the same length, diameter, and placement in test aquariums. Siphon flow for tests and controls were started at the same time. Only those males which displayed the search behavior within 5 minutes after end of siphon flow were considered to have responded positively.

The results (Table 1) indicate that a sexually attractive substance was emitted which functioned as a releasing stimulus evoking the "Aufspuren" (*7*) or search "phase" of the males' behavior. In addition, tests were done on 14 premolt females but not on controls, and the results were positive. On two occasions, water that a premolt female had been in was siphoned into a tank containing both juvenile and adult males. The search behavior was never demonstrated by the juvenile males. Several of the juvenile males subsequently passed through the pubertal molt (*2*) and 5 weeks later, when tested, displayed search behavior when exposed to water from a premolt female.

In preliminary experiments, there was no regularity of the response of males to water in which females had been kept for periods of less than 2 hours. This indicated that release of the attractant was intermittent.

An obvious possible source of the attractant was the urine of premolt females. The method of testing for pheromone substance in the urine was similar to methods used previously. Each premolt female was placed in a bucket of sea water for 2 hours and then removed. The excretory-pore areas were dried with acetone and capped with molten paraffin. The female was then placed in another bucket of sea water for 2 hours. The water from each bucket was siphoned into adjacent tanks containing pairs of adult males as before. The experiment was replicated three times on different days with different crabs. In these replicates (Table 2), none of the males exposed to water from a female with capped excretory pores displayed the search behavior. The paraffin caps of the excretory pores were removed, and subsequent tests gave positive male responses, indicating that the pheromone had again been released into the water.

These experiments indicate that a pheromone in the form of a sex attractant permits males to detect the premolt condition of *P. sanguinolentus* females. This does not eliminate an important role of submissiveness or other behavior on the part of the female in mating. These experiments also indicate that the pheromone is released through the excretory pores. Origin of the pheromone, its chemical nature, and the way it is detected by males remain to be determined.

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8 November 1965

Starch-Deficient Maize Mutant Lacking Adenosine Diphosphate Glucose Pyrophosphorylase Activity

Abstract. *The maize mutant* shrunken-2 *synthesizes only 25 to 30 percent as much starch as normal maize; it completely lacks adenosine diphosphate glucose pyrophosphorylase activity in both endosperm and embryo tissue. Identification of the mutant block indicates that the greater portion of starch in the endosperm of normal maize is synthesized by way of enzyme systems that utilize adenosine diphosphate glucose as a substrate, and that the latter is formed chiefly by adenosine diphosphate glucose pyrophosphorylase.*

The *shrunken-2* (sh_2) mutant in maize is characterized by kernels with poorly developed, shrunken endosperms (*1*); the kernels are unusually sweet, with a high concentration of sucrose and a low starch content in mature kernels: 16 and 25 percent compared with 1.4 and 65 percent, respectively, in normal maize (*2*). Since the dry weight of an sh_2 kernel (0.139 g) is less than that of a normal kernel (0.185 g), an sh_2 kernel synthesizes approximately 29 percent as much starch as a normal kernel. We have found that this mutant lacks ADPG (adenosine diphosphate glucose) pyrophosphorylase activity; this enzyme catalyzes formation of ADPG from glucose-1-phosphate and adenosine triphosphate (ATP) (*3*).

Enzymes that catalyze reactions conceivably intervening between sucrose and starch have been assayed in this mutant and also in normal stock. For sucrose synthetase, hexokinase, phosphoglucoisomerase, phosphoglucomutase, and uridine diphosphate phosphokinase and pyrophosphorylase, the sh_2 mutant has activities approximately equal to normal when they are calculated on the basis of fresh weights of protein in crude homogenates. On a per kernel basis the mutant has higher than normal activity for these enzymes because of the greater fresh weight of the sh_2 kernel. For the starch granule–bound ADPG, uridine diphosphate glucose–starch glucosyl transferase, the mutant has higher than normal activity per milligram of starch because of its greater number of starch granules. The invertase activity of the mutant is equal to normal on a per kernel basis, and thus lower on a fresh-weight basis. No ADPG pyrophosphorylase activity is detectable in mutant preparations.

For preparation of ADPG pyrophosphorylase and uridine diphosphate glucose pyrophosphorylase, isolated embryos or endosperms from kernels collected 22 days after self-pollination were homogenized for 3 minutes with an equal weight of chilled 0.05*M* phosphate buffer at *p*H 7.0, strained through muslin, and centrifuged for 20 minutes at 22,000*g*. From the supernatant, the fraction, precipitating at 25- to 40-percent or at 25- to 65-percent $(NH_4)_2SO_4$ saturation, was collected by centrigugation for 20 minutes at 18,000*g*, suspended in 3 ml of cold distilled water,

