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Genetics of honey-bee colony defense

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INTRODUCTION

The title of a 1964 *Bee World* article 'The spread of a fierce African bee in Brazil' (by Nogueira-Neto) pinpointed the major problem associated with the spread of hybrids of *Apis mellifera scutellata* and European subspecies in South America — their aggressiveness. Following the initial introduction of *A. m. scutellata* in 1956 (Kerr 1967), there were increasingly more reports in the media of attacks by bees, and deaths of livestock and people. These stories directed public attention to an undesirable behavior of this honey-bee type that was already known to scientists and beekeepers. The scientists who imported the original queens to Brazil had taken precautions to prevent the free movement of the African bees until studies could be undertaken to produce selected stocks that would not express this undesirable behavior (Kerr 1967).

A genetic approach to dealing with the problem of the extreme defensiveness of the Africanized bees has pervaded the scientific work and literature since before the introduction. Nogueira-Neto (1964) reported on initial plans to do selection experiments, and Kerr (1968) again proposed a genetic solution to the then-existing problem. The Committee on the African Honey Bee made several references to the production of stocks that hybridized with the Africanized type and to the selection of more manageable phenotypes within the Africanized population (Michener 1972). The genetic approach was one of the major recommendations of this committee.

COLONY DEFENSE

The term 'defensive behavior', or 'colony defense', is more correctly used than 'aggressiveness'. The term aggressiveness implies active initiation of the behavior by the bees, and is therefore not correct. The response of a colony must be triggered by a stimulus in the environment. It is not initiated by the build-up of action-specific energy (Lorenz 1957), nor does it involve an active search pattern to find something to attack, as in hunting.

Any work with colony defense in honey bees must consider the complexity of the behavior. This is not a simple, predictable response elicited in complete form by a single stimulus in the environment, but a sequence of several possible and variable responses by individual worker bees that is coordinated into a group response by many colony members. The type and level of stimulation required for expression of the behavior may vary from time to time and from colony to colony, depending on genetic makeup, previous experience, and current status.

Collins *et al.* (1980) have proposed a generalized model of the behavior which divides it into four major steps: alertation, search, orientation, and attack. Within each of the steps, there may be several possible responses, and the intensity of the response may also vary.

The bee's first response to a disturbing stimulus is to become alerted. This may involve a distinctive posture with raised abdomen, extended sting, and waving antennae as described by Maschwitz (1966). The extension of the sting releases alarm pheromone which communicates the alert to other members of the hive. The coordination of the individual responses into a group response may be enhanced by the alerted bee actively recruiting her hivemates. She does this by running into the hive with the sting still extended and spreading the alarm odor into areas where there are many more possible defenders. A third possible response by a disturbed bee is to simply flee from the area of danger.

In the presence of continued stimulation (the same stimulus or a different one), an alerted bee may move into the second step of the sequence and begin more rapid random movements within her immediate area. This 'searching' behavior may continue until she encounters a stimulus to which she can orient (step three), or until her motivation for defense decreases and she stops defending and goes on to another activity. Depending on her level of excitation, the searching can also carry her out of the colony and for some distance into the surrounding area.

Once an appropriate stimulus for orientation, usually visual, has been encountered, the worker can direct her movement towards the source of stimulation and move on to the final step of actual attack. Attack can also take several forms. The most obvious is stinging, but because this results in the death of the defender, it may not be the most efficient. In many instances, the threat of a flying, buzzing bee, or group of bees, may be sufficient to deter an intruder. The bee may also bite, burrow into fur, ears, or nose, or pull hair. At this stage also the release of alarm pheromone by the attacking bees, or from an implanted sting, and the presence of many orienting defenders will provide coordination for a group attack.

EXPERIMENTAL MEASUREMENT

Work by Free (1961), Maschwitz (1966), and others focused on the stimuli which provoked defensive behavior in European honey bees. The presence of a sting and associated tissue, including alarm pheromone, caused bees to become alerted and exit from the hive ready to attack (Maschwitz 1966). Dark moving objects attracted attack, and stinging was enhanced by odors and the texture of the surface (Free 1961). The relative effectiveness of the different stimuli was assessed by the number of stings in a target object, a small ball of cotton and muslin, or filter paper.

