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A non-policing honey bee colony (*Apis mellifera capensis*)

Received: 8 March 2002 / Accepted: 20 August 2002 / Published online: 21 September 2002
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Abstract In the Cape honey bee *Apis mellifera capensis*, workers lay female eggs without mating by thelytokous parthenogenesis. As a result, workers are as related to worker-laid eggs as they are to queen-laid eggs and therefore worker policing is expected to be lower, or even absent. This was tested by transferring worker- and queen-laid eggs into three queenright *A. m. capensis* discriminator colonies and monitoring their removal. Our results show that worker policing is variable in *A. m. capensis* and that in one colony worker-laid eggs were not removed. This is the first report of a non-policing queenright honey bee colony. DNA microsatellite and morphometric analysis suggests that the racial composition of the three discriminator colonies was different. The variation in policing rates could be explained by differences in degrees of hybridisation between *A. m. capensis* and *A. m. scutellata*, although a larger survey is needed to confirm this.

Introduction

In South Africa two honey bee subspecies occur, the African bee *Apis mellifera scutellata* and the Cape bee *A. m. capensis*. The two are separated by a hybrid zone (Hepburn et al. 1998). The Cape bee is native to the south of the Cape Province and the African bee to the rest of the country. Despite mixing of the subspecies in the hybrid zone, this zone has apparently been stable for decades with neither increasing its range (Hepburn and Crewe 1990, 1991). Unlike other honey bee species, where worker-laid eggs are haploid and develop into males, eggs laid by Cape bee workers are diploid and develop into females (Anderson 1963). The thelytokous production of females by workers has important consequences for within-colony relatedness and the occurrence of conflict over reproduction (Greeff 1996). One consequence is the predicted reduction or absence of worker policing, the differential removal of worker-laid eggs by other workers (Ratnieks 1988), because workers are equally related to both queen- and worker-laid eggs. Hence there is no relatedness advantage to raising the queen's offspring over worker's offspring. There could, however, be other advantages to worker policing, for example when worker reproduction lowers colony efficiency.

There are numerous anecdotal reports of worker-laid eggs in queenright colonies of *A. m. capensis* (Moritz et al. 1999). In most cases these report brood above the queen excluder. However, *A. m. capensis* workers rapidly activate their ovaries when above a queen excluder (personal observation) because it apparently creates a situation which is similar to queenlessness. Therefore, these observations cannot be taken as proof for the absence of worker policing.

To directly assess worker policing in *A. m. capensis* we transferred worker-laid and queen-laid eggs into the brood chamber of three queenright *A. m. capensis* discriminator colonies and monitored their removal. We used DNA microsatellite and morphometric analysis to investigate the relationship between the racial composition of the colonies and their policing behaviour.

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Methods

Study site and origin of colonies and eggs used

All colonies used originated from the Berg River, 90 km north of Stellenbosch, Cape Province, South Africa. The egg transfers were performed in Stellenbosch in December 2000. Two queenless splits were made from *A. m. capensis* colonies by dividing the colonies into a queenright (containing the queen) and a queenless (without the queen) part. Only the resulting queenless colonies were used and served as a source of worker-laid eggs. Five queenright *A. m. capensis* colonies served as a source of queen-laid eggs. Although we cannot fully exclude the possibility that some of the eggs collected from these queenright colonies were laid by workers, we reduced this possibility by collecting eggs from the brood chamber (that part of the colony where the queen is present). *A. m. capensis* workers are more likely to lay eggs above the queen excluder, where the queen is not present.

Bioassay

To determine the removal rates of worker- and queen-laid eggs, we introduced both egg-types into cells in three queenright discriminator colonies using standard techniques (Oldroyd and Ratnieks 2000; Ratnieks and Visscher 1989). However, we used worker cells instead of drone-cells because eggs laid by *A. m. capensis* workers are diploid and are normally laid in worker cells. We did not divide our discriminator colonies with a queen-excluder to avoid the possibility that workers activated their ovaries. Instead, we placed the frame with transferred eggs in the middle of the broodnest. To control against the possibility that the queen laid eggs in cells from which eggs had been removed we monitored an empty row for freshly laid eggs. Eggs were never found in these rows. Because the queen lays eggs in batches this strongly suggests that no egg laying by the queens occurred in our test cells. Eggs (0–24 h old) were removed from their cells using a needle and immediately transferred to the experimental comb. In total, 130 queen- and worker-laid eggs were transferred per discriminator colony during five consecutive days. The number of eggs remaining was determined 2, 6 and 18–22 h later. On each day, each discriminator colony was given eggs from the same source colony. Hence, even if some of the queen-laid eggs were in fact laid by workers, this will not affect our results because we are interested in differences in policing rates among the three discriminator colonies.

