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Heritabilities of honey-bee alarm pheromone production

ABSTRACT: **Honey-bee queens (*Apis mellifera* L.) were mated to single drones from either European (Baton Rouge, Louisiana, USA) or Africanized (Maturin, Monagas, Venezuela) honey-bee colonies. Stings and heads from worker-bee offspring of these matings were collected in methylene chloride and later analyzed by gas chromatograph for 12 sting-associated alarm pheromones. Heritability values of 0.48 to 1.98 were estimated for 10 of the pheromones. Two were not estimable due to negative variance components. Genetic correlations were significant and positive between all sting pheromones except octyl acetate, indicating common genetic regulation. Octyl acetate was genetically correlated with 2-heptanone produced by the mandibular glands.**

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AS PART of a major project to reduce the impact of the northward-spreading Africanized honey bee (*Apis mellifera* L.) on North American agriculture, estimates were made of heritabilities for a number of characters that differ between the Africanized honey bee and European honey bees currently used in the United States. These estimates were needed to assess the feasibility of a selection program to alter the Africanized phenotype, and for the design of the selection program itself. The Africanized bee is smaller[7], builds a smaller comb[12], has differences in its foraging behavior[11], swarms more frequently[17], is more defensive of its colony[5] and produces more of some of the sting alarm pheromone components (Collins et al., ms. in prep.). The differences in foraging, swarming, and colony defense make the Africanized bee uneconomical and undesirable for use by U.S. beekeepers.

Because the honey bee is a social, haplodiploid organism, there are a number of constraints on the method for estimation of heritability (h^2). Drones (males) develop from unfertilized eggs and are haploid; while diploid females (nonreproductive workers and reproductive queens) develop from fertilized eggs, depending on the treatment they receive as larvae. In addition, queens normally mate with 7–17 drones[1,16], utilizing the stored semen throughout their lives (up to five years). Since all the semen from a single drone is genetically identical, drones represent gametes from a queen[9], and a more usual diploid situation can be imposed if matings are viewed as between a virgin dam-queen and a sire-queen through her drones. Many of the economically important characters, like colony defense, are dependent on the social interaction between many individual workers. Therefore, under normal conditions the basic unit of selection is the colony, a collection of super-sib families having the same dam-queen but different sire-queens.

Rothenbuhler[14] proposed using highly inbred queens (homozygous at many loci) and mating them with one drone (identical sperm) to obtain workers that are genetically very uniform (coefficients of relationship greater than 0.75). Rinderer[10] proposed using this technique to produce an array of inbred dam-queens mated to several sire-queens and calculating heritabilities from the between sire-queen variance components.

Heritabilities for comb cell size, hoarding behavior (a laboratory measure of honey production), colony defense and response to an alarm pheromone, isopentyl acetate (IPA), in the laboratory are previously reported[6]. Because of the importance of alarm pheromones in initiating and mediating defensive behavior, and their differing levels of production in the two ecotypes (Collins et al., ms. in prep.), estimation of the h^2 of production also is important. This article reports on such estimates for 11 major alarm pheromones associated with the sting (butyl acetate, isopentyl acetate, 2-methyl butanol, hexyl acetate, 1-hexanol, 2-heptyl acetate, 2-heptanol, octyl

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acetate, 1-octanol, 2-nonyl acetate and 2-nonanol) and one from the mandibular gland of the head, 2-heptanone[2,3,15].

Materials and Methods

Based on the colony response in a standardized test of defensive behavior[4], six European sire-queens were chosen from colonies in Baton Rouge, Louisiana, U.S., and six Africanized sire-queens were chosen from colonies in Maturin, Monagas, Venezuela. The European colonies are representative of various commercial stocks available in the U.S., mixtures of several *A.m.* subspecies. The Africanized colonies are descendants of hybrids between *A.m. scutellata* from Africa and various European subspecies, all imported to Brazil. The two least defensive, two most defensive, and two intermediate colonies of each race were used as a source of drones. Drones (which can mate only once) from each of these 12 sources were used to singly inseminate nine virgin dam-queens per source, three queens each from three inbred lines obtained from USDA-ARS bee labs in Wisconsin and Louisiana. Each dam-queen headed a small colony consisting of 1 kg (ca. 9000) of her worker-daughters on 3–4 standard (20 × 43 cm) combs. These colonies also were used to estimate h^2 for measures of honey production and colony defense, and comb cell size as reported by Collins et al[5].

