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**Original article**

# Quantitative analysis of the mandibular gland components of the dwarf honey bee (*Apis florea* Fabricius)

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(Invited paper)

**Abstract** – Workers and mated queens of the dwarf honey bee (*Apis florea*) from Sri Lanka were quantitatively analyzed by gas chromatography-mass spectrometry for the following nine major mandibular gland components: methyl *p*-hydroxybenzoate (HOB), 8-hydroxyoctanoic acid (8-HOAA), 4-hydroxy-3-methoxyphenylethanol (HVA), (*E*)-9-oxodec-2-enoic acid (ODA), (*E*)-9-hydroxydec-2-enoic acid (9-HDA), 10-hydroxydecanoic acid (10-HDAA), (*E*)-10-hydroxydec-2-enoic acid (10-HDA), decanedioic acid (C10:0 DA) and (*E*)-dec-2-enedioic acid (C10:1 DA). Queens and workers were significantly different in most of the mandibular gland components analyzed. The major component in mated queens was 10-HDA and in workers was 8-HOAA. Queens and workers contained no detectable HOB or HVA. The mandibular gland compositions of queenless and queenright workers were similar.

***Apis florea* / honey bee / mandibular gland / pheromone**

## 1. INTRODUCTION

*Apis florea* is an open-nesting species of honey bee native to Asia. Plettner et al. [5] have recently analyzed the mandibular gland components of both female castes of five honey bee species including *A. florea*. They found distinct blends of the mandibular gland components of both castes for each honey bee species. However, only one mated *A. florea* queen was analyzed in that study, and they did not analyze queenless

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workers. The object of this study was to provide further information on the mandibular gland components in *A. florea* of mated queens and workers in colonies with and without queens.

In *A. mellifera*, both female castes produce distinctive blends of compounds in their mandibular glands. Functionalized aliphatic acids predominate in both castes but in mated queens, compounds functionalized at the penultimate (ω-1) position of the chain [(*E*)-9-oxodec-2-enoic acid (ODA) and the two enantiomers of (*E*)-9-hydroxydec-2-enoic acid (9-HDA)] predominate, whereas in workers, functionalization at the terminal (ω) position [10-hydroxydecanoic acid (10-HDAA) and (*E*)-10-hydroxydec-2-enoic acid (10-HDA)] predominates. The caste-selective biosynthesis of these acids in *A. mellifera* has recently been investigated [4, 6]. In addition, *A. mellifera* queen mandibular glands produce two aromatic compounds [methyl *p*-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA)] that, along with ODA and 9-HDA, make up the queen mandibular pheromone in *A. mellifera* [7]. This pheromone accounts for the retinue attraction and much of the chemical communication attributed to the queen. The functions of the components of the mandibular gland in workers are attributed to food preservation and larval nutrition [9].

In addition to the above compounds, the following compounds were also quantified: 8-hydroxyoctanoic acid (8-HOAA), decanedioic acid (C10:0 DA) and (*E*)-dec-2-enedioic acid (C10:1 DA). These are biosynthetically related to the major functionalized acids but are not known pheromone components in any *Apis* species [4, 6]. This set of nine mandibular gland components provides a basis for comparing the biosynthetic status of bees of different castes and species.

The mandibular gland components are not completely defined by caste determination. Upon queenlessness, workers of some species of honey bees can develop into false-queens, producing queen-like mandibular gland components and attracting a retinue of workers. *A. mellifera ligustica* and *A. m. carnica* workers can occasionally become false-queens [3] and *A. m. capensis* workers readily become false-queens [1]. Whether queenless *A. florea* workers can develop into false-queens is unknown.

## 2. MATERIALS AND METHODS

### 2.1. Collection of specimens

Bees of unknown age were collected from 5 colonies in October and November of 1996 in Makandura, Sri Lanka. All queens collected were mated, laying and heading normal colonies. Workers were collected from colonies 1, 2, and 3 several days after de-queening the colonies and queen cells were destroyed to prevent queen rearing. We found eggs from laying workers 11 days after de-queening in colony 1, but found no eggs from laying workers when we sampled workers in colonies 2 and 3 after being queenless for 10 and 8 days respectively. Colony 4 was a small colony that had been queenless for some time. It contained laying workers, emerging drone brood, and sexually mature adult drones. This suggests that this colony represented a very late stage of queenlessness. The ovarian development of workers from these colonies has been reported elsewhere [2]. Dissection of the mandibular glands could not be completed in the field, so bees were decapitated and the heads sealed in glass ampoules with 100 μL methanol and shipped to Simon Fraser University. Upon arrival, samples were stored at –20 °C until extracted.

