

Reproductive traits and mandibular gland pheromone of anarchistic honey bee workers *Apis mellifera* occurring in China

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Abstract – In honey bee colonies, workers, in particular of “anarchistic” lineages, can activate their ovaries and lay eggs, even in the presence of the queen. We identified three queenright colonies showing typical signs of worker reproduction. To characterize this new lineage, we extracted the mandibular gland and analyzed it using gas chromatography. The total amounts of the five main components of the mandibular gland, namely methyl p-hydroxybenzoate (HOB), 9-oxo-2(E)-decanoic acid (9-ODA), (S)-9-hydroxy-(E)-2-decenoic acid (9-HDA), 10-HDA, and 10-hydroxydecanoic acid (10-HDAA) were significantly higher in the mandibular gland profiles of workers with activated ovaries (AWs, $8.88 \pm 1.71 \mu\text{g}$) compared to workers with inactivated ovaries (IAWs, $4.00 \pm 2.09 \mu\text{g}$). Furthermore, the chemical profiles of IAWs were dominated by the “worker substances” 10-HDA (34.64 ± 8.19 %) and its precursor 10-HDAA (22.88 ± 4.95 %), while the chemical profiles in AWs were dominated by the precursor of the queen substance 9-HDA (40.04 ± 7.55 %). The ratios of two precursor substances 10-HDAA/9-HDA of IAWs were more worker like (>1.0) whereas AWs were more queen like (≤1.0). These results suggest that the mandibular pheromones of anarchistic workers resemble a more queen-like reproductive active profile and that these workers may represent a reversion to a more basal reproductive phenotype.

Apis mellifera / anarchistic colony / ovary activation / egg production / worker policing / worker mandibular gland pheromone

1. INTRODUCTION

Like many eusocial insects, the honey bee *Apis mellifera* L. is characterized by extreme reproductive division of labor between the queen and

workers (Michener 1974). Under normal conditions, the queen is usually the sole female reproductive active individual in a colony, while workers refrain from reproducing despite having functional ovaries and in general, can lay only haploid male eggs (arrhenotoky) (Winston 1987; Hepburn and Radloff 1998; Hoover et al. 2003; Slessor et al. 2005). In wild-type colonies of European subspecies, approximately 0.01 % of workers lay 7 % of all male eggs, of which only one egg in 1000 is reared and the other are removed by other workers; the so-called worker

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policing (Ratnieks 1993; Visscher 1996). However, effective worker policing is not present in a rare strain of *A. mellifera* “anarchistic” colonies, in which a large number of worker-laid eggs are reared (Oldroyd et al. 1994; Montague and Oldroyd 1998; Châline et al. 2002). In anarchistic colonies, approximately 1 % of workers show highly activated ovaries and frequently lay unfertilized eggs. That proportion of workers can be increased up to 9 % in selectively bred colonies in the presence of a laying queen (Montague and Oldroyd 1998; Oldroyd and Osborne 1999; Oldroyd et al. 1999). This results in the unusual situation that majority of males are offspring of workers rather than the queen in these colonies (Oldroyd et al. 1994; Montague and Oldroyd 1998; Barron et al. 2001), which is similar to the natural situation in the Cape honey bee where the laying workers significantly contribute to the population (Moritz et al. 1998). In the Cape honey bee, *Apis mellifera capensis* from South Africa, workers are able to produce diploid offspring via thelytokous parthenogenesis (Onions 1914; Lundie 1954; Anderson 1963; Verma and Rutner 1983). It has been suggested that workers discriminate between queen-laid and worker-laid eggs by the presence or absence of an egg-marking pheromone (Ratnieks 1995), and the Dufour gland has been suggested as a potential origin (Katzav-Gozensky et al. 1997) since laying workers mimic the composition of the queen Dufour’s gland secretion (Sole et al. 2002). Thus, eggs laid by anarchistic workers are more acceptable presumably due to their produced queen-like secretions, including esters, from their Dufour’s glands (Oldroyd and Ratnieks 2000; Martin et al. 2004). However, the exact mechanism or pheromone involved in discrimination of worker- and queen-laid eggs has not been determined.