Foraging age workers were collected in plastic bags from the entrance of each colony and frozen. The stings and heads from three samples of 10 bees each per colony were collected over ice in 1 ml pesticide-grade methylene chloride (with sodium sulfate as a drying agent), sealed in a septum vial and then kept in a freezer until analysis. Samples were analyzed using a Perkin-Elmer Sigma 3 gas chromatograph fitted with two glass columns, (6′ × 1/4″ OD, 2 mm ID, packed with SP1000 on 80/100 mesh Chromasorb W, with 10 percent loading) run as dual column compensation beginning at 70°C for 3 minutes and rising 5°C/min to 120°C. Amounts of components were calculated by electronic integration on a Varian CDS 111.

Replicate observations on each colony were used to calculate a colony mean, the best estimate of the colony value. Data for 11 sting-associated alarm pheromones and one produced in the head (2-heptanone) were analyzed using a mixed model least squares procedure as described by Harvey[8]. The linear model assumed was:

$$Y_{ijkl} = \mu + T_i + S_{j(i)} + Q_k + TQ_{ik} + SQ_{jk(i)} + e_{l(ijk)}$$

where:

Y_{ijkl} = response of the l^{th} colony in the k^{th} queen line, j^{th} sire-queen and i^{th} bee type

μ = overall mean

T_i = fixed effect of the i^{th} bee type (Africanized or European) (i = 1,2)

$S_{j(i)}$ = random effect of the j^{th} sire-queen nested in the i^{th} bee type (j = $1, \ldots, s$)

Q_k = fixed effect of k^{th} queen line (k = $1, \ldots, q$)

TQ_{ik} = fixed interaction effect of the i^{th} bee type with the k^{th} queen line

$SQ_{jk(i)}$ = random interactions of the j^{th} sire-queen with the k^{th} queen line, nested in the i^{th} bee type

$e_{l(ijk)}$ = random residual effect of the l^{th} colony in the k^{th} queen line, j^{th} sire-queen and i^{th} bee type assumed NID $(0, \sigma^2)$.

Rinderer[10] showed that the sire-queen variance component estimated one quarter of the additive genetic variance. Heritability was estimated as:

$$\frac{4\sigma_S^2}{\sigma_S^2 + \sigma_{SQ}^2 + \sigma_e^2}$$

where

σ_S^2 = estimates of sire-queen variance component

σ_{SQ}^2 = estimate of the sire-queen by queen line in bee-type variance component

σ_e^2 = estimate of the colony in queen line, sire-queen and bee type variance component.

Genetic correlations were computed by dividing the covariance component for sire-queen for two traits by the geometric mean of the sire-queen variance components for the two traits.

Table I. Least-squares mean levels (± SD) (μg/worker bee) of 12 alarm pheromone components

Component	X ± SD
Butyl acetate	0.12 ± 0.22
Isopentyl acetate	1.26 ± 0.83
2-Methyl butanol	0.34 ± 0.15
Hexyl acetate	0.13 ± 0.15
1-Hexanol	0.01 ± 0.02
2-Heptyl acetate	0.10 ± 0.10
2-Heptanol	0.04 ± 0.03
Octyl acetate	0.06 ± 0.09
1-Octanol	0.48 ± 0.49
2-Nonyl acetate	0.11 ± 0.17
2-Nonanol	0.05 ± 0.08
2-Heptanone*	1.23 ± 0.70

* This compound is from the mandibular glands, all others are associated with the sting

Table II. Estimates of heritability and genetic and phenotypic correlations for production of 12 alarm pheromone components*

