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Scientific Note

A scientific note on the aliphatic esters in queen honey bees[1]

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The organization and regulation of honey bee behaviour and physiology are mediated through primer and releaser pheromones from the queen, workers, and brood. Aliphatic methyl and ethyl esters in brood, first identified in the context of *Varroa* mite attractants (Le Conte et al., 1989) have been attributed to a multitude of effects on both worker behaviour and physiology (Le Conte et al., 2001; Pankiw et al., 2004, and references therein). In the course of identifying new components of the queen retinue pheromone, we found methyl and ethyl palmitate (MP and EP), palmitoleate (MPL and EPL), and oleate (MO and EO), and ethyl stearate (ES) in queens (Keeling et al., 2003). Of these, only MO was found to be a new queen retinue pheromone component. However, we recognized that the other esters might have other pheromonal roles within the colony like those found for the largely identical esters found in the brood, which also include methyl stearate (MS), and methyl and ethyl linoleate (ML and EL) and linolenate (MLN and ELN). Recently, an ester was found to mediate a worker-worker interaction; EO in foragers delays the onset of foraging by nurse bees (Leoncini et al., 2004). These esters, originally called "brood esters", have become important sociochemicals within the colony. We report here the quantities of esters found in queens. We examined virgin and mated queens because quantitative differences between these reproductive states are known for some queen pheromone components (Keeling et al., 2003). These data provide the basis for future studies of the importance of these esters in the queen.

Queens originated from queen stock (closest to *Apis mellifera ligustica* L.) imported from New Zealand, Australia, or Hawaii. Analysis of MO and EO in whole mated queens used mated/laying queens at least one year old from established colonies. Analysis of esters in the body sections of virgin and mated queens used queens collected in the course of another experiment from recently established colonies screened for 11 days to prevent foraging and fed only water and sugar water (Ledoux et al., 2001). Homogenized whole queens or body sections were extracted, derivatized, and analyzed for MP, EP, MPL, EPL, ES, MO, and EO by gas chromatography-mass spectrometry as previously described (Keeling et al., 2003). Peaks at the correct retention indices were integrated relative to an internal standard over diagnostic mass spectral peaks.

Whole mated/laying queens contained 3820 ± 830 and 630 ± 250 ng/bee of MO and EO respectively (n = 10). All esters were easily detectable in all body sections except for MPL, which could not be detected here but has been found in other queens (Keeling et al., 2003). The quantity of esters in the body sections of virgin and mated/laying queens were similar and were found mainly in the abdomen (Tab. I). Only EP and ES were significantly more abundant in the heads of mated versus virgin queens, but this is likely of little biological significance since the head contains a minor quantity of these esters relative to the other body sections. Depending on the individual queen, either methyl or ethyl esters predominated over the other. The large variation in the quantities and methyl/ethyl ratios seen in this study could be attributed to queen age, reproductive or nutritive state, colony size, or colony status. Whether some of these esters in the queen synergise with queen mandibular pheromone and/or have a cooperative role with brood or forager esters as releaser and primer pheromones in regulating colony behaviour and physiology awaits further research. Further studies are also needed to determine if queens biosynthesize the esters themselves or acquire it from worker contacts. The latter is unlikely because nurse bees, which attend the queen, do not biosynthesize appreciable levels of EO and they have negligible

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Table I. Esters in body sections of virgin and mated/laying queens compared to foragers and larvae reported previously.

	ng/bee (mean ± SE)									
	Virgin Queen			Mated/Laying Queen			Larvae			Forager[d]
Ester	Head	Thorax	Abdomen	Head	Thorax	Abdomen	Drone[a]	Worker[b]	Queen[c]	
MP	6 ± 2	5 ± 3	3 ± 3	3 ± 2	9 ± 5	3 ± 3	260	12	16	1.3
EP	29 ± 9*	72 ± 28	230 ± 60	55 ± 8	68 ± 16	200 ± 50	90	9	15	n.d.
MPL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EPL	180 ± 60	410 ± 160	800 ± 300	290 ± 60	500 ± 130	1000 ± 300	n.d.	n.d.	n.d.	n.d.
MS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	260	74	7	n.d.
ES	8 ± 3*	18 ± 7	19 ± 9	25 ± 2	20 ± 4	25 ± 1	80	18	9	0.5
MO	27 ± 6	34 ± 10	49 ± 15	53 ± 12	49 ± 7	60 ± 13	70	77	191	0.7
EO	730 ± 240	1700 ± 600	3400 ± 1300	1300 ± 200	1500 ± 300	3500 ± 800	30	25	126	60
ML	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	30	7	5	n.d.
EL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10	3	3	n.d.
MLN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	590	97	56	3.0
ELN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	180	65	50	n.d.

a. Hexane-washed 8 day-old drone larvae (Le Conte et al., 1989).
b. Pentane and dichloromethane-washed 8 to 9 day-old worker larvae, estimated from Le Conte et al. (1994).
c. Hexane-washed 9 day-old queen larvae (Le Conte et al., 1995).
d. *Iso*-hexane washed foragers (Leoncini, 2002), c.f. 62 ng/bee of EO in 2-methylpentane extracts of homogenized workers (Leoncini et al., 2004).
* Significant difference between queen types for the same body section (*t*-test, $P < 0.05$, $n=7$ for each queen type).
n.d., not detected.

amounts of the other esters (Leoncini et al., 2004). Although there is only one queen, some esters in the queen are many fold more abundant than those found in individual larvae or foragers (above and Tab. I). Further studies are needed to establish if the queen is a biologically significant source of these socio-chemicals and what roles they play within the colony. Recent cage experiments suggest they are not involved in inhibiting ovarian development in workers (Hoover et al., 2003).

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Note scientifique sur les esters aliphatiques des reines d'abeilles (*Apis mellifera*).

Eine wissenschaftliche Notiz über die aliphatischen Ester der Bienenkönigin (*Apis mellifera*).

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