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MANDIBULAR GLAND COMPONENTS OF EUROPEAN AND AFRICANIZED HONEY BEE QUEENS (*Apis mellifera* L.).

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Abstract—The composition of the five-component honey bee queen mandibular gland pheromone (QMP) of mated European honey bee queens was compared to those of virgin and drone-laying (i.e., laying only haploid unfertilized eggs that develop into males), European queens and Africanized mated queens. QMP of mated European queens showed significantly greater quantities of individual components than all queen types compared, except for a significantly greater quantity of 9-hydroxy-(*E*)-2-decenoic acid (9-HDA) found in Africanized queens. Glands of European drone-laying queens contained quantities intermediate between virgin and mated queens, reflecting their intermediate reproductive state and age. QMP ontogeny shifts from a high proportion of 9-keto-(*E*)-2-decenoic acid (ODA) in young unmated queens to roughly equal proportions of ODA and 9-HDA in mated queens. A biosynthetic shift occurs after mating that results in a greater proportion of 9-HDA, methyl *p*-hydroxybenzoate (HOB), and 4-hydroxy-3-methoxyphenylethanol (HVA) production, accompanied by a decreased proportion of ODA. Africanized QMP proportions of ODA and 9-HDA were significantly different from European queens. A quantitative definition of a "queen equivalent" of QMP is proposed for the various queen types, and a standard queen equivalent for mated European honeybee queen mandibular gland pheromone is adopted as 200 μg ODA, 80 μg 9-HDA, 20 μg HOB, and 2 μg HVA.

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INTRODUCTION

One of the most interesting aspects of honey bee biology is the variability found within and between races of *Apis mellifera*. Bees vary in behavioral, morphological, and physiological characteristics. Much of the worldwide success of this species is attributed to the variation among colony members providing adaptive advantages in different habitats (Seeley, 1985; Winston, 1987, 1992). A great deal is known about the phenotypic and genotypic variability among worker bees within colonies, but the variability among queen honey bees is not as widely studied. Here we present a detailed characterization and comparison of inter- and intraracial variation of queen mandibular gland pheromone.

Queen mandibular gland pheromone (QMP) is a blend of the three abundant aliphatic acids, 9-keto-(*E*)-2-decenoic acid (ODA), and 9-hydroxy-(*E*)-2-decenoic acid (9-HDA), present as two optical isomers [*R*-(−) and *S*-(+)], and two aromatic compounds; methyl *p*-hydroxybenzoate (HOB), and 4-hydroxy-3-methoxyphenylethanol (HVA) (Slessor et al., 1988). As a primer pheromone, QMP has been shown to delay swarming (Winston et al., 1991), suppress queen rearing (Pettis et al., 1995; Winston et al., 1989, 1990), inhibit worker juvenile hormone (JH) biosynthesis in the laboratory (Gast, 1967; Hildebrandt and Kaatz, 1990; Kaatz et al., 1992), and regulate foraging age of adult worker bees and suppress JH titers in colonies (Pankiw et al., unpubl. data). QMP also has some releaser pheromone functions, including retinue formation around the queen (Slessor et al., 1988; Kaminski et al., 1990), attraction of workers to swarms (Winston et al., 1989), stimulation of pollen foraging (Higo et al., 1992), calming of queenless workers (Naumann et al., 1990), and attraction of foragers to crops (Currie et al., 1992a,b).

In this study we extracted QMP from the glands of various European and Africanized queen types and compared the quantities and ratios of individual components. The ontogenetic development of the QMP signal also is presented, and a definition of one queen equivalent is proposed that reflects racial and reproductive differences, while allowing for the natural variation found among individuals.

The only previous descriptions of the five-component blend of QMP in *Apis mellifera* L. have been done by Slessor et al. (1988, 1990). The study presented here analyzes a greater number and range of queen types and permits a more complete description of QMP in *Apis mellifera*. The queen types examined here include European mated, virgin, and drone-laying queens, representing various female honey bee reproductive types, and mated Africanized queens. Africanized bees are derived from South African bees accidentally released in

South America. The rapid expansion of Africanized honey bees throughout South and Central America can be attributed to their propensity to higher swarming rates with smaller swarm sizes (Winston et al., 1983). Since QMP is important to colony cohesion and plays a role in the timing of swarming both in European and Africanized honey bees (Pettis et al., 1994; Winston et al., 1991), QMP difference may influence differences in swarming behavior between these races.

