Apidologie 41 (2010) 54–61
© INRA/DIB-AGIB/EDP Sciences, 2009
DOI: 10.1051/apido/2009047

Available online at:
www.apidologie.org

Original article

Hetero-specific queen retinue behavior of worker bees in mixed-species colonies of *Apis cerana* and *Apis mellifera**

Ming-Xian YANG[1,2], Ken TAN[2], Sarah E. RADLOFF[3], Christian W.W. PIRK[4], H. Randall HEPBURN[1,2]

[1] Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, Republic of South Africa
[2] Eastern Bee Research Institute, Yunnan Agricultural University, Heilongtan, Kunming, Yunnan Province, P.R. China
[3] Department of Statistics, Rhodes University, Grahamstown 6140, Republic of South Africa
[4] Department of Zoology and Entomology, Social Insect Research Group, University of Pretoria 0002 Pretoria, Republic of South Africa

Received 18 March 2009 – Revised 5 May 2009 – Accepted 6 May 2009

Abstract – The retinue behavior of worker bees of *Apis cerana cerana* and *Apis mellifera ligustica* in two types of mixed-species colonies were studied to determine whether observed behaviors are pre- or post-speciation developments. In *A. cerana* queen-led mixed colonies, almost equal numbers of *A. cerana* workers (53.4 ± 7.4) and *A. mellifera* workers (51.2 ± 8.1) attended the *A. cerana* queen; while in *A. mellifera* queen-led mixed colonies, the *A. mellifera* queen attracted significantly fewer (47.8 ± 5.9) *A. cerana* workers than *A. mellifera* workers (51.9 ± 4.6). Thus about 100 workers in total were attracted to each queen. In pure *A. cerana* and *A. mellifera* colonies, the queen attracted 105.8 ± 9.1 and 107.8 ± 11.2 workers, respectively, there being no significant difference between them. Only the pheromones 9-ODA, 9-HDA and 10-HDA of the queens were significantly different and the workers did not show avoidance behavior to either hetero-specific queen. Both species of workers were attracted by the queens and engaged in retinue behavior, suggesting that the retinue response was not related to a specific queen pheromone or colony environment. This non-specific queen retinue behavior in the mixed colonies indicates that the queen pheromones can be transmitted among the workers from the two species without any obstacles. We conclude that retinue behavior itself as well as the pheromones of the queens that induce this behavior are both primitive, conserved traits that preceded speciation in apine bees.

***Apis cerana* / *Apis mellifera* / retinue behavior / queen pheromones**

1. INTRODUCTION

Retinue behavior is fundamental and crucial to the biology of social insects because queens must be attended to ensure functionality of colonies. "Retinue" behavior [= "court behavior", Allen, 1955, 1960; = "attending behavior", Sakagami, 1958; Velthuis, 1972] refers to the behavior of worker honeybees, *Apis spp.*, that form a loose circle facing in toward their queen. These bees feed and frequently lick her, but soon leave the circle to be replaced by others (Ribbands, 1953; Allen, 1955). As the queen moves over the comb, most of the workers who encounter her show a distinct interest, and extend their antennae and palpate her (Butler, 1954; Sakagami, 1958). Naumann et al. (1991) demonstrated that these retinue bees obtain pheromones from the body of the queen and pass them trophallactically to other workers. Although the wax combs in honeybee colonies also play a role in queen pheromone transfer (Hepburn, 1998),

---

Corresponding author: Sarah Radloff, s.radloff@ru.ac.za; Ken Tan, eastbee@public.km.yn.cn
* Manuscript editor: Stan Schneider

the retinue bees are the principal transmitters (Naumann et al., 1991). Consequently, as the first group of receivers and messengers, the retinue bees play a key role in pheromonally transmitting the queen's presence throughout the colony (Seeley, 1979; Naumann et al., 1991; Pankiw et al., 1995).

The pheromones of a queen which attract workers and induce retinue behavior include secretions from the mandibular glands and Dufour's gland (Slessor et al., 1988; Kaminski et al., 1990; Pankiw et al., 1995; Katzav-Gozansky et al., 2003). In retinue bioassays with *A. cerana* workers, only three constituents of the mandibular gland pheromones were sufficient to elicit full retinue behavior (Plettner et al., 1997). Although *A. cerana*, is a sister-species of *A. mellifera*, having diverged only about 3 million years ago (Arias and Sheppard, 1996, 2005), some behavioral traits and morphological characteristics of the two species are very similar indeed, and, clearly are highly conserved, pre-speciation traits. Among them are pheromones of their respective queens, which share most, but not all, chemical constituents of the mandibular glad pheromonal bouquet (Plettner et al., 1997). For example methyl oleate, coniferyl alcohol, and linolenic acid appear unique to *A. mellifera* (Keeling et al., 2003). However, reciprocal assays to assess whether retinue behavior can be induced within a heterospecific context, such that *A. cerana* queens attract *A. mellifera* workers and *A. mellifera* queens *A. cerana* workers remain to be performed.