For the reproductive phenotype to arise, three predispositions facilitating worker reproduction have to be present. First, reproductive active workers must be less affected by the queen and brood pheromones that normally suppress their ovary activation and eggs-laying behavior (Barron and Oldroyd 2001), and second they should increase the survival of their eggs either by laying more acceptable eggs, which avoid being removed by other workers (worker policing)

(Ratnieks and Visscher 1989; Oldroyd and Ratnieks 2000) by laying away from her and the central brood nest (Neumann et al. 2003). However, the third characteristic has not been reported in anarchistic honey bees and which is often associated with reproductive active workers in the Cape honey bee (Petty 1922; Moritz et al. 1999, 2001, 2008; Martin et al. 2002a, b; Pirk et al. 2002, 2012; Zheng et al. 2010)—a queen-like pheromonal bouquet (Hoover et al. 2005b).

The queen mandibular gland pheromone (QMP) is a complex mixture of 9-oxo-2(E)-decenoic acid (9-ODA), the two enantiomers of 9-ODA’s biosynthetic precursor, (R)- and (S)-9-hydroxy-(E)-2-decenoic acid (9-HDA), and two aromatic compounds methyl p-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA) (Slessor et al. 1988), which is central to the regulation of queen-worker and worker-worker conflict resolution (Plettner et al. 1993; Moritz et al. 2000, 2004). QMP is dominated by the compound of 9-ODA, the so-called queen substance, which is essential for the inhibition of ovary activation and egg-laying behavior in workers (Hoover et al. 2003; Dietemann et al. 2007; Strauss et al. 2008). Anarchistic workers are less inhibited by the queen pheromones than wild-type workers, and it has been suggested that this is due to a reduced sensitivity to queen- and larvae-produced signals (Oldroyd et al. 2001; Hoover et al. 2005b). The worker mandibular gland secretion under queenright conditions is dominated by the “worker substances” 10-hydroxy-2(E)-decenoic acid (10-HDA) and 10-hydroxydecanoic acid (10-HDAA) (Crewe 1982; Plettner et al. 1993). However, workers are able to shift the synthesis towards the queen pathway and queens can also produce worker substances (Yusuf et al. 2015). Workers mostly change the pheromonal bouquet under queen-less conditions, in particular workers of *A. m. capensis* quickly develop into “pseudoqueens” which lay eggs and produce queen-like gland secretion dominated by the queen substance (9-ODA) in their mandibular glands (Crewe and Velthuis 1980; Moritz et al. 2000; Simon et al. 2001). Pseudoqueen-produced pheromones play an important role in establishing reproductive dominance hierarchies among queen-less workers,

because 9-ODA that is produced in regular colonies by a functional queen has been shown to inhibit ovary activation and suppress the production of a queen-like pheromone signal in other workers (Crewe 1988; Moritz et al. 2000, 2004, 2008; Dietemann et al. 2007). Thus, the ratios of 9-ODA/(9-ODA + 9-HDA + 10-HDA + 10-HDAA) and 10-HDAA/9-HDA have been used as strong indicators of individual fecundity (Plettner et al. 1993; Moritz et al. 2004; Hoover et al. 2005a; Schäfer et al. 2006).

Anarchists, as a special case of worker reproduction, provide an experimental resource for investigating the proximate mechanisms underlying the worker reproduction in wild-type colonies. However, anarchy is a complex syndrome, which is influenced by pheromones, worker policing, queen and worker genetic component, and other factors. The majority of research of the anarchistic traits is based on the selectively bred line maintained by the University of Sydney, which reliably show higher levels of anarchistic phenotype (Oldroyd et al. 1999; Oldroyd and Osborne 1999).

In 2012, we first saw drone eggs and larvae appeared above the queen excluder in several queenright *A. mellifera* L. colonies, in Shanxi province, China. In order to confirm their anarchistic traits, we investigated the following: (1) the percentage of worker with stage 4–5 activated ovaries (Hess 1942; Pirk et al. 2010) (2) and if worker-laid eggs are able to escape worker policing. In order to further understand the role of the worker mandibular gland secretions in the acquisition of reproductive status and in the proximate mechanisms underlying the anarchistic syndrome, (3) we investigated the composition of mandibular gland profiles of workers with inactivated ovaries (IAWs) and workers with activated ovaries (AWs) sampled in three colonies with typical anarchistic traits and determined if AWs produce queen-like substances in their mandibular gland secretions.