Table 1. Specific activities of ADPG pyrophosphorylase, uridine diphosphate glucose (UDPG) pyrophosphorylase, and starch phosphorylase in embryo and endosperm preparations from sh_2 and normal maize; incorporation into starch granules per microgram of protein in enzyme preparations. Embryo preparations from both sh_2 and normal maize were fractions precipitated by 25- to 65-percent $(NH_4)_2SO_4$ saturation. The endosperm preparation from normal maize was the fraction precipitated by 25- to 40-percent $(NH_4)_2SO_4$ saturation, while that of sh_2 mutant was from 25- to 65-percent $(NH_4)_2SO_4$ saturation. For the pyrophosphorylase assays, the reaction mixtures contained 10 μmole of tris-HCl at *p*H 7.4, 0.6 μmole of Mg^{++}, 0.1 μmole of ATP (or uridine triphosphate), 0.1 μmole of glucose-1-phosphate (70,000 count/min), and 10 μl of crude enzyme solution containing 39 μg of protein for sh_2 endosperms, 35 μg for normal endosperms, 20 μg for sh_2 embryos, and 30 μg for normal embryos [by the method of Lowry *et al.* (*4*)]; total volume 50 μl. Phosphorylase activity was measured under the same conditions except that ATP was omitted. The reaction was coupled with starch granule–bound glucosyl transferase by use of 3 mg of sh_2 endosperm starch granules. Incubation, 60 minutes at 37°C. The reaction was stopped by addition of 0.5 ml of 50-percent ethanol. Washing with 50-percent ethanol was repeated five times before the samples were counted in a gas-flow counter (Nuclear-Chicago).

Phosphorylase	Incorporation (count/min)			
	Embryo		Endosperm	
	sh_2	Normal	sh_2	Normal
ADPG pyro-	0	10	0	300
UDPG pyro-	364	255	131	127
Starch			6	6

and dialyzed against distilled water for 14 hours at 4°C.

In most tests of ADPG pyrophosphorylase and uridine diphosphate glucose pyrophosphorylase activity, the system was assayed by coupling with the starch granule–bound glucosyl transferase and by measuring incorporation of radioactivity into the starch granules. In all such tests, sh_2 starch granules were used, since they were more active per unit weight than those of normal maize.

Table 1 shows the specific activities of ADPG pyrophosphorylase, uridine diphosphate glucose pyrophosphorylase, and starch phosphorylase for the sh_2 mutant and for normal maize. Endosperm preparations from the sh_2 mutant and from normal maize have about the same uridine diphosphate glucose pyrophosphorylase activity; this is also true of embryo preparations. The embryo preparation is, however, more active than the endosperm preparation for both genotypes. The sh_2 mutant completely lacks ADPG pyrophosphorylase activity in both embryo and endosperm preparations. By contrast with uridine diphosphate glucose pyrophosphorylase activity, ADPG pyrophosphorylase activity in normal embryos is only about 3 percent of that of the endosperm. Tests for ADPG pyrophosphorylase activity in sh_2 have usually been made with the fraction precipitating between 25- and 65-percent $(NH_4)_2SO_4$ saturation, but negative results have also been obtained with the unfractionated homogenate and with the fractions precipitating between 0- and 25-percent and between 65- and 100-percent $(NH_4)_2SO_4$ saturation.

Phosphorylase activity in both sh_2 and normal preparations is low (about 0.3 percent incorporation), indicating that little of the incorporation observed in the assays of ADPG and uridine diphosphate glucose pyrophosphorylase activity could be due to starch phosphorylase.

Lack of ADPG pyrophosphorylase activity in the sh_2 preparation was confirmed by paper-chromatographic separation of reaction mixtures similar to those described in Table 1 but with starch granules omitted. The reactions were stopped by heating in a water bath at 100°C. After addition of 0.1 ml of water, each mixture was centrifuged. An authentic sample of ADPG and 30 μl of each reaction mixture were applied to acid-washed Whatman No. 1 paper and developed by the solvent system of Paladini and Leloir (*5*) for 18 hours at *p*H 3.8 in a descending chromatogram. The normal sample showed an ultraviolet-absorbing spot with high radioactivity (194 counts per minute per microgram of protein when corrected to the total volume of the reaction mixture) at the level of the known sample of ADPG. The sh_2 sample had neither an ultraviolet-absorbing spot nor radioactivity at the same level.

Ghosh and Preiss (*6*) have reported 58-fold enhancement of ADPG pyrophosphorylase activity in spinach-chloroplast preparations by 3-phosphoglyceric acid. Tests with normal ADPG pyrophosphorylase preparations from endosperm tissue have shown only slightly enhanced activity (10 percent) when 3-phosphoglycerate is present, and sh_2 preparations still lack activity.

The lack of ADPG pyrophosphorylase activity results in accumulation of sucrose. At 22 days (Table 2) the mutant contains only 16 percent starch while normal maize contains 67 percent. The mutant contains more than normal reducing sugars; it contains 27 percent sucrose in contrast with the normal 2.4 percent. No attempt has been made to measure the concentrations of the hexose phosphates in the two types.

Recondo and Leloir (*7*) reported that ADPG was much more active for starch synthesis by a starch granule–bound glucosyl transferase than was uridine diphosphate glucose. We have reported that preparations of starch granules from normal maize endosperms transferred glucose from ADPG 2.5 to 3 times as rapidly as from uridine diphosphate glucose (*8*). Akatsuka and Nelson later found that a soluble α-1-4 glucan synthetase from endo-

Table 2. Reducing sugars, sucrose, and starch in endosperms of sh_2 mutant and of normal maize 22 days after fertilization; they were measured by the methods of Somogyi (*15*), Roe (*16*) (after heating with alkali), and McCready *et al.* (*17*), respectively.