Mixed-species colonies offer an intriguing model to investigate the behavioral relationships of the two species, and to suggest which features are ancestral to the common ancestor of *A. cerana* and *A. mellifera* and which may have preceded speciation. As examples, while there are dialectical differences in the waggle dances of different species (Lindauer, 1957; Dyer and Seeley, 1991; Dyer, 2002), it has recently been demonstrated independently that heterospecific dance communication is operative in both *A. cerana* and *A. mellifera* (Su et al., 2008; Tan et al., 2008). Returning to pheromones, it remains to be seen whether heterospecific retinue behavior is shared in *A. cerana* and *A. mellifera*, and if so, would such behavior aid in the dispersal of the queen pheromones, or would the 'guest' species in such mixed colonies avoid the host queen in order to escape pheromonal control (Moritz et al., 2001; Neumann and Moritz, 2002). A plausible theoretical background for possible differences in heterospecific retinues would lead to the hypothesis that we would expect no differences in the proportions of *A. cerana* and *A. mellifera* workers attending heterospecific queens versus conspecific queens. In which case, a complete lack of differences would indicate that retinue behavior had developed prior to speciation; and small differences would indicate very recent changes in the system. The results could indicate whether any aspects of retinue behavior are pre- or post-speciation developments.

2. MATERIALS AND METHODS

2.1. Honeybee colonies

The experiments and observations were conducted with colonies of *Apis cerana cerana* and *Apis mellifera ligustica* at an apiary of Yunnan Agricultural University, Kunming, China. In order to avoid differing amounts of queen pheromones owing to possible age effects, all queens tested were between 300-330 days old (Pankiw et al., 1995) and all queens had headed their colonies for 10 months.

2.2. Organization of the mixed colonies

Two types of mixed colonies were established: mixed colonies containing worker brood of both *A. cerana* and *A. mellifera* and were headed by *A. cerana* queens; and mixed colonies containing worker brood of both *A. cerana* and *A. mellifera* and were headed by *A. mellifera* queens. Sealed brood of each species about to emerge as young adults was introduced into the colonies of the other species. Four colonies each of *A. cerana* and *A. mellifera* with an active egg-laying queen and populations of medium strength (4000–6000 workers for *A. cerana* and 6000–8000 individuals for *A. mellifera*) were chosen as parental colonies to maintain the sealed pupae until emergence. One empty comb and another one with pollen and honey were added to each of these colonies. The colonies were checked daily and the time when the empty combs had been filled with newly laid eggs was recorded so we knew when the developing bees would eclose as young adults. These combs were kept in the parental colonies until they developed into capped pupae and were then transferred into incubators.

Then the, three *A. mellifera* and three *A. cerana*, colonies were chosen as host colonies for establishing mixed-species colonies. These colonies were small, about 1500 individuals, mostly young adults (the older field bees having been eliminated by relocating the hives). These host colonies also had equal numbers of their own sealed pupae about to emerge, so a cohort of workers of the same age of both species could be obtained at the same time. Three days before the young adults would emerge, these brood frames were introduced into heterospecific host colonies i.e. one *A. mellifera* comb was put into each of the three *A. cerana* colonies and one *A. cerana* combs into each of the *A. mellifera* colonies. Newly emerged young adult bees are readily accepted by the host colonies and so the mixed colonies are constituted (Tan et al., 2006). Three pure *A. mellifera* colonies and three *A. cerana* colonies served as control groups and each contained enough newly emerging adult workers of the same age as those which were introduced into interspecific colonies.

Although the mixed colonies were constituted by an unequal number of host (adult + emerging) and introduced (only emerging) workers, this ought not to have an effect on retinue composition because queen attendance by workers is strongly age-dependent, with 3–9 days being the age range for intense contact with the queen (Seeley, 1979).

