that these may not be difficult to obtain, if a sensitive assay for detection of antibody in hybridoma supernatants is available. For example, Legrain et al. (11) obtained in BALB/c mice 17 monoclonal antibodies against a single BALB/c idotype, and in every case the idotype—anti-idotype reaction was specifically inhibited by antigen. Similar observations have been made by others (12, 13). It should be pointed out that the two monoclonal antibodies do not have to be raised in a single strain of mice. Instead, monoclonal antibody 2 could originate in mice of a different allotype, or even in another animal species, for example, in rats.

Some of the attractive features of the 4i-assy are shared with those of IRMA performed with monoclonal antibodies. For example, the antigen does not have to be purified or labeled, and most labeled antibodies have a long shelf life. Also, because the antibodies are products of hybridomas, they are homogeneous and can be obtained in unlimited amounts. However, most variants of commonly used immunoradiometric methods, such as the sandwich or two-site IRMA (14, 15), require a minimum of two separate epitopes, whereas the 4i-assy requires only a single epitope.

This unique characteristic may be particularly attractive when the antigen is a small polypeptide, or weakly immunogenic, or when one is attempting to identify a portion of an antigen associated with an epitope. For example, the epitope of Pb44 recognized by 3D11 seems to be involved in the interaction of sporozoites with their target cell (16), and in protective immunity against malaria (1, 2). The isolation of a fragment of Pb44 bearing this epitope could be important for the development of a malaria vaccine. This and similar undertakings may be greatly facilitated by the availability of a sensitive and epitope-oriented assay, such as the one described here.

From the point of view of those interested in malaria and other vector-transmitted diseases, the present results indicate that the 4i-assy is sensitive enough to detect a relatively small number of sporozoites in crude extracts of mosquitoes, and might therefore be used in epidemiological surveys.

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6. This antiserum was prepared by immunizing rabbits with 3D11 incorporated in Freund's adjuvant and absorbing their sera several times with normal BALB/c immunoglobulin immobilized on Sepharose 4B beads.
9. It is difficult to determine precisely the sensitivity of this immunoassay because we do not have an independent measurement of the concentration of Pb44. However, assuming that the dimensions of Pb44 are 3 by 10 by 10 nm, and that it is positioned with the long axis perpendicular to the surface of the parasite forming a packed monolayer, we calculate that a single sporozoite (cylinder with surface area of 3.5 x 10^6 m^2) could have a maximum of 3.9 x 10^6 molecules of Pb44 on its surface. If ten times as many molecules bearing the same epitope are present inside the parasite, the total number is 4.2 x 10^6 molecules. We find that the assay consistently detects the contents of 100 parasites in a volume of 15 μl. Therefore, if our assumptions are correct, the sensitivity of this 4i-assy is about 200 pmole of Pb44 per liter.
17. This investigation received financial support from the NIDF/World Bank/WHO Special Programme for Research and Training in Tropical Medicine, from the Rockefeller Foundation, and from the Agency for International Development.
18. To whom reprint requests should be addressed.
has occurred (10). Two independent lines of evidence support the assumption that underground consorts represent copulations. First, the date of weaning varied directly with the mother’s date of estrus and underground consortship in 1978, 1979, and 1980 (P < .001 for each year; Kendall rank correlation test) (10). Second, paternities determined from an electrophoretic analysis of blood proteins agree closely with those inferred from behavioral observations (8, 10).

Black-tails, like other sciurid rodents (11), separate by sex before the age of first breeding (Fig. 1). Females usually remain in the natal coterie for life, but males usually depart 12 to 14 months after weaning. One result is that adult females have little opportunity to breed with sons, nephews, or cousins.

A young black-tail male attempts to acquire a breeding coterie, and, if successful, remains there until he dies, is evicted by an invading male, or departs, apparently, on his own initiative. Since males regularly live to be 4 to 5 years old (12), an older male may sometimes live in the same coterie with his 2-year-old daughter. If the avoidance of extreme inbreeding is important, then an adult male should not remain in the same breeding coterie for more than two consecutive years. In 87 of 92 (94.6 percent) observed residencies, an adult male remained in the same coterie for only 1 or 2 years, precluding father-daughter inbreeding. Of the nine adult males that changed coteries after the second breeding year (13), seven would have been in the same coterie with breeding daughters in the third year if they had not transferred to another coterie in the colony. By contrast, of the five adult males (5.4 percent) that remained in the same breeding coterie for three consecutive years (Table 1), four did not have any breeding daughters at home in the third year; the fifth male had two 2-year-old daughters in his coterie in the third year, but father-daughter inbreeding was behaviorally avoided. The findings of Table 1 suggest that the dispersal of older males may be an evolutionary response to the possibility of father-daughter inbreeding.

A black-tail female sometimes first breeds as a yearling. If the avoidance of extreme inbreeding is important, then a yearling female should only breed if her father is no longer in her natal coterie. Yearling females whose fathers were not in the natal coterie were more likely to come into estrus and copulate than were yearling females whose fathers were still in the natal coterie (Table 2) (14). This difference was evidently unrelated to weight: yearling females that copulated were neither lighter nor heavier than were yearling females that did not (P > .100, Mann-Whitney U tests: 1978, U (6, 4) = 16; 1979, U (12, 1) = 1.5; and 1980, U (6, 7) = 29).