The first attempts to quantify aspects of the whole sequence of colony defense were made by Stort (1974) with Africanized and Italian honey bees. He also used a small moving ball, this time of black leather, to stimulate the bees' defense and to later count the number of stings. He measured the time to become aroused, time to the first sting in the leather ball, the number of stings in the gloves (soft leather) of the observer, time to calm, and distance followed.

Later comparative studies of Africanized and European honey-bee types by Collins *et al.* (1982) used a similar procedure to arouse the bees to defend (Collins & Kubasek 1982). They attempted to elicit the four steps of colony defense outlined in the model of Collins *et al.* (1980). The test began with the release of an alarm pheromone, isopentyl acetate (Boch *et al.* 1962). Further stimulation, vibration of the colony, was provided by an 18 g projectile hitting the hive. In the final portion of the test, two dark leather targets (4 cm×4 cm) were mechanically waved in front of the colony for the bees to attack. The characters measured were: the time to respond to the alarm pheromone, the time to respond to the targets, the number of stings in the targets, and the number of bees responding at three times during the test.

Both Stort (1974) and Collins *et al.* (1982) (Fig. 1) confirmed that the two populations of honey bees (Africanized and European) exhibited distinct differences in their level of colony defense, and that the Africanized type was significantly more defensive. The only comparisons that could be made between the two studies were for the number of stings in the leather targets. The amount of stinging seen in the Brazilian population studied by Stort was 0.32 or 0.26 stings per cm^2, less than that in the Venezuelan population tested by Collins *et al.* — 0.85 stings per cm^2. This difference could be the result of genetic differences in the two groups, the additional interbreeding of the spreading population with feral European bees in northern South America, or greater responsiveness by the bees to the stimuli in the Collins–Kubasek test.

Collins & Rothenbuhler (1978) studied one aspect of defense, communication of alarm by pheromones in isolated groups of young bees in laboratory cages. The European bees used differed in their level of colony defense in the field, and also showed different responses to isopentyl acetate (IPA). The more defensive bees responded faster, in larger numbers, and with more vigor than the less defensive bees.

Collins *et al.* (1987b) repeated this assay test with Africanized and European bees in Venezuela and found that the Africanized bees did not respond more quickly, but they did so in greater numbers and much more intensely. In addition, they continued to respond for a longer time.

MENDELIAN ANALYSES

In addition to a comparison of Africanized and European (Italian) bees, Stort evaluated the behavior of several F_1 colonies and backcrosses to both parental types (1975a,b,c, 1976, 1980). Both these studies and later ones by Collins (unpublished) used an approach proposed by Rothenbuhler (1960) in which inbred queens were instrumentally inseminated with a single drone to produce colonies of workers of similar genotype. Stort proposed a number of hypotheses for the inheritance of the various aspects of colony defense measured by his test. Fig. 2 depicts the specific genes and their relationships proposed for the character 'number of stings in the