2. MATERIALS AND METHODS

Eggs and larvae were found above the queen excluder in ten queenright *A. mellifera* L. colonies, in the apiary of Shanxi province, China, of which three were transported to Hangzhou and the University's apiary. All of them were suspected of being "anarchistic"

colonies. Furthermore, three colonies not showing any features of anarchistic activities were used as discriminator colonies.

2.1. Experiment 1. Ovary activation analysis

Honey bee abdomens were dissected according to Dade (1977) and assigned as being inactivated (ovaries thread-like and lacking defined ova, stages 1–3) or fully activated (clearly defined ova, stage 4, or at least one egg present in ovarioles, stage 5) (Hess 1942; Pirk et al. 2010). Sixty workers per anarchistic colony ($n=3$) were obtained from drone combs above the queen excluder, and the procedure was replicated in 3-day intervals for four times in June 2012, resulting in 720 workers being dissected and scored.

2.2. Experiment 2. Egg-laying behavior analysis

Each anarchistic colony ($n=3$) was split into two halves (queenright half: the area below queen excluder, and queen-excluded half: the area above queen excluder) using a queen excluder, which allows passage freely of workers but not of the queen. In order to measure the reproductive output of the queen and workers in terms of drones eggs produced within colonies, one drone comb was put in each half. After 8 h, drone combs were removed from the colonies and the eggs in each half were counted.

To compare the number of worker-laid eggs between the two halves and to evaluate the potential contribution of workers to the eggs laid in the queenright half, we placed one drone comb each in a plastic queen excluder boxes above and below the queen excluder. Again, the number of worker-laid eggs was counted in each half after 8 h, and this was replicated on three or four times within 1 week. Analysis for statistical differences of the egg production between queenright half and queen-excluded half was performed using Student's *t* test. Data are presented as means and standard deviations.

2.3. Experiment 3. Worker policing analysis

2.3.1. Sources of eggs

Three wild-type queenright colonies (WT1, WT2, WT3) are the sources of wild-type queen-laid eggs (WTQE), three wild-type queen-less colonies (WTL1,

WTL2, WTL3) as the sources of wild-type worker-laid eggs (WTWE), three anarchistic queenright colonies (AC1, AC2, AC3) as the sources of anarchistic queen-laid eggs (ACQE), and anarchistic worker-laid eggs (ACWE). These colonies ($n=9$) were divided into three groups; the first group WT1, WTL1, AC1; the second group WT2, WTL2, AC2; and the third group WT3, WTL3, AC3. Each group provided four different types of eggs, including WTQE, WTWE, ACQE, and ACWE.

2.3.2. Discriminator colonies

To assay the removal rates of eggs from various sources, we used three wild-type colonies (WT1, WT2, WT3) and three anarchistic colonies (AC1, AC2, AC3) as discriminator colonies. Each hive was separated into two parts by a queen excluder, in which the queen was confined to the bottom, yielding queen-excluded areas above the queen excluder and queenright areas below.

2.3.3. Egg removal bioassays

To perform the assay, we transferred 20 eggs from each source of eggs; the four different sources (WTQE, WTWE, ACQE and ACWE) into drone cells of the same test comb that has been left overnight in their corresponding discriminator colonies. All of these test eggs were collected from their original drone cells by using sterilized toothpick. A new toothpick was used for each row. After transferring, the test comb with 80 eggs was sandwiched between brood combs that previously placed in the center of the upper chamber of each discriminator colony (Pirk et al. 2002). Eggs were not introduced in their colony of origin to avoid any interferences of nestmate recognition (Pirk et al. 2007). Drone combs with four types of eggs were placed in the discriminator colonies ensuring that combs and eggs were originating from other colonies than the discriminator colonies. The number of remaining eggs was counted 1, 2, 4, and 6 h after introduction, and it was repeated four times on separate days.

