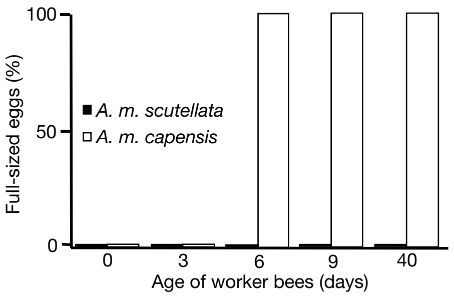

Figure 1 Ovary activation in *A. m. scutellata* and parasitic *A. m. capensis* workers fostered in a queenright *A. m. scutellata* colony. One hundred each of newly emerged *A. m. scutellata* and parastic *A. m. capensis* workers were marked and introduced into a queenright *A. m. scutellata* colony. Ten workers of each type were dissected on introduction and after 3, 6, 9 and 40 days to determine if full-sized eggs were present in their ovaries.

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that is, they produce more parasitic workers. As a result the parasitic *A. m. capensis* workers increase in number within a host colony. This leads to the death of the host colony on which they depend. An important factor causing the death of a colony seems to be the dwindling numbers of *A. m. scutellata* workers that perform foraging duties (*A. m. capensis* workers are greatly under-represented in the foraging force of an an infected colony) owing to death of the queen, and, before queen death, competition for egg laying between *A. m. capensis* workers and the queen[13]. Unfortunately, bee-keeping practices such as collecting wild swarms, seasonal movement of colonies by truck, and the close siting of hives in apiaries facilitate the horizontal spread of the *A. m. capensis* workers between colonies and provide a continuing source of hosts.

The mechanisms enabling intracolony parasitism that we document above are necessary for the parasitism to occur because worker policing is normally extremely efficient at killing worker-laid eggs, and because normal workers rarely activate their ovaries in queenright colonies[5–8]. The parasitic *A. m. capensis* workers appear to have no specific advantage that aids them in horizontal transmission. Guards at entrances of *A. m. scutellata* colonies reject non-nestmate *A. m. capensis* workers with the same probability as non-nestmate *A. m. scutellata* workers[12]. However, special mechanisms facilitating horizontal transmission are probably unnecessary because entrance guards usually accept a large proportion of non-nestmates attempting to enter[14,15], and workers frequently 'drift' among colonies within an apiary[16].

To our knowledge this is the first example of parasitism of eusocial colonies by self-replicating workers. However, this was perhaps foreseen by William Hamilton (ref. 17) who noted that "An ability of females to lay unfertilized eggs which develop into females would open another possible avenue for selfish selection". This parasitism is analogous to cancer in that it is by same-species, self-replicating units that do not respond to normal regulatory processes and proliferate to overwhelm the collective[18]. It is also analogous to a normal infectious disease in that the replicating workers are transmitted horizontally between hosts rather than arising *de novo* within each host colony. □

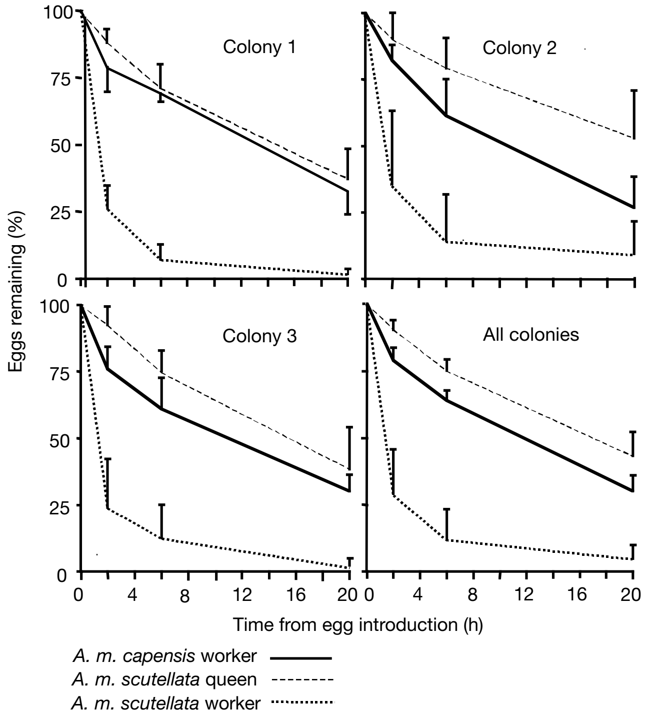

Figure 2 Removal rates of eggs (mean plus s.d.) laid by *A. m. scutellata* queens (dashed line), *A. m. scutellata* workers (dotted line) and parasitic *A. m. capensis* workers (solid line) when introduced into three queenright *A. m. scutellata* discriminator colonies (see Methods for detailed experimental procedure).