Microsatellite and morphometric analysis

From each discriminator colony, adult workers were collected in 90% ethanol. In addition, workers were collected from two *A. m. capensis* colonies originating from Cape Point in the Cape Peninsular National Park (colonies C1 and C2). Because the origin of these two colonies is as remote from *A. m. scutellata* populations as possible, they are the least likely to be hybrids and therefore served as a reference for *A. m. capensis* markers. From each colony 100 workers were analysed using the microsatellite loci A8 (Oldroyd et al. 1995), A24 (Estoup et al. 1995) and A28 (Estoup et al. 1994). These loci were used because their allele frequencies are significantly different between *A. m. capensis* and *A. m. scutellata* [χ^2 test using the combined allele frequencies of Estoup et al. (1995) and K.E. Clarke (www.bio.usyd.edu.au/Beelab2/Data_1.htm#data_1; <0.005, df values for each locus: A8: 11, A24: 11, A28: 17].

For each individually-labelled bee, one leg was used for genetic analysis and the rest of the bee was used for morphometric analysis. Thirty-six morphological characters were measured from 51 (colony 1), 42 (colony 2), 49 (colony 3), 50 (colony C1) and 47 (colony C2) bees following Ruttner (1988). Data were analysed using multivariate principal components analysis and stepwise linear discriminant analysis. Wilks' λ test was used to compare multivariate population means between the morphoclusters. The Mahalanobis squared distances (d^2) between the clusters were calculated separately.

DNA was extracted using a 5% Chelex solution (Walsh et al. 1991) and analysed using protocols given in Oldroyd et al. (2000). Individual bees originating from the three discriminator colonies and the two *A. m. capensis* colonies (C1 and C2), were assigned to either *A. m. capensis* or *A. m. scutellata* reference populations based on Nei's genetic distance from these populations using the program GeneClass (Cornuet et al. 1999). The *A. m. capensis* reference population consisted of bees sampled from 17 colonies originating from swarms caught in Cape Point in 1993. The *A. m. scutellata* reference population consisted of bees from 25 colonies collected in 1984 and 36 collected in 1993, all from around Johannesburg or further north. These reference populations are the samples of Clarke et al. (2001) excluding 16 *A. m. capensis* samples originating from Stellenbosch.

Results

Not all discriminator colonies removed worker-laid eggs. Figure 1 shows that two of the discriminator colonies removed worker-laid eggs rapidly but did not remove all queen-laid eggs (repeated-measures ANOVAs after arcsine square root transformation, colony 2: $F=51.206$, $df=1$, $P<0.001$, colony 3: $F=23.206$, $df=1$, $P=0.001$). In colony 1 worker and queen-laid eggs were treated equally ($F=4.766$, $df=1$, $P=0.06$), hence this colony did not police.

The genetic distances of the three discriminator colonies and the two *A. m. capensis* colonies (C1 and C2) was compared with the reference populations of *A. m. capensis* and *A. m. scutellata*. This yields the relative genetic distance of the three discriminator colonies and the two colonies from Cape of Good Hope to the reference *A. m. scutellata* and *A. m. capensis* populations and enables the comparison of the three discriminator colonies with the Cape of Good Hope colonies (Fig. 2). The difference among the five colonies is statistically different (ANOVA, $n=318$, $F=5.049$, $df=4$, $P=0.001$). Fisher's LSD test shows that colonies 2 and 3 are different from both *A. m. capensis* colonies C1 ($P=0.09$ and $P=0.011$) and C2 ($P=0.001$ and $P\leq 0.001$), whereas colony 1 is only different from colony C1 ($P=0.02$).

In a principal components analysis of the 36 morphometric characters measured, 12 principal components with eigenvalues >1 were isolated: PC 1, characters associated with size (5), (6), (7), (8), (9), (10), (11), (12), (15), (16), (17) and (18) had loadings between 0.54 and 0.78 and accounted for 17.03% of the variance; PC 2, wing venation characters (30) and (26) had loadings in the range 0.50–0.58 and accounted for 8.48% of the variance; PC 3, pigmentation (32), (33) and (35) had loadings between 0.53 and 0.67 and accounted for 7.14% of the variance; PC 4, size (19), wing venation (23) and pigmentation (34) had loadings between 0.495 and 0.69 and accounted for 6.31%; PC 5 to PC 12 had only one character with loadings >0.44 in each component. The 12 principal components accounted for 70.74% of the variance in the data. A graph of the principal component 1 scores (representing size) and principal component 2 scores (representing angles of venation) revealed three statistically separate but not distinct morphoclusters, colony C1, colony C2, and the three experimental colonies.