2.3.4. Statistical analysis

Survival data of (WTQE, WTWE, ACQE, and ACWE) were analyzed using a Cox regression survival analysis (Collett 1994) as implemented in SPSS. For

this analysis, eggs removed at 1, 2, 4, or 6 h were complete data points whereas remaining eggs after 6 h were treated as censored data. Egg source, day, and discriminator were used as variables in the Cox regression survival model, and we used it to calculate the likelihood ratio for the survival rate of the different egg sources after adding the factors of day and discriminator. The survival function was modeled without ("Null") and then with (overall) source of eggs, day, and discriminator colony (overall) as factors, and the improvement of the ability of the model to describe the data tested with a χ^2 test. The model then tested the effect of adding source of eggs, day, or discriminator as factors. Lastly, we presented a pairwise comparison between the survival eggs of four different sources. We presented the data graphically as the mean proportion (\pm standard deviations) of eggs remaining at each time period.

2.4. Experiment 4. Workers' mandibular pheromone analysis

Pheromone compositions analysis was conducted at Pretoria University, South Africa. Ten heads of IAWs and AWs each colony ($n=3$) were removed and stored in 200 μ l dichloromethane for at least 24 h to extract compounds of their mandibular glands. Half of the extracts were evaporated to dryness under a stream of nitrogen and analyzed by gas chromatography (Zheng et al. 2010). Chromatograms were recorded using an Agilent 6890 GC and peak areas quantified using HP ChemStation software. HOB, 9-ODA, HVA, 9-HDA, 10-HDA, and 10-HDAA were identified based on the retention times of synthetic compounds and their retention time relative to the internal standards (Simon et al. 2001). Their relative mass ratios (RMRs) were measured relative to tetradecane. The standard solution containing all of six compounds was run daily to ensure that RMRs were within the limit of the variability found in the series of standard runs.

We calculated the absolute amounts (micrograms) and relative amounts (their percentage composition) of HOB, 9-ODA, HVA, 9-HDA, 10-HDA, and 10-HDAA in IAWs and AWs. The ratio of 9-ODA/(9-ODA + 9-HDA + 10-HDA + 10-HDAA) (Moritz et al. 2004; Hoover et al. 2005a; Schäfer et al. 2006) and 10-HDAA/9-HDA (Plettner et al. 1993) were used as indicators of individual fecundity. Analysis for statistical differences between IAWs and AWs were performed

using Mann-Whitney U test. Data are presented as means and standard deviations.

3. RESULTS

3.1. Experiment 1. Ovary activation analysis

A total of 720 workers were randomly collected from each colony for ovary activation analysis. The mean percentage of AWs in three anarchistic colonies was 6.25–49.58 %, and the mean percentage of workers with at least one mature egg appeared in their ovarioles (stage 5, Pirk et al. 2010) was 3.00–21.25 % (Table I).

3.2. Experiment 2. Egg-laying behavior analysis

According to analysis of variance, the mean rate of egg production of queenright half (222.83 ± 85.91), to which potentially the queen and workers contributed, was significantly higher than that of the queen-excluded half (11.42 ± 21.38) within 8 h in anarchistic colonies ($t = 8.273$, $df = 12.358$, $P < 0.001$) (Figure 1). The mean rate of egg production of workers in queenright half (13.00 ± 20.72) was lower than that of queen-excluded half (25.67 ± 36.88) in anarchistic colonies (Figure 2).

From that, we can calculate that the contribution of the queen was on average 209 eggs or 94 % of the eggs laid in the queenright section of the hive.

3.3. Experiment 3. Worker policing

A Cox regression analysis showed that there were significant differences in the removal rates for the four different sources of eggs ("Source of eggs" $P < 0.001$). The experimental day did not significantly affected the removal rates ("Day of trial" $P > 0.05$) nor did discriminator colony ("Discriminator" $P > 0.05$) (Table II).

In anarchistic discriminator colonies, WTWE were removed significantly faster than ACWE ($P < 0.001$); however, ACWE were removed at a similar rate as WTQE ($P = 0.182$) and ACQE ($P = 0.290$) (Figure 3a, Table II).

In wild-type discriminator colonies, the pairwise comparisons showed that the four source eggs were treated significantly different: ACQE were removed significant less than WTQE ($P < 0.05$) and WTWE were removed fastest whereas ACQE were removed significantly slower than WTQE ($P < 0.001$) (Figure 3b, Table II).

In both anarchistic and wild-type discriminator colonies, WTWE were removed faster than other

Table I. The percentage of workers with activated ovaries (AWs) and at least one mature egg appeared in their ovarioles in three anarchistic colonies.