2.3. Monitoring the retinue behavior in the mixed-species colonies

Once the mixed colonies were settled, the introduced workers were adults about a fortnight old. In our observations, only those workers that attended a queen for at least 5 seconds were regarded as retinue bees (modified from Pankiw et al., 1995) because the queens were allowed to roam freely on the frames. Queen retinue behavior of the workers was recorded with a video camera for five minutes in each of the mixed and control colonies once a day for seven days. Therefore, it was possible to very accurately count the numbers of bees of each species in a particular retinue at any given time. Using a 5 sec contact paradigm for retinue bee recognition and a viewing window of 5 min over 7 days, the retinue data set was just about 420 observations per colony. We took the videos between 1400 h–1700 h in the afternoon.

The queens were allowed to roam freely on the comb during which one group of retinue bees were left behind and new ones formed a new retinue circle. The colonies were kept in normal standard hives so that we were able to take videos only by opening the hive and taking out the combs carefully, but no matter how gentle we were, all the queens stopped egg-laying and were seen roaming in our video clips. So, the five minutes cumulative numbers are obviously greater than what one might see at any instant. Therefore numbers were derived from worker turnover around the queen. We did not mark the bees individually in the hive because we could not know which bees would join a retinue, but we were able to eliminate pseudo-replication counts by replaying the video clips in a lower speed.

2.4. Pheromone analysis

After the experiment, the queens from the two types of mixed colonies were decapitated and the mandibular pheromones were extracted in 200 μL dichloromethane (DCM). The samples were then evaporated to dryness under a stream of nitrogen. The residues were re-dissolved in 10 μL of an internal standard solution (octanoic acid and tetradecane in dichloromethane; 0.38 and 0.25mg/mL, respectively) and 10 μL of derivatizing agent (bis-trimethylsilyltrifluoroacetamide). One μL of this solution was injected into a gas chromatograph (Hewlett Packard 6890) using routine analytical conditions (Dietemann et al., 2006). The following components, 9-keto-(E)-2-decenoic acid (9-ODA; "queen substance"), 10-hydroxy-(E)-2-decenoic acid (10-HDA; "worker substance"), methyl *p*-hydroxybenzoate (HOB), 10-hydroxydecanoic acid (10-HDAA), and 9-hydroxy-2-(e)-decenoic acid (9-HDA) were quantified using peak areas and the relative mass ratios calculated relative to tetradecane (Dietemann et al., 2006). The amount of 9-ODA relative to other components was quantified as 9-ODA / (9-ODA + 10-HDA + 10-HDAA). This ratio is an index of the 'queenliness' of honeybee mandibular pheromone: queens have a greater proportion of 9-ODA whereas workers have a greater proportion of 10-HDA (Hoover et al., 2005; Moritz et al., 2000).

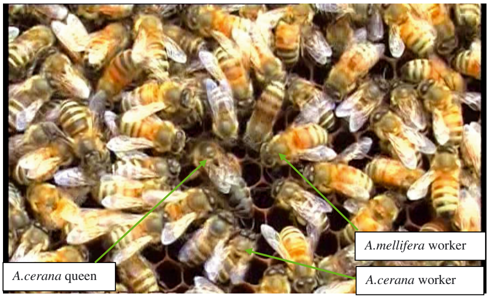

Figure 1. *A. cerana* queen attended by *A. mellifera* and *A. cerana* worker bees in an *A. cerana* queen-led mixed colony.

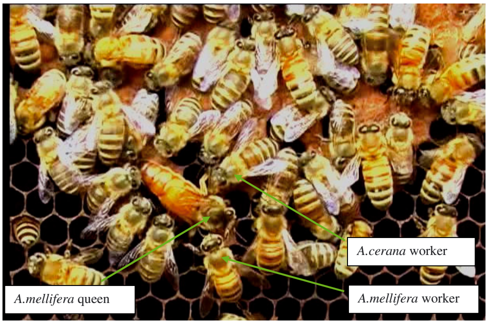

Figure 2. *A. mellifera* queen attended by *A. cerana* and *A. mellifera* worker bees in an *A. mellifera* queen-led mixed colony.

2.5. Data analysis

Independent samples T-tests were used to compare the mean number and proportions of the retinue workers to different queens. Homogeneity of the variances between groups was checked using Levene's test. Differences in the proportions of each component of queen pheromones were tested using independent T-tests, and a multivariate ANOVA test was used to test for overall differences in mandibular gland components between *A. cerana* and *A. mellifera* queens. The means and standard deviations of each variable were calculated. All tests were performed using Statistica© (StatSoft, 2008).