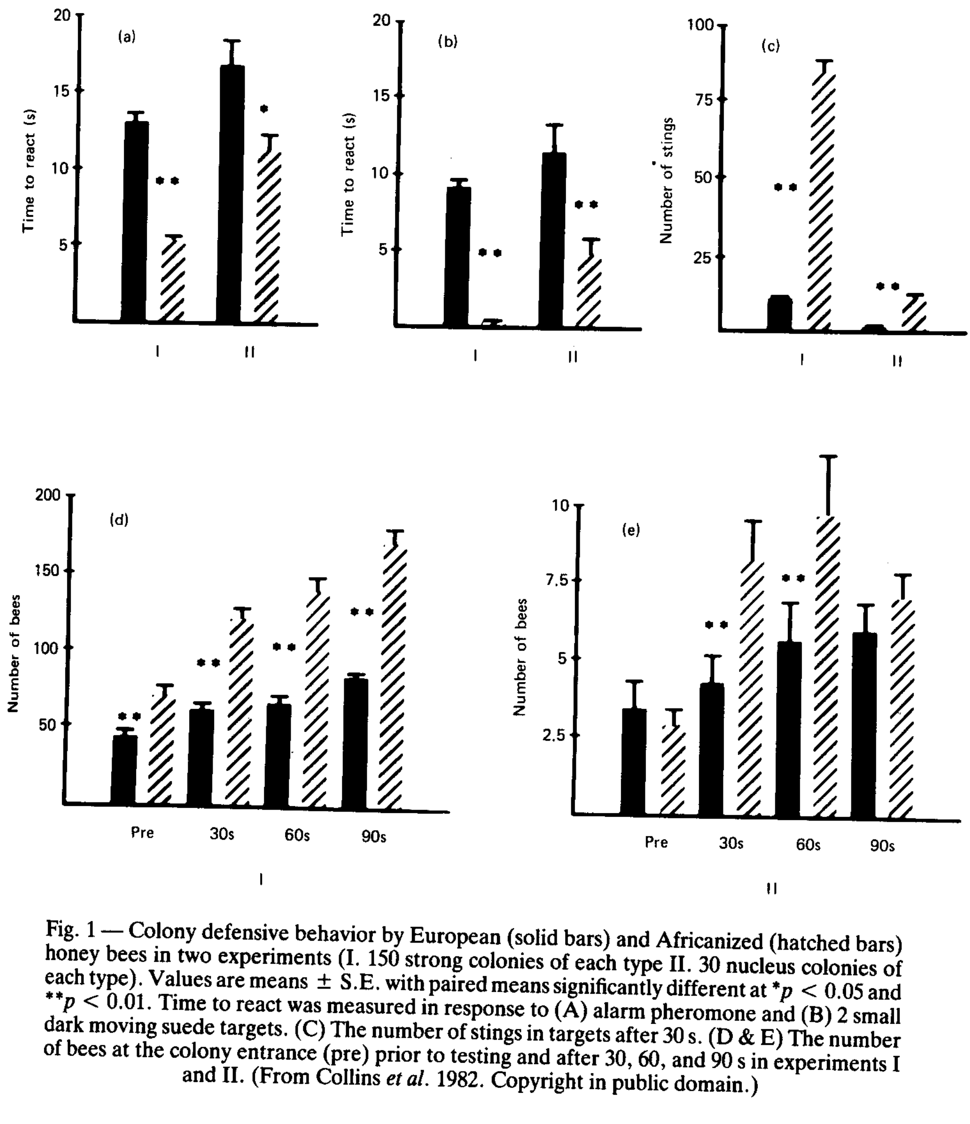

Fig. 1 — Colony defensive behavior by European (solid bars) and Africanized (hatched bars) honey bees in two experiments (I. 150 strong colonies of each type II. 30 nucleus colonies of each type). Values are means ± S.E. with paired means significantly different at $^*p < 0.05$ and $^{**}p < 0.01$. Time to react was measured in response to (A) alarm pheromone and (B) 2 small dark moving suede targets. (C) The number of stings in targets after 30 s. (D & E) The number of bees at the colony entrance (pre) prior to testing and after 30, 60, and 90 s in experiments I and II. (From Collins *et al.* 1982. Copyright in public domain.)

gloves of the observer'. In this instance, a two-gene model was proposed, with gentleness dominant. A second two-gene model was proposed for the number of stings in the leather ball, with defensiveness dominant. Of the other characters measured, time to become aggressive and time to first sting showed dominance over the Africanized, defensive phenotype and observer persecution behavior showed dominance over the gentle, Italian phenotype. No specific genes were proposed for these traits.

