Although accumulation of NaCl from saline soils may act as a defense of plants against herbivores in areas where excretory water is not readily available (*17*), we suggest that the opposite adaptation is more common: relative exclusion of sodium from the tissues of most land plants may help defend them against grazing by making it difficult for the grazers to obtain as much of this ion as they need.

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5. When a butterfly landed on a tray and remained on the surface for less than 15 seconds, a sampling visit was scored. Puddling was scored when a butterfly remained on the surface for 15 seconds or longer. "Butterfly minutes" represent the product of the number of butterflies times the number of minutes each "puddled." Through binoculars it was possible to see proboscises extended, in some cases before the butterflies landed.
6. Samples of sand (20 to 30 g) were scraped from the surface of each tray and weighed; after being dried at 110°C, the samples were weighed again (to calculate moisture content) and then extracted five times with twice-distilled water. The aqueous extracts were made up to 100 ml each, and samples were analyzed for sodium in a Perkin-Elmer model 403 atomic absorption spectrophotometer, alongside distilled water controls. The sodium content of distilled water washings (100 ml) of 25 g of the untreated sand was not detectably different from that of the distilled water controls (0.028 to 0.057 mg/liter).
7. Surface mud (about 5 mm deep) was scraped from approximately 5 cm^2 around each of four points where butterflies had been observed to puddle within the previous 2 hours. One portion of the combined samples was oven-dried to determine the moisture content (23.7 percent); a second portion was extracted five times with distilled water; combined supernatants after centrifuging were concentrated by vacuum distillation, and the volume was adjusted to 100 ml with distilled water; a third portion of mud was extracted five times with ethanol; combined supernatants were evaporated to dryness under vacuum and the residue taken up in 100 ml of distilled water. Sodium analyses of the extracts and controls were performed by J. S. Eaton with the atomic absorption spectrophotometer.
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9. The amino acids included in the mixture were lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and tryptophan. Total amino acid concentrations in the tray solutions were found by the method above to be $1.83 \times 10^{-4}M$ (amino acid mixture) and $4.28 \times 10^{-5}M$ (casein hydrolyzate).
10. Butterflies were homogenized in 10 percent sucrose, 0.1*M* tris-HCl, *p*H 7.0, and extracted four times with 5 percent trichloroacetic acid, and the suspension was counted in toluene-based counting fluid (Packard Tri-Carb scintillation counter). In the final pellet, 90 percent of the radioactive material was solubilized after incubation with protease (Worthington).
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Modulation of the Copulatory Sequence of the Male Rat by a Schedule of Reinforcement

Abstract. *Copulating male rats were permitted a maximum of seven intromissions in which to ejaculate. This experimental constraint of the male rat's sexual behavior produced an increase in the number of sessions in which the male ejaculated before the seventh intromission. This species-specific behavior pattern is therefore susceptible to environmental conditioning.*

The sexual behavior of the male rat consists of three distinguishable classes of copulatory responses: mounts, intromissions, and ejaculations. During each of these responses, the male rat grasps the receptive female's flanks with his forelegs and begins a series of pelvic thrusts. During mounts, these pelvic thrusts do not lead to vaginal penetration; during intromissions and ejaculations, however, vaginal penetration is achieved. After approximately 5 to 15 intromissions, a copulatory sequence is terminated by the occurrence of the ejaculatory response.

Because the reflexive components of its copulatory behavior are so readily apparent (*1*), learning has not been considered important in most descriptions of the male rat's mating activities. The present experiment was designed to assess whether rats could learn to modulate the stereotypic pattern of its copulatory sequence. Such a demonstration would support the argument that the structure of this behavior is not controlled solely by endogenous factors.

In order to determine whether learning could contribute to the control of sexual behavior, a schedule of reinforcement was used: for one group of male rats (experimental group), the opportunity to ejaculate was dependent on its occurrence within the first seven intromissions of the copulatory series. Each rat was given free access to a receptive female until the occurrence of the ejaculatory response or of seven intromissions, whichever occurred first. If ejaculation is a more potent reinforcer than intromission, and if the male rat can learn to modulate its copulatory behavior in order to produce reinforcement (that is, ejaculation), then he should learn to ejaculate with fewer preceding intromissions.

In addition to the experimental group, two control groups were used. The rats in the maturation-experience (M-E) control group, which were the same age as the males in the experimental group, were given free access to receptive females with the same weekly frequency as the experimental group; however, these males were always permitted to intromit until ejaculation. By comparing the number of intromissions preceding ejaculation (intromission frequency) for the males in this group with those of the experimental group, it should be possible to determine whether changes in the experimental group's intromission frequencies were generally due to maturation and sexual experience or to the specific contingencies of this experiment.