3. RESULTS

3.1. Queen retinue behavior between colonies

In *A. cerana* queen-led mixed colonies, almost equal proportions of *A. cerana* workers (0.51 ± 0.04) and *A. mellifera* workers (0.49 ± 0.04) attended the *A. cerana* queens (Fig. 1) and the results were not significantly different (t = 1.32, df = 20, *P* = 0.202, Tab. I). In *A. mellifera* queen-led mixed colonies a significantly smaller proportion of *A. cerana* workers, (0.48 ± 0.04), than *A. mellifera* workers, (0.52 ± 0.04), attended the *A. mellifera* queen (Fig. 2) (t = 2.71, df = 20, *P* = 0.014, Tab. I).

Comparing the number of retinue bees to different types of queens, in *A. cerana* queen-led mixed colonies, 53.4 ± 7.4 *A. cerana* workers were attracted by the *A. cerana* queen, whilst *A. mellifera* queens in *A. mellifera* queen-led mixed colonies attracted significantly fewer *A. cerana* worker bees (47.8 ± 5.9, t = 2.74, df = 40, *P* = 0.009). As for the *A. mellifera* worker bees, 51.2 ± 8.1 attended *A. cerana* queens and 51.9 ± 4.6 attended *A. mellifera* queens, there was no significant difference (t = 0.33, df = 40, *P* = 0.744).

When the total numbers of workers (*A. cerana* + *A. mellifera*) attracted to a retinue of *A. cerana* queens were compared with those attracted to *A. mellifera* queens, in *A. cerana* queen-led mixed colonies, an average of 104.6 ± 13.3 workers were observed in retinues around the *A. cerana* queen, while in *A. mellifera* queen-led mixed colonies, the *A. mellifera* queen attracted 99.7 ± 7.8 retinue bees. The values are not significantly different. (t = 1.49, df = 40, *P* = 0.145). In pure *A. cerana* and *A. mellifera* colonies, the queen attracted 105.8 ± 9.1 and 107.8 ± 11.2 workers, respectively, there being no significant difference between them (t = 0.62, df = 40, *P* = 0.538).

There was a significant difference between the mean number of workers that *A. mellifera* queens attracted, 99.7 ± 7.8, in *A. mellifera* queen-led mixed colonies and *A. mellifera* queen attracted in pure colonies, 107.8 ± 11.2 (t = 2.74, df = 40, *P* = 0.009). There was no significant difference between the mean number of workers that *A. cerana* queens attracted, 104.6 ± 13.3 in *A. cerana* queen-led mixed colonies and *A. cerana* queen attracted in pure colonies, 105.8 ± 9.1, (t = 0.34, df = 40, *P* = 0.736). A final point of interest is that

Table I. Mean number and proportion of retinue bees (± SD) attracted to the queens for each group.

	A. cerana queen-led mixed colonies		*A. mellifera* queen-led mixed colonies		Pure *A. cerana* colonies	Pure *A. mellifera* colonies
Col.	*A. cerana* retinue	*A. mellifera* retinue	*A. cerana* retinue	*A. mellifera* retinue	*A. cerana* retinue	*A. mellifera* retinue
1	56.4 ± 6.4	54.9 ± 6.4	49.4 ± 6.6	50.4 ± 3.1	101.9 ± 4.9	103.9 ± 7.6
2	46.6 ± 4.6	42.9 ± 4.7	44.4 ± 5.7	51.0 ± 5.0	109.1 ± 10.0	107.3 ± 12.5
3	57.3 ± 6.0	55.9 ± 5.9	49.4 ± 4.6	54.1 ± 5.1	106.4 ± 11.0	112.1 ± 12.6
x ± SD	53.4 ± 7.4	51.2 ± 8.1	47.8 ± 5.9	51.9 ± 4.6	105.8 ± 9.1	107.8 ± 11.2
Proportion	0.51 ± 0.04	0.49 ± 0.04	0.48 ± 0.04	0.52 ± 0.04		

workers showed no ovarian activity or egg-laying under the host queens.

3.2. Pheromones

The proportional values of the pheromonal components of the queens in the two types of mixed colonies were analyzed, and the results are shown in Table II. The results of multivariate ANOVA procedures to test for differences in proportional values of mandibular gland components between *A. mellifera* and *A. cerana* showed a significant overall difference (Wilk's lambda: F = 741.6, df = 4, 1, P = 0.027, Tab. II). Two of these components, HOB and 10-HDAA did not differ between species; however, 9-ODA, 9-HDA and 10-HDA differed significantly (9-ODA: t = 6.5, df = 4, P = 0.003; 9-HDA: t = 7.4, df = 4, P = 0.002; 10-HDA: t = 3.5, df = 4, P = 0.024). The ratio of pheromonal components 9-ODA/(9-ODA+10-HDA+10-HDAA) was significantly higher in *A. cerana* queens than in *A. mellifera* queens (t = 3.0, df = 4, P = 0.041, Tab. II).

