histochemically stained axons in the human spinal cord (Fig. 3), which is consistent with binding of antibodies to neurofilaments. None of the disease categories was associated with higher titers of antibodies to the 200-kD NFP or to axons than those found among the healthy controls.

In summary, a large sample of healthy people and patients with neurological diseases had in their serum antibodies against the 200-kD NFP, and this NFP was the only protein in homogenates of neural tissues against which most of the healthy subjects had serum antibodies. The neurofilaments are the intermediate filaments of neurons and are composed of three proteins of 200, 150, and 68 kD. The 68-kD protein forms the backbone of the neurofilaments and the 200- and 150-kD proteins appear to be more peripherally located (*13*, *14*). It is also possible that the 200-kD NFP gives rise to smaller protease-resistant fragments that retain immunoreactivity, as seen in transsected nerves (*15*). Antibodies to cytoskeletal components, including the intermediate filaments, are commonly found in the serum of healthy people (*16*), and so are antibodies to many other autoantigens (*5*). These autoantibodies may even serve a physiological role in the normal catabolism of tissues or as vehicles for transporting blood-borne substances (*17*).

This does not, however, explain the relatively high titer of antibodies to the 200-kD NFP compared with other autoantigens in the homogenates of neural tissues used in our work. In the rat the 200-kD NFP appears late in development (*18*); according to the clonal selection theory (*3*), the immune system could therefore have less tolerance for it than proteins that appear at an earlier stage. We did not find increased titers of antibodies to the 200-kD NFP associated with any of the diseases (Table 1). This is in contrast to the results of Sotelo *et al.* (*19*) and Bahmanyar *et al.* (*20*), who found a higher incidence of antibodies to "neurofilament antigens" in serum from patients with various neurological diseases than in serum from healthy individuals, but agrees with the results of Elizan *et al.* (*21*), who did not find any such difference. However, Elizan *et al.* did observe a greater incidence of serum antibodies to neurofilaments in people over 70 years of age. We found the same incidence of antibodies against the 200-kD NFP in the serum in all age groups.

It is difficult to compare our data with those of the aforementioned investigators because they used immunohistochemical staining of axons to detect antibodies to neurofilaments or neurofilament antigens, whereas we used immunoblots to detect antibodies to the 200-kD NFP, and the methods differ substantially in their sensitivity and specificity. Recently, Bahmanyar *et al.* (*22*) showed that a chimpanzee that was inoculated intracerebrally with brain tissue from a patient with Creutzfeldt-Jakob disease (CJD) and that developed CJD 14 months later had serum antibodies to the 200-kD NFP after onset of the disease but not before the inoculation. However, our results underscore the need for caution when interpreting the finding of antibodies to neurofilaments in the serum of patients with neurological diseases.

KARI STEFANSSON
LINDA S. MARTON
MICHAEL E. DIEPERINK
Department of Neurology and Pathology and Brain Research Institute, University of Chicago, Chicago, Illinois 60637
GABOR K. MOLNAR
Department of Neurology, University of Turku, Turku, Finland
WILLIAM W. SCHLAEPFER
Department of Pathology, University of Pennsylvania, Philadelphia 19104
CATHY M. HELGASON
Department of Neurology and Pathology and Brain Research Institute, University of Chicago