The Collins–Kubasek defense assay was also used to test colonies of each type and ones headed by European queens mated in areas of Africanization (F_1 hybrids) (Collins, Rindered & Tucker, unpublished). In this instance, the hybrids were largely intermediate to the two parental types for the characters measured, indicat-

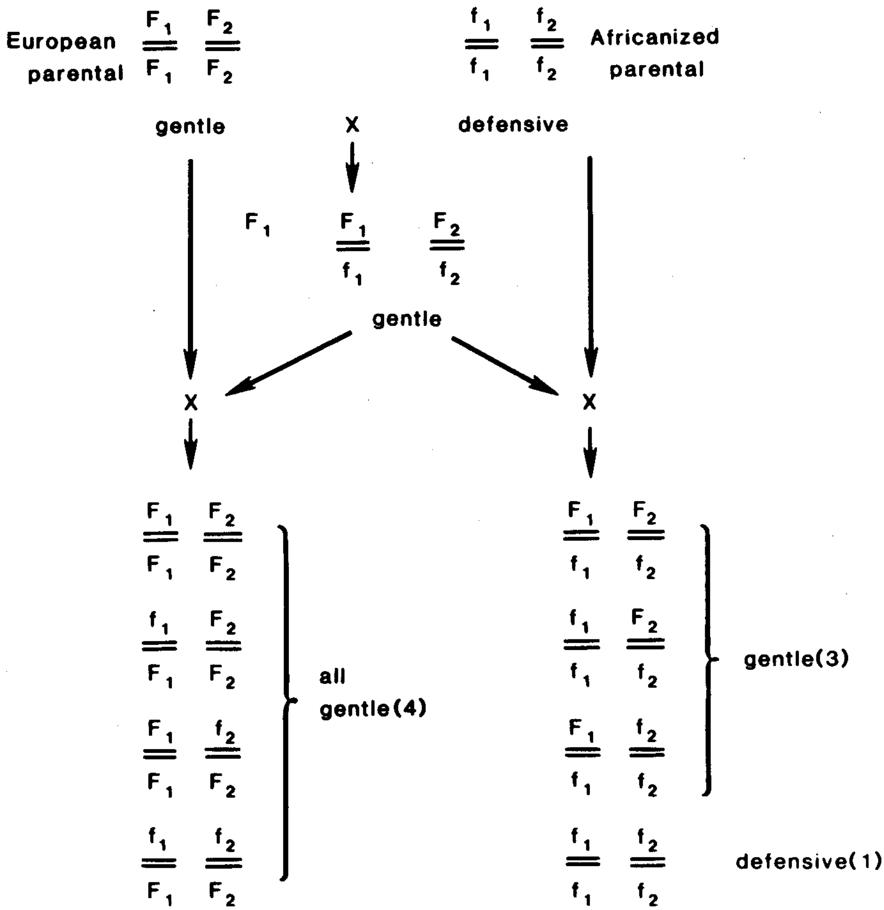

Fig. 2 — Hypothesis for the inheritance of the character 'number of stings in the gloves of the observer' (after Stort 1975b).

ing an additive mode of inheritance, rather than one involving dominance. However, the subspecies of the European parental type was important; crosses with *A. m. caucasica* were more defensive than crosses with *A. m. ligustica*.

A number of single-drone insemination crosses between inbred Africanized and European bees also showed variable results depending on the specific crosses made, but the majority of the characters were intermediate to the two parental phenotypes. None of these results were in conflict with the conclusions of Stort. However, it was clear that a more useful approach to the genetic study of colony defense was a quantitative, rather than Mendelian, analysis.

The earlier work by Collins & Rothenbuhler (1978) on response to alarm pheromone had also been extended to an evaluation of the F_1 and backcrosses of the two inbred European lines (Collins 1979). For the three aspects of response measured, a more responsive phenotype was dominant. Two or three loci were estimated to control each of the three traits.

Comparisons between Africanized and European bees were also made using the same test procedure (Collins unpublished). The speed of the reponse was not significantly different for the two bee types, but the Africanized bees were more vigorous in their response and remained alerted for a longer time.

