

Frequencies of Restriction Fragment-Length Polymorphisms Indicate That Neotropical Honey Bee (Hymenoptera: Apidae) Populations Have African and West European Origins

H. GLENN HALL¹ AND MARGARET A. McMICHAEL²

Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611

Ann. Entomol. Soc. Am. 94(5): 670-676 (2001)

ABSTRACT Frequencies are reported for restriction fragment-length polymorphisms (RFLPs) at a highly polymorphic nuclear locus in Old and New World honey bee populations. The distribution of these (RFLPs) alleles (composed of *MspI* and *DdeI* variants) had been found previously to be discontinuous among groups of Old World honey bee subspecies, which included *A. mellifera mellifera* L. (west European), *A. m. ligustica* Spinola, *A. m. caucasica* Gorbachev (east European), and *A. m. scutellata* Lepeletier (African). In this study, ancestry in New World bees was inferred from allele identities and frequencies at this locus in combination with mitochondrial DNA types. In bees from the United States, collected before the invasion of African bees, east and west European alleles were found at frequencies of 83 and 17%, respectively, which is consistent with previously identified nuclear and mitochondrial DNA markers. Colonies from two neotropical countries, Mexico and Honduras, had African mitochondrial DNA and high frequencies of African nuclear DNA alleles. Consistent with previous findings, east European alleles were absent or detected at low frequencies in these colonies. However, west European alleles were found at frequencies from 26 to 31%. These results suggest that queen offspring of the African queens first introduced into Brazil mated with west European drones, incorporating neutral markers that have since remained in the expanding population of feral African bees. The results point to little paternal introgression from managed east European colonies encountered by the African bees spreading through the neotropics.

KEY WORDS *Apis mellifera mellifera*, *Apis mellifera caucasica*, *Apis mellifera ligustica*, African and European honey bees, allele frequencies, introgression

HONEY BEE, *Apis mellifera* L., subspecies group into four major evolutionary lineages: east (and southeast) European (C), west (and north) European-Saharan African (M), sub-Saharan-African (A), and near-eastern (O). These groups were first established through morphometrics (Ruttner et al. 1978, Ruttner 1988). The classification of subspecies into lineages C, M, and A has been substantiated by allozyme frequencies (Badino et al. 1982, Cornuet 1986, Sheppard and Huettel 1988), mitochondrial DNA (mtDNA) restriction sites (Cornuet and Garnery 1991, Smith 1991), mtDNA sequences (Garnery et al. 1992), nuclear DNA microsatellites (Estoup et al. 1995), and unique sequence nuclear DNA restriction fragment-length polymorphisms (RFLPs) (McMichael and Hall 1996). Recent molecular evidence placed the west Saharan subspecies, *A. m. intermissa* Buttel-Reepen, within the A lineage rather than the M lineage (Garnery et al. 1992, Franck et al. 1998) and placed *A. m. caucasica* Gorbachev within the C lineage (McMichael and Hall 1996).

The first honey bee introduced to North America was *A. m. mellifera* L., from western and northern Europe, in 1622 (Sheppard 1988, 1989). *Apis mellifera ligustica* Spinola, *A. m. carnica* Pollmann, and *A. m. caucasica* from eastern Europe were introduced in the mid- to late 1800s (Pellet 1938, Oertel 1976, Kent 1988, Sheppard 1989). East European bees, particularly *A. m. ligustica*, became predominantly used for beekeeping due to their docility and productivity. *Apis mellifera scutellata* Lepeletier from South Africa were brought to Brazil in the late 1950s. Since then, African swarms established self-sustaining feral populations that expanded through most of the neotropics and into the subtropics of South and North America. In neotropical regions, the resident European bees, largely confined to managed apiaries, were subject to African paternal introgression and were replaced consequently by "Africanized" progeny.

DNA markers have been effective in revealing processes involved in the expansion of the African bee population in the New World (Hall 1991, 1992a, 1999). With mtDNA, the feral neotropical population was shown to be composed of unbroken African matrilineal swarms spreading as swarms (Hall and Muralidharan 1989, Smith et al. 1989, Hall and Smith 1991). Nuclear DNA RFLPs, characteristic of east European bees, were

¹ E-mail: hgh@gnv.ifas.ufl.edu

² Current address: Department of Entomology, Louisiana State University, Baton Rouge, LA, 70803.

virtually absent in feral African colonies, indicating that there had been limited paternal introgression from managed European colonies (Hall 1990). However, frequencies of African alleles at another locus were lower in feral neotropical populations than in South African populations (Hall 1992b, Hall 1998), suggesting greater levels of African-European hybridization than that observed in the earlier study. Paternal introgression specifically from west European bees into the feral African population could account for these different findings. Markers characteristic of each of these three subspecies groups would allow that hypothesis to be evaluated and would provide a more comprehensive view of hybridization in the neotropical populations.

