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.

LES GREENBERG
Department of Entomology, University of Kansas, Lawrence 66045

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)].
2. P. W. Sherman, *Am. Nat.* **113** (No. 6), 925 (1979).
3. S. W. T. Batra, *Insectes Soc.* **11**, 159 (1964); *Univ. Kans. Sci. Bull.* **46**, 359 (1966).
4. E. M. Barrows, *Behav. Biol.* **15**, 57 (1975); ———, W. J. Bell, C. D. Michener, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2824 (1975); W. J. Bell, M. D. Breed, K. W. Richards, C. D. Michener, *J. Comp. Physol.* **93**, 173 (1974).
5. P. F. Kukuk, M. D. Breed, A. Sobti, W. J. Bell, *Behav. Ecol. Sociobiol.* **2**, 319 (1977).
6. W. J. Bell and W. A. Hawkins, *J. Comp. Physiol.* **93**, 183 (1974).
7. W. J. Bell, *ibid.*, p. 195.
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].
13. W. W. Averhoff and R. H. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 591 (1976).
14. S. W. T. Batra, *J. Kans. Entomol. Soc.* **38**, 367 (1965).
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 (χ^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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Table 2. Gestation length (median days ± range) and fecundity in prenatally stressed and nonstressed female offspring. Data are expressed in means ± standard error.

Females	N	Length of gestation (days)	Litter		Dead pups (N)	
			N	Weight (g)	Term	Day 10
Prenatally stressed	33	24 ± 3*	9.7 ± 0.1†	6.6 ± 0.2†	1.0 ± 0.1*	2.8 ± 0.1‡
Nonstressed	22	22 ± 1	11.7 ± 0.8	7.4 ± 0.1	0.6 ± 0.1	1.2 ± 0.1

*$P < .01$. †$P < .05$. ‡$P < .001$.

an 18 by 8 cm semicircular Plexiglas cage under four incandescent lights which produced a surface illumination of 4280 lm/m² and a surface temperature of 34°C. Three 45-minute stress periods beginning at 10:00 a.m. daily were alternated with 45-minute rest periods in the home cage. Eighteen control females remained unhandled in the home cage.

The pregnant females were observed daily to determine the day of birth of their offspring. On day 21 after birth, prenatally stressed and nonstressed offspring were weaned, segregated by sex, and housed two per cage. At 60 days of age, the animals were housed individually. Starting at 90 days of age, 93 prenatally stressed and 72 nonstressed randomly selected females weighing approximately 250 g were examined by vaginal smear daily for stages of the estrous cycle. About 2 weeks later, after confirmation of cyclic regularity, females in proestrus were placed in the home cages of stud males for mating and were removed 2 hours later. They were examined immediately by vaginal smear for the presence of sperm. For the following 15 days they were examined for confirmation of pregnancy, as evidenced by persistent vaginal diestrus and abdominal palpation, or for premature termination of pregnancy, as indicated by vaginal hemorrhage or spontaneous abortions. The uteri of females with interrupted pregnancies were examined on day 22 of pregnancy, the expected day of birth. For 33 prenatally stressed and 22 nonstressed females selected at random, the numbers of viable and of dead offspring and the weight of living pups were recorded on the day of birth. For the first 20 days postpartum, observations were made daily at 10:00 a.m. and 2:00 p.m. for the incidence of lactation (the percentage of pups with abdomens distended by milk) and for the frequency of nursing behavior (female crouching over young).