4. DISCUSSION

4.1. Queen pheromones

Our results show that the proportional values of three of the pheromonal components from *A. mellifera* and *A. cerana* queens (9-ODA, 9-HDA and 10-HDA) differed significantly (Tab. II). The proportional values for *A. mellifera* queens obtained here are within the range of those reported in the literature (Crewe and Velthuis, 1980; Slessor et al., 1988; Naumann et al., 1991; Pankiw et al., 1995; Hoover, 2005). Quantitative analysis of the amounts showed that *A. cerana* queens have significantly less of the QMS components than *A. mellifera* (Tab. II). This result is consistent with previous investigations (Plettner et al., 1997; Free, 1987). These results confirm that there are high levels of variation between individuals, and possibly between different races. Possible environmental effects in the production of queens' pheromones are ruled out because comparisons of the heterospecific queen pheromones do not differ from those of normal queens for each species.

We argue that the basic queen signaling mechanism is conserved and queen pheromones and retinue formation preceded speciation in *Apis* because workers of both species respond to heterospecific queens. However, there is a pheromonal nuance because *A. cerana* workers responded less to *A. mellifera* queens and there are significant differences in the proportions of 9-ODA, 10HDA, 9HDA and in the ratio of 9-ODA/(9-ODA+10-HDA+10-HDAA) that could have led to differences in retinue responses. The queen pheromones appear to be quantitatively different between queens and could be 'interpreted' as different pheromonal "dialects". This would appear to be a parsimonious explanation for the differences in the attractiveness of queens for *A. cerana* workers, but begs the question for the *A. mellifera* workers. Nonetheless, this leaves unanswered questions such as (1) what does it mean if retinues of similar proportions are measured

Table II. Mean ± SD weight (μg) and proportional values of mandibular gland components of mixed colonies *A. cerana* and *A. mellifera* queens (N = 3, each).

Component	*A. cerana*		*A. mellifera*		*P**
	weight (μg)	proportion	weight (μg)	proportion	
4-methyl-hydroxy-benzoate (HOB)	49.98 ± 17.48	0.16 ± 0.06	38.71 ± 7.05	0.08 ± 0.01	0.103
9 -keto-2(*E*)-decenoic acid (9-ODA)	232.07 ± 27.55	0.71 ± 0.04	244.13 ± 30.27	0.52 ± 0.03	0.002
9-hydroxy-2(*E*)-decenoic acid (9-HDA)	31.92 ± 10.80	0.10 ± 0.03	147.47 ± 21.54	0.31 ± 0.04	0.002
10-hydroxydecanoic acid (10-HDAA)	6.17 ± 3.51	0.02 ± 0.01	13.71 ± 9.50	0.03 ± 0.02	0.512
10-hydroxy-2(*E*)-decenoic acid (10-HDA)	4.26 ± 0.99	0.01 ± 0.00	25.91 ± 10.29	0.06 ± 0.02	0.024
Multivariate test of all components					0.027†
Ratio:9-ODA/9-ODA+ 10-HDA+10-HDAA	0.96 ± 0.02		0.86 ± 0.05		0.041

* Probability from univariate t−tests (df = 4).
† Wilk's lambda (df = 4,1).

in the two species while the queens of one of these species produce more pheromone? and (2) why do *A. mellifera* queens attract fewer workers in mixed colonies compared to pure colonies?

4.2. Queen retinue behavior

Workers form a retinue around the queen in all honeybee species thus far examined (Verheijen-Voogd, 1959; Free, 1987; Plettner et al., 1997). But, bioassay-guided identification of retinue-active compounds has only been done in *A. mellifera* (Kaminski et al., 1990; Plettner et al., 1997; Keeling et al., 2003). So, the exact compounds responsible for retinue behavior in *A. cerana* are unknown (Plettner et al., 1997, Keeling et al., 2001). Under experimental conditions, Plettner et al. (1997) found that the retinue response of *A. cerana* workers to QMP blends with and without HVA did not differ significantly, suggesting that HVA is not required for maximal worker attraction in *A. cerana*. However, this result can not exclude the possibility that this component is not necessary for *A. mellifera* workers to be attracted to exhibit retinue behavior. Because cuticular hydrocarbons also play a role in the recognition systems of insects (Singer, 1998), and especially so in honeybees (Breed, 1998) this possibility must be addressed. We discount any importance of cuticular hydrocarbons in retinue behavior in this case because queens being superseded do not attract retinues because of a pheromonal insufficiency (Slessor et al., 1988) while pheromonally queen-like workers (pseudoqueens) do (Moritz et al., 2000).