HERITABILITY AND CORRELATION

One of the recommendations of the Committee on the African Honey Bee (Michener 1972) was for the selection of specific stocks for barrier areas and to improve bees in areas of Africanization. Before a selection program was attempted, it was useful to predict the probable success of such a venture. For a trait to be modified, there must be phenotypic variation present in the population selected. The variation that is present must be due at least in part to underlying differences in genotype. The genetic parameter that indicates the proportion of the total variation that is genetic in nature and amenable to selection is called *heritability*, and can be estimated in a number of ways.

The estimation of heritability in honey bees has a number of limitations, as discussed in Rinderer (1977). For a colonial behavior such as colony defense, the colonies measured for the estimation need to be headed by inbred queens inseminated by a single drone. This ensures that the workers in the colony will be almost identical genetically. The estimates of h^2 by Collins *et al.* (1984, 1987a) used an array of such colonies, with inbred European queens, and an analysis of variance of each trait. The h^2 was estimated from the drone parent (sire queen) variance component only. The values determined for colony defense, and response to pheromone, are presented in Table 1. Although the values were variable from trait to trait, they were

Table 1 — Heritability (h^2) estimates for Africanized honey bee characters associated with colony defensive behavior (from Collins *et al.* 1984)

	$h^2 \pm$ std. dev.
Response to alarm pheromone†	
initial activity level	0.12 ± 0.01
time to react	0.31 ± 0.01
Colony defense‡	
time to respond to target	0.59 ± 0.31
number of stings	0.57 ± 0.24
number of bees responding at:	
30 s	0.71 ± 0.01
90 s	0.17 ± 0.01

†Test of small groups of bees in a cage (Collins & Rothenbuhler 1978).
‡Test of entire colonies (Collins & Kubasek 1982).

of sufficient magnitude to predict probable success in a selection program.

In addition to the heritability of a trait, the correlation of that trait with related ones is important so that selection for more gentle bees does not adversely affect other characters such as honey production. Or, as is the case with defensive behavior, a closely correlated, but more easily measured, character could be used for the actual selection. A number of studies determined the correlation of various measures of colony defense with other morphological, physiological, and behavioral traits. Significant correlations were found for the amount of 2-heptanone produced and defensive behavior, but not the amount of IPA (Kerr *et al.* 1974, Boch & Rothenbuhler 1974) except for the number of bees responding after 90 s (Collins *et al.* 1987a). Two other alarm pheromones were also correlated with this trait, 2-heptyl acetate and 2-nonyl acetate. Fewer *sensilla placodea* were found in the more aggressive Africanized bees (Stort & Barelli 1981), but the size of the sting (Stort & Chaud-Netto 1978) and abdominal color (Stort 1978) were not related to colony defense. No correlations were found with hygienic behavior (Rothenbuhler 1964) or with response to *Nosema apis* (Rinderer *et al.* 1983), although it had been thought that disease resistance and defensive behavior were related expressions of general vigor.

SELECTION PROGRAM

On the basis of estimates of h^2, Collins *et al.* (unpublished) carried out a genetic selection program beginning with only Africanized bees (caught swarms in an Africanized area). Colony defense was measured by the procedure of Collins & Kubasek (1982). After two generations of selection, there were significant differences between the two lines (more defensive and less defensive bees) for speed of response to the target, number of bees responding, and the number of stings. However, the magnitude of the change in behavior was much less in the less defensive direction than in the more defensive direction. For example, the realized h^2 for total stings was 0.87 in the more defensive line, and 0.10 in the less defensive line. This difference is probably due to the low frequency of alleles for the less defensive phenotype in the Africanized population, and could be overcome in a selection program by the inclusion of European honey bees in the selected population.

The conclusion reached by the slow success of selecting for less defensive colonies encourages our efforts to prevent Africanization of commercial honey bee stocks rather than to select for a more manageable type after Africanization. Certainly, genetic selection programs will need to be used in conjunction with management for reproductive isolation to maintain desirable phenotypes in our honey-bee stocks.

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