Egg laying and egg removal by workers are positively correlated in queenright Cape honeybee colonies

_Apis mellifera capensis_

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Abstract – Queenright _Apis mellifera capensis_ colonies exhibit egg laying by workers in periods of both low and high egg removal. To reproduce workers should lay in times of low egg removal to increase survival of their eggs. Were this so, a negative correlation between egg laying and removal would be expected. Egg removal rates for queen (N=240) and worker-laid (N=240) eggs and egg laying by workers were tested in queenright colonies. Worker-laid eggs were removed significantly faster than queen-laid eggs; but significant differences in egg laying by workers occurred among colonies. Egg laying and removal are positively correlated and co-dependent. Egg removal appears triggered by the number of worker-laid eggs. Intercolonial variation for laying worker egg number and egg removal rates may explain the phenotypic variation in worker reproduction in queenright Cape honeybee colonies.

Apis mellifera capensis / egg removal / laying workers / worker reproduction / worker policing

1. INTRODUCTION

With few exceptions (Oldroyd et al., 1994), worker-laid eggs are removed by other workers in queenright colonies of the European subspecies of _Apis mellifera_ L. (Ratnieks and Visscher, 1989). This seems to be based on relatedness grounds (Ratnieks, 2000), because laying workers usually produce male offspring (Free, 1987). However, laying workers of the Cape honeybee (_Apis mellifera capensis_ Eschscloltz) produce female offspring (Onions, 1912; Neumann et al., 2000; Hepburn and Radloff, 2002; Radloff et al., 2002), leading to predictions that egg
removal is either not expressed at all, or is less expressed in this subspecies (Greeff, 1996). Nevertheless, queenright Cape honeybee colonies exhibit worker policing (Neumann, Pirk, Ratnieks, unpublished data), indicating that removal of worker-laid eggs can also be based on colony efficiency grounds.

Brood above the queen excluder is more frequently observed in queenright colonies of Cape honeybees (Pettey, 1922; personal observations) than in other subspecies of *A. mellifera* (Visscher, 1996). It has been shown that such brood is actually worker-derived (Moritz et al., 1999), indicating successful worker reproduction despite the presence of a queen and egg removal. Indeed, thousands of queenright colonies of the neighbouring subspecies *Apis mellifera scutellata* Lepeletier were taken over by laying *A. m. capensis* workers (Allsopp and Crewe, 1993; Martin et al., 2002), showing that *A. m. capensis* workers are facultative social parasites (for details see Hepburn and Allsopp, 1994; Neumann et al., 2001; Calis et al., 2002; Moritz, 2002; Neumann and Hepburn, 2002; Reece, 2002; Wossler, 2002). These observations strongly indicate that laying workers of *A. m. capensis* are able to evade worker policing, but what potential strategies, if any, could these laying workers use to increase the survival of their eggs?

Earlier observations showed (Pirk, Neumann, Hepburn, Radloff, unpublished data) that worker policing is subject to environmental variation within colonies of *A. m. capensis*, thus leading to periods of low egg removal rates under unfavourable weather conditions within a colony. Because worker policing is only exercised against eggs (Ratnieks and Visscher, 1989), worker-laid eggs need only survive three days after oviposition, which might fit well in a time window of low egg removal. So one potential strategy of laying *A. m. capensis* workers to evade worker policing, which we designate as hypothesis 1 could be that they are able to evaluate periods of low egg removal and lay their eggs during this time window. In this case one would expect a negative correlation between worker egg laying and the removal of worker-laid eggs by other workers, because workers should lay more eggs when there is less egg removal by other workers and vice versa.

But, the same environmental factors which affect egg removal behaviour may also reduce egg-laying activity (hypothesis 2). This might be simply due to the generally reduced activity of workers during periods with unfavourable weather conditions (Riessberger et al., 1998). Alternatively, periods with unfavourable weather conditions may also reduce the survival chances of worker-derived offspring because nurse bees change from the care of young larvae to the care of older larvae during such periods (Blaschon & Crailsheim, 2001). This may easily outweigh the risk of being removed by other workers. Thus, unfavourable weather conditions may not only reduce the activity of egg removal behaviour by workers but also of egg laying by workers. In this particular case one would expect a positive correlation between worker egg laying and removal of worker-laid eggs by other workers, because laying worker activity and egg removal behaviour are affected in the same way.

