nitrogen demand generated by the seed from the nitrogen supplies available from the soil. Increasing the nitrogen uptake rate to 6 kg ha^{-1} day^{-1} increases the supply to 24 mg of nitrogen per gram of photosynthate and leaves the division between the crop groups nearly unaffected, with only chick pea moving below the line.

For the crop species in the top part of Fig. 1 to sustain the calculated rates of seed biomass production, we hypothesize that the remaining nitrogen demand must be obtained from the vegetative plant parts. Experimental observations of nitrogen mobilization and translocation from leaves to seeds during seed development support this hypothesis (*3*). Since the pool of nitrogen and protein in vegetative tissues will be continually depleted, the vegetative tissues must eventually lose physiological activity as the nitrogen levels decrease and the plant becomes "self-destructive." Many of these self-destructive species in the top part of Fig. 1 exhibit leaf senescence and abscission during seed development. Final seed yield of these species may also be closely tied to the self-destructive characteristic. Since the duration of the seed development period is obligatorily linked to productive vegetative tissues, if seed development eventually impairs the physiological activity of vegetative tissues, seed production in the self-destructive species is inherently limited to a finite length of time for seed-fill. Therefore, the duration of the seed development period is intimately tied to the rate of nitrogen uptake by the self-destructive crop during seed-fill. A low rate of uptake results in a large nitrogen demand and leads to extensive nitrogen translocation to the seeds from vegetative tissue, a shorter period of seed development, and lower total yield (*4*).

We expect that the self-destructive species would be unresponsive to nitrogen fertilization unless it increased directly the rate of nitrogen uptake by the plant during seed-fill. The inability of the self-destructive crops to respond to nitrogen fertilizer is aggravated because many of these species support symbiotic nitrogen fixation, which tends to decrease in proportion to nitrogen fertilization. In soybeans the yields of a nodulating genotype were essentially unchanged by nitrogen fertilization, while the yields of a nonnodulating isoline increased with fertilization, reaching those of the nodulating genotype (*5*).

For the species in the lower portion of Fig. 1 seed growth may be sustained by a relatively small rate of uptake of nitrogen during the seed-filling period without the necessity of nitrogen redistribution within the plant. However, these species may also translocate nitrogen during seed development and the total amount of nitrogen available from the soil is often finite, so that self-destruction may also develop in the species in the lower portion of Fig. 1. Nitrogen fertilization may at least partially alleviate the limitation of nitrogen availability in the soil and minimize the effects of the self-destructive process in these species. This agrees with the well-established phenomenon that nitrogen fertilization of small grains increases yield by postponing senescence.

Our analysis of the chemical composition of crop seeds shows the potential importance of nitrogen availability and supply on crop yields. The seed yields of crop species identified as self-destructive may be significantly inhibited by insufficient rates of nitrogen supply. The high rates of nitrogen uptake demanded by the seeds of these species may cause the rapid translocation of nitrogen from the vegetative plant parts. The destruction of proteins in vegetative plant parts leads to a loss in physiological activity and senescence of the plant, and a shortened period for seed development. The remaining species are not generally limited by the potential rate of nitrogen supply from the soil but rather the total amount of available nitrogen.

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Rapid Food-Aversion Learning by a Terrestrial Mollusk

Abstract. *The terrestrial slug* Limax maximus *can learn to avoid new palatable food if* CO_2 *poisoning is paired with ingestion of the new food. Some animals learn in one trial and remember without error for 3 weeks. Avoidance most commonly consists of complete rejection of the unsafe food, based on olfactory cues. This preparation offers the opportunity for detailed neurophysiological analysis of a rapid-onset learning mechanism of long duration.*

There are specialized mechanisms which provide for very rapid and long-lasting learning. Imprinting and song-learning by birds are classical examples. Such rapid learning has been documented for invertebrates in studies of wasp nest-site recognition (*1*), honey bee communication (*2*), and octopus visual discrimination tests (*3*). Because invertebrate preparations are proving increasingly useful in studies of the cellular basis of learning (*4*), it would be advantageous to document a rapid-onset learning mechanism of long duration in an invertebrate whose nervous system is amenable to detailed neurophysiological analysis. The terrestrial slug, *Limax maximus*, is such an organism (*5*).