Times ($n = 12$)	Colony ($n = 3$)	AWs (%)	$\bar{X} \pm SD$	Workers with at least one mature egg (%)	$\bar{X} \pm SD$
1	AC1	10.00	16.25 ± 7.50	6.67	7.25 ± 4.72
2		26.67		23.33	
3		11.67		6.67	
4		16.67		11.67	
1	AC2	3.33	49.58 ± 14.23	3.33	21.25 ± 7.59
2		8.33		8.33	
3		11.67		8.33	
4		1.67		0	
1	AC3	58.33	6.25 ± 4.59	33.33	3.00 ± 2.45
2		61.67		45.00	
3		30.00		18.33	
4		48.33		45.00	

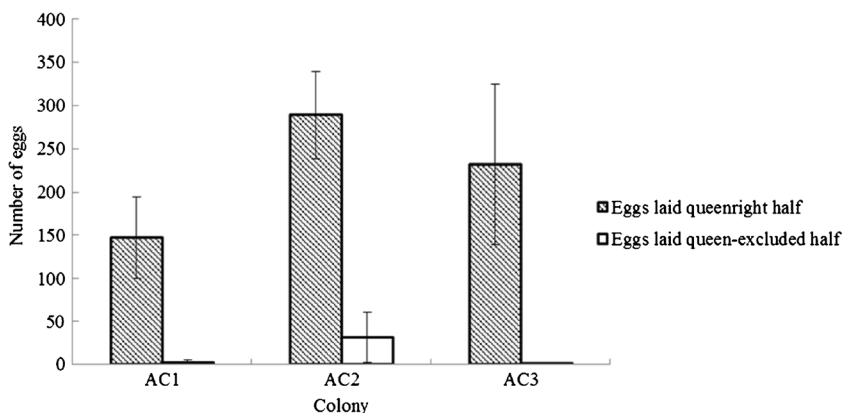


Figure 1. The rate of egg production of queenright half and queen-excluded half in three anarchistic colonies (AC). The two parts were separated by a queen excluder, so the queen was the only individual confined to on art of the hive. Mean and standard deviations are shown.

egg sources, while the survival rates of ACWE were intermediate between those of queen-laid eggs (WTQE, ACQE) and WTWE. In addition, the anarchistic colonies were less discriminatory than wild-type colonies towards the four sources of eggs (Figure 3).

3.4. Experiment 4. Workers' mandibular pheromone analysis

The total amounts of all identified compounds were significantly higher in AWs ($8.88 \pm 1.71 \mu\text{g}$, $n=30$) compared to IAWs ($4.00 \pm 2.09 \mu\text{g}$, $n=28$) ($P < 0.05$, Table SI). Furthermore, the absolute amounts of HOB and 9-HDA were significantly higher in AWs compared to IAWs ($P < 0.05$,

whereas the remaining four compounds were not significantly different between the two groups (lowest P value = 0.565) (Figure 4, Table SI).

The relative amounts of 10-HDAA and 9-HDA were significantly different between IAWs and AWs ($P < 0.001$) with 10-HDA ($34.64 \pm 8.19\%$) and 10-HDAA ($22.88 \pm 4.95\%$) being the dominant components in the profile of IAWs, while AWs was dominated by 9-HDA ($40.04 \pm 7.55\%$), the queen substance, 9-ODA was a relatively minor component in both IAWs ($5.14 \pm 3.24\%$) and AWs ($2.09 \pm 1.14\%$), and was not significantly different between the two groups, similar to the remaining three components (lowest P value = 0.063) (Figures 4 and 5, Table SI). The ratio of 9-ODA/(9-ODA + 9-