We have described alleles found in Old and New World honey bees at a highly polymorphic, anonymous, nuclear locus, called 178, corresponding to a randomly cloned fragment of honey bee DNA (McMichael and Hall 1996). The alleles were composed of pairwise, coupled (*cis*), combinations of *MspI* and *DdeI* RFLPs, most of which were identified in individual drones (haploid males). In principal coordinate analyses, the alleles were found to cluster exclusively into three groups corresponding to three of the four main honey bee lineages (samples from the lineage O were not included in the study). In that study, the distribution of the alleles at locus 178 in New World populations had been reported: west and east European alleles were found in U.S. bees; west European, east European, and African alleles were found in neotropical bees. Here, frequency data are reported for the same 86 alleles, obtained primarily from drone samples. The allele frequencies reveal a level and specificity of African and European introgression not observable in the previous RFLP studies.

Materials and Methods

Honey bee samples used for this study were obtained as larvae or pupae. Previously described protocols were followed for the collection and preservation of the brood samples (Hall 1986, 1990). The samples used previously for the identification of the RFLP alleles at locus 178 (McMichael and Hall 1996) were included in determining the allele frequencies. These samples were workers (diploid females) from Old World European populations and drones from Old World African and New World populations. The number of colonies sampled and the total number of alleles identified in each population are listed in Table 1. Samples from Europe were provided by B. Vaissiere (INRA, Avignon, France) and J.-M. Cornuet (INRA, Montpellier, France). Samples from South Africa were collected from four locations in the Transvaal, in January 1990 by H.G.H., with the assistance of G. Pretorius (Plant Protection Institute, Pretoria), R. Crewe (University of Witwatersrand, Johannesburg), and beekeepers of South Africa. Samples from Honduras, as feral swarms or managed colonies established from feral swarms, were obtained from 1990 to 1992 by H.G.H. and A. Suazo (Escuela Agrícola Panamericana,

Zamorano). Samples from Mexico were obtained in 1988 by H.G.H., as feral swarms from Tapachula, Las Choapas, and San Andrés Tuxla, or as managed colonies established from feral swarms from Tapachula. The swarms came from bait hives maintained by the Secretariat of Agriculture and Hydrologic Resources (SARH). Samples from the United States were from managed colonies from Florida, collected by H.G.H.; Kansas, provided by O. Taylor (University of Kansas); Hawaii (queens from Kona Queen Company); and Arizona, as a closed breeding population representing U.S. bees (Page et al. 1982, Severson et al. 1986), provided by J. Martin, G. Waller, G. Loper, and E. Erickson (Carl Hayden Bee Research Center, USDA-ARS, Tucson).

The procedures used for total DNA isolations and for nuclear DNA RFLP analyses with clone 178 as a probe on blots are described in a previous report (McMichael and Hall 1996). The characterization and naming of the alleles are also in the earlier report. MtDNA types (west European, east European, African) of the colonies used in this study either had been determined in previous studies (Hall and Muralidharan 1989, Hall 1990, Hall and Smith 1991) or were determined more recently for this study by polymerase chain reaction (PCR)-RFLP procedures (Crozier et al. 1991, Hall and Smith 1991).

DdeI variants were difficult to identify in workers due to fragment superimposition, precluding the determination of allele frequencies. Only in the Old World European worker samples could most of the *MspI* and *DdeI* variants be identified. Because both alleles of an individual worker could not always be identified, allele frequencies were estimated as the fraction of the total number of alleles identified. Honey bee queens mate with a number of drones from distant colonies. Thus, the genotype of workers from a colony represent the local population, although the maternal genotype, and possibly some paternal genotypes would be overrepresented.

Because of the larger number of alleles found in the New World, especially in the African populations, and the difficulty of identifying *DdeI* variants in workers, allele frequencies came from haploid drone brood. Frequencies were calculated as a fraction of the total number of alleles detected, as described by Hall (1992b). Drones are parthenogenetic progeny of the queen, thus alleles in drones from the same colony reflect her genotype. At least two drones were analyzed per colony. When two alleles were found (heterozygous queen), each was counted once. When only one allele was found, the allele was counted once (if six or more drones per colony had been analyzed, which was not done in this study, one allele found would have been counted twice; a >98% probability that the queen was homozygous). The total number of alleles expected to be found for frequency counts would be twice the number of colonies tested, i.e., the two alleles of all queens.