Methods

Study organisms

All *A. m. scutellata* colonies, eggs and worker bees were from eight colonies moved into Pretoria from a region free of the *A. m. capensis* parasitism in October 2000, just before the study. All *A. m. capensis* eggs and workers were obtained from infected colonies provided by a bee-keeper in the Pretoria area. These colonies showed the typical symptoms of the *A. m. capensis* problem: colony dwindling, multiple eggs per cell, and dark-coloured bees (*A. m. scutellata* workers are more yellow than the parasitic *A. m. capensis* workers). In addition a subsample of our *A. m. capensis* bees ($n = 5$) are genetically the same as parasitic *A. m. capensis* workers from across northern South Africa (P. Kryger, personal communication).

Egg laying and worker policing by *A. m. scutellata*

Following standard methods[6], each *A. m. scutellata* study colony was queenright and housed in two hive boxes with a queen excluder between the upper and lower box (the queen was in the lower box). One test frame per colony containing thousands of worker cells and 500–900 empty drone cells was placed in the upper box and sandwiched between two frames containing open brood. The drone cells in each frame were checked daily and the locations of any eggs were noted[6]. The five eggs observed (see Table 1) were all removed within 24 h. This procedure shows that both egg laying by workers and worker policing occurs in *A. m. scutellata*.

Egg-removal rates

Following standard methods[5,9,10] we transferred 600 *A. m. scutellata* queen-laid eggs, 600 *A. m. scutellata* worker-laid eggs and 600 parasitic *A. m. capensis*-laid eggs into worker- and drone-sized cells in three test frames (that is, 20 eggs × 3 discriminator colonies × 5 days × 2 cell sizes per egg source). Test frames were placed into unrelated *A. m. scutellata* discriminator colonies above a queen excluder and sandwiched between two frames containing open brood. Egg removal was quantified by inspecting cells after 2, 6 and 20 h. The difference among all egg sources after 2 h was highly significant ($P < 0.001$). No significant difference between cell type and day or interaction between any of the above parameters was found (three-way analysis of variance (ANOVA)). The data were arcsine-transformed to satisfy the assumption of normality because most observations were close to the limits.

We obtained *A. m. capensis* eggs from infected *A. m. scutellata* colonies. We verified that the eggs were laid by *A. m. capensis* workers and not an *A. m. scutellata* queen as follows: only *A. m. capensis* workers were emerging from cells; there was an irregular pattern of eggs in cells (with multiple eggs in some cells), as is typical in a colony with egg-laying workers; no *A. m. scutellata* queen was seen in any colony despite several hive inspections each.

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Dynamic coding of behaviourally relevant stimuli in parietal cortex

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A general function of cerebral cortex is to allow the flexible association of sensory stimuli with specific behaviours. Many neurons in parietal[1,2], prefrontal[3,4] and motor[5–7] cortical areas are activated both by particular movements and by sensory cues that trigger these movements, suggesting a role in linking sensation to action. For example, neurons in the lateral intraparietal area (LIP) encode both the location of visual stimuli and the direction of saccadic eye movements[8,9]. LIP is not believed to encode non-spatial stimulus attributes such as colour[10,11]. Here we investigated whether LIP would encode colour if colour was behaviourally linked to the eye movement. We trained monkeys to make an eye movement in one of two directions based alternately on the colour or location of a visual cue. When cue colour was relevant for directing eye movement, we found a substantial fraction of LIP neurons selective for cue colour. However, when cue location was relevant, colour selectivity was virtually absent in LIP. These results demonstrate that selectivity of cortical neurons can change as a function of the required behaviour.

