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 idiotype, and in every case the idiotype–anti-idiotype 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-assay 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-assay 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-assay 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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Prairie Dogs Avoid Extreme Inbreeding

Abstract. *Black-tailed prairie dogs (Rodentia: Sciuridae:* Cynomys ludovicianus*) live in colonies composed of contiguous but separate family groups called coteries. During the 6 years that individuals in a colony were observed, they almost never mated with close genetic relatives. Inbreeding is avoided in four ways: (i) a young male usually leaves his natal coterie before breeding, but his female relatives remain; (ii) an adult male usually leaves his breeding coterie before his daughters mature; (iii) a young female is less likely to come into estrus if her father is in her coterie; and (iv) an estrous female behaviorally avoids mating with a father, son, or brother in her coterie.*

Although inbreeding may sometimes be advantageous (*1, 2*), biologists generally assume that most organisms outbreed (*3*). The reasons, extent, and mechanisms of outbreeding, however, are rarely understood (*4*). Particularly in social species, the details of how and to what extent inbreeding is avoided may provide insights into significant biological and social questions (*5*). To acquire extensive knowledge of exact genetic relationships among social individuals requires long-term field studies. From a 6-year study of 200 marked individuals under natural conditions, I report four mechanisms of outbreeding in a highly social species, the black-tailed prairie dog (Sciuridae: *Cynomys ludovicianus*).

Black-tails are large, diurnal rodents. At Wind Cave National Park, Hot Springs, South Dakota, where I study them, they mate in February and March, and weaned juveniles first emerge from their natal burrows in May and June. The area of my study colony is about 500 m by 130 m (6.6 hectares) and in late spring of each year contains 142.9 ± 33.6 (mean ± standard deviation) yearlings and adults (≥ 2 years) and 72.4 ± 36.9 juveniles arranged into 24.2 ± 1.47 family groups called coteries. A coterie typically contains one adult male, three to four adult females, and several yearlings and juveniles of both sexes. Males and females first breed as 2 year olds, although females occasionally breed as yearlings. Individuals remain within well-defined contiguous territories and are usually amicable toward members of their own coterie and hostile toward members of other coteries (*6*).

From 1975 through 1980, all young were marked with ear tags and fur dye before they mixed with young from other litters (*7*), so that exact genetic relationships through common female ancestors and probable genetic relationships through common male ancestors are now known for more than 90 percent of all colony residents (*8*). With three to six field assistants in February and March 1978, 1979, and 1980, I observed the colony with binoculars from three towers (5 m) for most (≥ 99 percent) of the daylight hours when prairie dogs were aboveground; we observed 94 periods of estrus, involving 74 different females.

Black-tail copulations usually occur underground during daylight hours, and only estrous females enter a burrow with adult males. Because there are specific behaviors associated with what we term an underground consortship (*9*), we are often able to pinpoint when a copulation has occurred (*10*). Two independent lines of evidence support the assumption that underground consortships 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.

Table 1. Dispersal of older black-tail males versus the possibility of father-daughter inbreeding. Differences shown are significant at $P = .060$ (one-tailed Fisher exact probability test).

Adult male in same breeding coterie	Father-daughter inbreeding in third year	
	Possible	Not possible
Only 2 years	7	2
3 years	1	4

Table 2. The effect of the father's presence in the natal coterie on the probability of copulation by yearling females. Differences shown are statistically significant at $P = .021$ (one-tailed chi-square test, $\chi^2 = 4.11$, d.f. = 1).

Copulation by yearling female	Father's status in coterie	
	Present	Departed
Does	2	13
Does not	26	37

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

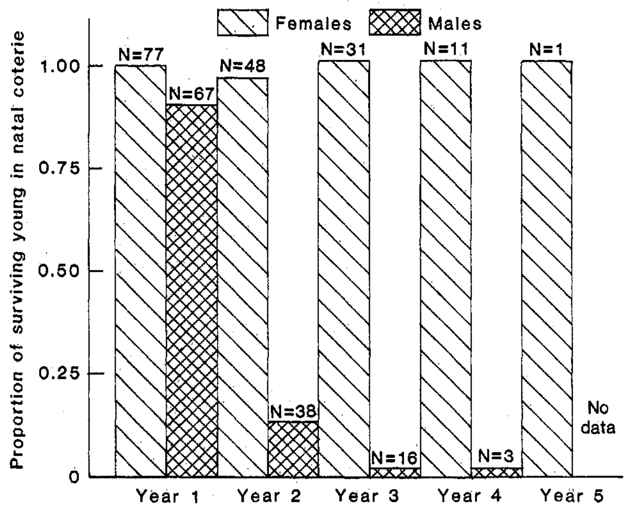

Fig. 1. Dispersal patterns of male and female black-tails. From 1975 through 1979, all 298 young (146 males, 152 females) from 107 litters were marked and their movements were followed. Numbers above each bar indicate the number of known survivors at the end of the indicated year. At the end of year 1, an individual had been weaned for 12 months; at the end of year 2, an individual had been weaned for 24 months; and so on. During the study period, a total of five females and ten males immigrated into the study colony and produced weaned offspring; all other females and males originated at the study colony. For each of the first 4 years, the sexual difference in dispersal was significant ($P \leq .008$, one-tailed chi-square test or one-tailed Fisher extract probability test).

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 (*1, 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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12. 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.
13. Adult males that disappeared after 2 years in the same breeding coterie may have died rather than dispersed; therefore, I did not use data from these males in Table 1.
14. Since the adult male sires most of the offspring born into his coterie during his residency (*6, 8, 10*), I assumed that the adult male in a female's natal coterie 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.
15. Of the other 92 estrous females, 20 (21.7 percent) copulated with a second adult male from a different coterie.
16. This nephew was the only male at the study colony known to breed as a yearling.
17. In cases one through six, the unrelated male chosen by the estrous female was not consistently either older or heavier than the available male relative.
18. The male in case eight moved to an adjacent coterie in his first year and then returned to his natal coterie as a 2 year old; no other male returned to his natal coterie after dispersing as a yearling.
19. Although the female in case eight weaned litters in the two previous years, she showed no sign of pregnancy (*6, 10*) when her 2-year-old son was back in her coterie, indicating that we did not simply fail to detect a subtle estrus.
20. Field assistants and I failed to detect several estrous periods in 1978, our first year of breeding observations; therefore, 1978 data are not included here.
21. This female weighed 860 g on 28 May 1979 when she was lactating and only 418 g on 7 May 1980, just before she disappeared, when she was not lactating.
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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 overlays 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 lagenar) comprises two fields (a central field surrounded by a peripheral field) with distinctly different receptor cells (hair cells) (*2*). On the utricular and lagenar maculae, the central fields are thin

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