References and Notes

1. F. M. Burnet, *The Clonal Selection Theory of Acquired Immunity* (Cambridge Univ. Press, London, 1959).
2. B. Guilbert, G. Dighiero, S. Avrameas, *J. Immunol.* **128**, 2779 (1982).
3. A. S. Doar and J. W. Fabre, *Clin. Exp. Immunol.* **45**, 37 (1981).
4. G. Dighiero, B. Guilbert, S. Avrameas, *J. Immunol.* **128**, 2788 (1982).
5. S. Avrameas *et al.*, *Ann. Immunol. (Inst. Pasteur)* **134D**, 103 (1983).
6. H. U. Lutz and G. Wipf, *J. Immunol.* **128**, 1695 (1982).
7. J. L. Fahey and E. W. Tarry, in *Handb. Exp. Immunol.* **1** (No. 8), 1 (1976).
8. A. F. C. Nisonoff *et al.*, *Arch. Biochem. Biophys.* **89**, 230 (1960).
9. R. A. Hague-Angeletti, H.-L. Wu, W. W. Schlaepfer, *J. Neurochem.* **38**, 116 (1982).
10. G. Fairbanks, T. L. Steck, D. H. Wallach, *Biochemistry* **10**, 2606 (1971).
11. K. Stefansson *et al.*, *Acta Neuropathol. (Berlin)* **59**, 255 (1983).
12. S. M. Hsu, L. Raine, H. Fanger, *J. Histochem. Cytochem.* **29**, 577 (1981).
13. R. K. H. Liem and S. B. Hutchinson, *Biochemistry* **21**, 3221 (1982).
14. M. Willard and C. Simon, *J. Cell Biol.* **89**, 198 (1981).
15. W. W. Schlaepfer *et al.*, *J. Neurochem.* **43**, 857 (1984).
16. P. Kurk and I. Virtanen, *J. Immunol. Methods* **67**, 209 (1984).
17. G. Pierre, *Immunol. Today* **4**, 337 (1983).
18. G. Shaw and K. Weber, *Nature (London)* **298**, 277 (1982).
19. J. Sotelo, C. J. Gibbs, Jr., D. C. Gajdusek, *Science* **210**, 190 (1980).
20. S. Bahmanyar *et al.*, *J. Neuroimmunol.* **5**, 19 (1983).
21. T. Elizan, J. Casals, M. D. Yahr, *J. Neurol. Sci.* **59**, 341 (1983).
22. S. Bahmanyar, *J. Neuropathol. Exp. Neurol.* **43**, 369 (1984).
23. H. Towbin and J. Gordon, *J. Immunol. Methods* **72**, 313 (1984).
24. Supported in part by grants from the Brain Research Foundation of the University of Chicago, the Muscular Dystrophy Association of America, and the Amyotrophic Lateral Sclerosis Society of America. We thank B. G. W. Arnason for his advice, encouragement, and support and M. Murphy for her excellent secretarial contribution.

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Male Reproductive Parasitism: A Factor in the Africanization of European Honey-Bee Populations

Abstract. *Africanized drone honey bees* (Apis mellifera) *migrate into European honey-bee colonies in large numbers, but Africanized colonies only rarely host drones from other colonies. This migration leads to a strong mating advantage for Africanized bees since it both inhibits European drone production and enhances Africanized drone production.*

Colonization by Africanized honey bees in the continental United States is a possibility within 5 years (*1*). This honey bee (*Apis mellifera scutellata*; formerly *A. m. adansonii*) (*2*) was introduced into Brazil in 1956 with the escape of 26 queens (*3*) and the possibly 250 drones with which they had mated in Africa. In less than 30 years the genetic materials from this bee have overwhelmed the vastly larger and more diverse gene pool of previously imported European honey bees (mainly *A. m. mellifera* and *A. m. ligustica*) (*4*) throughout most of South America and lately much of Central America. Taxonomic (*5*) and behavioral (*6*) descriptions of the Africanized population are quite similar to descriptions of the parental African bee population (*2*, *3*, *7*, *8*) in spite of interbreeding with European bee stocks. After a few years the European bee is largely replaced by the Africanized bee (*9*), which retains fully its several objectionable characteristics including severe stinging (*6*) and poor honey production (*10*).

The rapid change of European bee colonies into Africanized colonies strongly suggests a mating advantage. An earlier study (*11*) used equal numbers of drones and did not demonstrate a mating advantage; rather, it showed a small amount of positive assortive mating (like-to-like) which, acting alone, would tend to enhance the genetic integrity of European apiaries. One of us (T.E.R.), while inspecting an experimental apiary of genetically marked European colonies in Venezuela, noticed that the majority of drones in the colonies did not carry the expected mark and were presumably Africanized. This observation led to a series of experiments to study the causes and magnitude of this parasitism and its effects on host-colony drone production.

