Journal of Apicultural Research 31(3/4): 119–123 (1992)

Nest usurpation, supersedure and colony failure contribute to Africanization of commercially managed European honey bees in Venezuela[1]

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(Received 4 September 1992, accepted subject to revision 2 December 1992, accepted for publication 17 March 1993)

Keywords: Africanized honey bees, European honey bees, *Apis mellifera*, honey bee colonies, usurpation, supersedure, colony failure, genetic change, mitochondrial DNA, morphology, neotropical region, Venezuela

SUMMARY

The fates of 58 colonies in 4 apiaries managed commercially for honey production in northwest Venezuela were determined during 15 months. All colonies had been previously requeened with marked European queens. Heritage of new queens (European daughter replacements versus Africanized usurpers) was traced by mitochondrial DNA and morphological characteristics of worker progeny. Three usurpations (5% of colony fates, at a rate of 4.7% of colonies per year) occurred. These colonies showed congruent African-derived mitochondrial DNA and Africanized morphology. Supersedures accounted for much of the introgression of African genes into the test apiaries. Twenty-eight colonies (48%) superseded their queens once, three (5%) had two supersedures and one (2%) had three supersedures.

All original and supersedure queens had European mtDNA. The worker progeny of original queens had European morphology, but the progeny of supersedure queens showed mixed morphology, with mostly intermediate and European morphology in first generation workers and intermediate and Africanized morphology in later generations. Sixteen (28%) of the European queens died without being successfully replaced by their colonies, and only seven (12%) survived the 15-month test. Hives left unoccupied after colony failure would have been available for colonization by Africanized swarms. Usurpations, supersedures and colony failures thus accounted for an annual Africanization rate (i.e. a decrease in the percentage of colonies with both European mitochondrial DNA and European morphology) of 53%.

[1]All editorial functions for this paper, including the selection of referees, have been undertaken by staff at IBRA headquarters

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INTRODUCTION

The process of Africanization of European honey bee colonies has numerous components, each contributing to increased difficulty for profitable and pleasurable beekeeping in an Africanized zone (Taylor, 1985; Rinderer & Hellmich, 1991). The most dramatic means of genetic change in an apiary is nest usurpation (also known as queen invasion or colony takeover), a form of permanent, facultative, social reproductive parasitism (Danka & Rinderer, 1988). A small cluster of workers with a queen (presumably Africanized) kills the resident queen of a managed European colony. As soon as remnant European workers die the usurped colony is fully Africanized; a hybrid transition (as occurs when a supersedure queen mates to Africanized drones) is bypassed. This process of introgression of African genes into European populations was first recognized as a potential problem for beekeepers through anecdotal reports (Michener, 1972). Its importance to apiculture lies in beekeepers unwittingly maintaining Africanized colonies, perhaps propagating queens from those colonies, and perhaps moving the colonies or propagated queens to non-Africanized zones. In addition, colonies may contribute further to local Africanization through swarming and drone production.

There is uncertainty over the magnitude of the role of nest usurpation in the process of Africanization. Beekeepers report that the incidence of colony takeovers ranges from rare (Gonçalves *et al.*,1974; Camazine, 1986) to frequent (Vergara, 1991; Vogel, personal communication, cited in Taylor, 1985). Only a few experimental approaches have addressed the issue. When established colonies were challenged with artificial swarms, swarms tended to be accepted by European colonies but not by Africanized colonies (Dietz *et al.*, 1989), especially when resident queens were confined in an excluder cage (Hellmich & Villa, unpublished data). Working in central Mexico at the onset of Africanization,Vergara (1991) observed an annual usurpation rate of 5.7%. Invasions were detected by frequent searches for marked queens in colonies maintained at several sizes, and with 13–25% of colonies maintained queenless. Queenless colonies were usurped at a significantly greater frequency than colonies with queens.

Measuring the frequency of usurpation is problematic because invasions are subtle and often indistinguishable from hybridization through supersedure (Taylor, 1985). Recent advances in the analysis of restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA) in honey bees generally permits differentiation of African versus European maternal lineage of a colony (Smith *et al.*, 1991). In thoroughly Africanized areas mtDNA thus can be used to help clarify the source of a new queen by decreasing misclassification (as usurpations) of morphologically Africanized progeny produced by a European supersedure queen mated to Africanized drones. Frequencies of African-derived mtDNA in feral Africanized populations have been measured at 1.00 in Venezuela (Hall & Muralidharan, 1989; Smith *et al.*, 1989) where this study was undertaken. Morphology of feral bees also is generally Africanized and shows only limited influence from European bees in Venezuela (Buco *et al.*, 1987).