More than twice the percentage of prenatally stressed females failed to become pregnant compared with the nonstressed control group ($\chi^2 = 5.65$, $P < .05$) (Table 1). In at least 19 of the 32 prenatally stressed rats that did not become pregnant, irregularities in estrous cycling were noted, including prolongation of the estrous-metestrous phase and possible pseudopregnancy, as suggested by persistent vaginal diestrus. Approximately three times the percentage of prenatally stressed females failed to maintain pregnancy compared with the nonstressed group ($\chi^2 = 16.42$, $P < .001$). Eleven prenatally stressed females with interrupted pregnancies showed vaginal hemorrhaging during the first trimester, and seven spontaneously aborted in the last trimester. Upon autopsy, three prenatally stressed females showed decaying fetal matter in the uterine horns; eight others had uterine implantation sites but no fetuses. By contrast, only one prenatally nonstressed female spontaneously aborted on or about day 11; the few nonstressed animals that had interrupted pregnancies showed indications of vaginal hemorrhage during the first trimester. Thus, more than twice as many nonstressed females gave birth to offspring compared to the prenatally stressed group ($P < .001$).

Table 2 shows that gestation was significantly prolonged in the prenatally stressed group that maintained pregnancy compared with the controls (median test, $\chi^2 = 8.86$, $P < .01$). The mean number of live offspring born to prenatally stressed females was lower than that in the nonstressed group ($t = 2.31$, $P < .05$), as was the mean body weight per litter ($t = 3.08$, $P < .05$). In addition, the mean number of dead offspring was significantly greater in the prenatally stressed group not only at term ($t = 2.87$, $P < .01$) but also at day 10 postpartum ($t = 11.5$, $P < .001$).

The prenatally stressed females lost a significantly greater percentage of litters by stillbirths or neonatal mortalities than did nonstressed females ($\chi^2 = 16.39$, $P < .001$) (Table 3). Only 49 percent of the litters born to prenatally stressed females survived by day 10 postpartum in contrast to a 100 percent survival rate of litters born to nonstressed animals ($\chi^2 = 14.07$, $P < .001$). The incidence of lactation among prenatally stressed females was low during the first few days postpartum: only 26 to 40 percent of their offspring received milk compared with almost 100 percent of the offspring in the nonstressed condition. Nevertheless, almost all of the mothers, regardless of prenatal treatment, engaged in nursing behavior. As expected, with the increase in neonatal mortality, the percentage of prenatally stressed females exhibiting nursing behavior declined considerably by day 10 postpartum. Therefore lactational dysfunction rather than a decrease in maternal attention appeared to be the primary cause of neonatal mortality.

Table 3. Percentage of litters with stillbirths, neonatal deaths, and survivors of prenatally stressed and nonstressed female offspring. $P < .001$, all groups.

Females	N	Stillbirths	Neonatal deaths	Survivors
Prenatally stressed	33	15	36*	49*
Nonstressed	22	0	0	100

*By day 10 postpartum.

The mechanism whereby prenatal stress disrupts reproductive activities in female offspring is not known. Cross-fostering was not used in the present experiment, and it is possible that the prepartal stress-induced disturbances in the behavior or lactational performance of the mother during the postnatal period were the primary causes of the reproductive deficits in the offspring. This possibility appears unlikely, however, because in previous studies, prepartally stressed mothers did not differ significantly from nonstressed mothers in latency of litter retrievals or the duration of nursing behavior regardless of whether they were rearing prenatally stressed or nonstressed offspring (*5, 6*).

Nevertheless, a second experiment was conducted in which all procedures were similar to those in the first experiment with the exception that cross-fostering was used between and within treatment groups. The 2 by 2 experimental design yielded the following treatment groups with their respective sample size: (i and ii) prenatally stressed females reared by prepartally stressed (SS) or nonstressed mothers (SN) ($N = 23$ and 16), and (iii and iv) nonstressed females reared by prepartally stressed (NS) or nonstressed mothers (NN) ($N = 11$ and 18). Within these four groups, the number of females not becoming pregnant after exposure to stud males was 9, 7, 3, and 4, respectively. The number of females with interrupted pregnancies was 8, 5, 1, and 0, whereas the number giving birth was 6, 4, 7, and 14. These differences were significant ($\chi^2 = 14.54$,

