which in turn oscillated the eyeball at about 4° per second over a range of 16° (Fig. 1). All other procedures were identical to those used on the other paralyzed kittens.

Figure 1g shows the results of this experiment. As in the kittens that were not paralyzed, binocular connections are clearly reduced: binocularly activated cells comprise 44 percent of the total. Therefore a central influence of gallamine triethiodide cannot be of primary importance in preventing the deprivation effect. Rather, the effect seems to depend upon activation of pathways associated with movement of the eye. It is possible that this activation by itself could cause a breakdown of binocular connections. To determine the role of eye movement alone, we paralyzed two kittens and oscillated their right eyes for 12 hours with both eyes occluded. Subsequent study of cortical cells showed that a large proportion (72 percent) were binocularly activated (Fig. 1h). Eye movement alone is therefore not sufficient to cause cortical changes during short-term monocular occlusion.

In conclusion, we have shown that a brief period of monocular deprivation can produce substantial alterations of binocular connections in the visual cortex of anesthetized kittens. It should be possible to monitor these changes in individual neurons. The effect does not occur in a paralyzed kitten unless an eye is mechanically moved while the stimulus is presented. Eye movement and visual stimulation are necessary conditions for cortical changes, but neither is sufficient to induce such changes alone. It is intuitively obvious that visual stimulation should be required, but the additional requirement of eye movement suggests that indirect pathways are also involved. A relationship between extraocular muscle function and changes in ocular dominance has been proposed (*6*). The extraocular muscles of the cat have spiral endings responding to stretch (*7*), and afferent responses from these muscles have been reported from recordings in the cerebellum (*8*). In addition, it has been shown that lateral geniculate activity is modulated by oculomotor centers (*9*) and that electrical stimulation of motor branches of extraocular muscles evokes some response in visual cortex (*10*). Some of the pathways suggested by these studies could be involved in a gating system that controls the degree to which striate cortex is receptive to visual input.

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## Genetic Component of Bee Odor in Kin Recognition

Abstract. *The primitively social sweat bee,* Lasioglossum zephyrum, *blocks the entry into its nest of most conspecifics from other colonies. Laboratory inbreeding of these bees produced lines which showed a positive linear relationship between the coefficient of relationship of bees tested and how often they permitted non-nestmates to pass them. The most probable mechanism is a genetically determined odor coupled with a learned component by which guard bees discriminate between odors of close kin and other bees.*

The concept of altruism in sociobiological theory is usually based on the relatedness of individuals; for example, the "inclusive fitness" of a sterile individual can be enhanced if a close relative is prolific (*1*). Given the many examples of altruism in animal behavior, the question arises whether there are specific mechanisms to ensure that related individuals cooperate with one another. In a recent review Sherman states that, although there are many examples of recognition of nestmates or siblings in animals, "recognition of more versus less similar siblings has not been demonstrated in any creature" (*2*). I infer such discrimination, however, because of evidence (presented below) of a genetic component for chemical recognition of relatives in a primitively social sweat bee, *Lasioglossum zephyrum*, which permits bees to recognize degree of relatedness even though they have not previously met. This species lives in burrows in soil guarded by one or more individuals that commonly exclude natural enemies as well as conspecifics from other nests (*3*).

Earlier work with this bee demonstrated the probable existence of individual or group odors involved in mating, nest defense, and nest recognition (*4*–*6*). Particularly relevant are Bell's findings that, among bees killed by freezing, nonresidents elicited aggressive responses by guards more often than residents did (*7*). Nonresident bees were also rejected when only far-red light was used, presumably simulating darkness to the bees. Bell concluded that contact chemoreception or olfaction were the modalities of recognition.

Bees were raised in the laboratory year-round in artificial nests consisting of burrows in a layer of soil between two sheets of glass; the nest entrances consisted of plastic tubes 4 mm in diameter (*8*). The two family lines used for inbreeding were originally collected from field sites about one-half mile apart (1 mile = 1.6 km). Additional colonies were started with pupae collected at widely separated locations. The latter were used to measure recognition between distantly related bees. All bees lived in mixed soil from a single site, and were fed from a single stock of *Typha* pollen and honey water.

New laboratory colonies were started with callow (young) bees which generally do not meet adults before digging out of their cells in their second day; for this experiment they were removed from their cells within 24 hours of eclosion. They were put into new nests one at a time until a colony of six sister bees was established. Each bee was marked on its thorax with a drop of enamel, a different color for each.