In the first experiment, an apiary in Venezuela was used with ten Africanized and ten European colonies standardized to fill two-story ten-frame Langstroth hives. Distinctive orientation marks painted on hive fronts and placement of the colonies in a serpentine row near a wood lot were used to reduce the movement of drones among colonies that arises from errors in orientation (*12*) and is known to occur infrequently with European drones (*13*). In addition, the Africanized and European colonies were alternated along the row, with Africanized colonies facing north and European colonies facing south, in order to further reduce orientation errors (*12*).

Africanized and European drones were reared in these and other colonies from eggs obtained from queens usually caged on drone comb. All adult drones emerged in an incubator and, within 2 days, were marked with paint and introduced into experimental colonies. The paint marks indicated age, geographical type (*14*), and colony into which the drones were introduced (home colony). Each colony received approximately 100 drones of each geographical type from several different sources during a 5-day period. Two weeks later we introduced a similarly raised and marked group of approximately 200 drones to each colony. At 2- to 3-day intervals after the first drones were introduced, each colony was thoroughly inspected, and all drones seen were recorded by paint-mark classification. When drones had migrated, their "host colony" was also recorded. These colony inspections were made only in the morning since drones do not normally take flights until afternoon.

Africanized drones established in Africanized colonies migrated to European colonies in large and vastly disproportionate numbers when compared to drones in any other category of drone type, home colony, and host colony (Fig. 1A) (*15*). Three factors contributed to the migration of these Africanized drones. First, European colonies hosted the large majority of drones that left their home colony (95 percent by day 21) (Fig. 1B). Second, a greater proportion of drones placed in Africanized home colonies migrated (Fig. 1C). Finally, a greater proportion of Africanized drones migrated (Fig. 1D).

In the second experiment, the effects of the direction that colony entrances faced were estimated. Half of the Africanized colonies and half of the European colonies, selected randomly, were turned at intervals during the course of 10 days so that their entrance directions were reversed. One additional group of drones was reared, paint-marked, and introduced into the 20 colonies. Again, each colony received approximately 100 Africanized and 100 European drones from several different sources. These colonies were inspected as before.

The effects of the direction of colony entrances were minimal. Nearly equal proportions of drones migrated from colonies with north-facing and south-facing entrances ($\chi^2 = 1.6$, d.f. = 1), and nearly equal proportions of drones from colonies facing these two directions migrated to Africanized and European colonies ($\chi^2 = 2.15$, d.f. = 1). A larger proportion of drones in the second experiment migrated by day 12 (experiment 1, 33.4 percent; experiment 2, 40.6 percent; $\chi^2 = 9.15$, d.f. = 1; $P < 0.001$) probably because all but two colonies had at least one adjacent colony facing in the same direction.

The major effects of the geographical type of colony seen in the first experiment were seen also in the second. The large majority of drones that left their home colony entered European colonies ($\chi^2 = 80.1$; d.f. = 1; $P < 0.0001$), and a greater proportion of drones originally placed in Africanized colonies migrated ($\chi^2 = 60.7$; d.f. = 1; $P < 0.001$). In the second experiment, the proportions of Africanized and European drones that migrated were not significantly different ($\chi^2 = 0.21$, d.f. = 1).

The simple movement of drones between colonies is relatively unimportant (*16*) unless their presence or absence influences the reproductive potential of