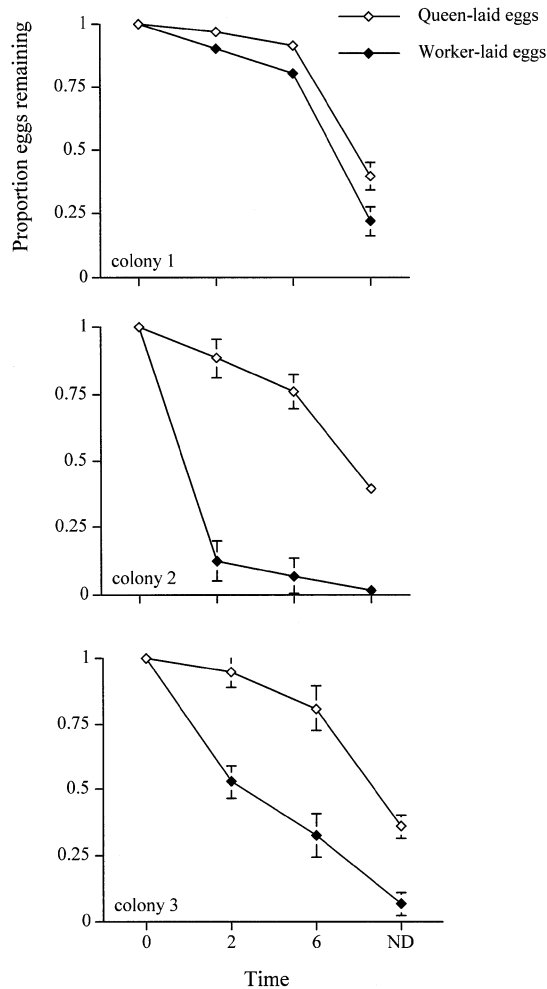


Fig. 1 Removal rates of eggs laid by *Apis mellifera capensis* workers and *A. m. capensis* queens when introduced into worker cells in the three discriminator colonies. The bars represent the standard errors of the means (five trials per colony). In some cases the error bars are very small and so are obscured by the data point. ND stands for Next Day, when the last assessment was performed (18–22 h after introduction)

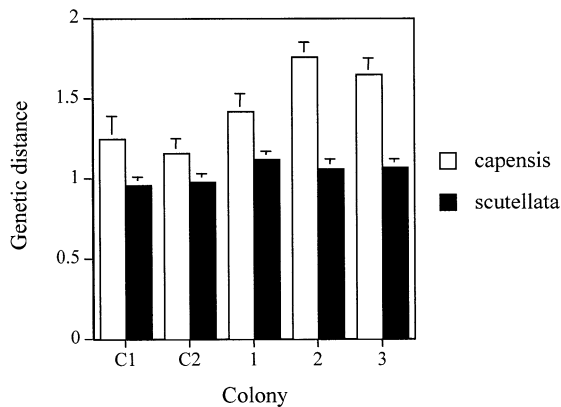


Fig. 2 Mean genetic distances (and standard error) of 100 bees of each colony to the reference *A. m. scutellata* (black bars) and *A. m. capensis* (white bars) populations (Clarke et al. 2001). C1 and C2 are the *A. m. capensis* colonies originating from Cape Point. Distances are significantly different among the five colonies. Colonies 2 and 3 are different from both C1 and C2 whereas colony 1 is only different from colony C2

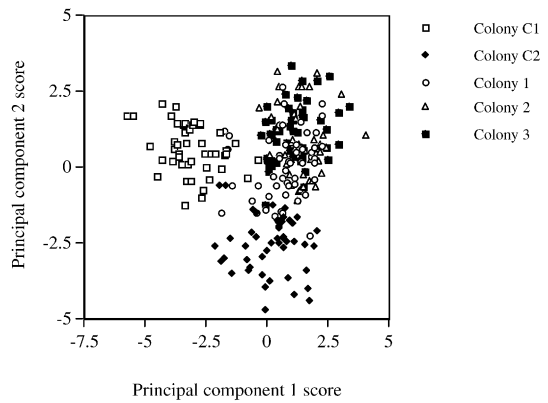


Fig. 3 Morphoclusters based on stepwise linear discriminant analysis using the 36 morphometric characters measured. Colonies C1 and C2 are different from the experimental colonies. Among the three experimental colonies, colony 1 is different from the other two

Table 1 Squared Mahalanobis distances (d^2) of the five colonies

	Reference <i>A. m. capensis</i> colonies		Discriminator colonies		
	C1	C2	1	2	3
C1	0.00000	19.9808	20.0768	23.4310	20.5298
C2		0.00000	14.8360	15.4164	15.4049
1			0.00000	12.6163	11.1800
2				0.00000	7.52341
3					0.00000

PC scores plots using the other principal components revealed less distinct morphoclusters.