For the populations *A. m. mellifera*, United States, neotropical African (Mexico and Honduras, with African mtDNA, pooled), and South Africa (Table 1),

the allele frequency distributions were evaluated for selective neutrality with programs provided in the Arlequin package (Schneider et al. 1997). Slatkin's exact test (Slatkin 1994) was used to compare the observed frequencies with the expected frequencies under selective neutrality based on Ewens sampling distribution.

Results

Frequencies of the 86 alleles of locus 178 found in samples of Old and New World populations are given in Table 1. The collective frequencies of west European, east European, and African alleles in New World populations indicate the relative contributions of these three groups of honey bee subspecies. In U.S. populations, east and west European alleles were found at collective frequencies of 83 and 17%, respectively. The frequency of east European alleles was comparable to that determined with other east European markers in an earlier study, in which the alternate alleles were assumed to represent west European ancestry (Hall 1990). The U.S. samples came largely from managed colonies, and these frequencies reflect the preferred use of east European bees for beekeeping. Managed colonies in southern Mexico, sampled about 15 mo after the African bee invasion, that had east European mtDNA, had only east and west European alleles at 63 and 37%, respectively. Thus, these colonies had not yet reared new queens that would have mated with African drones. East European mtDNA and one east European allele at locus 178 were found in a single swarm from Honduras.

In neotropical colonies with African mtDNA, from Honduras and Mexico, the alleles at locus 178 were primarily African (Table 1). Virtually no east European alleles were found. In contrast, west European alleles were found at substantial levels (26–31%). Three of the colonies tested were feral swarms from southern and central Mexico, which, at the time of collection, were just behind the expanding front of the African population. All three queens in these colonies were heterozygous for an African and a west European allele.

For the populations tested, the allele distributions were found not to deviate significantly from those predicted under selective neutrality (Slatkin's exact test, $P \leq 0.95$; *A. m. mellifera*, $P = 0.3879$; U.S., $P = 0.4055$; New World African, $P = 0.8290$; South African, $P = 0.8831$).

Discussion

The alleles at locus 178 were found to be distributed discontinuously among the samples tested that belonged to three of the four main honey bee lineages: east European, west European, and African (McMichael and Hall 1996). The primary conclusions of the current study come from the collective frequencies of alleles within these groups, because the frequencies of individual alleles were subject to sampling error. Testing was largely limited to haploid drones to

identify the complex allelic band patterns. Drones reared in the same colony reflect the genotype of the mother queen, and, thus, the number of samples are, in effect, limited to the number of colonies. Sixty characters define the alleles: *MspI* and *DdeI* sites plus insertions and deletions, which could generate a very large number of possible combinations. Many of the alleles were found only once, and many probably remain to be found. Nevertheless, the frequencies of the individual alleles that were found indicate that a small proportion are the most common and a larger proportion are rare. These frequency distributions would be expected for neutral alleles.

As a consequence of the sampling, a minor proportion of the alleles at locus 178 were found in both the Old and New World. The small overlap is particularly notable for the African alleles. The ancestry of the alleles found only in the New World was based on their clustering with alleles from the Old World (McMichael and Hall 1996). Evidently, sampling for this study selected subsets from the Old World different from subsets that were imported to the New World. Sampling errors of the New World populations were probably much lower. Because of genetic bottlenecks resulting from importations, total numbers of alleles may be much less. For example, the west European alleles found in the United States were the same as those found in Mexico, and most of the African alleles found in Mexico were also found in Honduras.

The alleles at locus 178 provide a more complete view of the contributions of honey bee subspecies groups to New World populations. Nuclear RFLP markers found previously were specific either for east European or African bees, but alternate alleles were common to two subspecies groups, and no alleles were specific for west European bees (Hall 1990, 1992b). Thus, a west European contribution to populations could not be detected and could only be inferred. In this study, a higher level of European alleles in feral neotropical African colonies was revealed than that seen with east European markers alone but that had been suggested by a later study using African markers (Hall 1992b). Consistent with the previous reports, east European alleles at locus 178 were nearly absent.