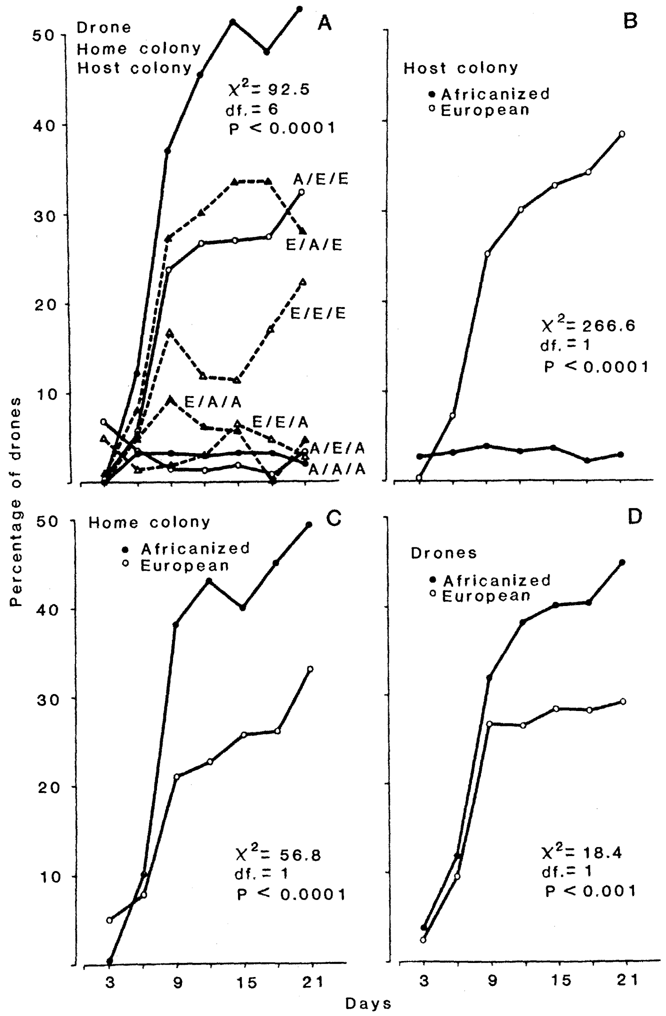

Fig. 1. The migration of adult drones (ages 3 to 21 days) among European and Africanized colonies in experiment 1. (A) The percentages of drones in four categories of drone type and home colony that migrated to other colonies (host colonies) of both types. A, Africanized; E, European; categories represent drone/home colony/host colony. (B) The percentages of all drones that migrated to European and Africanized host colonies. (C) The percentages of drones in Africanized and European colonies that migrated. (D) The percentages of Africanized and European drones that migrated. Numbers of drones in each category of drone type and home colony are between 229 and 361.

colonies. For the third experiment, we prepared an apiary of European bees in Baton Rouge, Louisiana, in order to estimate these possible influences. This apiary contained large colonies (50,000 to 60,000 bees) with laying queens, no immature drones, and three frames of empty drone comb. Adult drones were reared in other colonies and added to experimental colonies when they were 1 to 6 days of age in groups of 0, 500, 1000, or 2000. These four treatments were used with four, seven, seven, and eight colonies, respectively. Hive entrances were screened in order to confine drones to the hives but permit normal worker bee activity. On the 14th day after the apiary was prepared the number of immature drones being reared by each colony was counted.

A statistically significant inverse linear relationship ($b = -0.648 \pm 0.287$) between the number of drones reared by the colonies and the number of drones added to them was found (Fig 2). The estimated slope implies that an adult drone in a colony has a depressing value of about −0.65 drones on the regulatory system that governs the production of additional drones. It also suggests that the loss of an adult drone stimulates the additional production of 0.65 drones. The migration of a single drone results in a numerical reproductive advantage of about 1.3 drones for the parent colony when both factors are considered.

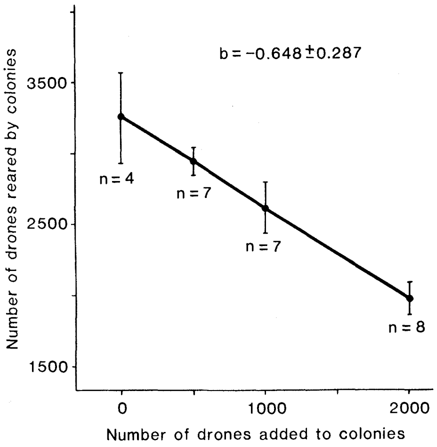

Fig. 2. The relationship between numbers of adult drones in colonies and the production of immature drones during 14 days. The slope and the mean (± standard error of the mean) are shown for the four points that are collinear.