### 2.2. EXTRACTION AND ANALYSIS

Although all compounds analyzed originate within the mandibular glands, the whole head was extracted because the analytes are leached from the intact mandibular

glands into the rest of the head and the shipping solvent [5]. Individual bee heads were macerated in the shipping solvent and extracted with additional methanol. Some ampoules contained several heads which were not extracted separately, but instead macerated and extracted in one batch. For statistical analysis, the samples consisting of a batch of several workers were each treated as a single source. Extracts were stored at –20 °C until analyzed.

To each extract, 5.53 μg of undec-10-enoic acid in methanol (10 μL) was added as an internal standard. A 10-μL portion (approx. 0.1 bee equivalents) of an extract was placed in a small (approx. 100 μL) ampoule fashioned from a Pasteur pipette and the solvent gently evaporated by reduced pressure. The residue was reacted overnight at room temperature in the sealed ampoule with 3 μL of neat bistrimethylsilyltrifluoroacetamide (BSTFA). The derivatized sample was then diluted with distilled hexane (20 μL), and a 2-μL portion (approx. 0.009 bee equivalents) was analyzed by splitless capillary gas chromatography-mass spectrometry (Varian 3400 GC-Varian Saturn ion trap MS, J&W Scientific DB-5ms column, 30 m × 0.25 mm ID × 0.25 μm film). The GC oven was programmed at 100 °C (1 min), 10 °C/min to 200 °C (6 min), and 25 °C/min to 250 °C (21 min), head pressure 120 kPa helium. The SPI injector was programmed at 80 °C (0.1 min), 100 °C/min to 250 °C (38.2 min). The mass spectrometer was operated in electron impact mode at 70 eV with a target value of 23 500, multiplier at 1 700 V and an ionization current of 10 μA. Standard solutions of methyl *p*-hydroxybenzoate (HOB), 8-hydroxyoctanoic acid (8-HOAA), 4-hydroxy-3-methoxyphenylethanol (HVA), (*E*)-9-oxodec-2-enoic acid (ODA), (*E*)-9-hydroxydec-2-enoic acid (9-HDA), 10-hydroxydecanoic acid (10-HDAA), (*E*)-10-hydroxydec-2-enoic acid (10-HDA), decanedioic acid (C10:0 DA) and (*E*)-dec-2-enedioic acid (C10:1 DA) derivatized with BSTFA were used to calibrate the response of the instrument with respect to the internal standard over a 200-fold range of concentration for each analyte. We used the mass spectrometer's software capability to integrate peaks over specific masses. Masses were chosen for each analyte based on the fragmentation patterns of the trimethylsilyl derivatives [typically $M^+$, $(M+1)^+$, and/or $(M-15)^+$] and the absence of adjacent peaks with similar masses as follows: undec-10-enoic acid (241 and 257), HOB (209 and 224), 8-HOAA (273 and 289), HVA (209 and 312), ODA (241, 256, and 257), 9-HDA (286 and 315), 10-HDAA (301 and 317), 10-HDA (315 and 331), C10:0 DA (331, 346, and 347), and C10:1 DA (329, 344, and 345). Retention indices [8] for these derivatives on the DB-5ms column were as follows: undec-10-enoic acid (1541), HOB (1492), 8-HOAA (1607), HVA (1701), ODA (1704), 9-HDA (1782), 10-HDAA (1800), 10-HDA (1851), C10:0 DA (1881), and C10:1 DA (1934). JMP software (SAS Institute Inc., 1996) was used for statistical analysis.

## 3. RESULTS

### 3.1. Queens

The mated queens analyzed here contained much more ODA, 9-HDA, and 10-HDA (Tab. I) than previously reported [5], and the queens had significantly more ODA than 9-HDA (Kruskal-Wallis, $\chi^2 = 5.33$, $df = 1$, $P = 0.02$). The diacid C10:1 DA was the second most abundant acid in the queen after 10-HDA. For the ω-hydroxy acids (10-HDA, 10-HDAA, and 8-HOAA), there was significantly more 10-HDA than the others (Kruskal-Wallis, pairwise comparisons, $P = 0.02$). However, 8-HOAA and 10-HDAA were not significantly different (Kruskal-Wallis, $\chi^2 = 3.00$, $df = 1$, $P = 0.08$). HOB and HVA were not detected in any of the queens (< 0.01 μg/queen).