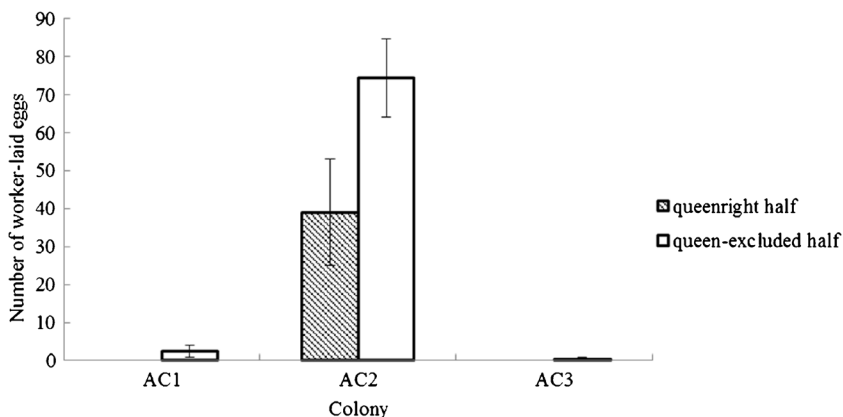


Figure 2. The rate of egg production of workers in queenright and queen-excluded halves of three anarchistic colonies (AC), when the queen was caged onto one drone comb. Mean and standard deviations are shown.

Table II. Likelihood ratio comparing the survival of eggs laid by wild-type queens (WTQE), anarchistic queens (ACQE), wild-type workers (WTWE), and anarchistic workers (ACWE) in anarchistic colonies ($n = 180$ for each egg source) and wild-type colonies ($n = 240$ for each egg source) worker policing bioassays.

Term	Anarchistic discriminator colonies				Wild-type discriminator colonies			
	-2 log likelihood	χ^2	df	P	-2 log likelihood	χ^2	df	P
Null	6436.147				10,897.387			
Overall	6416.886	19.274	3	<0.001	10,859.700	37.590	3	<0.001
Source of eggs		19.195	1	<0.001		34.441	1	<0.001
Day of trial		0.057	1	0.811		1.277	1	0.259
Discriminator		0.075	1	0.784		2.272	1	0.132
Pairwise comparison of eggs source:								
WTQE vs ACQE		0.074	1	0.786		4.526	1	0.033
WTQE vs ACWE		1.780	1	0.182		17.556	1	<0.001
ACQE vs ACWE		1.118	1	0.290		38.669	1	<0.001
WTQE vs WTWE		30.350	1	<0.001		101.960	1	<0.001
ACQE vs WTWE		27.738	1	<0.001		132.645	1	<0.001
ACWE vs WTWE		19.443	1	<0.001		43.047	1	<0.001

The likelihood ratios compared the survival of four different sources of eggs (WTQE, WTWE, ACQE, and ACWE). The survival function was modeled without ("Null") and then with ("overall") source of eggs, day, and discriminator colony (overall) as factors, and the improvement of the ability of the model to describe the data tested with a χ^2 -test. The model then tested the effect of adding source of eggs, day, or discriminator as factors. Lastly, we presented a pairwise comparison between the survival eggs of four different sources

HDA + 10-HDA + 10-HDAA) showed no significant differences between IAWs and AWs (ratio IAWs=0.057±0.074, AWs=0.024±0.037, $P=0.421$), whereas the ratio of 10-HDAA/9-HDA was significantly higher in IAWs than in AWs (ratio IAWs=1.490±1.319>1.0, AWs=0.501±0.700<1.0, $P<0.001$) (Table SI).

4. DISCUSSION

In our colonies, the rates of workers with fully activated ovaries (3–21 %) were higher than previous naturally occurring anarchistic colonies (around 1 %; Montague and Oldroyd 1998; Oldroyd et al. 1999), which was similar to typical selectively bred colonies (3–9 %; Oldroyd and Osborne 1999; Barron and Oldroyd 2001) and queen-less colonies of *Apis mellifera ligustica* (5–24 %; Miller and Ratnieks 2001).

The rate of egg production of queenright half was significantly higher than that of queen-excluded half workers, and even when subtracting the number of eggs potentially laid by workers in

the queenright part, it still suggests that the majority is queen derived. Anarchistic workers were prone to lay eggs in queen-excluded half revealed that anarchists reproduction still influenced by the queen pheromones inhibitory effects but need higher levels, because they could avoid suppression increasing the spatial distance to the pheromone source. A behavior has also been seen in other laying workers (Moritz et al. 2001; Pirk et al. 2002), which might be facilitated by spatial differences in worker policing behavior (Neumann et al. 2003).