Several species of vertebrates, including man, possess a specialized learning mechanism which can associate food-related sensory cues with the internal consequences of ingestion. Rats will rapidly and specifically associate a new odor or taste with intestinal malaise produced experimentally (*6*). Such learning shows a rapid onset, high resistance to extinction, specificity to olfactory and gustatory inputs, occurrence with long ($>$ 1 hour) delays between sensory input and internal consequence, and resistance to disturbance by electroconvulsive shock (*7*). Birds have a similar learning mechanism but use visual cues to form aversions to new foods associated with gastric upset (*8*).

The terrestrial pulmonate slug, *Limax maximus*, is a generalized herbivore faced with the problem of assessing both nutritional value and potential toxicity of foods. The selectivity of food plant selection by wild slugs has been amply demonstrated. Gain (*9*) offered *Limax* 194 different food items, of which 155 (80 percent) were totally rejected. Preferences for the 39 acceptable foods varied, with mushroom (*Boletus edulis*) and carrot root (*Daucus carota*) being most avidly eaten. Frömming (*9*) showed that *Limax* would accept only 31 percent of 110 varieties of higher plants. Potato tubers (*Solanum tuberosum*) and cucumber seed pods (*Cucumis sativa*) were eaten more readily than any of the other plant leaves, roots, or fruits offered. Below

Table 1. Comparison of several behavioral measures of learning between control and trained slugs.

Treatment	Days mushroom eaten < potato (%)	Mushroom meals rejected (%)	Meals gassed (%)	Duration mushroom refused (days)	Rapid learners* (%)
Zero-delay gas	60†	58	28	3.0	50
1-hour-delay gas	30‡	41	38	2.5	25
3-hour-delay gas	20§	32	43	1.4	0
No gas	10¶	30	0	1.5	0

*Animals refusing mushroom for six consecutive days after one or two trials. †Significant difference on days 9, 11, 12, 14, 16, and 18. ‡Significant difference on days 9, 13, and 16. §Significant difference on days 17 and 18. ¶ Significant difference on day 12.

I show that *Limax* can rapidly learn to associate noxious stimuli with ingestion of a novel, palatable food, resulting in rejection of the new food although ingestion of a safe food continues.

The slugs in these experiments included both collected and cultured animals, weighing 6 to 12 g. They were housed individually in ventilated plastic containers with several layers of moist filter paper. The cages were cleaned and the filter paper was changed daily. Pieces of food were weighed and placed on a dry region of the cage floor. The animal was then placed within 2 cm of the food and, if inactive, was stimulated to crawl toward the food by gently prodding its tail. During the first daily feeding, the food was left in the cage for 1 hour, then removed and reweighed after blotting up excess saliva or mucus. During the second daily feeding, the food was removed and reweighed immediately after the animal clearly stopped feeding and started to move away from it, or at the end of 1 hour if the animal did not eat. Evaporation controls were run with no animal in the container and showed that weight changes due to water adsorption or loss were negligible for the foods used in these experiments (potato, -0.009 ± 0.01 g; mushroom, +0.014 ± 0.036 g; cucumber, -0.021 ± 0.014 g). Control and experimental groups were matched as to weight and previous history.