Inbred lines were developed by first observing these new colonies until queen determination was made (*9*). These young queens readily mated in small cages (16 by 12 by 13 cm) containing males from the same parental colony (presumably brothers). After a single mating, the females were returned to their original nests. At least 5 days were allowed for a new colony to establish itself before any tests were made. These inbred lines did not seem more difficult to raise than others, nor did any obvious deleterious traits develop.

For the test, all the bees of a given colony were removed and placed individually into a series of different nest entrances with guard bees. A guard can effectively block the entrance to prevent an intruder from entering (*10*). Such introductions were made into nests that contained either sisters, aunts, nieces, first cousins, or distantly related bees. The guard in each case was expected to show a binary decision—either permitting the introduced bee to pass or actively preventing it from doing so (*11*). If the introduced bee voluntarily turned to leave or if the guard's response was ambiguous, the test was repeated.

In many cases more than one guard bee was present near the nest entrance. If an introduced bee passed the first bee, its interaction with the next bee in line was also recorded. If the first guard rejected the intruder, it would usually pursue it into an empty tube, thus also permitting the intruder to be reintroduced to interact with the next in line.

In all, bees of 14 different degrees of genealogical relationship were tested against one another. Eleven of these represented instances where the tested bees had not previously met or been in the same nest. The remaining three cases involved sister bees which, although they had not previously met, were in the same nest until removed from their cells. In laboratory colonies, adults occasionally open cells and antennate the brood. Such interactions could influence my results, at most, only for the three groups of sisters.

The results are summarized in Fig. 1. To calculate $r$, the average coefficient of relationship, two basic presumptions were necessary: (i) the original collected bees were outbreeding, and (ii) the males used for mating were queen rather than worker produced (*12*). Error due to failure of either assumption would result, respectively, in an under- or overestimate of the values calculated for $r$, but would not alter the overall conclusions. The data points in Fig. 1 show the percentage of introduced bees that passed guards, summed over all nests tested for each relationship. The data for the two family lines were pooled since their separate regression coefficients were not significantly different ($P$ = .12).

No significant difference was found between the first and subsequent guard bees' reactions to a single intruder ($\chi^2$ = .05, $n$ = 1095), nor did a comparison of the first time an intruder was used with the last time show any significant change in guard behavior ($\chi^2$ = .06, $n$ = 568). It was not uncommon to have one or more bees from one nest pass a guard bee while one or more of her sisters could not pass the same guard.

The acceptance rate for sisters where $r$ = .75, .80, and .86 was 70.2, 81.1, and 94.6 percent, respectively. Thus, although the sister data may be confounded by the fact that sisters shared nests as brood, a contingency table analysis gives a $\chi^2 = 17.8$, with $P < .001$, indicating that even for sister data there is good evidence for the presence of a genetic component.

Has an analogous recognition system been described for another species? In *Drosophila* there is both behavioral and chemical proof of a chemical recognition system which is most directly involved with mating phenomena such as the "rare male" effect, in which males with uncommon odors are preferred by the females (*13*).

Colonies of *L. zephyrum* can be started with pupae or callows of diverse genetic background; nestmates apparently learn each other's odors early in their lives. Kukuk *et al.* (*5*) have suggested that guarding bees learn (that is, habituate) the odors of nestmates, thereafter permitting them to leave and enter the nest. However, the unfamiliar odor of an intruder elicits the guarding response. The genetic component is therefore in odor production and apparently not in the perceptual system. In a natural situation, nestmates are likely to be close kin while intruders would not be. But, as my data illustrate, closely related but nonresident bees should be allowed in because of odor similarities.

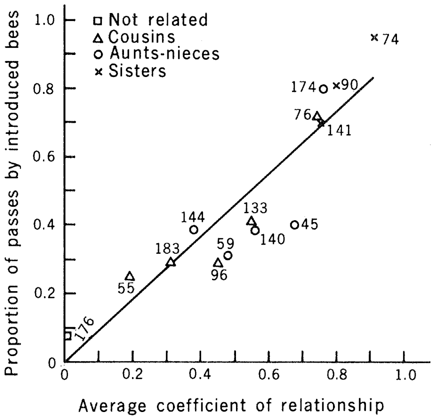

Fig. 1. Linear regression of frequencies with which introduced bees passed guarding bees on the average coefficient of relationship of the tested bees. Each data point was weighted by the number of interactions for that relationship, shown next to each point. A total of 593 introduced bees was used to obtain the 1586 interactions shown. The regression line is described by the equation $Y = 0.07 + 0.924\ X$ and the Pearson product-moment correlation coefficient for the data = .93. The regression coefficient is significant at $P \ll .001$. The data points were also analyzed with a nonparametric test, Kendall's coefficient of rank correlation, and found to be significant at $P < .001$. If one assumes worker-produced males and therefore cousin matings, the regression equation becomes $Y = 0.02 + 0.941\ X$, significant at $P \ll .001$. If the sister data are excluded from the regression analysis, the equation is $Y = 0.03 + 0.821\ X$, signficant at $P < .001$. For the latter, Kendall's coefficient of rank correlation is significant at $P < .001$. The data points show a better fit to a power function, but there are not enough points to show that it is significantly better.