In general, the efficiency of egg removal behavior seems sufficiently high enough to remove the majority of the worker-laid eggs (Ratnieks 1993; Visscher 1996; Pirk et al. 2003). However, the eggs laid by workers from our anarchistic colonies seems to be more acceptable since in both anarchistic and wild-type discriminator colonies, ACQE were removed less than other three sources of eggs, while the survival rates of ACWE were intermediate between queen-laid eggs (WTQE, ACQE) and WTWE. All of the four

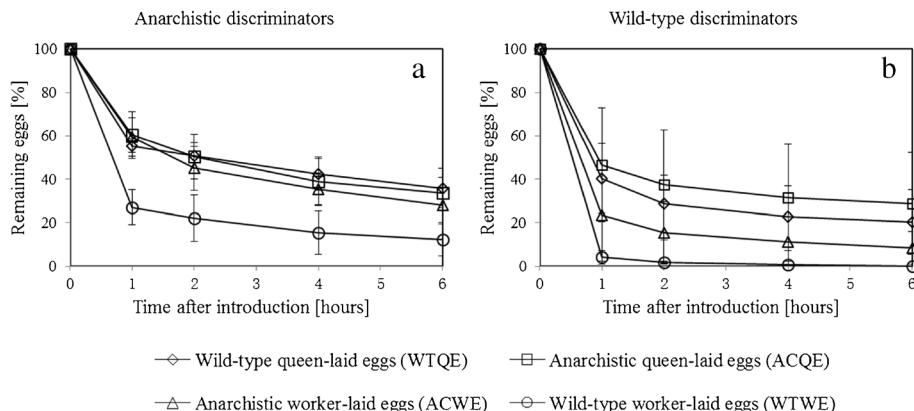


Figure 3. Removal rates of eggs laid by wild-type queens (*WTQE*) and workers (*WTWE*) and anarchistic queens (*ACQE*) and workers (*ACWE*) when introduced into three unrelated anarchistic (a) and wild-type (b) queenright discriminator colonies, respectively. Values were the means of all 3 or 4 days for all discriminators ($n=3$). The bars represented the standard errors of the means.

sources of eggs, especially WTWE were rejected more strongly in wild-type discriminator colonies than in anarchistic discriminator colonies. These results strongly support previous studies showing that anarchistic workers evade egg policing by laying more acceptable eggs (Oldroyd and Ratnieks 2000) and that worker policing seems to be reduced in anarchistic colonies. However, the proximate mechanism underlying the discrimination of worker and queen-laid eggs in honey bees and, in particular, in anarchistic colonies has not been determined, besides a potential egg-marking pheromone (Ratnieks 1995), egg viability might also play a role (Pirk et al. 2004).

The analysis of the mandibular gland products of workers collected from these three colonies showed that anarchistic workers had ability to

produce queen-like signal to establish their reproductive dominance. When comparing with literature data, the total amounts in both IAWs and AWs were higher than in *A. mellifera* queenright workers ($2.59 \pm 0.62 \mu\text{g}$), while they were less than *A. mellifera* queen-less workers ($10.73 \pm 2.30 \mu\text{g}$) (Tan et al. 2012). Since pheromonal dominance can translate into trophallactic dominance and greater ovarian activation potential (Schäfer et al. 2006), it would suggest that our anarchistic workers have a higher reproductive potential than the “standard” *A. mellifera* queenright workers and perhaps even than laying workers of other anarchistic lines or other European subspecies, but most likely not laying workers of African subspecies (Zheng et al. 2010; Yusuf et al. 2015). The extracts of IAWs

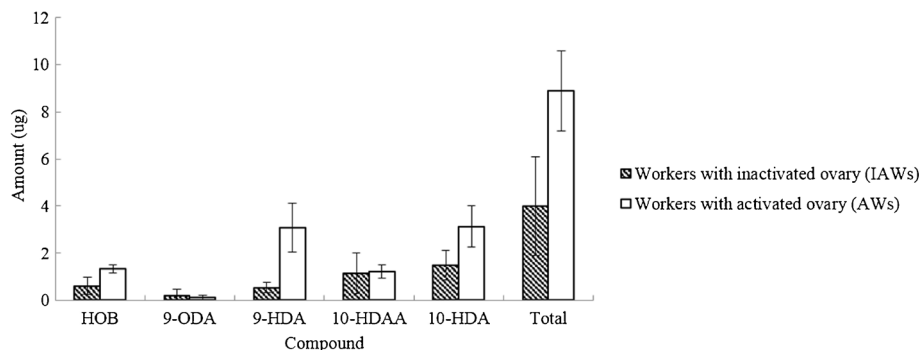


Figure 4. Comparison of the absolute amount (micrograms, mean±SD) between workers with inactivated ovary (*IAWs*) and workers with activated ovaries (*AWs*) sampled in three anarchistic honey bee colonies.