A second control group, called the frustration (F) control group, was used to evaluate the noncontingent effects (for example, frustration) which might follow the interruption of a rat's copulatory sequence. Each

member of the F control group was "yoked" to a member of the experimental group. If a rat in the experimental group ejaculated on a given day, its partner from the F control group was permitted to intromit until ejaculation. If the male from the experimental group did not ejaculate, its paired F control male was permitted only one intromission and was then removed from the chamber. Thus, during those days when its experimental partner ejaculated, so did the F control male; and conversely, when the rat in the experimental group was removed prior to ejaculation, so was its paired F control rat.

Twenty-one sexually naive, albino rats about 90 days old were randomly assigned to one of the three groups (experimental, M-E control, F control). All subjects were tested every Monday, Wednesday, and Friday until 20 sessions were completed.

Stimulus females were brought into behavioral estrus with subcutaneous injections of 0.1 mg of estradiol benzoate (Schering, Progynon) 72 hours before testing and 1.0 mg of progesterone (Schering, Poluton) 3 hours before testing. Each female, like each male, was permitted only one copulatory series per test day. Between testing sessions, male and female rats were segregated, and kept in a reverse 14-hour light, 10-hour dark cycle (lights on at 6:30 p.m.) with food and water freely available.

A Plexiglas chamber with a wooden floor (18 by 18 by 24 inches) (46 by 46 by 61 cm) served as the experimental space. Mounts, intromissions, and ejaculations were recorded on event-pen recorders and scored in terms of intromission frequencies and the times between successive intromissions (inter-intromission intervals).

Figure 1 presents for each group the percentage of trials during which rats ejaculated within seven intromissions. Each data point is the average performance of the seven animals composing each group for blocks of four mating sessions. To obtain the percentage measure for the experimental and M-E control groups, the number of trials during which ejaculation occurred within seven intromissions was divided by 28 (7 subjects × 4 sessions). For the F control group, the number of ejaculations occurring on or before seven intromissions was divided by the number of sessions in each four-session block in which the F control males were permitted to ejaculate. Since only one male in the experimental group ejaculated within seven intromissions, there was only one instance of unrestricted access during the first four-session block for the F control group; therefore, the first data point for the F control group is presented during the second block of sessions.

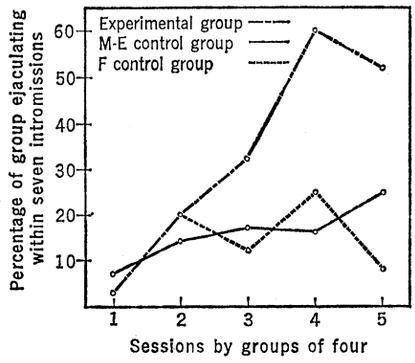

Fig. 1. Percentage of trials during which ejaculation occurred within seven intromissions as a function of sessions summed over four-session blocks.

Figure 1 illustrates, across sessions, the tendency of the males in the experimental group to ejaculate after progressively fewer intromissions. While there was a significant increase in the percentage of these males ejaculating across sessions, there was no equivalent change in the behavior of the M-E controls [$P < .05$ versus $P < .35$ for the two groups, Friedman Two-way Analysis of Variance in Siegel (*2*)]. Across all sessions, the median percentage of animals ejaculating on or before the seventh intromission was greater for the experimental group (41 percent) than for the M-E control (13 percent) and F control (14 percent) groups. The difference between the experimental and M-E control group medians was significant at $P = .037$ [Mann-Whitney U test in (*2*)]. The difference between the experimental and F control groups was significant at $P = .1$ [Sign test in (*2*)].

These data demonstrate that rats can modulate the occurrence of the ejaculatory response within the copulatory sequence. Moreover, this modulation does not appear to be a consequence of general maturation or sexual experience (M-E control group) or of the noncontingent interruption of the copulatory sequence (F control group). Rather, the reduction in the experimental group's average intromission frequency appears to be a direct response to making the opportunity for ejaculation dependent on the number of prior intromissions.

Clearly, the number of intromissions preceding ejaculation seems to be a dimension of behavior sensitive to outcome-contingent control. What is the mechanism by which this occurs? One possibility is that the male rats lower their intromission frequencies by increasing their mean inter-intromission intervals. This hypothesis is based on the well-documented finding that lengthening the inter-intromission intervals by experimentally enforcing a minimum inter-intromission interval on a pair of copulating rats reliably reduces the number of intromissions preceding ejaculation (*3*).

The data from the present experiment are consistent with these "enforced interval" studies. The median inter-intromission interval during the first four sessions was 20 seconds for the experimental group and 25 seconds for the M-E controls. During the first four sessions, the differences between the two groups were not significant ($P = .16$, Mann-Whitney U test). By the last four sessions, however, this difference was statistically significant ($P = .022$, Mann-Whitney U test).