Alternatively a third hypothesis emerges, but not necessarily mutually exclusive of hypothesis 2, that laying workers may not be able to evaluate periods of low egg removal periods and egg removal is simply triggered by the number of worker-laid eggs present in the colony, leading to a positive correlation between egg laying and egg removal. Clearly, it is not possible to distinguish between hypothesis 2 and 3 because both predict a positive correlation between egg removal and number of worker-laid eggs.

Here we test these three hypotheses by evaluating egg laying and removal of worker-laid eggs by workers in queenright Cape honeybee colonies.
2. METHODS

Two queenless and four queenright A. m. capensis colonies were obtained from Port Elizabeth and placed in a test apiary in Grahamstown, South Africa. All colonies were unrelated to avoid any bias derived from nestmate recognition on egg removal behaviour (Pirk et al., 2001), and housed in 10-frame standard Langstroth hives with two brood boxes. The colonies were given two days to settle to avert absconding (Hepburn et al., 1999). Then, three test combs (A, B, C) were placed in the brood nest of each of three queenright test colonies two days before the experiments began to avoid any potential impact of the introduced comb (Breed et al., 1995) on egg removal behaviour.

The A test combs were used to evaluate the level of egg removal for queen and worker-laid eggs (Ratnieks and Visscher, 1989; Ratnieks, 1993). The level of egg removal was measured as the proportion of eggs removed from the total number of eggs that were transferred expressed as a percentage. One queenright and two queenless colonies were used as egg sources. Twenty queen and 20 worker-laid eggs were transferred daily for four days on each of the A test combs, which were then again sandwiched between two brood frames of the test colonies (Fig. 2) according to standard methods for evaluating egg removal rates (Ratnieks and Visscher, 1989; Ratnieks, 1993; Oldroyd and Ratnieks, 2000). After 24 hours the A test combs were briefly removed, all remaining eggs were counted and then removed before transferring a new set of eggs onto the comb.

The queens of the three test colonies were placed in small wooden cages [8 cm × 4 cm × 2.5 cm] with gauze mesh [mesh width = 2 mm], to allow feeding by workers but preventing the queens from egg laying (Fig. 1). These cages were attached in a frame of empty comb (B) and returned to the middle of the brood nest (Fig. 2). The B

Figure 1. The queen cage of frame B. The B frame was sandwiched between two brood frames in the bottom box next to empty combs A and C (Fig. 2), so that the queen was still present in the brood nest and workers had partial access to the queen.
combs were not moved in any way during the experiment to keep the disturbance of the colony and the queen to a minimum.

Another empty test frame (C) was also placed in each of the colonies to evaluate egg laying by workers (Fig. 2). From the next morning onwards worker-laid eggs in each of the test colonies were counted once daily at 09:00 after removing the C test frames on four sequential days. During counting, sheets of transparent films were placed over the C combs and the position of each egg was recorded by using a reference grid to avoid double counting and to count only eggs laid by workers within a 24 hour period.

Simultaneously on four sequential days, the level of egg removal for queen and worker-laid eggs (A test combs) and the egg laying behaviour of workers were evaluated (C test combs). It is assumed that egg removal and egg laying could equally occur on both A and C combs, so that any difference in the numbers of egg between the combs would a be systematic error.

Mann Whitney U-tests were used to compare the level of egg removal of queen and worker-laid eggs on the A test combs after 24 hours. A $\chi^2$-test was performed to test the difference in the number of worker-laid eggs between the colonies. The same test was used to compare the level of egg removal of worker laid eggs between the three colonies. Both tests were performed to investigate possible intercolonial variation. A Spearman rank order correlation was calculated for the number of worker-laid eggs and the level of egg removal of worker-laid eggs in the test cells.

### 3. RESULTS

On the A test combs a total of 240 queen-laid and 240 worker-laid eggs were tested for egg removal rates on a daily basis (Tab. I). Worker-laid eggs were removed significantly faster than queen-laid eggs (Mann-Whitney U-test: $Z = 2.22, P < 0.026$). There were no differences in the levels of egg removal of worker-laid eggs among the colonies ($\chi^2 = 1.37; df = 2, n.s.$), but there was variation within each test colony (Tab. I).

A total of 57 eggs laid by workers were recovered from the C combs in the test
colonies (Tab. I). Significant differences were found between the colonies in the number of worker-laid eggs (χ² = 30.74; df = 2, P < 0.0001). The number of worker-laid eggs that were laid and the level of egg removal of worker-laid eggs were significantly positively correlated (Fig. 3).