In our study, we tested the responses of workers of both species to hetero-specific queens, and found that three pheromonal components of the queens were significantly different, 9-ODA, 9-HDA and 10-HDA (cf. Tab. II). The other compounds of the QMP are very similar, and the workers did not show any obvious avoidance behavior to either of the hetero-specific queens. Both species were attracted by the queens, engaged in retinue behavior, licked the queens and showed normal grooming and feeding behavior. These results suggest that the retinue response was not related to a specific queen pheromone or colony environment, and this is consistent with the results of other investigations (Pankiw et al., 1994; Hoover et al., 2005). This non-specific queen retinue behavior in the mixed colonies indicates that the queen pheromones can be transmitted among the workers from the two species without any obstacles, irrespective of possible "suppressive agents" (Fletcher and Ross, 1985) or "honest signals" (Peeters et al., 1999; Strauss et al., 2008). Workers showed no ovarian activity or egg-laying under the host queens (Tan et al., unpubl. data). We conclude that retinue behavior itself as well as the pheromones of the queens that induce this behavior are both ancestral, conserved traits that preceded speciation in apine bees.

ACKNOWLEDGEMENTS

Financial support was granted to Mingxian Yang by the Yunnan Agricultural University of China.

Comportement de cour des ouvrières d'abeilles envers la reine de l'autre espèce dans des colonies mixtes d'*Apis cerana* et *Apis mellifera*.

***Apis cerana* / *Apis mellifera* / comportement de cour / ouvrière / phéromone de la reine**

Zusammenfassung – Hofstaatverhalten von Arbeiterinnen in gemischten *Apis cerana/Apis mellifera*-Völkern. Mittels reziproker Versuchsansätze untersuchten wir, ob Hofstaatverhalten in einem heterospezifischen Kontext ausgelöst werden kann, d.h., ob *Apis cerana* Königinnen attraktiv sein können für *Apis mellifera* Arbeiterinnen und umgekehrt, ob dies auch bei *A. mellifera* Königinnen und *A. cerana* Arbeiterinnen der Fall ist. Diese Versuche führten wir in zwei Mischvölkern bestehend aus *A. cerana* und *A. mellifera* Arbeiterinnen durch. In Mischvölkern mit einer *A. cerana* Königin fanden wir etwa gleiche Proportionen von *A. cerana* (53,4 ± 7,4) und *A. mellifera* Arbeiterinnen (51,2 ± 8,1) im Hofstaat. In Mischvölkern mit einer *A. mellifera* Königin hingegen waren dort signifikant weniger *A. cerana* (47,8 ± 5,9) als *A. mellifera* Arbeiterinnen (51,9 ± 4,6) verrtreten (Tab. I). Die Arbeiterinnen beider Arten zeigten ein völlig normales Hofstaatverhalten gegenüber den heterospezifischen Königinnen und wir fanden keine offensichtliche Aggression oder ein Vermeidungsverhalten, obwohl sich die Königinnenpheromone der beiden Arten in einigen Punkten unterscheiden.

Als Erklärung für die proportionalen Unterschiede in der Teilnahme am Hofstaatverhalten nehmen wir leichte Abweichungen in den Pheromonen der beiden Arten an. Wir untersuchten deshalb die relative Anteile der Komponenten des Königinnenpheromons in den beiden Typen der Mischvölker (Tab. II). Die Ergebnisse der Multivarianzanalyse der proportionalen Anteile der Mandibeldrüsenkomponenten zeigten einen signifikanten Unterschied zwischen *A. mellifera* und *A. cerana* (Wilk's lambda: F = $741,6$, df = $4,1$, $P = 0,027$, Tab. II). Während für zwei Komponenten, HOB und 10-HDAA, kein Unterschied zu sehen war, waren diese für 9-ODA, 9-HDA und 10-HDA signifikant (9-ODA: t = $6,5$, df = 4, $P = 0,003$; 9-HDA: t = $7,4$, df = 4, $P = 0,002$; 10-HDA: t = $3,5$, df = 4, $P = 0,024$). Der Quotient der Pheromonkomponenten zueinander [9-ODA/(9-ODA+10-HDA+10-HDAA)] war signifikant höher für *A. cerana* als für *A. mellifera* Königinnen (t = $3,0$, df = 4, $P = 0,041$, Tab. II). Für beide Arten waren die Königinnen jedoch attraktiv, die Arbeiterinnen bildeten eine Hofstaat, beleckten die Königin und zeigten normales Putz- und Fütterungsverhalten.