In order to determine the probabilities of correct classification of the bees into the three morphoclusters and to ascertain the degree of distinctness of the morphoclusters, a stepwise linear discriminant analysis using the 36 morphometric characters was carried out (Fig. 3). Listed are the characters that entered the discriminant functions according to their discriminatory power: (30), (1), (24), (21), (28), (27), (13), (16), (6), (3), (8), (33), (34), (36), (26), MJI, (35) (31), (2) and (32). The linear discriminant functions obtained correctly classified 96.0% of the bees from colony C1; 93.6% from C2; and 90.9% from the experimental colonies. Multiple vector means of the three colony clusters were significantly different (Wilks' test for the multivariate equality of means, $\lambda=0.0912$, $df=40,434$, $P<0.0001$). The Mahalanobis squared distances between the centroids of the morphoclusters are given in Table 1.

These results confirm the microsatellite results in that the bees from colony C1 and C2 are morphometrically different from those from the three experimental colonies. More importantly, the bees originating from the non-policing colony 1 are morphometrically different from the bees of colonies 2 and 3 ($d^2 =7.52$ for colony 1 and $d^2 =12.6$ and 11.2 for colonies 2 and 3, respectively).

Discussion

Lack of policing, using the same experimental set-up, has not been previously recorded in honey bees despite a

large number of studies across the genus. Policing of worker-laid eggs has now been reported from 46 *Apis* colonies [24+4+4 *A. mellifera* colonies: (Oldroyd and Ratnieks 2000; Ratnieks 1995; Ratnieks and Visscher 1989), three *A. m. scutellata* colonies (Martin et al. 2002), six *A. cerana* colonies (Oldroyd et al. 2001), and five *A. florea* colonies (Halling et al. 2001)]. When reduced policing occurs, as in anarchistic bees (Oldroyd and Ratnieks 2000) and *A. m. capensis* workers parasitising *A. m. scutellata* colonies (Martin et al. 2002), it is due to eggs that evade policing probably because the workers that lay the eggs mimic the queen's egg marking pheromone. Although anarchistic discriminator colonies seem to be somewhat less accurate in their policing behaviour (Oldroyd and Ratnieks 2000), they still remove worker-laid eggs. In our case, however, all eggs transferred into the discriminator colonies came from the same worker-laying or queenright colonies. Therefore, the non-policing of colony 1 is due to the discriminating colony, not due to the eggs it was given. This makes our colony 1 unique.

Unlike *A. m. capensis*, *A. m. scutellata* is expected and observed to police worker-laid eggs (Martin et al. 2002). Does this mean that our non-policing colony does not police because it is more *capensis*-like than the other two discriminator colonies? Both our microsatellite data and the morphometrics data point in that direction: colony 1 was more similar to the *A. m. capensis* references than the other two colonies used.

It is tempting to suggest that the variance in policing rates observed is related to the degree of hybridisation in our discriminator colonies, with our non-policing colony representing pure *A. m. capensis* and the other two being somewhat hybridised with *A. m. scutellata*. However, because *A. m. scutellata* and *A. m. capensis* are subspecies, and the genetic differences between them are subtle, making it impossible to unambiguously distinguish *A. m. capensis* and *A. m. scutellata* and their hybrids on a per colony basis via genetic or morphometric means. Nonetheless two sorts of data (morphometrics and microsatellite allele frequencies) support the interpretation of a correlation between genotype and policing behaviour.

Alternatively, non-policing could be due to other reasons. Because there are no relatedness benefits from preferring the queen's offspring over worker's offspring, and the cost of worker reproduction is likely to be low when workers produce new workers, selection for or against policing might be weak, leading to large variation among *A. m. capensis* colonies. So the question is: does the policing rate depend on the amount of hybridisation between *A. m. capensis* and *A. m. scutellata* or is there simply more variation in policing rates in *A. m. capensis* due to the weaker selection on policing in this subspecies? Our data do not allow us to say which of the two hypotheses is the most plausible. What our data do show is the need for further studies in which larger numbers of *A. m. capensis* colonies are bioassayed.

Acknowledgements We thank Johan Calis for his assistance in Stellenbosch and Kylea Clarke and Ben Oldroyd for their help with the genetic analysis. M.B. was supported by a postdoctoral

fellowship from the research network "Social Evolution" financed by the European Commission via the Training and Mobility of Researchers programme. Further financial support was obtained from the Australian Research Council. The experiments comply with the current laws of the country in which they were performed.

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