Since Africanized colonies export half or more of their Africanized drones to European colonies and accept almost no foreign drones, they gain an important reproductive advantage. Minimally, Africanized colonies produce a disproportionately large share of a mixed drone population. This would enhance the positive assortive mating tendencies (*11*) of Africanized bees and impair those of European bees. Over several generations this condition alone would result in complete Africanization. Maximally, a few Africanized colonies could produce all or nearly all of the drones in a mating area if they produce large numbers of drones earlier in the season as certain of our observations suggest. In this case, complete Africanization would result in two to three generations. Where male reproductive parasitism rests on this continuum probably varies as Africanized populations grow. Nonetheless, throughout the process of Africanization, reproductive parasitism results in a strong mating advantage for Africanized colonies.

Knowledge of Africanized-bee reproductive parasitism suggests that drone-control technologies in commercial European mating areas can contribute to a continuing supply of commercial European bee stocks despite Africanization. Methods can be devised to exclude Africanized drones from European colonies and efforts made to destroy Africanized drones. The availability of such bee stocks will substantially reduce the costs of Africanization for American agriculture (*6*). Drone-control technologies might also be useful to slow the large-scale spread of Africanized bees although the expense of such an enterprise might be prohibitive.

THOMAS E. RINDERER
RICHARD L. HELLMICH, II
ROBERT G. DANKA
ANITA M. COLLINS
Agricultural Research Service, Bee Breeding and Stock Center Laboratory, Baton Rouge, Louisiana 70820

References and Notes

1. O. R. Taylor, *Bee World* **58**, 19 (1977).
2. F. Ruttner, *Proceedings of the 25th International Congress on Apiculture* (Apimondia, Bucharest, 1975), p. 325.
3. W. E. Kerr, *S. Afr. Bee J.* **39** (No. 1), 3 (1967).
4. E. Oertel, *Proc. La. Acad. Sci.* **9**, 71 (1945).
5. H. V. Daly and S. S. Balling, *J. Kans. Entomol. Soc.* **51**, 857 (1978).
6. A. M. Collins, T. E. Rinderer, J. R. Harbo, A. B. Bolten, *Science* **218**, 72 (1982).
7. C. D. Michener, *Annu. Rev. Entomol.* **20**, 399 (1975).
8. D. J. C. Fletcher, *ibid.* **23**, 151 (1978).
9. C. D. Michener (chairman), *Report of the Committee on the African Honey Bee* (National Technical Information Service, Springfield, Va., 1972), pp. 8–92.
10. T. E. Rinderer, A. M. Collins, K. W. Tucker, *J. Apic. Res.*, in press.
11. W. E. Kerr and D. Bueno, *Evolution* **24**, 145 (1970).
12. S. C. Jay, *J. Apic. Res.* **4**, 167 (1965); *ibid.* **5**, 103 (1966); *ibid.*, p. 137; *ibid.* **7**, 37 (1968).
13. C. G. Butler, *Bee World* **20**, 140 (1939); T. P. Livenetz, *Pchelovodstvo* **1**, 25 (1951).
14. The European bees were from North America. Such bees have mixed subspecies representatives in their ancestries. Africanized bees are descendants of *A. m. scutellata* imports and their hybrids with various subspecies representatives previously imported to Brazil. Neither the European nor Africanized bees in this study can correctly be called race, subspecies, stock, or line representatives. We use the term geographical type to indicate that the bees showed major characteristics typical of descriptions for temperately or tropically adapted bees.
15. All χ^2 values in Fig. 1 and in the text are calculated from numbers collected when the adult age of drones was 12 days. The same drones were seen repeatedly throughout the experiment which violates the additivity assumptions necessary to calculate sums of χ^2's for drones of different age groups and χ^2's for sums of drones from all age groups. Day 12 was chosen because it is the first day in the experiment after the drones were all old enough to fly. The χ^2's calculated for older drones are, in all cases, consistent with the values and conclusions presented.
16. In addition to direct reproductive advantages, Africanized colonies gain energetically since they do not feed many of the drones they produce.
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