Although there are changes in the quantitative nature of the male rat's ejaculatory series as a function of age and amount of sexual activity (*4*), the pattern of this species-specific behavior is quite stable over time. Young males quickly develop the adult pattern, and the response topography does not seem to be a function of specific sexual experience. In view of this fact, it is not surprising that much previous work has been directed toward a description of the endogenous neural and hormonal mechanisms controlling this behavior. Nevertheless, experimental modification of highly organized, stereotypic behavior patterns has been demonstrated in other animals. The learning of song dialects by white-crowned sparrows (*5*) and the reduced probability of aggression displayed in Siamese fighting fish following punishment (*6*) are two examples. As in these instances, the male rat's copulatory behavior is sensitive to outcome-contingent control. In all of these cases, the sources of control of the stereotyped behavior are not solely endogenous in origin.

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Solar Energy by Photosynthesis: Manganese Complex Photolysis

In a recent article (*1*) a reference was made to the photolysis of a binuclear manganese complex [reference 20 in (*1*)] in which oxygen evolution was measured by a Teflon-coated silver-gold electrode [figure 7 in (*1*)]. In an attempt to quantify this effect it has become apparent that most, if not all, of the change in slope of the apparent oxygen concentration is the result of a small temperature change (0.4°C) on the oxygen permeability of the membrane. Upon illumination in a visible band the thermostated sample solution is warmed slightly by thermal de-excitation of the excited state of the complex. This in turn increases the diffusion rate of atmospheric oxygen across the Teflon membrane, causing a change in the apparent oxygen concentration as sensed by the electrode.

In light of these results, we are seeking alternate evidence for oxygen evolution by the binuclear manganese complex.

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2. This work was performed under the auspices of the U.S. Atomic Energy Commission.

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Ecological Genetics and Natural Selection in Mollusks

Jones (*1*) has raised again the controversial question as to whether polymorphism in shell color and pattern in the land snail *Cepaea* is correlated with climate. He provides evidence suggesting that in *C. nemoralis* there is a correlation between gene frequencies at the shell color locus and mean summer temperature, but he could find no correlation with climate for other loci. Following earlier workers, Jones acknowledges that there is a correlation between polymorphism and habitat in some (but not all) English populations of *C. nemoralis*, and that there are situations in which frequency-dependent selection by predators occurs. The possibility of heterozygote advantage is also entertained. There remain, however, many inexplicable variations in the frequency of the phenotypes that are not correlated with obvious environmental features. These "area effects" occur over what are claimed to be ecologically similar environments, and thus far have defied explanation.

There is an extensive literature on polymorphism in *Cepaea*, some of it cited by Jones (*1*), but two features of the ecology of the snails, population size and population density, both potentially important in the interpretation of genetic diversity, have been neglected by most workers. Estimates of population size would seem desirable if genetic drift is to be accepted or rejected as a major factor affecting gene frequencies, and estimates of population density would seem relevant in view of the correlations that have already been established between density and polymorphism in other species of molluscs.

Thus in *Donax rugosus*, a common bivalve of the sandy shore in West Africa, polymorphism correlates well with population density, the diversity of phenotypes being greater at high as compared to low densities (*2*). In the African land snail *Limicolaria martensiana*, polymorphism also increases with density, and in the Kampala area of Uganda there is little or no polymorphism where the snails exist at densities of less than 1 per square meters; but where they occur at densities in excess of 100 per square meter, polymorphism is maximal (*3*).

The association of genetic diversity with population density in *Donax* and *Limicolaria* can be interpreted theoretically in terms of frequency-dependent apostatic selection by predators like birds. Such predators are likely to acquire search images of their prey; and at high densities where predation is heavy, phenotypes that stand out or contrast may be at a selective advantage.

That there are variations in population density in *Cepaea nemoralis*, even in an apparently uniform environment, has been demonstrated (*4*). If, then, density can vary, why should not the genetic diversity also be affected? Perhaps, too, what appears to us as a uniform environment is to a snail immensely varied; this would certainly explain variations in density over quite small areas. I do not mean to imply that variation in population density (and indeed in population size) are necessarily of profound importance in understanding polymorphism in *Cepaea*, but it would certainly be worth looking to see if there are density effects.

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Owen suggests that population density may have an effect in controlling gene frequencies in populations of *Cepaea* and of other mollusks. In the light of the recent important theoretical work (*1*) on possible relations between density and the genetic structure of populations this suggestion is an interesting one. Some information is already available on population density in *C. nemoralis* (*2*), and it is clear even to the casual collector that this species shows enormous local variations in abundance. However, it is difficult to estimate real population densities in many land mollusks (including *Cepaea*) by simply making collections because very frequently a large proportion of the population is buried beneath the surface of the