METHODS AND MATERIALS

Queen Collection and Mandibular Gland Component Analysis

Queens were immediately placed on Dry Ice upon collection, then into labeled Eppendorf tubes, and stored at −70°C prior to dissection, except where stated otherwise. Mandibular glands were extirpated, then extracted in 2 × 50 μl of methanol containing 10-undecenoic acid (0.22 mg/ml) as an internal standard. Two-microliter aliquots of the extracts were derivatized with BSTFA (Slessor et al., 1990), diluted with hexane and analyzed by splitless capillary gas chromatography on an HP5890 gas chromatograph equipped with a flame ionization detector (FID), and a 30-m DB-1 fused-silica capillary column. The FID was calibrated with standards of known concentration, and these FID responses were used to calculate the amount of each component in the mandibular gland extracts.

European Mated Queens. Mandibular gland extract data from 125 1- and 2-year-old mated queens of Simon Fraser University stock most similar to *Apis mellifera ligustica* were combined to establish a baseline mated queen mandibular gland component profile. Data from 10 queens have not been reported previously, and the remainder are from Slessor et al. (1990), Pankiw et al. (1994), and Pettis et al. (1995).

Virgin Queens. Ten virgin queens of Simon Fraser University stock most similar to *Apis mellifera ligustica* were reared until 2 weeks old and then collected in July and August 1992. Mandibular gland component analysis results from these queens were combined from seven queens from Slessor et al. (1990) for a total of 17 queens.

European Drone-Laying Queens. Twenty-two queens of mixed stock most similar to *Apis mellifera ligustica* were narcotized two times with CO_2 seven days after emergence and instrumentally inseminated with insect saline solution to induce haploid male egg laying. They then were collected from five-frame nucleus colonies at 8 weeks of age on August 21 and 22, 1991, as above. Queens were dissected from May 20 to June 6, 1992.

Africanized Mated Queens. Twenty mated Africanized queens were obtained from Mexico in 1992. Morphometric (Daly and Balling, 1978) analysis of the progeny confirmed the racial status of these queens. Queens were transported

on Dry Ice by overnight courier from Mexico and received March 3, 1992. The queens were stored at −70°C prior to dissection from June 22 to 29, 1992.

Proportions and Ratios

To develop an ontological profile of queen mandibular gland components, the results reported in Slessor et al. (1990), Pankiw et al. (1994), Pettis et al. (1995), and this study were combined. Ratios of HDA, HOB, and HVA to ODA were calculated to develop a definitive formula of a QMP queen equivalent.

Statistical Analysis

Analysis for statistical differences between European mated and all other queen types were performed using the one-tailed t test Satterthwaite method for unequal variances (Snedecor and Cochran, 1980). Analysis for statistical differences between European mated and all other queen types for proportion and ratio data were by the Kruskal-Wallis test (Conover, 1980). SAS was used for all statistical analyses. The level of significance throughout was at the 0.05 level, unless stated otherwise.

RESULTS

Quantities. Glands of European mated queens contained statistically greater quantities of all QMP components compared to virgin, drone-laying, and Africanized queens (Figure 1), except for a statistically similar quantity of 9-HDA in Africanized and European mated queens (Figure 1).

Proportions and Ratios. Queen mandibular gland biosynthetic capabilities appear to be ontogenetic (Figure 2); an examination of individual QMP component proportions to the total amount of QMP reveals a reproductive shift in the pheromonal "bouquet" (Figure 2). Virgin queen glands contained high proportions of ODA and lower 9-HDA proportions. Drone-laying queen ODA and 9-HDA proportions were intermediate between virgin and mated queens. Mated queen glands contained significantly greater proportions of 9-HDA compared to virgin queens (Figure 2). Africanized queen ODA proportions were significantly lower, and 9-HDA higher, compared to mated European queens.

The aromatic component (HOB and HVA) proportions changed significantly with reproductive status (Figure 2). Virgin and drone-laying queen glands contained significantly lower proportions of the aromatic components. Africanized queen gland aromatic proportions were similar to mated European queens (Figure 2).