The composition of the neotropical population resulting from the African bee invasion has been intensely debated (Hall 1992a, Rinderer et al. 1993, Hall 1999). The Africanization of European apiaries primarily by African paternal introgression has not been questioned, but the extent of European paternal introgression into the feral African population has not been resolved. Morphometric evidence suggests that neotropical bees are African-European hybrids (Buco et al. 1987), but in regions of the neotropics where African bees have been long established, both managed and feral colonies are more African than European, based on morphology, behavior, and genotype (Fletcher 1988, Taylor 1988, Lobo et al. 1989, Hall 1990, Lobo and Krieger 1992). Subspecies proportions determined from allozyme frequencies of malate dehydrogenase (MDH) pointed specifically to a west European ancestry ranging from 16% in Central Amer-

ica to 26% in bees from southern Brazil (Lobo et al. 1989, Lobo and Krieger 1992). These percentages must be interpreted in light of allele-sharing between European and African bees and unknown ancestral gene frequencies (Sheppard 1988, Lobo et al. 1989, Lobo and Krieger 1992). Nevertheless, these results with allozymes are consistent with our DNA findings for locus 178, indicating a substantial west European contribution to the neotropical African population.

The presence of west, but not east, European markers in the feral African population may be the result of larger population sizes of west relative to east European bees in the area where African bees were released or in other areas of the neotropics into which African bees spread (Sheppard et al. 1991). Italian and German farmers brought *A. m. ligustica* and *A. m. mellifera*, respectively, to their settlements in south and southeastern Brazil (Gonçalves 1974, Lobo et al. 1989, Gonçalves et al. 1991) and Argentina (Kerr et al. 1982). *Apis mellifera caucasica* and *A. m. carnica* were also introduced (Ruttner 1986, Kent 1988). In contrast to the United States, the contribution of *A. m. ligustica* in the neotropics was minor despite considerable importation (Kent 1988), although this subspecies was found concentrated in certain areas: Argentina (Kerr et al. 1982, Sheppard et al. 1991), Costa Rica (Kent 1988, Spivak 1991), and Mexico, particularly the Yucatan (Kent 1988, Rinderer et al. 1991). There is little indication that significant introductions of east European bees were made or were successful in central and northeastern Brazil (Lobo et al. 1989), the Guianas, Suriname (Taylor 1977), or Panama (Roubik 1982, Boreham and Roubik 1987). New World African bees may have descended primarily from daughter queens of the introduced queens distributed in large numbers to beekeepers (Rinderer et al. 1993). If so, considerable hybridization might have occurred with the resident European bees, which at that time in that area of Brazil were predominantly the west European *A. m. mellifera* (Kent 1988, Lobo et al. 1989, Lobo and Krieger 1992).

After African bees enter an area, European bees and their hybrids seem to disappear over time. Selection in tropical environments is probably largely responsible, although other hybrid deficiencies, for example lower metabolic capacities (Harrison and Hall 1993), might also reduce their survival. Despite the predominance of west European bees in most of the neotropics and the concentration of east European bees in other areas before the African bee invasion, virtually no European mtDNA, west or east, is found in the feral African population (Hall and Muralidharan 1989, Smith et al. 1989, Hall and Smith 1991). Thus, even after repeated backcrossing with African drones, strongly "Africanized" European matrilineal bees do not become adapted to the tropics and do not survive in the feral population. A low level of east European nuclear DNA markers was found in feral African colonies as they first encountered European populations along the expanding front, but apparently such hybrids did not persist in more established African populations. After initial hybridization of the introduced African bees with *A. m.*

mellifera in Brazil, subsequent selection could have eliminated most European genes but may have left genes or markers that were neutral, perhaps such as the 178 west European alleles, which remained in the genetic pool at a relatively constant frequency.

Acknowledgments

We thank Drion Boucias for reviewing the manuscript and Dorothy Prowell for reviewing an earlier version. We are grateful to the people mentioned in the *Materials and Methods* for providing samples of honey bees. This research was supported by the USDA National Research Initiative Competitive Grants Program. Florida Experiment Station Journal Series No. R-08138.