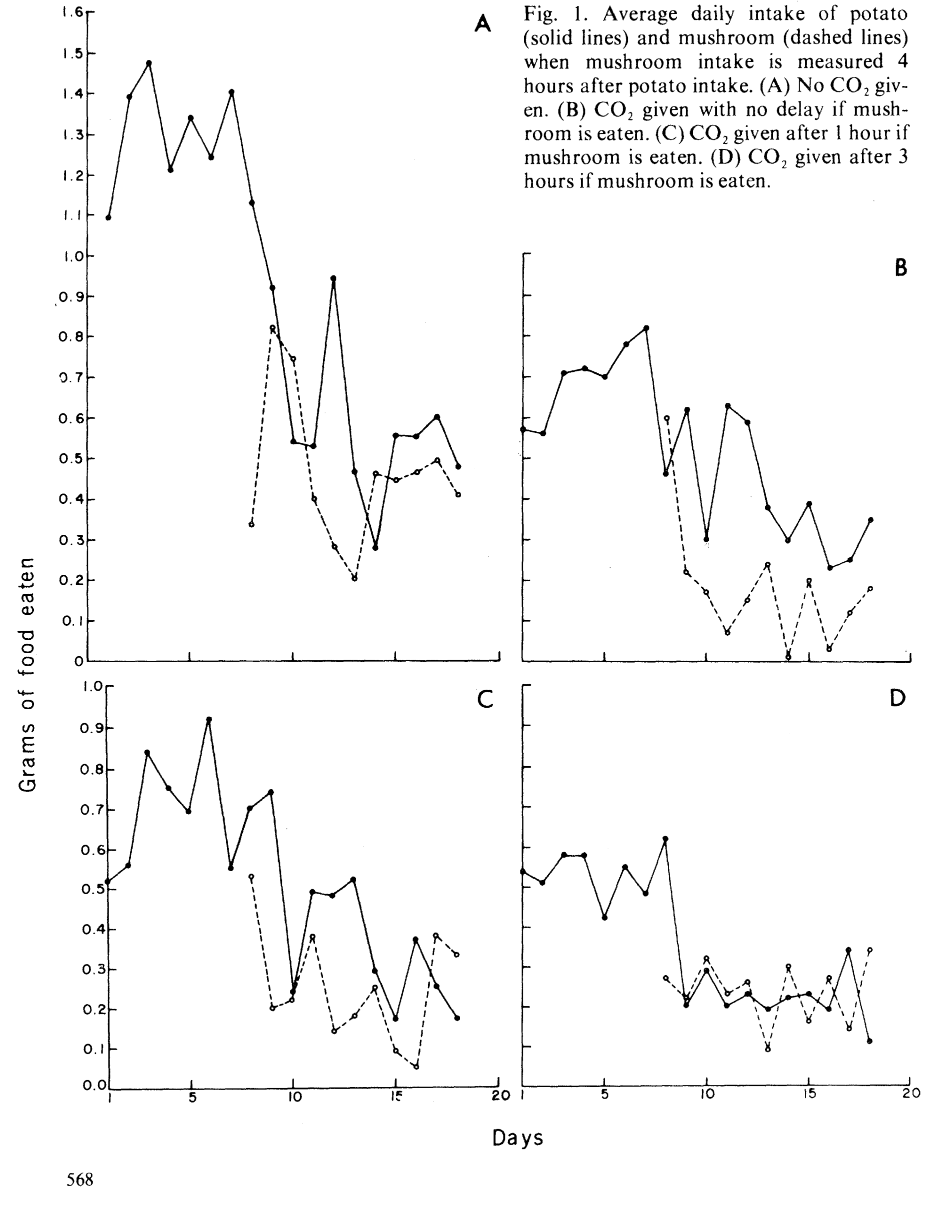

Fig. 1. Average daily intake of potato (solid lines) and mushroom (dashed lines) when mushroom intake is measured 4 hours after potato intake. (A) No CO_2 given. (B) CO_2 given with no delay if mushroom is eaten. (C) CO_2 given after 1 hour if mushroom is eaten. (D) CO_2 given after 3 hours if mushroom is eaten.

In the first experiment, slugs were fed potato once daily for 1 hour, and their intake was measured quantitatively. On the eighth day, the animals which had eaten consistently for the first 7 days were selected and randomly assigned to either the control group or the training group. This initial selection eliminated 10 to 15 percent of the slugs. On the eighth day, control animals were offered mushroom (*Agaricus campestris*) 4 hours after the potato feeding, and mushroom intake was measured. Once these dual feedings commenced, no further selection of control or experimental animals occurred. The data for five control slugs are shown in Fig. 1A. From day 9 to day 18, mushroom intake was not significantly different from potato intake (Wilcoxon matched-pairs signed-ranks test, two-tailed, $P = .18$). When tested day by day, on only 10 percent of the days was mushroom intake significantly less than potato intake (Mann-Whitney U test, one-tailed, $\alpha < .05$). Slugs to be trained ($N = 8$) were fed potato and mushroom on the same schedule, but each time an animal ate a meal of mushroom (intake ≥ 0.1 g), it received a 5-minute exposure to CO_2. Carbon dioxide was applied by flushing the animal's cage with humidified CO_2 for 1 minute. If intake was less than 0.1 g, no CO_2 was given. Mushroom intake over the training period was significantly less than potato intake (Wilcoxon test, $P < .0001$). Mushroom intake was significantly reduced on the second day of training (Fig. 1B). On 60 percent of the days, mushroom intake was significantly less than potato intake (U test, $\alpha < .05$). This experiment wherein mushroom intake is associated with CO_2 exposure has been replicated with three additional groups of four slugs each. These groups had significantly depressed mushroom intake on 63, 39, and 63 percent of the training days, respectively.