Measured simultaneously, changes in mtDNA and morphology therefore can be used to determine rates of usurpation and supersedure in European colonies kept in an Africanized zone. The relative contributions of these processes to Africanization then can be estimated. We used this approach with colonies managed commercially for honey production; test colonies thus typified the majority of colonies likely to be threatened by Africanization in the USA. Estimating the magnitude of the threat of invasions will help gauge the beekeeping management and research efforts needed in North America to mitigate this mechanism of Africanization.

MATERIALS AND METHODS

The 15-month study (January 1989 to April 1990) was conducted at sites within 30 km of Acarigua (9° 33' N, 69° 12' W), Venezuela. The area has a mixture of dry tropical forest and farmland; elevation is 150–190 m, annual rainfall is 1 400–2 000 mm, mean annual temperature is 27°C, and mean annual temperature minima and maxima are 21°C and 33°C, respectively (Ministerio del Ambiente y de los Recursos Naturales Renovables, Venezuela, 1979). There is a pronounced wet season (typically April through September) and dry season (October through March), with honey storage and reproductive swarming by honey bees during the dry season. Africanized bees have been present since 1978.

Sixty-seven commercially managed colonies were requeened in mid-December 1988 with marked European queens obtained from three commercial sources in the USA. Test colonies were distributed in four apiaries separated by 5–43 km. In late January 1989, initial samples of worker progeny of the European queens were collected and observations of queen fates were begun. Most progeny samples consisted of post-teneral (2- to 3-day old) workers collected after eclosion from a caged brood comb in an incubator (35°C); a final sampling of remaining colonies in April 1990 used workers collected directly from combs. Thirty or more workers were stored in ethanol for morphological assessment and 20 or more were stored frozen (–4°C in the field, then at –70°C) for mtDNA assessment.

A local beekeeper experienced with Africanized and European bees managed the colonies for honey production. Monthly colony inventories were made to ascertain queen status, and routine management

TABLE 1. Fates of test queens and morphological and mtDNA characteristics of 58 colonies with European queens used for honey production in Portuguesa, Venezuela, during the period from January 1989 to April 1990. Morphology and mtDNA classification for worker progeny of each original queen were European.

Fate of queens during 15 months	*n* (% of total fates)	Final and (intermediate) worker mtDNA	Final and (intermediate) worker morphology
Survived	7 (12%)	European	European
Died without replacement	16 (28%)	no final sample	no final sample
Usurped	3 (5%)	African	Africanized
Superseded			
1st generation	28 (48%)	European	6 European 3 Africanized 19 intermediate
2nd generation	3 (5%)	(1st gen: European) 2nd gen: European	(1st gen: 1 European 2 intermediate) 2nd gen: 3 intermediate
3rd generation	1 (2%)	(1st gen: European) (2nd gen: European) 3rd gen: European	(1st gen: Africanized) (2nd gen: not sampled) 3rd gen: Africanized

was conducted. Beginning in April 1989, inventories at 3-month intervals included estimates of colony size (combs covered with bees, unsealed brood, sealed brood, honey and pollen) and nectar flow conditions. When a queen change was observed, the new queen was marked for identification purposes and a progeny sample was collected. When a colony was found to be queenless it was left in place, at risk to be parasitized by swarms, for at least one month. Queenless units were removed only when combs were in danger of being destroyed by wax moths.

Worker mtDNA was assessed by extracting total nucleic acids from five degastered bees per colony (Sheppard & McPheron, 1991), digesting with the restriction enzyme *Eco*RI, separating the fragments with agarose gel electrophesis, and visualizing patterns with ethidium bromide stain and UV illumination (Maniatis *et al.*, 1982). For morphometric assessment, samples of 10 workers per colony were dissected and mounted on microscope slides. Measurements of wing, hindleg and sternite features taken from projected images were submitted to multivariate discriminant analysis procedures (Daly & Balling, 1978; Daly *et al.*, 1982) simplified and modified to include expanded baselines of European and Africanized standards (Rinderer *et al.*, 1993; discriminant functions are available from the USDA Honey-Bee Breeding, Genetics and Physiology Laboratory). Assignment to European or Africanized genotypic classes was based on a probability of ≥ 0.98 if the progeny of a queen were sampled once. If progeny were sampled twice (because all remaining colonies were resampled when field work was concluded in April 1990), classification was made if both samples had probabilities of ≥ 0.98, or if the samples had probabilities of ≥ 0.99 and ≥ 0.95. All other cases were deemed morphologically intermediate. Voucher samples are archived at the USDA Honey-Bee Breeding,Genetics and Physiology Laboratory.

RESULTS

Fates were determined for 58 of the 67 European colonies during the 15-month test (six colonies were lost due to theft or insecticides, two apparent supersedure queens died before producing progeny, and one sample was mishandled). Three nest usurpations (5% of colony fates) were documented based on congruence of African-derived mtDNA and Africanized morphology (table 1). These usurped colonies were the only cases showing a shift to African mtDNA from European mtDNA.