References Cited

- Badino, G., G. Celebrano, and A. Manino. 1982. Genetic variability of *Apis mellifera ligustica* Spin. in a marginal area of its geographical distribution. *Experientia* 38: 540–541.
- Boreham, M. M., and D. W. Roubik. 1987. Population change and control of Africanized honey bees (Hymenoptera: Apidae) in the Panama canal area. *Bull. Entomol. Soc. Am.* 33: 34–38.
- Buco, S. M., T. E. Rinderer, H. A. Sylvester, A. M. Collins, V. A. Lancaster, and R. M. Crewe. 1987. Morphometric differences between South Am. Africanized and South African (*Apis mellifera scutellata*) honey bees. *Apidologie* 18: 217–222.
- Cornuet, J.-M. 1986. Population genetics, pp. 235–254. In T. E. Rinderer [ed.], *Bee genetics and breeding*. Academic, London.
- Cornuet, J.-M., and L. Garnery. 1991. Mitochondrial DNA variability in honeybees and its phylogeographic implications. *Apidologie* 22: 627–642.
- Crozier, Y. C., S. Koulianos, and R. H. Crozier. 1991. An improved test for Africanized honeybee mitochondrial DNA. *Experientia* 47: 968–969.
- Estoup, A., L. Garnery, M. Solignac, and J.-M. Cornuet. 1995. Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics* 140: 79–695.
- Fletcher, D.J.C. 1988. Relevance of the behavioral ecology of African bees, to a solution to the Africanized-bee problem, pp. 55–61. In G. R. Needham, R. E. Page, M. Delfinado-Baker, and C. E. Bowman [eds.], *Africanized honey bees and bee mites*. Ellis Horwood, Chichester, UK.
- Franck, P., L. Garnery, M. Solignac, and J.-M. Cornuet. 1998. The origin of west European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data. *Evolution* 52: 1119–1134.
- Garnery, L., J. M. Cornuet, and M. Solignac. 1992. Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. *Mol. Ecol.* 1: 145–154.
- Gonçalves, L. S. 1974. The introduction of the African bees (*Apis mellifera adansonii*) into Brazil and some comments on their spread in South America. *Am. Bee J.* 114: 414, 415, and 419.
- Gonçalves, L. S., A. C. Stort, and D. De Long. 1991. Bee-keeping in Brazil, pp. 359–372. In M. Spivak, M. Breed, and D.J.C. Fletcher [eds.], *The "African" honey bee*. Westview, Boulder, CO.