Between-group comparisons were made by using potato intake minus mushroom intake difference values, so that variations between animals or groups in intake levels which affected both foods simultaneously would not influence the comparison. Based on these difference values the no-gas control group was very significantly different from the gas with zero-delay group (U test, $P < .00003$).

To establish the associative nature of the learning and test for the delayed reinforcement effect characteristic of vertebrate taste-aversion learning, two additional groups of animals were trained. For one group ($N = 8$) the application of CO_2 was delayed 1 hour from the cessation of eating mushroom, while for the other group ($N = 8$) CO_2 application was delayed by 3 hours. The group learning curves are shown in Fig. 1, C and D. As the application of CO_2 is increasingly delayed from mushroom ingestion, mushroom rejection approaches closely the level shown by slugs not receiving CO_2. For the 3-hour-delay group mushroom intake was not significantly different from potato intake (Wilcoxon test, $P = .3$). Also, for the 3-hour-delay group 20 percent of the training days showed significantly depressed mushroom intake, versus 10 percent for the no-gas group and 60 percent for the gas with zero-delay group.

The control group shown in Fig. 1A was obtained from the same laboratory culture as the animals shown in Fig. 1, B to D, but they were run at a different time than the groups shown in Fig. 1, B to D, which were run concurrently.

Between-group comparisons showed that the 3-hour-delay group was very different than the zero-delay group (U test, $P < .00003$) and the 1-hour-delay group (U test, $P = .001$). The two control groups, no-gas and 3-hour-delay, were not different from each other (U test, $P = .1$), nor was the 1-hour-delay group different from the zero-delay group (U test, $P = .29$).

The variability of the data shown in Fig. 1, A to D, arises from several sources, including variation in the number of animals eating a meal and the size of the meal. The variance in the data was examined by calculating the coefficient of variation ($V = \sigma/\bar{x}$ times 100) for each average daily intake value of potato and mushroom. The average values of V (days 9 through 18) for the zero-delay group are: potato, 76; mushroom, 160; for the 1-hour-delay group: potato, 96; mushroom, 133; for the 3-hour-delay group: potato, 103; mushroom, 108; for the no-gas group: potato, 78; mushroom, 102. This variability necessitated replication of the basic experiment several times and with several variations to ensure the reproducibility of the effect.

Table 1 summarizes additional behavioral measures of learning. For example, the extent of mushroom aversion can be represented by the frequency with which mushroom is rejected. A meal of mushroom is defined as intake ≥ 0.1 g. Rejection is scored if mushroom intake is < 0.1 g and potato intake 4 hours previously is ≥ 0.1 g. If both mushroom and potato intake are < 0.1 g the trial is not counted. Using these criteria, I found that slugs trained to avoid mushroom by immediate CO_2 application rejected it significantly more often (58 percent) than slugs with CO_2 delayed 3 hours after mushroom intake (32 percent) or given no gas at all (30 percent). The average numbers of consecutive days of mushroom refusal for the no-gas and 3-hour-delay groups, respectively, are similar (1.5 and 1.4 days) and are significantly different from the zero-hour-delay group (3.0 days).

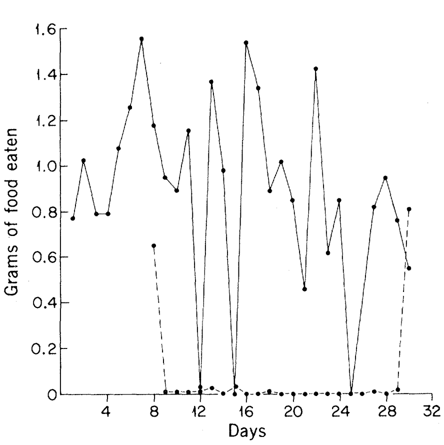

Fig. 2. Intake of potato (solid lines) and mushroom (dashed lines) by individual slug. Mushroom meal was punished by CO_2 exposure.