### 4. DISCUSSION

The data confirm earlier findings that worker honeybees in queenright colonies of *A. m. capensis* are able to recognise and remove worker-laid eggs (Pirk et al., 2001). Moreover, this study shows a significant

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**Table I.** Numbers of worker-laid eggs and number of eggs remaining or removed after 24 hours in the queenright *A. m. capensis* test colonies on four sequential days. Means and standard deviations are shown.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Day</th>
<th>Test comb A</th>
<th>Worker eggs remaining (Removal rates [%])</th>
<th>Queen eggs remaining (Removal rates [%])</th>
<th>Worker-laid eggs</th>
</tr>
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<tr>
<td>1</td>
<td>1</td>
<td>0 (100)</td>
<td>6 (70)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3 (85)</td>
<td>6 (70)</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>4 (80)</td>
<td>3 (85)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6 (70)</td>
<td>9 (55)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± sd 3.25 ± 2.5 (83.75 ± 12.5) 6 ± 2.45 (70 ± 7.5) 9.25 ± 11.3

<table>
<thead>
<tr>
<th>Colony</th>
<th>Day</th>
<th>Test comb C</th>
<th>Worker eggs remaining (Removal rates [%])</th>
<th>Queen eggs remaining (Removal rates [%])</th>
<th>Worker-laid eggs</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1</td>
<td>0 (100)</td>
<td>6 (70)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0 (100)</td>
<td>4 (80)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3 (85)</td>
<td>3 (85)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5 (75)</td>
<td>6 (70)</td>
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<td></td>
</tr>
</tbody>
</table>

Mean ± sd 2 ± 2.45 (90 ± 12.25) 4.75 ± 2.65 (76.25 ± 7.5) 4.25 ± 4.27

<table>
<thead>
<tr>
<th>Colony</th>
<th>Day</th>
<th>Test comb C</th>
<th>Worker eggs remaining (Removal rates [%])</th>
<th>Queen eggs remaining (Removal rates [%])</th>
<th>Worker-laid eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2 (90)</td>
<td>6 (70)</td>
<td>0</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
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<td>8</td>
<td>8 (60)</td>
<td>10 (50)</td>
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<td>4</td>
<td>5</td>
<td>7 (55)</td>
<td>9 (55)</td>
<td>0</td>
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</tr>
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</table>

Mean ± sd 5.0 ± 2.9 (75 ± 17.56) 8.75 ± 2.06 (56.75 ± 10.3) 0.75 ± 0.95

**Figure 3.** Number of worker-laid eggs (C comb) and removal rate (%) of worker-laid eggs (A comb) after 24 hours for three queenright *A. m. capensis* colonies on four sequential days. The number of worker-laid eggs and the level of egg removal are significantly positively correlated (Spearman Rank Order Correlation: rₚ = 0.6, P < 0.039).
colony variation in egg laying by workers among the three discriminator colonies (Tab. I), but no significant differences for egg removal behaviour among them. The data also show that egg removal and egg laying by workers are positively correlated in the Cape honeybee (Fig. 3), indicating that egg removal and egg laying are co-dependent.

The standard method for evaluating egg removal behaviour in queenright honeybee colonies uses non-nestmate queen and worker-laid eggs (Ratnieks and Visscher, 1989; Ratnieks, 1993; Oldroyd and Ratnieks, 2000). Thus, nestmate recognition for eggs (Visscher, 1986) affects egg removal estimates by overestimating the removal rates of alien worker-laid eggs compared to native worker-laid eggs (Pirk et al., 2001). Although alien worker-laid eggs (transferred into the colony) were compared with native worker-laid eggs (laid in the colony) in this study, this would simply result in a systematic error that is the same for all colonies and would not affect the correlation between egg removal and egg laying.

The positive correlation between the number of worker-laid eggs and egg removal rates clearly indicates that Cape honeybee laying workers do not specifically lay eggs during periods of low egg removal rates. Hypothesis 1 can thus be rejected. Hypothesis 2 is based on the coincidence of periods of low egg removal rates with low egg laying rates and predicts a positive correlation between removal and egg laying. Also if egg removal, is triggered by the number of eggs found in the combs there would be a positive correlation between removal and egg laying (hypothesis 3). Thus, hypotheses 2 and 3 both fit our data but we cannot discriminate between the two. We consider hypothesis 3 as slightly more likely because less steps are involved to explain the observed correlation.