How could a chemical recognition system of this type evolve? The evolution of the appropriate effectors and receptors has to be explained. It seems probable that many insects (and other animals) have distinctive odor mixtures produced by glands or simply as metabolic byproducts. In a social context, these odors could then be one way in which animals differentiate individuals. *Lasioglossum zephyrum* will attack vigorously any object with the odor of a mutillid parasite on it (*14*); thus one possibility is that the receptor systems which originally evolved to detect odors of other species (and particularly parasites) were a preadaptation for kin recognition within the species.

The inclusive fitness of a nonreproductive worker in a social insect colony is enhanced if it is closely related to the egg layer. In highly social insects, such as honey bees or ants where most evidence indicates environmentally determined nest odors (*15*), the queens are physiologically distinct from workers, with caste determination occurring in larval stages. In the primitively social sweat bee, *L. zephyrum*, caste determination occurs in the adult stage—that is, probably any of the workers can become queens (*3*). Therefore, joiners from other colonies could potentially become egg layers. If these joiners were not closely related to the workers, the workers' inclusive fitnesses would diminish and consequently there should be selection for any basis of discrimination that makes their acceptance less likely. In contrast, joiners that are close kin should be permitted to enter since they would add to the productivity of the colony (*16*), and any future offspring would pose little threat to the inclusive fitnesses of the acceptors. Acceptance of joiners would be beneficial in establishing new aggregations in situations where single foundresses might be subject to high predation. Also genetic homogeneity among workers may possibly reduce friction over caste roles and thereby increase overall nest productivity. These findings lend credence to the concept of the parasocial route to evolution of sociality in bees, which proposes that in early stages adults (perhaps related) may aggregate to cooperatively care for brood. In showing some inbred cousins ($r = .74$) with acceptance rates as high as those for outbred sisters ($r = .75$), my results suggest such aggregate units could arise in the context of locally inbreeding colonies of solitary bees.

Finally, field observations suggest that there may be significant inbreeding in local populations of *L. zephyrum* (*17*). One large and isolated population of *L. zephyrum* discovered by Batra almost 20 years ago is still prospering in the same general vicinity (*13*). Such populations would have enlarged family units, perhaps of benefit to such a system. For example, homogeneity of odors in a population could increase the net productivity because of reduced worker agonism. But the odors of parasites should be more easily detected as being foreign. Other explanations of the significance of the odors are possible and may be discovered by field studies.

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### References and Notes

1. E. O. Wilson, *Sociobiology* (Belknap, Cambridge, Mass., 1975) defines "altruism" as "self-destructive behavior performed for the benefit of others." "Inclusive fitness" is defined as "the sum of an individual's own fitness plus all its influence on fitness in its relatives other than direct descendants; hence the total effect of kin selection with reference to an individual." Much of the original theoretical work has been done by W. D. Hamilton [*J. Theor. Biol.* **7** (No. 1), 1 (1964); *Ann. Rev. Ecol. Syst.* **3**, 193 (1972)].
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6. W. J. Bell and W. A. Hawkins, *J. Comp. Physiol.* **93**, 183 (1974).
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8. The basic rearing techniques were described by C. D. Michener and D. J. Brothers [*J. Kans. Entomol. Soc.* **44**, 236 (1971)]. E. M. Barrows described laboratory matings [thesis, University of Kansas, Lawrence (1975)]. I improved the rearing techniques to keep the bees active through the winter and to selectively mate the young queens with given males.
9. Several sequences of queen-backing and worker-following was the criterion. See D. J. Brothers and C. D. Michener [*J. Comp. Physiol.* **90**, 129 (1974)] for a description of behaviors.
10. A description of guard bee behavior toward intruders was given by Bell and Hawkins (*6*).
11. Although I have not analyzed the extent of graded responses, they were clearly evident. For example, the response to a distant relative was usually much stronger than to a close relative even when both intruders were blocked.
12. "Coefficient of relationship" is defined by E. O. Wilson [in (*1*)] as "the fraction of genes identical by descent between two individuals." A clear statement of the rules used for calculating these coefficients is presented by J. F. Crow and M. Kimura [*An Introduction to Population Genetics Theory* (Harper & Row, New York, 1970), pp. 68–73].
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15. E. O. Wilson, *The Insect Societies* (Belknap, Cambridge, Mass., 1971), pp. 272–277.
16. See discussion in C. D. Michener, *The Social Behavior of the Bees* (Belknap, Cambridge, Mass. 1974), pp. 241–253. Theories based on inclusive fitness usually assume queen monogamy; otherwise the relationships between individuals are diminished. E. M. Barrows [*Behav. Biol.* **15**, 57 (1975)] states that *L. zephyrum* usually mates only once in a given day. My own work in mating bees tends to confirm that most queens mate only once; in fact, if a mating pair is approached by a second male, the female invariably starts to struggle to break away from the males. Inbreeding in an aggregation would reduce the necessity for queen monogamy.
17. A comparison of adults collected as pupae from distant sites showed a significantly greater acceptance rate for non-nestmates within a site than between the sites ($\chi^2 = 6.1$, $P = .987$, $n = 102$). These data could be influenced by the fact that the pupae were raised in different soil. However, laboratory tests where pupae were raised in different soil did not give significant results [L. Greenberg, thesis, University of Kansas (1979)]. Furthermore, within a small aggregation of bees, separated by less than 15 cm, I found high acceptance rates between some of the neighboring colonies, indicating that at least some neighboring colonies were probably closely related.
18. I thank G. Buckle, B. Smith, C. Gilbert, and P. Decelles for assistance in various aspects of this project; and C. D. Michener, W. J. Bell, and R. Jander for critical review of this manuscript. Partially supported by NSF grants BNS 78-07707 and BNS 75-07654 (C. D. Michener and W. J. Bell, principal investigators). Contribution 1730 from the Department of Entomology, University of Kansas, Lawrence.