The ratios of 9-HDA, HOB, and HVA to ODA in the glands for the various

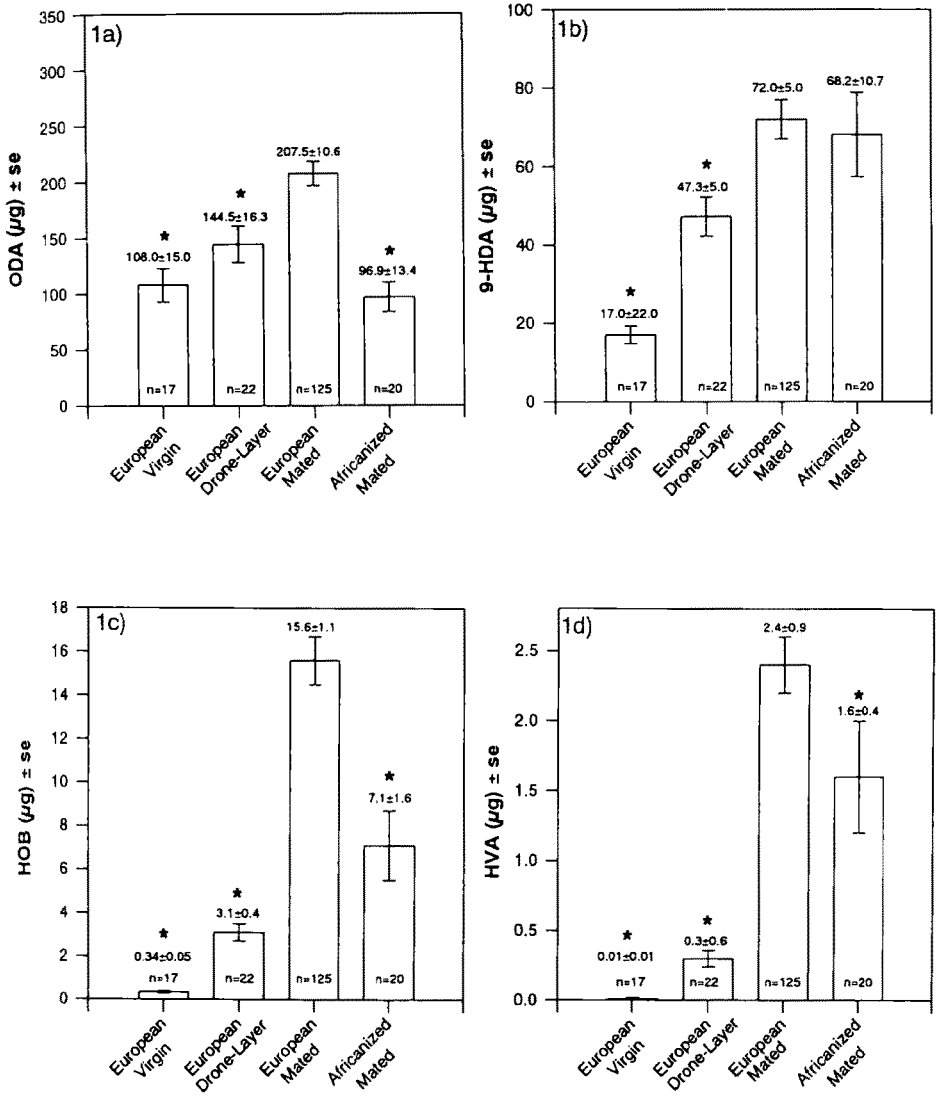

FIG. 1. Whole gland quantities of individual queen mandibular pheromone components. An asterisk above a bar indicates a statistically significant difference from the mated European queen mean at the 0.05 level; (a) ODA, 9-keto-(*E*)-2-decenoic acid; (b) 9-HDA, 9-hydroxy-(*E*)-2-decenoic acid; (c) HOB, methyl *p*-hydroxybenzoate; (d) HVA, 4-hydroxy-3-methoxyphenylethanol.

