Laying-worker Production of Drones in Mixed Colonies of Africanized and European Honey Bees (Hymenoptera: Apidae)

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ABSTRACT Queenless honey-bee colonies (*Apis mellifera* L.) with mixed populations of Africanized and European workers produced 15-fold more Africanized drones than European drones. Drones developed from eggs laid by previously nonreproductive workers. First eclosion of Africanized drones preceded that of European drones by 2.4 days. Laying workers of both bee types developed more rapidly when colonies had Africanized queens and brood before queenlessness than when colonies had European queens and brood. The percentage of European drones of the total number of drones that eclosed each day changed significantly in a curvilinear manner over 15 days, starting near 0, increasing to a high near 8, and then decreasing to near 0.

KEY WORDS *Apis mellifera*, laying worker, arrhenotoky, drone, Africanized, queenless

QUEENLESS COLONIES of bees that are unsuccessful in gyne production often produce male offspring by arrhenotoky. Usually these colonies are destined to die and the males are their only chance for reproduction. This occurs in the honey bee, *Apis* spp., and also has been reported in some halictines, *Bombus*, and occasionally in *Trigona* (Michener 1974).

Speed of laying-worker development varies greatly among subspecies of the western honey bee. Workers of the African subspecies, *Apis mellifera capensis* (Escholtz), *A. m. intermissa* (von Buttel-Recpen), and *A. m. scutellata* (Lepeletier) [formerly *adansonii* (Latreille)], develop ovaries and oviposit more quickly than workers of the European subspecies, *A. m. ligustica* (Spinola), *A. m. carnica* (Pollmann), and *A. m. mellifera* L. (Ruttner & Hesse 1981). A mixture of pheromones produced by the mandibular glands of the queen inhibits development of worker ovaries (Butler & Fairey 1963). A principal component has been identified as *trans*-9-oxo-2-decenoic acid (9-ODA) (Barbier & Lederer 1960, Butler et al. 1961). Crewe (1982) found that in *A. m. capensis*, where laying workers develop rapidly, the quantity of 9-ODA compared with the other components of the mandibular gland secretions is high. In *A. m. scutellata*, where laying workers develop less rapidly, the proportion of 9-ODA is lower; and in *A. m. mellifera*, where laying workers develop slowly, the proportion of 9-ODA is lowest. Crewe hypothesized that different blends of 9-ODA with other components of the queen's mandibular gland are correlated "with the ease with which laying workers develop," and that these component blends are relatively subspecies specific.

The Africanized honey bee stems from mated queens of the African subspecies, *A. m. scutellata*, that were brought to Brazil and subsequently went feral with swarms (Michener 1975). The purpose of this study was to compare Africanized and European laying-worker production of drones. Information regarding various components of reproductive biology of the Africanized honey bee is fundamental in explaining the past colonization success. Also, reproductive traits may suggest techniques useful in the regulation of the Africanized bee.

Materials and Methods

The experiment was conducted near Sarare, Venezuela. Twelve colonies with mixed populations of Africanized and European honey bees were made by putting equal weights (380 g) of each bee type into each hive (25-liter, five-frame nucleus). European honey bees, predominately *A. m. ligustica*, were selected from "golden" stock that produced yellow workers and yellow drones. Africanized honey bees were selected from colonies that produced black workers and black drones that were easily distinguished from the European stock. Half the colonies were given laying Africanized queens and a comb (44 by 23 cm) 70% filled with mixed-aged (<9 days old) Africanized brood; and half of the colonies were given laying European queens and a comb filled with 70% mixed-aged European brood. Combs with young brood were selected to avoid eclosion of bees while they were in the colonies. Queens were put under hardware cloth push-in cages. In addition to the comb of brood, each

Table 1. Mean (±SE) number of Africanized (A) and European (E) drones per colony, numbers of colonies sampled (n), and t test probabilities for each day (date of initial eclosion of drones was adjusted to day 1 for each colony)