**Table I.** Summary of the analysis of mandibular gland components.

| | N | Amount (μg/bee), mean ± SE | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 8-HOAA | ODA | 9-HDA | 10-HDAA | 10-HDA | C10:0 DA | C10:1 DA |
| **Colony 1** | | | | | | | | |
| Queen | 1 | 0.8 | 13.9 | 5.4 | 0.5 | 105 | 0.3 | 30.6 |
| Queenright workers | 7 | 11.9 ± 1.9 | 1.2 ± 0.6 | 1.4 ± 0.3 | 0.5 ± 0.1 | 8.8 ± 1.3 | 0.7 ± 0.1 | 5.3 ± 1.2 |
| Workers 3 days queenless | 6 | 8.7 ± 4.4 | 0.3 ± 0.2 | 1.1 ± 0.6 | 0.4 ± 0.2 | 5.7 ± 2.2 | 0.7 ± 0.3 | 5.0 ± 1.9 |
| Workers 11 days queenless | 9 | 11.1 ± 2.3 | 0.6 ± 0.2 | 1.3 ± 0.4 | 0.5 ± 0.1 | 6.5 ± 1.0 | 0.7 ± 0.1 | 4.9 ± 0.8 |
| **Colony 2** | | | | | | | | |
| Queen | 1 | 0.7 | 15.5 | 10.3 | 0.9 | 93 | 0.4 | 9.3 |
| Workers 10 days queenless | 2 | 7.4 ± 0.1 | 0.2 ± 0.1 | 1.0 ± 0.1 | 0.4 ± 0.1 | 4.4 ± 1.1 | 0.6 ± 0.1 | 3.4 ± 0.3 |
| **Colony 3** | | | | | | | | |
| Queen | 1 | 1.2 | 11.6 | 3.1 | 0.2 | 87 | 0.7 | 22.8 |
| Workers 8 days queenless | 6 | 14.8 ± 2.4 | 0.2 ± 0.0 | 2.0 ± 0.2 | 0.5 ± 0.1 | 8.1 ± 1.1 | 0.9 ± 0.2 | 5.6 ± 0.7 |
| **Colony 4** (Queenless colony with laying workers) | | | | | | | | |
| Batch of 20 workers | 1 | 13.8 | 0.8 | 1.6 | 1.0 | 10.3 | 0.9 | 5.9 |
| Batch of 10 workers | 1 | 22.2 | 0.7 | 2.5 | 1.2 | 14.3 | 1.5 | 8.7 |
| Workers | 3 | 25.0 ± 2.5 | 0.3 ± 0.0 | 3.7 ± 0.4 | 0.9 ± 0.1 | 11.4 ± 1.1 | 1.1 ± 0.1 | 4.7 ± 0.2 |
| **Colony 5** (Normal but small colony) | | | | | | | | |
| Queen | 1 | 1.6 | 13.1 | 3.1 | 0.4 | 69 | 0.6 | 12.5 |
| Batch of 20 queenright workers | 1 | 10.6 | 1.2 | 1.3 | 0.8 | 7.1 | 0.5 | 3.9 |
| Batch of 20 queenright workers | 1 | 9.5 | 0.7 | 1.4 | 0.5 | 6.9 | 0.6 | 4.6 |
| Queenright workers | 9 | 16.7 ± 1.0 | 0.7 ± 0.2 | 1.8 ± 0.2 | 0.9 ± 0.0 | 11.8 ± 0.7 | 1.1 ± 0.1 | 6.4 ± 0.7 |
| **All queens** | 4 | 1.1 ± 0.2 | 13.5 ± 0.8 | 5.5 ± 1.7 | 0.5 ± 0.1 | 88 ± 7 | 0.5 ± 0.1 | 18.8 ± 4.9 |
| **All queenless workers** | 28 | 13.1 ± 1.6 | 0.4 ± 0.1 | 1.7 ± 0.2 | 0.6 ± 0.1 | 7.4 ± 0.7 | 0.8 ± 0.1 | 5.1 ± 0.5 |
| **All queenright workers** | 18 | 14.1 ± 1.1 | 0.9 ± 0.3 | 1.6 ± 0.2 | 0.7 ± 0.1 | 10.1 ± 0.7 | 0.9 ± 0.1 | 5.7 ± 0.6 |
| **All workers** | 46 | 13.5 ± 1.0 | 0.6 ± 0.1 | 1.7 ± 0.2 | 0.6 ± 0.0 | 8.5 ± 0.6 | 0.8 ± 0.1 | 5.4 ± 0.4 |