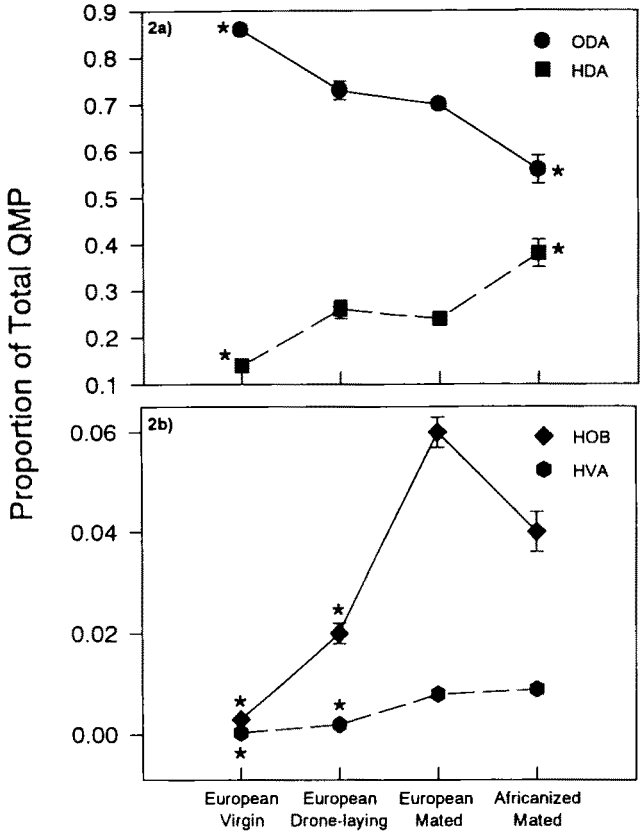

FIG. 2. Ontogenetic and reproductive profile of QMP extracted from glands. An asterisk indicates a statistically significant difference from the mated European queen mean at the 0.05 level. Invisible standard error bars are less than the size of the symbol.

queens are summarized in Table 1. The gland ratio of 9-HDA/ODA was significantly higher in Africanized than European mated queens and significantly lower in the virgin queens. The virgin and drone-laying queen glands contained significantly lower ratios of HOB/ODA, and HVA/ODA compared to the European queens, whereas Africanized queen glands contained similar ratios.

Table 2 demonstrates examples of calculated queen equivalents of glandular QMP based on the mean ODA values from Figure 1a.

Frequency Distributions. Frequency distributions of QMP component quantities of the mated European queens (Figure 3) indicated a wide range of variability in the composition of this pheromone.

TABLE 1. MEAN RATIOS OF QMP COMPONENTS TO ODA FOR VARIOUS QUEEN TYPES

Queen type	Ratios		
	9-HDA/ODA	HOB/ODA	HVA/ODA
European mated	0.40	0.09	0.01
Africanized mated	0.75[a]	0.07	0.02
European drone-laying	0.40	0.03[a]	0.002[a]
European virgin	0.20[a]	0.004[a]	0.0004[a]

[a]Significant ($P < 0.0002$) difference from mated European queen.

TABLE 2. CALCULATED VALUES FOR ONE QUEEN EQUIVALENT OF QMP

Queen type	Queen equivalent QMP components (μg)[a]			
	Mean ODA	Calculated 9-HDA	Calculated HOB	Calculated HVA
European mated	208 (200)	83 (80)	19 (20)	2 (2)
Africanized mated	97 (100)	39 (40)	7 (10)	2 (2)
European drone-layer	145 (145)	58 (60)	4 (5)	0.4 (1)
European virgin	108 (100)	22 (20)	0.8 (1)	0.7 (1)

[a]Values in parentheses are proposed "standard" queen equivalent values.

DISCUSSION

We define one queen equivalent (QE) based on the mean ratios of 9-HDA, HOB, and HVA quantities to ODA for the various queen types (Tables 1 and 2). A standard queen equivalent for the mated European queens, derived by rounding off from values in Table 2 is: 200 μg ODA, 80 μg 9-HDA, 20 μg HOB, and 2 μg HVA. Proportions of the total amount of QMP also may be used to achieve similar queen equivalent values. The primary purpose for proposing a queen equivalent definition is to reflect racial and reproductive differences, while incorporating the variation found in nature. Previously reported definitions of one queen equivalent used for synthetic formulations were: (1) 150 μg ODA, 55 μg 9-HDA, 13 μg HOB, and 1.5 μg HVA (Slessor et al., 1988), and (2) 250 μg ODA, 150 μg 9-HDA, 20 μg HOB, and 2 μg HVA (Slessor et al., 1990). These two queen equivalent values were formulated based on the most current knowledge of mean queen equivalent QMP component values at the time and fit well within the distributions reported here (Figure 3).