Day	A	E	n	$P > t$
1	4.64 ± 1.67	0.18 ± 0.21	11	0.025
2	17.50 ± 3.24	0.33 ± 0.26	12	0.001
3	36.08 ± 5.70	1.58 ± 1.05	12	0.001
4	44.58 ± 5.80	2.42 ± 0.75	12	0.001
5	40.42 ± 4.48	3.50 ± 0.91	12	0.001
6	41.33 ± 5.73	4.33 ± 1.06	12	0.001
7	30.17 ± 3.06	3.08 ± 0.77	12	0.001
8	32.54 ± 5.50	2.55 ± 0.68	11	0.001
9	33.90 ± 4.76	2.55 ± 0.82	11	0.001
10	32.40 ± 4.54	1.70 ± 0.52	10	0.001
11	27.60 ± 4.04	0.90 ± 0.35	10	0.001
12	20.78 ± 2.15	1.56 ± 0.71	9	0.001
13	16.60 ± 4.21	0.60 ± 0.40	5	0.005
14	16.60 ± 2.68	0.40 ± 0.40	5	0.001
15	9.25 ± 0.95	0.00	4	0.001

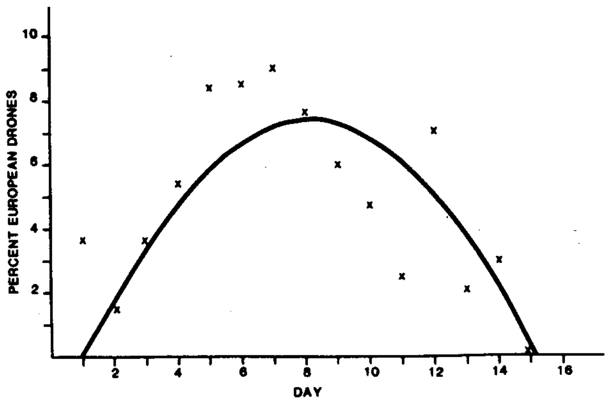

Fig. 1. Percentage of drones eclosing daily that were European. Day represents the number of days following first drone eclosion for each colony. A regression curve ($y = -0.0253 + 0.0243x - 0.0015x^2$, where y = percent European drone and x = day) was fitted to the points.

colony was given a comb of honey and an empty comb.

Queens and brood were removed from the colonies after 7 days. Newly eclosed drones were removed from the colonies each day and identified as Africanized or European based on body color. Three days after queens and brood were removed 60 workers were sampled from each colony and identified as Africanized or European. When the experiment was terminated, 36 days later, all the bees were frozen and 500 workers were sampled from each colony and identified.

Student's t test (modified for unequal variances [Snedecor & Cochran 1980]) was used to analyze daily drone-eclosion data. Analysis of variance was used to analyze first-eclosion data; drone type and queen type were used as blocking factors. Analysis of variance with subclass regression was conducted on drone percentages over eclosion days; queen type and colonies nested within queen type were used as blocking factors (SAS Institute 1981).

Results

Fifteen-fold more Africanized drones (4,293) than European drones (286) were produced by laying workers in the mixed-population queenless colonies. Drone-type differences were highly significant each of the 15 days that drones eclosed (Table 1). When the colonies were sampled they had similar numbers of Africanized and European workers at the beginning of the experiment (percent Africanized workers, 50.7 ± 2.0), but there were significantly more Africanized workers than European workers at the end of the experiment (percent Africanized workers, 55.9 ± 1.5) ($P < 0.005$). Proportions of Africanized bees in the mixed colonies were significantly higher at the end of the experiment than they were at the beginning ($P < 0.05$).

First eclosion of drones occurred earlier in colonies that had had Africanized queens and brood ($\bar{x} = 30.4 \pm 1.1$) than in colonies that had had European queens and brood ($\bar{x} = 33.8 \pm 1.1$) ($P < 0.006$). First eclosion of Africanized drones preceded that of European drones by 2.4 days ($\bar{x}_A = 30.9 \pm 1.1$, $\bar{x}_E = 33.3 \pm 1.1$) ($P < 0.04$). This pattern was evident for both Africanized ($\bar{x}_A = 29.0 \pm 1.1$, $\bar{x}_E = 31.8 \pm 1.1$) and European ($\bar{x}_A = 32.8 \pm 1.1$, $\bar{x}_E = 34.8 \pm 1.1$) queen types.

Disparity in drone production was not constant through the experiment, but changed significantly in a curvilinear manner (Fig. 1). The percentage of European drones of the total number of both types that eclosed was near 0 the first eclosion day. The percentage increased to 7.9 on day 8 and decreased to near 0 again on day 15 (linear, $P < 0.0001$; quadratic, $P < 0.0002$).