Our data show that the occurrence of worker-derived brood above the excluder is unlikely to reflect a specific strategy of laying workers to evade periods of high egg removal. We rather conclude that a combination of intercolonial variation in the number of eggs laid by workers, their egg removal rates and the ability of policing workers to remove worker-laid eggs may explain the phenotypic variation for successful worker reproduction in queenright Cape honeybee colonies.

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Résumé – La ponte et l’élimination des œufs par les ouvrières sont positivement corrélées chez les colonies avec reine de l’abeille du Cap (Apis mellifera capensis). Chez les colonies d’abeilles domestiques (Apis mellifera L.) européennes les œufs pondus par les ouvrières sont éliminés par d’autres ouvrières, c’est ce qu’on appelle le « worker policing » ou maintien de l’ordre par les ouvrières. Ce phénomène repose théoriquement sur le fait que les mâles produits par les ouvrières sont moins apparentés aux ouvrières que les mâles produits par la reine, du fait de l’accouplement multiple de la reine. Les ouvrières pondeuses de l’abeille du Cap produisent des œufs femelles diploïdes qui peuvent donner soit des ouvrières, soit des reines. Cela conduit à l’hypothèse que l’élimination des œufs ne s’exprime pas, ou s’exprime moins chez A. m. capensis comparé aux races à reproduction arrhénotoque. Néanmoins les abeilles du Cap présentent un comportement de maintien de l’ordre par les ouvrières, indiquant que ce comportement peut reposer sur des bases d’efficacité au sein de la colonie.
Dans les colonies de l’abeille du Cap on observe régulièrement du couvain au-dessus de la grille à reine, couvain issu d’ouvrières pondueuses, ce qui indique que les ouvrières réussissent à se reproduire malgré la présence d’une reine et l’élimination des œufs. Ces observations sont de forts indices que les ouvrières pondueuses d’*A. m. capensis* sont capables d’échapper au maintien de l’ordre par les ouvrières mais l’on peut se demander de quelles stratégies, si elles existent, ces ouvrières pondueuses usent pour augmenter la survie de leurs œufs.

Le processus d’élimination du couvain d’ouvrières est influencé par les conditions du milieu. Lorsque les conditions météorologiques sont défavorables, l’élimination des œufs pondus par les ouvrières est réduite, si bien qu’il y a dans les colonies alternance de périodes à forte élimination d’œufs et de périodes à faible élimination d’œufs. Notre première hypothèse est que les ouvrières, principalement durant les périodes de faible élimination d’œufs, pondent pour augmenter les chances de survie de leur propre couvain. La seconde hypothèse est que ces mêmes facteurs du milieu qui influencent le comportement d’élimination des œufs sont aussi préjudiciables à l’activité de ponte. La troisième hypothèse est que les ouvrières pondueuses ne peuvent pas différer les périodes à forte élimination d’œufs des périodes à faible élimination, si bien que l’élimination des œufs par les ouvrières dépend du nombre d’œufs pondus par les ouvrières. Pour tester ces hypothèses nous avons évalué chez trois colonies du Cap possédant une reine la ponte et l’élimination des œufs pondus par les ouvrières (Tab. I). La corrélation significativement positive entre le comportement de ponte et l’élimination des œufs pondus par les ouvrières montre que ces deux comportements ne sont pas indépendants l’un de l’autre (Fig. 3). On peut donc éliminer l’hypothèse 1 selon laquelle les ouvrières pondueuses pondent durant les périodes de faible élimination d’œufs. L’hypothèse 2 est basée sur la coïncidence de périodes à faible élimination d’œufs et à faible taux de ponte et prédit une corrélation positive entre l’élimination des œufs et leur ponte. Si l’élimination des œufs est déclenché par le nombre d’œufs présents dans les rayons, l’élimination des œufs et leur ponte doivent être également corrélées positivement. Ainsi nos résultats confirment les hypothèses 2 et 3 mais ne peuvent trancher entre elles deux.

**Apis mellifera capensis** / ouvrière ponduese / reproduction des ouvrières / élimination des œufs / régulation sociale


**Apis mellifera capensis / legenden Arbeiterinnen / Arbeiterinnenreproduktion / Entfernen von Arbeiterinneneiern / worker policing**

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