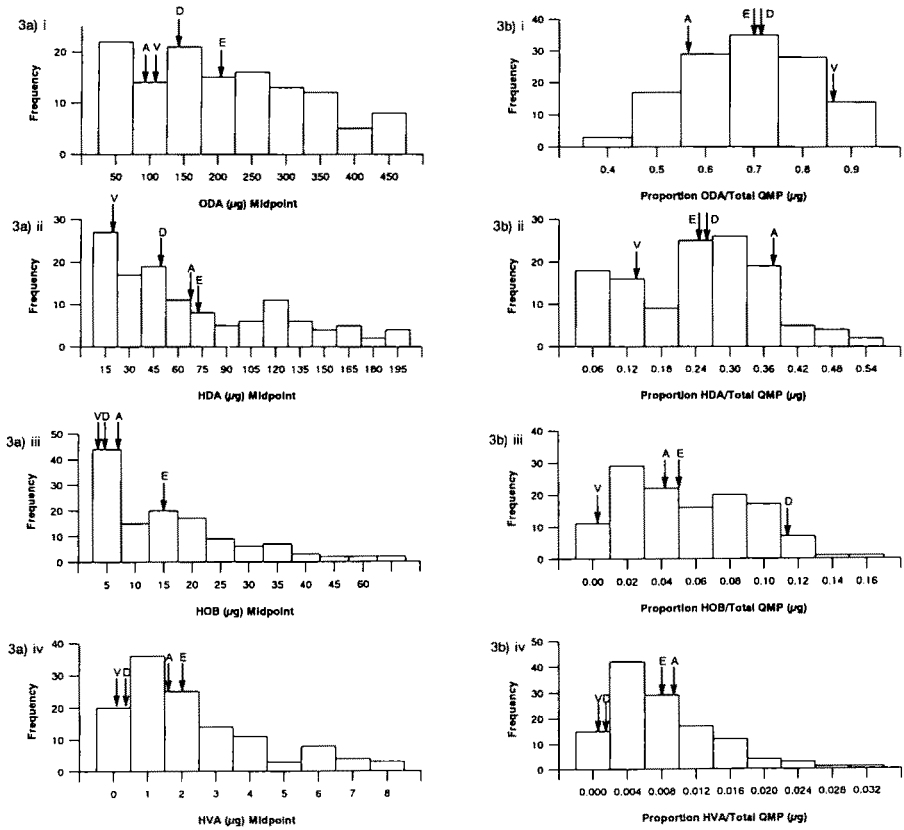

FIG. 3. Frequency distribution histograms of quantity and proportions of QMP components of 125 mated European queens: A = mean, Africanized mated queens; D = mean, European drone-laying queens; E = mean, European mated queens; V = mean, European virgin queens. Midpoint represents the median value of a range, e.g., in Figure 3a)i, a midpoint of 50 µg includes amounts of ODA greater than or equal to 0 µg and less than 75 µg ($0 \leq 50 < 75$).

The first queen equivalent definition was more reflective of mean ratios than the second (Table 1).

The comparisons between European and Africanized mated queens reveal some differences in the quantities and proportions of QMP components in these two honey bee races. However, this trait would not be reliable for classifying individual queens into a race category due to the high variability between individual queens (Figure 3). For all variables, Africanized means fall within high

frequency categories of European means, except perhaps the quantity of 9-HDA. Crewe (1982) reported differences in the proportion of mandibular gland components among *A. m. adansonii*, *A. m. mellifera*, and *A. m. capensis*, but discriminant analysis indicated that gland components were not dependable predictor variables for grouping inbred lines. Methodological differences between previously published African queen mandibular gland component quantities or percentages (Crewe, 1982; Crewe and Velthuis, 1980; Allsopp, 1988) and results reported in this study make comparisons unrealistic, but comparative studies would be of interest in the future.

Queens of either race may head colonies composed of workers of both races (Winston, 1992), and thus it appears that workers will accept racially different queen signals. Further, a European-like synthetic blend of QMP is equally effective at preventing queen rearing in colonies of either European or Africanized bees (Pettis et al., 1994). Nevertheless, the lower total quantity of QMP found in Africanized queens may contribute to the tendency for Africanized colonies to swarm with smaller parent colony sizes. The higher proportion of 9-HDA in Africanized queen glands (Figures 1 and 2) may reflect an adaptation to greater swarming activity in this race, because 9-HDA is an important component for swarm clustering behavior (Winston et al., 1982).

Unmated virgin queens not only produce significantly lower quantities of QMP components, but the proportions of components are significantly different from mated queens (Figures 1 and 2). The older (8 weeks) drone-laying queen glands contained ODA and 9-HDA proportions similar to the European mated queens, but the younger (6–14 days old) virgin queen proportions were significantly different from the mated queens. The older drone-laying queen ODA and 9-HDA proportions may reflect ontogenetic development and enzymatic capabilities of the gland. Conversely, the significantly lower proportions of the aromatic components represent a nonreproductive status (Figure 2) (Slessor et al., 1990). Virgin queens were producing a significantly different signal from the mated queens, suggesting an important biosynthetic change occurs with mating that cannot be explained by age alone, as seen by the intermediate signal produced in drone-laying queens. The essence of the different signals is a combination of quantity and proportion.

The wide variation in the quantity of QMP components suggests a generic signal indicating the individual is a queen, but there are important ontogenetic and reproductive differences in the signal as well. More will need to be known about individual QMP component biological and behavioral influences before any inferences can be made concerning the meaning of total or relative presence of any component in the blend.

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