- Hall, H. G. 1986. DNA differences found between Africanized and European honeybees. *Proc. Natl. Acad. Sci. U.S.A.* 83: 4874–4877.
- Hall, H. G. 1990. Parental analysis of introgressive hybridization between African and Eur. honeybees using nuclear DNA RFLPs. *Genetics* 125: 611–621.
- Hall, H. G. 1991. Genetic characterization of honey bees through DNA analysis, pp. 45–73. *In* M. Spivak, M. Breed, and D.J.C. Fletcher [eds.], *The "African" honey bee*. Westview, Boulder, CO.
- Hall, H. G. 1992a. DNA studies reveal processes involved in the spread of New World African honeybees. *Fla. Entomol.* 75: 51–59.
- Hall, H. G. 1992b. Further characterization of nuclear DNA RFLP markers that distinguish African and European honeybees. *Arch. Insect Biochem. Physiol.* 19: 163–175.
- Hall, H. G. 1998. PCR amplification of a locus with RFLP alleles specific to African honey bees. *Biochem. Genet.* 36: 351–361.
- Hall, H. G. 1999. Genetic and physiological studies of African and European honey bee hybridization: past, present and into the 21st century, pp. 52–59. *In* R. Hoopinger and L. J. Connor [eds.], *Apiculture for the 21st century*. Wicwas, Cheshire, CT.
- Hall, H. G., and K. Muralidharan. 1989. Evidence from mitochondrial DNA that African honeybees spread as continuous maternal lineages. *Nature (Lond.)* 339: 211–213.
- Hall, H. G., and D. R. Smith. 1991. Distinguishing African and European honeybee matrilineages using amplified mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* 88: 4548–4552.
- Harrison, J. F., and H. G. Hall. 1993. African-European honeybee hybrids have low non-intermediate metabolic capacities. *Nature (Lond.)* 363: 258–260.
- Kent, R. B. 1988. The introduction and diffusion of the African honeybee in South America. *Yearbook of the Association of Pacific Coast Geographers* 50: 21–43.
- Kerr, W. E., S. De Leon, and M. Dardo. 1982. The southern limits of the distribution of the Africanized honey bee in South America. *Am. Bee J.* 122: 196–198.
- Lobo, J. A., and H. Krieger. 1992. Maximum likelihood estimates of gene frequencies and racial admixture in *Apis mellifera* L. (Africanized honeybees). *Heredity* 68: 441–448.
- Lobo, J. A., M. A. Lama, and M. A. Mestriner. 1989. Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L.). *Evolution* 43: 794–802.
- McMichael, M., and H. G. Hall. 1996. DNA RFLPs at a highly polymorphic locus distinguish European and African subspecies of the honey bee *Apis mellifera* L. and suggest geographic origins of New World honey bees. *Mol. Ecol.* 5: 403–416.
- Oertel, E. 1976. Bicentennial bees. Early records of honey bees in the eastern United States. *Am. Bee J.* 116: 70, 71, 114, 128, 156, 157, 214, 215, 260, 261, and 290.
- Page, R. E., E. H. Erickson, and H. H. Laidlaw. 1982. A closed population breeding program for honey bees. *Am. Bee J.* 122: 350–355.
- Pellet, F. C. 1938. *History of American beekeeping*. Collegiate Press, Ames, IA.
- Rinderer, T. E., J. A. Stelzer, B. P. Oldroyd, S. M. Buco, and W. L. Rubink. 1991. Hybridization between European and Africanized honey bees in the neotropical Yucatan peninsula. *Science* 253: 309–311.
- Rinderer, T. E., B. P. Oldroyd, and W. S. Sheppard. 1993. Africanized bees in the U.S. *Sci. Am.* 269: 84–90.
- Roubik, D. W. 1982. Africanized honey bees confirmed in Panama. *Am. Bee J.* 122: 322.
- Ruttner, F. 1988. *Biogeography and taxonomy of honey bees*. Springer, Berlin.
- Ruttner, F. 1986. Geographic variability and classification, pp. 23–56. *In* T. E. Rinderer [ed.], *Bee genetics and breeding*. Academic, London.
- Ruttner, F., L. Tassencourt, and J. Louveaux J. 1978. Biometrical-statistical analysis of the geographic variability of *A. mellifera* L. *Apidologie* 9: 363–381.
- Schneider, S., J.-M. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin, version 1.1. Software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Severson, D. W., R. E. Page, and E. H. Erickson. 1986. Closed population breeding in honey bees: a report on its practical application. *Am. Bee J.* 126: 93–94.
- Sheppard, W. S. 1988. Comparative study of enzyme polymorphism in United States and European honey bee (Hymenoptera: Apidae) populations. *Ann. Entomol. Soc. Am.* 81: 886–889.
- Sheppard, W. S. 1989. A history of the introduction of honey bee races into the United States. *Am. Bee J.* 129: 617–619, 664–667.
- Sheppard, W. S., and M. D. Huettel. 1988. Biochemical genetic markers, intraspecific variation, and population genetics of the honey bee, *Apis mellifera*, pp. 281–286. *In* G. R. Needham, R. E. Page, M. Delfinado-Baker, and C. E. Bowman [eds.], *Africanized honey bees and bee mites*. Ellis Horwood, Chichester, UK.
- Sheppard, W. S., T. E. Rinderer, J. A. Mazzoli, J. A. Stelzer, and H. Shimanuki. 1991. Gene flow between African- and European-derived honey bee populations in Argentina. *Nature (Lond.)* 349: 782–784.
- Slatkin, M. 1994. An exact test for neutrality based on the Ewens sampling distribution. *Genet. Res.* 64: 71–74.
- Smith, D. R. 1991. Mitochondrial DNA and honey bee biogeography, pp. 131–176. *In* D. R. Smith [ed.], *Diversity in the genus Apis*. Westview, Boulder, CO.
- Smith, D. R. 1991. African bees in the Americas: insights from biogeography and genetics. *Trends Ecol. Evol.* 6: 17–21.
- Smith, D. R., O. R. Taylor, and W. W. Brown. 1989. Neotropical Africanized honey bees have African mitochondrial DNA. *Nature (Lond.)* 339: 213–215.
- Spivak, M. 1991. The Africanization process in Costa Rica, pp. 137–155. *In* M. Spivak, M. Breed and D.J.C. Fletcher [eds.], *The "African" honey bee*. Westview, Boulder, CO.
- Taylor, O. R. 1977. The past and possible future spread of Africanized honeybees in the Americas. *Bee World* 58: 19–30.
- Taylor, O. R. 1988. Ecology and economic impact of African and Africanized honey bees, pp. 29–41. *In* G. R. Needham, R. E. Page, M. Delfinado-Baker, and C. E. Bowman [eds.], *Africanized bees and bee mites*. Ellis Horwood, Chichester, UK.

Received for publication 13 November 2000; accepted 18 May 2001.