Two rhesus monkeys were trained on a delayed saccade paradigm (Fig. 1). Monkeys fixated a spot in the centre of the screen, viewed a peripheral cue for 500 ms, held fixation with no cue present for 1 s, and then directed their eyes from the fixation point to one of two identical targets. The peripheral cue appeared in one of two colours and one of two locations. Two complementary colours were chosen at random for each cell. Of the two locations used, one was centred on the cell's 'response field' (mapped independently using a standard delayed-memory-saccade task, see Methods) and the other location was placed 180° opposite, at the same distance from the fixation point. In interleaved blocks of trials, the rule for determining the appropriate target was switched. One rule was to direct the saccade on the basis of cue colour (for example, 'red means saccade left' and 'green means saccade right'), regardless of the cue location. The alternative rule was to direct the saccade to the cue location ('cue on left means saccade left', 'cue on right means saccade right'), regardless of the cue colour. These two rules are termed 'colour-relevant' and 'location-relevant'. In the colour-relevant task, colour-saccade pairings were reversed every 40 trials (for example, to 'red means saccade right' and 'green means saccade left'), so that selectivity for colour could be analysed independently from selectivity for saccade direction. The colour-relevant task included antisaccades (eye movements away from cue) as well as prosaccades (eye movements towards cue) to require the animals to rely on colour alone to perform the task (Fig. 1). Prosaccade trials in the colour-relevant task matched exactly (in cue colour, cue location and saccade direction) prosaccade trials in the location-relevant task—the only difference was the animal's strategy for choosing. For example, in all trials where a red cue appeared on the left and the animal correctly made a leftward eye movement, the animal was correct on colour-relevant trials because the cue was red, and on location-relevant trials because the cue was on the left. Thus we were able to determine the effect of the behavioural rule on the stimulus selectivity of the neurons.

Figure 2 shows data recorded from 69 LIP neurons during the location-relevant task. Selectivity for cue location (Fig. 2a, c, e) and colour (Fig. 2b, d, f) was examined in three ways. First, normalized population-response histograms were constructed, averaged across all cells. For each cell, trials were sorted by preferred (black line) and non-preferred (grey line) cue location (Fig. 2a) and colour (Fig. 2b). Because the preferred colour was defined as the colour that gave the greater response in this task, colour selectivity was compared to a chance selectivity level computed by a bootstrap technique (see Methods). Although the population of cells was strongly selective for cue location (Fig. 2a), no colour selectivity above chance was observed (Fig. 2b). Two measures were used to quantify the colour and cue-location selectivity. First, transmitted information[12,13], which indicates the reliability of differences in firing rate between two sets of trials, was separately calculated for cue location and colour (Fig. 2c, d). Second, an analysis of variance (ANOVA) was calculated for each unit to evaluate the significance of modulation by cue location or colour ($\alpha = 0.05$; Fig. 2e, f). Both of these analyses were calculated using spike counts in each of five 500-ms epochs of the trial: baseline (B), cue visible (C), early delay (D1), late delay (D2), and perisaccade (S) (see Methods). In the location-relevant task, up to 49% of neurons showed significant selectivity for cue location in any single time period (Fig. 2c, e) while no more than 13% of neurons showed significant selectivity for colour (Fig. 2d, f).

Responses of neurons to corresponding prosaccade trials of the colour-relevant task were analysed with the same methods (Fig. 3). As in the location-relevant task, population responses showed substantial modulation by cue location (Fig. 3a), but unlike in the

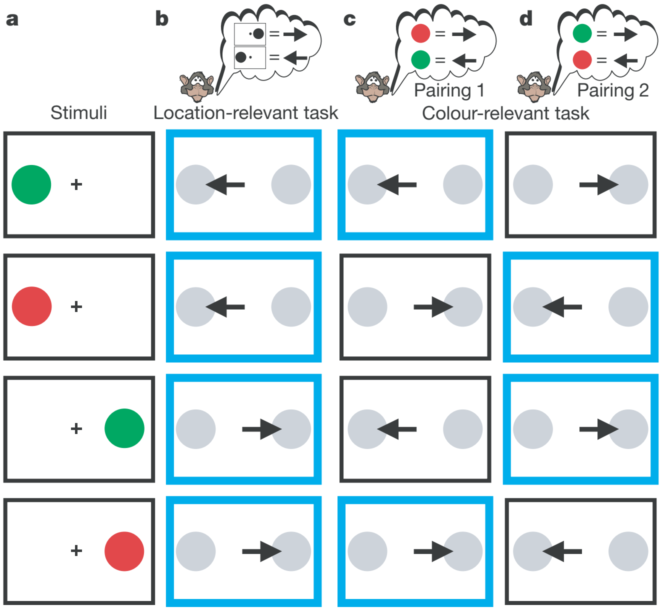

Figure 1 Design of experiment. On each trial, the animal was shown a visual cue (**a**) and after a delay given two identical saccade targets. For the location-relevant task (**b**), the location of the cue, regardless of its colour, indicated which target to choose. For the colour-relevant task (**c**, **d**) the colour of the cue, regardless of its location, indicated which target to choose. Colour-saccade pairings were alternated in blocks of trials yielding all possible pairings of colour, cue location, and saccade direction. In each row, prosaccade trials (blue boxes) are identical in cue colour, cue location and saccade direction in both colour-relevant and location-relevant tasks. Cartoons indicate the behaviour rules that the trained monkeys applied for each task.

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