$P < .01$), as were the following comparisons: SS versus NS, SS versus NN, SN versus NS, and SN versus NN ($\chi^2 = 4.21$, 11.2, 4.49, and 17.67, respectively; $P < .05$). Median gestation length in days (± range) was 24 ± 3, 23 ± 2, 22 ± 0, and 22 ± 0, respectively. The mean number of offspring per treatment (± standard error) was 8.2 ± 0.8, 8.0 ± 1.1, 11.7 ± 0.7, and 11.4 ± 0.4, respectively. The mean weight per pup in grams (± S.E.) was 5.8 ± 0.3, 5.5 ± 0.6, 7.6 ± 0.3, and 7.8 ± 0.2. The latter differences were significant ($F = 8.83$ and 11.40; $P < .01$), as were the following paired treatment means according to the Scheffé test: SS versus NS, SS versus NN, SN versus NS, and SN versus NN ($P < .05$).

Of the total number of litters born (6, 4, 7, and 14), the number of litters with neonatal deaths by day 10 postpartum was 4, 3, 0, and 0, respectively; the number that survived virtually intact was 1, 0, 7, and 14, respectively. Overall differences between the number of litters with neonatal deaths and the number surviving by postpartum day 10 were significant ($\chi^2 = 19.15$ and 26.94, respectively; $P < .001$), as were the following comparisons: SS versus NS, SS versus NN, SN versus NS, and SN versus NN ($\chi^2 = 6.74$, 11.67, 7.22, and 12.60, respectively, and 9.48, 15.56, 11.0, and 18.0; $P < .01$). Thus, in the cross-fostering experiment, the prenatally stressed groups differed from the nonstressed groups independent of rearing condition. Prenatal stress therefore seems to affect later reproduction not by disrupting postnatal rearing conditions but by altering the fetus, possibly by changing the hormonal milieu.

Under severe environmental stress, sexual differentiation in some mammalian species is believed to take place in the presence of large amounts of steroids, some of which are secreted by the adrenal glands (*7*). Disturbances in gonadal and adrenal hormones during perinatal sexual differentiation can disrupt reproduction in female offspring by decreasing sexual receptivity or by inducing gonadotropic or ovarian irregularities or by both means (*8*). Prenatal stress therefore may influence the exchange of gonadal and adrenal hormones between the mother and fetus or the balance of these hormones in the fetus alone during a critical stage of hypothalamic differentiation, thereby producing reproductive dysfunctions in adulthood.

LORRAINE ROTH HERRENKOHL
Department of Psychology,
Temple University,
Philadelphia, Pennsylvania 19122