Diese Ergebnisse weisen darauf hin, dass die Hofstaatbildung nicht auf ein spezifisches Königinnenpheromon oder die Volkssituation zurückzuführen ist, und diese Interpretation stimmt mit bereits publizierten Ergebnissen überein (Pankiw et al., 1994; Hoover et al., 2005). Das nicht artspezifische Hofstaatverhalten in gemischten Kolonien deutet darauf hin, dass Königinnenpheromon von Arbeiterinnen über die Artgrenzen hinweg ohne Hindernisse und unabhängig von möglichen "suppressiven Faktoren" (Fletcher and Ross, 1985) oder "ehrlichen Signalen" (Peeters et al., 1999; Strauss et al., 2008) weitergegeben werden kann. Wir schliessen daraus dass das Hofstaatverhalten selbst, ebenso wie die Könginnenpheromone, die dieses Verhalten induzieren, anzestrale, konservierte Merkmale sind, die vor der Artenaufspaltung der apinen Bienen entstanden sind.

***Apis cerana* / *Apis mellifera* / Hofstaatverhalten / Königinnenpheromone**

REFERENCES

Allen M.D. (1955) Observations of honeybees attending their queen, Br. J. Anim. Behav. 3, 66–69.

Allen M.D. (1960) The honeybee queen and her attendants, Anim. Behav. 8, 201–208.

Arias M.C., Sheppard W.S. (1996) Molecular phylogenetics of honeybee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequence, Mol. Phylogenet. Evol. 5, 557–566.

Arias M.C., Sheppard W.S. (2005) Phylogenetic relationships of honeybees (Hymenoptera: Apinae: Apini) inferred from nuclear and mitochondrial DNA sequence data, Mol. Phylogenet. Evol. 37, 25–33.

Breed M.D. (1998) Recognition pheromones of the honey bee, Bioscience 48, 463–470.

Butler C.G. (1954) The method and importance of the recognition by a colony of honeybees (*A. mellifera*) of the presence of the queen, T. Roy. Entomol. Soc. London 105, 11–29.

Crewe R.M., Velthuis H.H.W. (1980) False queens: a consequence of mandibular gland signals in worker honeybees, Naturwissenschaften 67, 467–469.

Dietemann V., Pflugfelder J., Härtel S., Neumann P., Crewe R.M. (2006) Social parasitism by honeybee workers (*Apis mellifera* capensis Esch.): evidence for pheromonal resistance to host queen′s signals, Behav. Ecol. Sociobiol. 60, 785–793.

Dyer F.C. (2002) The biology of the dance language, Ann. Rev. Entomol. 47, 917–949.

Dyer F.C., Seeley T.D. (1991) Dance dialects and foraging range in three Asian honey bee species, Behav. Ecol. Sociobiol. 28, 227–233.

Fletcher D.J.C, Ross K.G. (1985) Regulation of reproduction in eusocial Hymenoptera, Ann. Rev. Entomol. 30, 319–343.

Free J.B. (1987) Pheromones of social bees, Chapman and Hall, London.

Hepburn H.R. (1998) Reciprocal interactions between honeybees and combs in the integration of some colony functions in *Apis mellifera* L., Apidologie 29, 47–66.

Hoover S.E.R., Winston M.L., Oldroyd B.P. (2005) Retinue attraction and ovary activation: responses of wild type and anarchistic honeybees (*Apis mellifera*) to queen and brood phromones, Behav. Ecol. Sociobiol. 59, 278–284.

Kaminski L.A., Slessor K.N., Winston M.L., Hay N.W., Borden J.H. (1990) Honeybee response to queen mandibular pheromone in laboratory bioassays, J. Chem. Ecol. 16, 841–850.

Katzav-Gozansky T., Soroker V., Kamer J., Schulz C.M., Francke W., Hefetz A. (2003) Ultrastructural and chemical characterization of egg surface of honeybee worker and queen-laid eggs, Chemoecology 13, 129–134.