### 3.2. Workers

The workers analyzed in this study contained similar amounts of the mandibular gland components as previously reported [5]. The ω-hydroxy acids (10-HDA, 10-HDAA, and 8-HOAA) were all significantly different from each other (Kruskal-Wallis, pairwise comparisons, $P < 0.0003$). Both ODA and 9-HDA were detected in workers. These are two components of *A. mellifera's* queen mandibular pheromone. Although small quantites of 9-HDA are found in workers of most *Apis* species, ODA is not usually detected in *A. mellifera* or *A. cerana* workers. HOB and HVA were not detected in any of the workers (< 0.01 μg/worker).

### 3.3. Queenright versus queenless workers

There were no significant differences for any compound between queenright, 3 days queenless, and 11 days queenless workers from colony 1 (Kruskal-Wallis, $df = 2$, $P > 0.05$). However, the laying workers from colony 4 were significantly different from the queenright workers from colonies 1 and 5 (pooled, $N = 18$) in the amounts of 8-HOAA, 9-HDA, and 10-HDAA present (Kruskal-Wallis, $df = 1$, $P < 0.05$). Whether these differences were due to long-time queenlessness or other factors is not clear. Comparison of all the queenright with all the queenless workers indicated significant differences in ODA, 10-HDAA, and 10-HDA (Kruskal-Wallis, $df = 1$, $P < 0.05$). Although statistically significant, the queenless workers still had distinctive worker-like blends and did not produce a queen-like blend of mandibular gland components. Due to limited sampling, we cannot ignore the possibility that these differences were due to colony variation and not to queen state. Analysis of bees of known age from several queenright and queenless colonies are needed for proper comparison.

### 3.4. Differences between queens and workers

The 10-HDA/9-HDA ratios of queens and workers were significantly different ($20 \pm 4$ and $6.3 \pm 0.6$ respectively, Kruskal-Wallis, $\chi^2 = 9.46$, $df = 1$, $P = 0.002$). This ratio did not change significantly as workers became queenless [all queenright ($7.0 \pm 0.8$, $N = 18$) versus all queenless ($5.8 \pm 0.8$, $N = 28$) workers, Kruskal-Wallis, $\chi^2 = 3.57$, $df = 1$, $P = 0.06$). In fact, the trend was away from a queen-like ratio. Queens were significantly different to the workers for all compounds except for 10-HDAA and C10:0 DA (Kruskal-Wallis, $P < 0.05$).

## 4. DISCUSSION

Our results for queenright workers are consistent with those reported previously [5]. The mandibular gland components of queenless workers are slightly different to queenright workers, but certainly do not appear to become queen-like. The amounts of the mandibular gland components in the workers from colony 4 (long-time queenless) were not very different from queenright workers even though this colony contained laying workers [2]. This suggests that *A. florea* workers do not become false-queens, contrasting with *A. mellifera capensis* workers that can rapidly begin producing queen-like mandibular gland components upon queenlessness [1]. However, North American honey bees (*A. m. ligustica* and *A. m. carnica*) also continue to produce worker-like mandibular gland components upon queenlessness, although occasionally a worker will become a false-queen, attracting a retinue of workers around her by producing components of the queen mandibular pheromone [3]. *A. florea* workers appear the most biosynthetically fixed among these species.

The queens in this study had significantly more ODA, 9-HDA, and 10-HDA than previously reported [5]. Also, the previous

study did not report analyses of 8-HOAA, C10:0 DA, and C10:1 DA in queens. We found that *A. florea* queens produce detectable amounts of 8-HOAA and C10:0 DA, and large amounts of C10:1 DA. Our results confirm that *A. florea* has a larger 10-HDA/9-HDA ratio for queens than workers; the only honey bee species in which this has been found.

The role all these compounds play, if any, in the pheromone communication of *A. florea* is unknown. However, this study confirms that the castes of *A. florea* have unique differences in the biosynthesis of mandibular gland components.