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## Prenatal Stress Reduces Fertility and Fecundity in Female Offspring

Abstract. *Female rats subjected to prenatal stress later experienced fewer conceptions, more spontaneous abortions and vaginal hemorrhaging, longer pregnancies, and fewer viable young than nonstressed rats. The offspring of the prenatally stressed rats were lighter in weight and less likely to survive the neonatal period. Prenatal stress may influence the balance of adrenal and gonadal hormones during a critical stage of fetal hypothalamic differentiation, thereby producing a variety of reproductive dysfunctions in adulthood.*

Severe behavioral and physiological stress during gestation, such as conditioned anxiety, crowding, immobilization, and temperature extremes, permanently modify structural or functional development of offspring in rats. Under certain conditions these stresses produce such physical abnormalities as cleft palate and harelip in mice or aberrant sexual behavior in male rats (*1, 2*). Prenatal influences on the offspring are believed to be mediated by a maternal response involving stress hormones such as epinephrine and corticosteroids from the adrenal glands and adrenocorticotropic hormone (ACTH) from the anterior pituitary gland (*1*). Thus, little doubt remains that the form and structure of the body as well as later behavior can be modified, sometimes adversely, by disadvantageous environmental conditions before birth.

Interest in prenatal stress influences on reproduction has been stimulated by Ward's discovery that stress during pregnancy feminizes and demasculinizes the sexual behavior of male offspring (*2*). Evidence suggests that prenatal stress may modify the neuroanatomical and biochemical organization of the brains of both males and females and turn the direction of male fetal brain development toward that of the female sex (*3, 4*). In female offspring prenatal stress increased concentrations of the neurotransmitter dopamine in the hypothalamic arcuate nucleus of adults (*4*). Because marked alterations in arcuate dopamine have been associated with abnormalities in the release of gonadotropic hormone from the anterior pituitary gland, we predicted that prenatal stress would disrupt estrous cycling in female offspring (*5*). I now report that prenatal stress affects other reproductive capabilities of female offspring.

Table 1. Fertility of prenatally stressed and nonstressed female offspring. Data given in percentages.

| Females | *N* | Not pregnant | Interrupted pregnancy | Births |
|---|---|---|---|---|
| Prenatally stressed | 93 | 34* | 31† | 35† |
| Nonstressed | 72 | 16 | 10 | 74 |

*$P < .05$. †$P < .001$.

In one experiment, 36 primiparous, pregnant Sprague-Dawley rats weighing about 250 g were obtained from Zivic-Miller (Allison Park, Pennsylvania) 1 week before they were subjected to stress. They were housed individually in 24 by 32 by 16 cm Fiberglas observation cages with San-i-cel bedding under a standard 12-hour light-dark cycle with lights on at 8:00 a.m., and they were maintained on a freely accessible diet of Purina chow and water. On days 14 through 22 of gestation (day 1 being the day of mating), 18 randomly selected females were subjected to the stress of heat, restraint, and bright light. Stress was applied by placing each animal in

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