Twenty-eight queens (47%) were superseded once, three colonies (5%) had two supersedures, and one colony (2%) had three supersedures. Seven (12%) of the original European queens survived the 15-month test. All original European queens and all supersedure queens had European mtDNA. Worker progeny of each of the original 67 queens had European morphology. Progeny of supersedure queens showed mixed morphology, with mostly intermediate and European morphology in first generation workers shifting to intermediate and Africanized morphology in later generations (table 1).

Sixteen (28%) of the European queens died without being successfully replaced by their colonies. In most of these cases colonies either dwindled before dying or their queens failed.

A usurpation rate of 4.7% per year is calculable based on three invasions over the course of 758 colony-months during which invasion of test colonies was possible. The three nest usurpations occurred in two of the four apiaries, during different seasons, and under different circumstances. Two strong colonies (each with 10 combs of adult bees and 6–7 combs of brood) in one apiary were invaded. One instance occurred in March 1989 (end of the nectar flow) and one in May 1989 (start of the rainy season). The third usurpation, in November 1989, involved a strong colony (10 combs with bees and eight combs with brood) whose resident queen had been killed accidentally during the October colony inspection (the only such death known). Two of the usurped colonies later absconded in the rainy season; such behaviour is associated with Africanized bees (Winston *et al.*, 1979). In all, 16 of 23 (70%) colony losses due to absconding or permanent queenlessness occurred in the late rainy season months of July, August and September.

DISCUSSION

The finding of 4.7% of European colonies being usurped annually is likely to be moderately troublesome for beekeepers trying to maintain genetic control of large numbers of colonies. The difficulty is compounded greatly when invasions are coupled with other threats of Africanization (see below). Annual inventories of colonies and corrective requeening would be crucial minimum steps toward stock control. Previous experiences (Vogel, personal communication, cited in Taylor, 1985; Rinderer & Hellmich, 1991; Danka, unpublished data), which need further investigation, have indicated that colonies which are of unusual composition (e.g. mating nuclei, queen banks) are prone to invasion; results presented here and by Vergara (1991) show that colonies managed conventionally for honey production also are at risk. Usurpation of about 5% of colonies each year in addition, predictably, has played, and will continue to play, a substantial role in the genetics of feral honey bee populations.

This test was conducted specifically to measure rates of colony usurpation in Africanized areas but had the added benefit of documenting the variety and extent of other fates of European colonies in the neotropics. Africanization in the test apiaries was particularly noteworthy when all contributory events (usurpation, supersedure and colony failure) are considered. A large proportion (83%) of European queens used in the test either died or were superseded within 15 months. This high rate of failure and replacement of European queens is evidence of the comparative astounding success of Africanized bees, viz., that European colonies in general often perform poorly in the neotropics (see also Gentry, 1990). In many cases beekeepers are challenged with simply keeping European colonies alive, and thus often welcome the enhanced vigour of hybridized bees. In our study, more than one-fourth (16 of 58, 28%) of the original European queens and nearly one-sixth (5 of 32, 16%) of first generation supersedure queens died without being replaced. This resulted in permanently queenless units which, if neglected, soon would have provided empty equipment available for habitation by Africanized swarms. Colonization by swarms within an apiary has the same effect of complete, immediate Africanization as does usurpation, for which it may be mistaken.

Supersedures accounted for 37 of 40 queen changes in our test, and occurred in 55% of experimental colonies. This is at least twice the general rate of supersedure of European queens as measured in temperate North America (Duff & Furgala, 1986; Sugden & Furgala, 1982). Supersedure and associated mating events clearly were responsible for the majority of the influx of Africanized genes into the European colonies. Progeny of only 22% of first generation supersedure queens and none of later generation queens were classified as European based on morphology (table 1). Of 35 colonies remaining when the test was terminated, only 12 (34%; seven original queens and five first generation queens) were identifiably European, based on having both European morphology and European mtDNA. Collectively then, usurpations, queen replacement and colony failure were responsible for Africanization manifested at a rate of 53% per year in the test apiaries (i.e. 66% of colonies remaining after15 months were non-European). These findings emphasize that managed colonies of desired stocks must be maintained through frequent, careful monitoring and requeening if Africanized bee production (through swarms, drones and queen grafts) and movement (of colonies) are to be minimized in North America.

ACKNOWLEDGEMENTS

We gratefully acknowledge the skill and perseverance of Alcides Escalona, our beekeeping co-operator. We thank J Anthony Stelzer for field assistance, data handling and mtDNA analyses, Rita Riggio for morphological measurements, and Sandra Kleinpeter for mtDNA analyses. Helpful discussion was provided by W Steven Sheppard, José Villa, Anita Collins and Steven Buco. This work was completed in co-operation with the Louisiana Agricultural Experiment Station.

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