Individual slugs vary in their pattern of response during the training procedure. Some animals refuse to eat the unsafe food on the second trial and continue to refuse it for several days, while continuing to eat the safe food. The behavior of such an animal is shown in Fig. 2. This animal ate a meal of mushroom and received CO_2 on the first training trial and then did not eat mushroom or receive CO_2 for 21 days. If we define rapid learning as total refusal to eat mushroom for 6 days after one or two training trials, then 50 percent of the slugs in Fig. 1B were rapid learners. Of the 12 additional animals run with zero delay between mushroom ingestion and CO_2, 33 percent showed one- or two-trial learning lasting 9 to 26 days. The remaining animals required three to six training trials before refusing mushroom. While these data serve to establish the range of patterns of responses displayed by individual animals during training, it can be seen from Table 1 that the "average" slug in the zero-delay group was not a rapid learner.

An alternative explanation of these data asserts that during the first 7 days the animal learns that potato is safe and that during training the animal is learning to avoid both foods, but longer conditioning times are required to begin to avoid the safe food. If this is so, intake of both foods should decline. Some reduction in potato intake over days 8 to 18 is evident in Fig. 1, B and C; however part of this reduction is due to adjustments in total caloric intake caused by access to two foods per day rather than one. The present experiments do not rule out the possibility of generalization of the learned avoidance of the unsafe food to the safe food; however, over the time periods used in my experiments the learned food aversion affects the unsafe food to a much greater extent than the safe food.

Two additional points are made by the data in Table 1. Some learning does occur with a 1-hour delay of the CO_2. All the behavioral measures of learning for the 1-hour-delay group are intermediate between the zero- and 3-hour-delay groups. Second, the legitimacy of the 3-hour-delay group as a control for sensitization and nonassociative processes depends on the frequency of CO_2 application in this group being equal to or greater than the CO_2 application frequency in the zero-hour delay learning group. The CO_2 application frequency was 1.5 times greater in the 3-hour-delay group (43 percent versus 28 percent). Because the greater frequency of gassing should have aggravated a sensitization process if it occurred, the equal ingestion of mushroom and potato by the 3-hour-delay group argues strongly for the associative nature of the learning in the zero-delay group.

The experiment has also been replicated with potato as the familiar, safe food and cucumber (mesocarp) as the novel, unsafe food. A new group of four slugs was fed potato once per day. On the eighth day they were offered cucumber 4 hours after the potato feeding. Dual feedings were continued for 18 days. Cucumber intake was significantly greater than potato intake on 15 of 18 days (83 percent). Another group of five slugs was fed potato and cucumber according to the same protocol, except that each time a slug ate a meal of cucumber, it received a 5-minute exposure to CO_2. Cucumber intake was greatly depressed so that on only 2 of 18 days (11 percent) was it greater than potato intake, which is significantly different from the control group (χ^2 two-sample test, $P < .001$).

When the trained slugs eat a meal of the unsafe food, do they eat a normal-sized meal? Defining a meal as intake ≥ 0.1 g (less than 2 percent of the meals measured in this study were less than 0.1 g), the mean meal sizes of potato, mushroom, and cucumber for the control and trained slugs were computed and compared. Animals trained to avoid mushroom take significantly smaller meals of mushroom (69 percent of control), whereas animals trained to avoid cucumber eat the same size meal of cucumber as do controls. This suggests that for the mushroom the plasticity is expressed both as a reduced efficacy of olfactory stimuli in initiating feeding and a reduced efficacy of gustatory stimuli in maintaining feeding, whereas for cucumber only the altered effectiveness of olfactory input is apparent.

Slugs are first alerted to the presence of potential food plants by olfactory cues (*10*). As the slug approaches food, the superior and inferior tentacles bearing olfactory receptors explore its surface closely. If the odor signals acceptability, the lips are everted and applied to the food. With the food items used in this study, control slugs invariably show radular rasping and ingestion following lip contact. Conditioned slugs occasionally move away after lip contact, but the more typical avoidance response is rejection based on olfactory cues alone. If conditioned slugs start eating the unsafe food, they consume a normal meal if it is cucumber, and a subnormal but substantial meal if it is mushroom.

Several parametric features of the learning experiment are critically important in determining the rate and duration of learning. The animal's hunger level, the nature of the aversive stimulus, and the attractiveness of the novel, unsafe food all interact to influence the level of learning achieved. My initial attempts to train slugs to avoid a highly attractive dog food by mixing the slug-poison metaldehyde into the dog food were unsuccessful, even though the slugs became very sick after ingesting small amounts of metaldehyde. It is possible that bitter tastes and surface irritants help to promote learned food aversions.