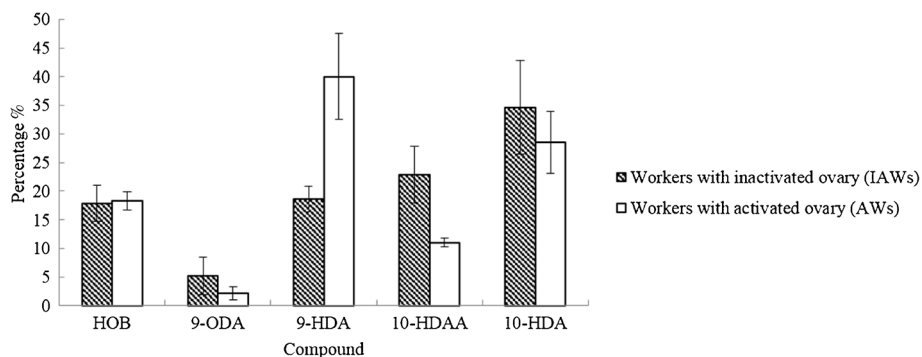


Figure 5. Comparison of the relative amounts (mean \pm SD) of the five compounds of mandibular glands between workers with inactivated ovaries (IAWs) and workers with activated ovaries (AWs) sampled in three anarchistic honey bee colonies.

had the typical worker-like profiles dominated by “worker substances” 10-HDA and 10-HDAA. AWs showed a more queen-like mandibular gland secretion profile, which is dominated by 9-HDA, the precursor of the major queen pheromone compound (9-ODA). In this study, the mandibular gland phenomenon signals of IAWs were more worker-like whereas that of AWs were queen like (Table S1). Our results are consistent with previous studies on reproductive active workers. We show that AWs produce more of 9-HDA similar to *A. m. capensis* workers by preferentially following the “queen-specific” synthetic pathway (Zheng et al. 2010), a physiological priming for pheromone production and reproduction (Plettner et al. 1996, 1998). Therefore, AWs just have to synthesize the final compound 9-ODA by oxidization of 9-HDA, to shift from a worker-like mandibular gland signal to a queen-like signal (Hepburn and Allsopp 1994) dominated by 9-ODA, which allows them to rapidly dominate reproduction (Ruttner and Hesse 1981; Zheng et al. 2010).

Our results confirmed anarchistic syndrome occurred in three *A. mellifera* queenright colonies of European subspecies origin sampled in China, which is observed only in the four other countries before, New Zealand, Australia, America, and the UK under natural conditions (Oldroyd et al. 1994; Ratnieks 1995; Montague and Oldroyd 1998; Châline et al. 2002). Moreover, our results show for the first time that the pheromonal signal of anarchistic workers is more queen-like, a feature

missing in all previous cases on anarchistic bees. The ratio of 10-HDAA to 9-HDA produced by honey bee worker mandibular glands could be considered a mechanism that explains worker “reproduction” in queenright colonies in *A. mellifera*. Understanding the mechanisms of worker reproduction will provide an inside in the evolution of sociality in general.

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Traits reproductifs et phéromone de la glande mandibulaire des ouvrières 'anarchistes' de l'abeille *Apis mellifera* présente en Chine

colonie anarchiste/ activation de l'ovaire/ production d'oeufs/ ouvrière/ 'policing'

Reproduktive Eigenschaften und Mandibeldrüsenpheromon von anarchistischen Arbeiterinnen der Honigbiene *Apis mellifera* in China

***Apis mellifera* / anarchistisches Bienenvolk / Aktivierung der Ovarien / Eiproduktion / „Policing“ durch Arbeiterinnen / Mandibeldrüsenpheromon der Arbeiterinnen**

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