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**Résumé – Analyse quantitative des composés de la glande mandibulaire de l'abeille naine *Apis florea* F.** Des ouvrières et des reines fécondées de l'abeille naine, *Apis florea*, ont été prélevées dans cinq colonies à Makandura, Sri Lanka. Les ouvrières ont été prélevées dans des colonies avec reine, des colonies orphelines depuis peu et des colonies orphelines depuis longtemps. On a procédé à des extraits de têtes dans le méthanol puis analysé quantitativement en chromatographie phase gazeuse-spectrométrie de masse les neuf composés majoritaires de la glande mandibulaire sous forme de dérivés triméthylsilysés : le *para*-hydroxybenzoate de méthyle, (HOB), l'acide 8-hydroxyoctanoïque (8-HOAA), le 4-hydroxy-3-méthoxyphényléthanol (HVA), l'acide (*E*)-9-oxodécène-2-oïque (ODA), l'acide (*E*)-9-hydroxydécène-2-oïque (q-HDA), l'acide 10-hydroxydécanoïque (10-HDAA), l'acide (*E*)-hydroxy-10-décène-2-oïque (10-HDA), l'acide décanedioïque (C10:0 DA) et l'acide (*E)*-décène-2-dioïque (C10:1 DA). Les reines et les ouvrières différaient significativement par la plupart des composés de la glande mandibulaire analysés (Tab. I). Le composé majoritaire chez les reines fécondées était le 10-HDA et chez les ouvrières le 8-HOAA. Le rapport 10-HDA/9-HDA était de 20 ± 4 pour les reines et de 6,3 ± 0,6 pour les ouvrières. *A. florea* est la seule espèce d'*Apis* pour laquelle ce rapport est plus élevé chez les reines que chez les ouvrières. La composition de la glande mandibulaire des ouvrières de colonies orphelines et de colonies avec reine était semblable, ce qui laisse à penser que, chez cette espèce, les ouvrières orphelines ne deviennent pas de fausses reines comme c'est le cas chez certaines autres espèces. Aucune quantité détectable de HOB et de HVA, composés présents chez la reine d'*A. mellifera*, n'a été trouvée chez les reines ni chez les ouvrières. Cette étude confirme que les castes d'*A. florea* présentent des différences dans la biosynthèse des composés de la glande mandibulaire qui sont propre à cette espèce.

***Apis florea* / phéromone / glande mandibulaire / reine / ouvrière**

**Zusammenfassung – Quantitative Analyse von Komponenten der Mandibeldrüse bei der Zwerghonigbiene (*Apis florea* F.).** Arbeiterinnen und begattete Königinnen der Zwerghonigbiene (*Apis florea*) wurden von 5 Völkern in Makandura, Sri Lanka gesammelt. Die Arbeiterinnen stammten aus weiselrichtigen Völkern sowie aus Bienenvölkern kurz nach der Entfernung der Königin und nach langer Weisellosigkeit. Die Köpfe wurden in Methanol extrahiert und nach folgenden 9 Komponenten wurde gaschromatographisch-massenspektrometrisch gesucht und bei Nachweis quantitativ analysiert: Methyl *p*-hydroxybenzoat (HOB), 8-Hydroxyoctansäure (8-HOAA), 4-Hydroxy-3-methoxyphenylethanol (HVA), 9-Keto-2(*E*)-decensäure (ODA), 9-Hydroxy-2(*E*)-decensäure (9-HDA), 10-Hydroxydecansäure

(10-HDAA), 10-Hydroxy-2(*E*)-decensäure (10-HDA), Decandicarbonsäure (C10:0 DA) und 2(*E*)-Decendicarbonsäure (C10:1 DA). Königinnen und Arbeiterinnen unterschieden sich signifikant in den meisten analysierten Komponenten der Mandibeldrüse (Tab. I). Die Hauptkomponente war bei begatteten Königinnen 10-HDA und bei Arbeiterinnen 8-HOAA. Das Verhältnis von 10-HDA/9-HDA betrug bei Königinnen 20 ± 4 und bei Arbeiterinnen 6,3 ± 0,6. Damit ist *A. florea* die einzige Bienenart, bei der ein größeres Verhältnis bei Königinnen als bei Arbeiterinnen nachgewiesen wurde. Die Zusammensetzung der Komponenten der Mandibeldrüse von weisellosen und weiselrichtigen Arbeiterinnen war ähnlich. Das spricht dafür, dass weisellose *Apis florea* Arbeiterinnen trotz Eilage, anders als bei einigen anderen Arten, keine für Königinnen typische Pheromone produzieren (false queens). Die Komponenten HOB oder HVA lagen sowohl bei Königinnen als auch bei Arbeiterinnen mengenmässig unter der Nachweisgrenze.

***Apis florea* / Honigbiene / Mandibeldrüse / Pheromon**

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