Plants contain a variety of noxious secondary substances such as cyanogenic glycosides and chlorogenic acid (*11*). In plants polymorphic for the concentration of these substances in leaf tissue, slugs preferentially feed on the morph with lowest concentration. It will be interesting to determine if ingestion of a plant containing a noxious secondary substance will alter later food selection.

Neural circuits involved in feeding behavior have been examined in a variety of gastropod mollusks, including *Limax* (*12*). The buccal ganglia contain the motoneurons responsible for radular rasping and ingestion. Chemical and electrical synaptic interactions among buccal motoneurons have been described, and some cerebral cells which synaptically excite the motoneurons have been identified. In *Limax*, olfactory input arises in the digitate ganglia of the superior tentacles and projects to the cerebral ganglia, as does gustatory input from the lips. A neurophysiological analysis of synaptic plasticity in *Limax* feeding circuitry is now feasible. It is further encouraged by the demonstration that in the gastropod *Pleurobranchaea* a neurophysiological correlate of learning could be found in vitro with brains from animals whose feeding response had been previously conditioned (*13*).

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Sea Anemone with Zooxanthellae: Simultaneous Contraction and Expansion in Response to Changing Light Intensity

Abstract. *Under increasing intensity of light, the normal tentacles of* Lebrunea *contract whereas the pseudotentacles expand; in decreasing light the reverse is true. This behavior may be correlated with greater numbers of zooxanthellae in the pseudotentacles. Simultaneous but opposite response of parts suggests adaptations toward photosynthesis by day and predation by night.*

Many genera of sea anemones contract in strong light (*1*), whereas only a few expand in response to the same stimulus (*2*). There appears to be no record of an actinian (or coelenterate) which simultaneously expands and contracts different body regions in response to changes in light intensity. Such a behavior pattern has been found, however, in the aliciid anemone *Lebrunea coralligens* H. W. Wilson 1890 (Fig. 1).

Lebrunea coralligens is a common inhabitant of shallow marine waters of the Caribbean. This anemone occupies small

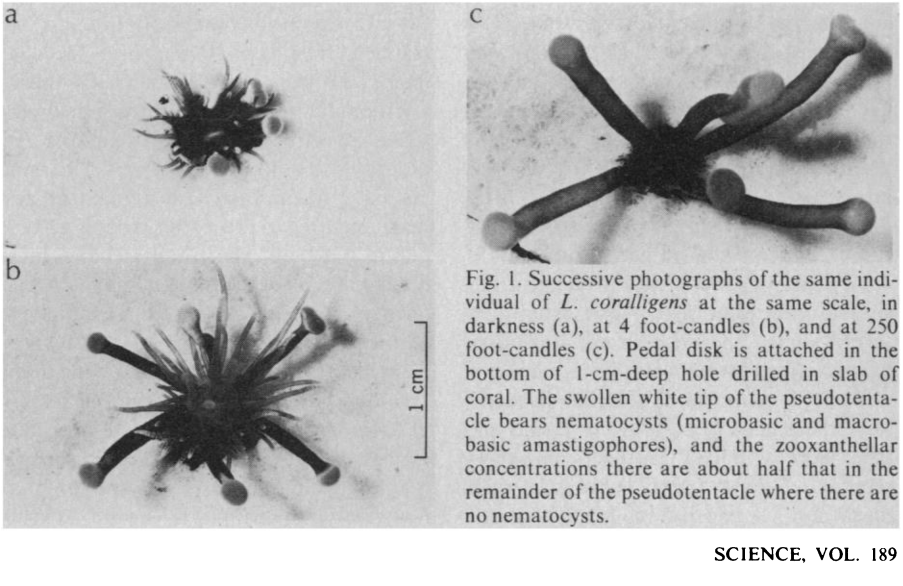

Fig. 1. Successive photographs of the same individual of *L. coralligens* at the same scale, in darkness (a), at 4 foot-candles (b), and at 250 foot-candles (c). Pedal disk is attached in the bottom of 1-cm-deep hole drilled in slab of coral. The swollen white tip of the pseudotentacle bears nematocysts (microbasic and macrobasic amastigophores), and the zooxanthellar concentrations there are about half that in the remainder of the pseudotentacle where there are no nematocysts.