Discussion

Greater production of Africanized drones in this experiment suggests that Africanized laying workers have a reproductive advantage over European laying workers when they are in the same colony. Possiblities for this advantage are 1) Africanized laying workers develop ovaries and oviposit more quickly than European laying workers; 2) Africanized laying workers inhibit ovary development of their nestmates; 3) Africanized workers establish dominance over European nestmates; and 4) Africanized workers live longer than European workers.

After queens were removed from African (*A. m. scutellata*) and European (*A. m. ligustica*) colonies, first oviposition of African workers preceded that of European workers by 17.8 days (Ruttner & Hesse 1981). If the Africanized bee is similar to its ancestor, then the latency difference found by Ruttner & Hesse should be similar to the first-eclosion difference found during the present experiment. (This assumes developmental times of the two drone types are not appreciably different.) However, first emergence of European drones was only 2.4 days later than that of the Africanized drones. Even though the difference is smaller than that found by Ruttner & Hesse, it still represents a reproductive advantage for Africanized workers.

Discrepancy between these results may be due to different testing conditions. Before queens were removed from the Ruttner & Hesse experiment they were free laying; so inhibition of worker ovary development by queen pheromones was normal. Our queens were confined to excluder cages. Such confinement probably interfered with the transfer of pheromones and, thus, reduced the inhibitory effects of the queens. Workers of the two subspecies could have been affected differently. Genetic differences between the Africanized bee and its *A. m. scutellata* ancestor also could account for the discrepancy; that is, the Africanized bee may be partly Europeanized.

Velthuis (1970) noted that workers with more developed ovaries inhibit ovary development of nestmates in queenless colonies of European bees (predominately *A. m. mellifera*). If ovary development of Africanized workers is faster than that of European workers, then Africanized workers with faster developing ovaries could inhibit ovary development of European workers. Such a pattern would lead to a reproductive advantage for Africanized laying workers because more Africanized workers than European workers would be reproductive. Additionally, European workers would help rear unrelated Africanized drones.

Hierarchies in worker dominance have been reported for honey bees and are particularly well developed in *A. m. capensis* (Moritz & Hillesheim 1985). Dominant bees are fed by subordinate bees; in the absence of a queen, the dominant bees become laying workers and the subordinate workers remain infertile (Korst & Velthuis 1982). If Africanized workers have a dominance hierarchy that is better developed than that of the European workers, then Africanized workers might be able to dominate European workers when they are in mixed colonies. Presently, we do not know if this dominance hierarchy is better developed for Africanized bees than for European bees. However, most characteristics of *A. m. scutellata* workers related to ovary development and egg production are more similar to *A. m. capensis* than to *A. m. ligustica* (Ruttner & Hesse 1981).

Africanized workers appear to live longer than European workers when the two bees are in the same colony. Similar results were found by Winston & Katz (1981), presumably because European workers begin foraging earlier in mixed colonies (Winston & Katz 1982). Shorter longevity of the European workers probably did not greatly influence production of European drones in this experiment. Most of the eggs were laid early in the experiment when the numbers of Africanized and European workers were similar. However, decreased percentages of European drones that emerged toward the end of the experiment (Fig. 1) may be related to the shorter longevity of European workers.

Laying workers of both bee types developed more rapidly when colonies had Africanized queens and brood than when colonies had European queens and brood before queenlessness. Crewe (1982) hypothesized that the speed of laying-worker development is positively correlated with the proportion of 9-ODA produced by the queen's mandibular glands. Thus, workers develop ovaries more quickly when their former queen produced high, rather than low, proportions of 9-ODA. The proportion of 9-ODA produced by the Africanized queen is unknown. If one assumes that this proportion is higher for Africanized queens than European queens, then the apparent queen effects found in this experiment would be expected. This is likely since African queens have higher proportions of 9-ODA than European queens (Crewe 1982).

Is the reproductive advantage displayed by Africanized laying workers only a biological peculiarity, or are there practical implications? Surveys of apiaries that have both Africanized colonies and European colonies indicate that many of the European colonies have at least a few Africanized bees that have drifted into them (unpublished data). Additionally, European virgin queens that take mating flights in Africanized areas probably will mate with Africanized drones; their worker progeny will be hybrids. In both of these scenarios, if the colonies become queenless, it is possible that Africanized drones will be produced at the expense of European drones. Drones are thought to play a significant role in Africanization (Rinderer et al. 1985, Taylor 1985). Perhaps drones from Africanized laying workers contribute to the success of the Africanized bee.

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