Keeling C.I., Otis G.W., Hadisoesilo S., Slessor K.N. (2001) Mandibular gland component analysis in the head extracts of *Apis cerana* and *Apis nigrocincta*, Apidologie 32, 243–252.

Keeling C.I., Slessor K.N., Higo H.A., Winston M.L. (2003) New components of the honey bee (*Apis mellifera* L.) queen retinue pheromone, Proc. Nat. Acad. Sci. 100, 4486–4491.

Lindauer M. (1957) Communication in swarm-bees searching for a new home, Nature 179, 63–66.

Moritz R.F.A., Crewe R.M., Hepburn H.R. (2001) Attraction and repellence of workers by the honeybee queen (*Apis mellifera* L.), Ethology 107, 465–477.

Moritz R.F.A., Simon U.E., Crewe R.M. (2000) Pheromonal contest between honeybee workers (Apis mellifera capensis), Naturwissenschaften 87, 395–397.

Naumann K., Winston M.L., Slessor K.N., Prestwich G.D., Webster F.X. (1991) Production and transmission of honey bee queen (*Apis mellifera* L.) mandibular gland pheromone, Behav. Ecol. Sociobiol. 29, 321–332.

Neumann P., Moritz R.F.A. (2002) The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? Behav. Ecol. Sociobiol. 52, 271–281.

Pankiw T., Winston M., Slessor K.N. (1994) Variation in worker response to honey bee (*Apis mellifera* L.) queen mandibular pheromone (Hymenoptera: Apidae), J. Insect Behav. 7, 1–15.

Pankiw T., Winston M.L., Slessor K.N. (1995) Queen attendance behavior of worker honey bees (*Apis mellifera* L.) that are high and low responding to queen mandibular pheromone, Insectes Soc. 42, 371–378.

Peeters C., Monnin T., Malosse C (1999) Cuticular hydrocarbons correlated with reproductive status in a queenless ant, *Diponera quadriceps*, Proc. R. Soc. Lond. B 266, 1323–1327.

Plettner E., Otis G.W., Wimalaratne P.D.C., Winston M.L., Slessor K.N., Pankiw T., Punchihewa P.W.K. (1997) Species- and caste-determined mandibular gland signals in honey bees (*Apis*), J. Chem. Ecol. 23, 363–377.

Ribbands C.R. (1953) The behavior and social life of honeybees, Bee Research Association, London.

Sakagami S.F. (1958) The false-queen: fourth adjustive response in dequeened honeybee colonies, Behaviour 13, 280–296.

Seeley T.D. (1979) Queen substance dispersal by messenger workers honeybee colonies, Behav. Ecol. Sociobiol. 5, 391–415.

Singer T.L. (1998) Roles of hydrocarbons in the recognition systems of insects, Am. Zool. 38, 394–405.

Slessor K.N., Kaminski L.A., King G.G.S, Borden J.H., Winston M.L. (1988) Semiochemical basis of the retinue response to queen honey bees, Nature 332, 354–356.

StatSoft Inc. (2008) STATISTICA, version 8.0. www.statsoft.com.

Strauss K., Scharpenberg H., Crewe R.M., Glahn F., Foth H., Moritz R.F.A. (2008) The role of the queen mandibular gland pheromone in honeybees (*Apis mellifera*): honest signal or suppressive agent? Behav. Ecol. Sociobiol. 62, 1523–1531.

Su S., Cai F., Si A., Zhang S., Tautz J., Chen S. (2008) East Learns from West: Asiatic honeybees can understand dance language of European honeybees, PLoS ONE 3, e2365.

Tan K., Hepburn H.R., He S.Y., Radloff S.E., Neumann P., Fang X. (2006) Gigantism in honeybees: *Apis cerana* queens reared in mixed-species colonies, Naturwissenschaften 93, 315–320.

Tan K., Yang M.X., Radloff S.E., Hepburn H.R., Zhang Z.Y., Luo L.J., Li H. (2008) Dancing to different tunes: heterospecific deciphering of the honeybee waggle dance, Natuwissenschaften 95, 1165–1168.

Velthuis H.H.W. (1972) Observations on the transmission of queen substances in the honey bee colony by the attendants of the queen, Behaviour 41, 105–128.

Verheijen-Voogd C. (1959) How worker bees perceive the presence of their queen, J. Comp. Physiol. A 41, 527–582.