References and Notes

1. W. R. Thompson, *Science* **125**, 698 (1957); J. J. Christian and C. D. LeMunyan, *Endocrinology* **63**, 517 (1958); C. H. Hockman, *J. Comp. Physiol. Psychol.* **54**, 679 (1961); K. Keeley, *Science* **135**, 44 (19 → M. W. Lieberman, *ibid.* **141**, 824 (1963); J. C. DeFries, M. W. Weir, J. P. Hegmann, *J. Comp. Physiol. Psychol.* **63**, 332 (1967); R. Wehmer, R. H. Porter, B. Scales, *Commun. Behav. Biol.* **5**, 211 (1970); S. Barlow, P. McEllhatton, P. Morrison, F. M. Sullivan, *Proc. Physiol. Soc. (London)* **239**, 55 (1974).
2. I. L. Ward, *Science* **175**, 82 (1972).
3. J. B. Whitney and L. R. Herrenkohl, *Physiol. Behav.* **19**, 167 (1977); J. A. Moyer, L. R. Herrenkohl, D. M. Jacobowitz, *Brain Res.* **121**, 385 (1977).
4. J. A. Moyer, L. R. Herrenkohl, D. M. Jacobowitz, *Brain Res.* **144**, 173 (1978).
5. L. R. Herrenkohl and J. A. Politch, *Experientia* **34**, 1240 (1978).
6. L. R. Herrenkohl and J. B. Whitney, *Physiol. Behav.* **17**, 1019 (1976); J. A. Politch, L. R. Herrenkohl, R. R. Gala, *ibid.* **20**, 91 (1978).
7. See reviews by K. Milkovic and S. Milkovic [in *Neuroendocrinology*, L. Martini and W. F. Ganong, Eds. (Academic Press, New York, 1966), vol. 1, pp. 371-405; and R. M. Rose, *Psychosom. Med.* **31**, 405 (1969)].
8. B. B. Turner and A. N. Taylor, *Endocrinology* **98**, 1 (1976); *J. Reprod. Fertil.* **51**, 309 (1977); R. E. Harlan and R. A. Gorski, *Endocrinology* **101**, 741 (1977); *ibid.*, p. 750.
9. I thank J. A. Politch, J. A. Moyer, and S. S. Carley for their technical assistance. I am especially indebted to R. R. Gala, Physiology Department, School of Medicine, Wayne State University, and D. M. Jacobowitz, Laboratory of Clinical Science, National Institute of Mental Health, for their encouragement and advice. I also thank I. L. Ward, Psychology Department, Villanova University, for encouragement and advice, and for critical reading of this manuscript. Research was supported by a Biomedical Science Support Grant awarded to the Interdisciplinary Graduate Program in Psychobiology at Temple University and by a grant-in-aid from Temple University.

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Release of Luteinizing Hormone in Male Mice During Exposure to Females: Habituation of the Response

Abstract. *Male mice release luteinizing hormone when exposed for a short time to a female. In this experiment, multiple blood samples were withdrawn by atrial cannulas from tethered males during either continuous or intermittent exposure to nonreceptive females. After an immediate, transient release of luteinizing hormone, continuous exposure to the same female was accompanied by only random, spontaneous elevations in plasma levels of this hormone. Successive presentations of the same female at 2-hour intervals elicited gradually diminishing luteinizing hormone responses. Exposing such unresponsive males to novel, diestrous females, however, dramatically stimulated their release of the hormone. These results demonstrate habituation of a socially induced, neuroendocrine response involving reproductive hormones.*

Males of many mammalian species secrete increased amounts of reproductive hormones when exposed to females of the same species (*1*, *2*). The precise nature of the relevant cues from the females is unknown in most cases, and the reproductive function served by the males' responses is not understood in any. Although short-term exposure of male house mice to either females or female urinary odor provokes immediate release of luteinizing hormone (LH) and then secretion of testosterone, male mice do not show elevated titers of these hormones during sustained cohabitation with females (*3*, *4*). Thus, this particular neuroendocrine response must be subject to either sensory adaptation, hypothalamo-hypophyseal depletion, or habituation. By analogy with neuromuscular terminology, habituation is defined here as the absence of the other two phenomena (*5*). In the experiment reported here, we observed that progressively fewer male mice release LH in response to repeated exposure to the same female. The males' LH responses resumed, however, upon the introduction of a novel female. Therefore, our data establish that habituation can occur in a socially induced, neuroendocrine response involving reproductive hormones.

The design of the study involved sequentially sampling blood from cannulated but freely moving male mice (*6*) during continuous or intermittent exposure to individual females. In more detail, 45 cannulated CF-1 males (*7*) were allowed to interact with nonreceptive females in the males' home cages; 15 males were used for each of three patterns of female exposure. During the pattern of continuous exposure, a female remained with each male throughout the test period without being disturbed. During the two patterns of repetitive exposure, the same female was placed in each male's cage three times, and then either that individual or an unfamiliar female was presented during the fourth sequence. In the latter two experimental conditions, females were placed in the cages every 2 hours for 90 minutes and then removed for 30 minutes. Five blood samples were withdrawn from the males at 5-minute intervals every 2 hours, always beginning before females were placed in the cages. The first two samples established the individuals' baseline levels of LH and the

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