In 9 of 94 cases (9.6 percent) of observed estrus, breeding close genetic relatives of the opposite sex were not separated by male dispersal. In cases one and two, the female copulated with her male relative but also with at least one other male from a different coterie who invaded the female’s home coterie on her day of estrus; in one of these cases a yearling female copulated with her father (≥ 4 years), and the other involved a 4-year-old female and her 2-year-old half-brother from the same mother (15). In cases three, four, and five, the home coterie contained two adult males (6), and the estrous female avoided her male relative and copulated exclusively with the unrelated male; these cases involved a 3-year-old female and her yearling nephew (16), a 5-year-old female and her 2-year-old son, and a yearling female and her 3-year-old father. In case six, a 2-year-old female avoided copulating with her father (≥ 4 years) by leaving her home coterie on the afternoon of her estrus and copulating exclusively with a male in an adjacent coterie before returning home (17). In case seven, a 3-year-old male died or dispersed the day before his mother (≥ 6 years) came into estrus, and he was never seen again; none of the other 88 adult males in 1978 through 1980 disappeared during the breeding season. In case eight, a female (≥ 5 years) whose 2-year-old son was the only adult male in her coterie (18) failed to come into estrus. She was only the second of 69 adult females observed in 1979 and 1980 that showed no estrus (19, 20); the other was a female (≥ 6 years) in poor physical condition during the breeding season who disappeared and presumably died shortly thereafter (21). In case nine, a 2-year-old female was in a coterie in 1979 where the only male was her 2-year-old brother or half-brother (22); this female copulated in both 1978 and 1980 but, even though she showed several signs of estrus (9, 10) in 1979, she was the only one of 94 estrous females that evidently did not copulate.

Black-tails thus avoid extreme inbreeding in the four specific ways described above, and these can be reduced.
to two general mechanisms: male dispersal and reluctance of females to copulate with male relatives. Existence of these mechanisms is easily understood if, as is the case with many plants and animals (23), inbreeding commonly leads to genetically inferior offspring. I have few data bearing on this issue. Of the two females that copulated with a male relative, one probably never gave birth and offspring of the other were found dead aboveground shortly after weaning. These two cases are inconclusive, since mortality is also high among young of outbred litters (6).

If prairie dogs avoid extreme inbreeding, then the frequency of heterozygotes at polymorphic loci should be higher than that expected under conditions of Hardy-Weinberg equilibrium (24). At the four polymorphic loci examined, Foltz and I found (25) that, as predicted, there was a consistent excess of heterozygotes in 1978, 1979, and 1980.

Behavioral and physiological avoidance of copulation with male relatives in the home coterie (a kind of female choice) is probably an evolved mechanism of outbreeding. Male dispersal patterns may also have evolved primarily to promote outbreeding. However, it is also possible that male dispersal patterns are secondary consequences of female choice (26): why should a male remain in a coterie if his female relatives there are unlikely to mate with him?

Numerous investigators have demonstrated one or two mechanisms by which individuals avoid inbreeding (4), but single mechanisms of outbreeding usually have alternative explanations (4, 2). Alternative explanations become less parsimonious when several different mechanisms all suggest the same conclusion. Four mechanisms are described for prairie dogs; except possibly for humans (5), so many mechanisms have not previously been implicated in the maintenance of outbreeding.

Even when individuals avoid mating with close genetic relatives such as parents, offspring, and siblings, inbreeding coefficients can be high if populations are small and isolated or if individuals regularly mate with more distant relatives such as nieces, nephews, and first cousins (1, 27). Black-tail colonies are usually large and there is regular immigration of males (6, 25) (Fig. 1). Whether individuals avoid mating with their more distant genetic relatives is not yet known.

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11. These behaviors include (i) a mating call by the male, (ii) the estrous female aboveground 10 to 30 minutes away that has not yet been visited, and (iii) postcopulatory behavior. These behaviors may also be associated with rare aboveground copulations (6). Each female enters once on one day only each year.


14. In 1978, for example, 16 of 25 (64.0 percent) adult males of known age at the study colony were 4 years old; in 1979, 5 of 28 (17.9 percent) adult males of known age were ≥ 5 years old.

15. Adult males that disappeared after 2 years in the same female were probably dead rather than dispersed; therefore, I did not use data from these males in Table 1.

16. Since adult females sire most of the offspring born into their coterie during his residency (6, 8, 10), I assumed that the adult male in a female's native colony in her year of birth was her father; for all 15 of the 28 cases in Table 2 for which the critical blood samples were available, the samples supported this assumption.

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Inner Ear: Dye Injection Reveals Peripheral Origins of Specific Sensitivities

Abstract. In the American bullfrog (Rana catesbeiana) tracing of functionally identified, dye-filled fibers of the eighth cranial nerve to their peripheral origins has provided the first precise functional overalays for the microstructural maps of inner ear sensory surfaces.

The inner ear of the frog comprises eight sensory surfaces and the various structures accompanying each of them (1). Certain features of those structures suggest the general class of sensitivity associated with each surface: the semicircular canal accompanying each of the three cristae implies sensitivity to rotational motion about a particular axis; the calciferous mass accompanying each of the three maculae implies sensitivity to gravity or to linear motion; and the intimate connections between the chambers of the basilar and amphibian papillae and the sound-conducting apparatus of the middle ear imply auditory sensitivity. These implications have been supported by electrophysiological and behavioral experiments of earlier investigators (Table 1).

None of the eight sensory surfaces is topographically uniform. For example, each macula (utricular, saccular, and lagena) comprises two fields (a central field surrounded by a peripheral field) with distinctly different receptor cells (hair cells) (2). On the utricular and lagena maculae, the central fields are thin
Editor's Summary

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**Prairie Dogs Avoid Extreme Inbreeding**
JOHN L. HOOGLAND (March 26, 1982)