	Butyl acetate	Isopentyl acetate	2-Methyl butanol	Hexyl acetate	1-Hexanol	2-Heptyl acetate	2-Heptanol	Octyl acetate	1-Octanol	2-Nonyl acetate	2-Nonanol	2-Heptanone
Butyl acetate	1.94 ± 0.66	0.99 ± 0.23	...†	0.99 ± 0.02	0.63 ± 0.33	0.81 ± 0.25	0.90 ± 0.28	0.04 ± 0.52	...	1.10 ± 0.09	0.63 ± 0.27	−0.14 ± 0.63
Isopentyl acetate	0.47	0.57 ± 0.55	...	0.95 ±0.20	1.07 ± 0.42	1.12 ± 0.16	1.71 ± 0.68	0.46 ± 0.59	...	1.01 ± 0.21	1.19 ± 0.31	0.16 ± 0.79
2-Methyl butanol	0.03	0.35	...	...	...	...	...	...	...	...	...	...
Hexyl acetate	0.92	0.59	0.07	1.98 ± 0.66	0.70 ± 0.31	0.89 ± 0.89	1.03 ± 0.21	0.15 ± 0.50	...	1.11 ± 0.08	0.71 ± 0.23	−0.10 ± 0.62
1-Hexanol	0.42	0.35	0.24	0.47	0.89 ± 0.82	1.42 ± 0.43	1.20 ± 0.25	0.51 ± 0.56	...	1.47 ± 0.44	1.29 ± 0.28	...
2-Heptyl acetate	0.48	0.71	0.32	0.63	0.43	0.67 ± 0.57	1.54 ± 0.51	−0.07 ± 0.66	...	1.17 ± 0.19	1.26 ± 0.26	−0.37 ± 0.81
2-Heptanol	0.40	0.53	0.31	0.57	0.59	0.65	0.54 ± 0.54	0.53 ± 0.55	...	1.23 ± 0.24	1.09 ± 0.21	−0.35 ± 0.75
Octyl acetate	0.14	0.26	0.03	0.22	0.24	0.19	0.35	0.48 ± 0.52	...	−0.09 ± 0.59	−0.27 ± 0.54	2.09 ± 2.04
1-Octanol	0.27	0.34	0.21	0.36	0.50	0.40	0.59	0.57	...	...	...	...
2-Nonyl acetate	0.71	0.56	0.12	0.77	0.61	0.57	0.61	0.31	0.52	0.96 ± 0.62	1.02 ± 0.09	−0.46 ± 0.67
2-Nonanol	0.50	0.33	0.24	0.50	0.62	0.42	0.57	0.21	0.50	0.71	1.07 ± 0.64	−0.92 ± 0.59
2-Heptanone‡	0.02	0.11	0.16	0.01	0.23	−0.01	0.23	0.57	0.45	0.21	0.27	0.59 ± 0.54

* Genetic correlations are above the diagonal, heritabilities are on the diagonal, and phenotypic correlations are below the diagonal
† Value not estimable due to negative variance components
‡ This compound is from the mandibular glands, all others are associated with the sting

Results and Discussion

Least-squares means and standard errors for the 11 alarm pheromones associated with the sting and 2-heptanone from the mandibular glands are presented in Table I. They lie within the ranges found in the two populations (Collins et al., ms. in prep.).

Table II lists the heritabilities, and genetic and phenotypic correlations for production levels. The h^2 values for butyl acetate, hexyl acetate, 2-nonyl acetate and 2-nonanol are very high (close to 1). Values for isopentyl acetate, 1-hexanol, 2-heptyl acetate, 2-heptanol, octyl acetate and 2-heptanone are intermediate (0.48–0.89); however, the standard errors associated with these h^2s are of equal magnitude to the h^2s, so there actually may be a greater proportion of variation due to the environment than the h^2 values indicate. These standard errors are greater than those associated with response to isopentyl acetate, comb-cell size, and measures of defensive behavior using the same matrix of colonies[6].

The h^2 values for 2-methyl butanol and 1-octanol were not estimable due to negative variance components, probably the result of greater environmental variability for these two components.

Keeping in mind the high standard errors associated with the h^2 estimates, the most likely candidates for inclusion in a selection program for defensive behavior would be butyl acetate, hexyl acetate, and the 2-nonyl moieties. However, further study of the function of the alarm pheromones in regulating colony defense, especially related to the differences between European and Africanized ecotypes, is necessary to evaluate the inclusion of such characters in a selection program.

The phenotypic and genetic correlations are also presented in Table II. All of the sting components, except octyl acetate, are highly genetically correlated, indicating common genetic regulation. Nothing is known about the specific biochemical pathways utilized for the production of these alarm pheromones, nor why such a plethora of compounds are produced when fewer might be equally effective and more energy efficient. The genetic correlations found here would suggest the existence of some commonality of pathway involving the same enzymes or precursors. Perhaps the less abundant components, including 1-hexanol, heptyl alcohol and 2-nonyl acetate (Blum et al., unpub. data) are simply breakdown products occurring after secretion of the pheromone onto the sting shaft.

Octyl acetate, the second most abundant pheromone[2], is not significantly genetically correlated with the other sting components. It may be synthesized separately from the others. A possible interpretation of this situation is that isopentyl acetate (having the highest level of production), and octyl acetate are the two primary biologically active sting alarm pheromones. However, no synergy has been found between these two, (Collins, unpub. data), a phenomenon that commonly occurs in pheromone complexes[13].

Finally, 2-heptanone, the alarm pheromone identified from the mandibular gland, was negatively correlated with the sting components, with the possible exception of octyl acetate, although the standard errors here are also high. This suggests that the two biochemical pathways may compete for precursors, but probably not enzymes as the synthesis occurs in two separate glandular areas.

Given the magnitude of the heritability estimates made for 10 of the alarm pheromone components, a selection program to reduce their level of production should be successful. The desirability of such a selection program would depend on the importance of the pheromone in affecting the intensity of colony defense.

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