Endosperm	Concentration (% dry wt)		
	Sugars	Sucrose	Starch
sh_2	3.63	27.34	16.25
Normal	1.60	2.35	67.14

Table 3. Radioactivity, from sucrose-C^{14}, incorporated by starch granules when preparations from sh_2 or normal endosperms were present; incorporation by starch granules per microgram of protein in enzyme preparations. The reaction mixture contained 10 μl of enzyme preparation (with 56 μg of protein for sh_2 and 90 μg for normal maize) in a total volume of 50 μl, including (μmole): 0.6 Mg^{++}, 5 NaF, 0.2 sucrose (51,000 count/min, uniformly labeled), 3 glycylglycine buffer at *p*H 7.5, and 0.15 ADP or UDP, where added. Enzyme preparations were made by homogenizing 15 g of endosperm with 30 ml of 0.05*M* phosphate buffer (*p*H 7); after centrifugation, the fraction precipitated by 15- to 65-percent $(NH_4)_2SO_4$ saturation was suspended in 3 ml of chilled water and dialyzed against water for 16 hours at 4°C. After centrifugation the supernatant was assayed. Coupling system and experimental conditions were as described in Table 1. UDP, uridine diphosphate.

Additions to sucrose	Incorporation (count/min)	
	sh_2	Normal
None	0.1	0.2
+ ADP (0.15 μmole)	13.8	10.6
+ UDP (0.15 μmole)	13.4	10.3
+ ADP (0.15 μmole) and UDP (0.15 μmole)	9.6	6.4

sperm preparations of normal maize or of the waxy, sugary, or amylose-extender mutants transfers the glucose moiety from ADPG and uridine diphosphate glucose at ratios of 30 : 1, 3 : 1, and 2 : 1 with phytoglycogen, amylopectin, and amylose as acceptors, respectively (*9*). Identification of ADPG in *Chlorella* (*10*), and its isolation from sweet corn (*11*) and from rice (*12*), indicate that ADPG could be a natural substrate for starch synthesis.

On the basis of experiments with rice Murata *et al.* (*13*) proposed that ADPG could be formed by way of a reversal of sucrose synthetase activity (sucrose + ADP → ADPG + fructose). Sucrose synthetase in maize has much less affinity for ADP than for uridine diphosphate, and the reaction is strongly inhibited by all the uridine-containing nucleotides (*14*). This does not seem to be an important pathway in maize for the formation of ADPG, but (Table 3), when sucrose-C^{14} and either ADP or uridine diphosphate are used as substrates, sh_2 and normal preparations do permit about 1.5-percent incorporation by starch granules. The relatively small amount of starch synthesis in sh_2 mutants may in part reflect a small amount of ADPG formation by way of sucrose synthetase; in part, the transfer of glucose from uridine diphosphate glucose by the soluble and starch granule–bound glucosyl transferases.

In the sh_2 mutant, which completely lacks ADPG-pyrophosphorylase activity, starch synthesis is substantially blocked; this indicates that starch synthesis in the normal maize endosperm proceeds largely by way of ADPG as a substrate, and that ADPG is chiefly synthesized through the action of ADPG pyrophosphorylase. It also indicates that only limited amounts of starch are formed with uridine diphosphate glucose as a substrate, and rules out the possibility that any substantial portion of starch synthesis proceeds by way of glucose-1-phosphate and starch phosphorylase.

Note added in proof: We have found that *brittle-2*, another mutant that accumulates sucrose, also lacks ADPG pyrophosphorylase activity. We have not yet succeeded in obtaining enzymic activity in mixtures of mutant extracts.

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18. Research supported by NSF grant GB-1073. Journal paper No. 2657 of the Purdue University Agricultural Experiment Station.

15 October 1965

Sponge: Effect on the Form of Reef Corals

Abstract. *The sponge*, Mycale laevis, *when encrusting the lower surfaces of flattened reef corals, induces marked peripheral folding of the host colonies. This relationship, though facultative, has advantages for both associates. The sponge has a continually enlarging substrate that is free from competitive sessile forms. The coral may benefit from an increased feeding efficiency as a result of water currents produced by the sponge and it is protected from invasion by boring forms, notably clionid sponges.*

The common West Indian sponge, *Mycale laevis* (Carter), grows frequently in close association with living corals in the fore-reef slope environment (*1*) of Jamaica. The sponge encrusts the lower surfaces of certain reef corals that have assumed a flattened colony form as a result of growing in dimly illuminated reef habitats. *Montastrea annularis* (E. and S.) is the most common coral involved in the association, but the sponge has also been found living with *Montastrea cavernosa* (Linn.), *Mycetophyllia lamarckana* (E. and H.), *Porites astreoides* (Lamarck), and *Agaricia agaricites* (Linn.).

Corals associated with *Mycale* assume a characteristic shape: the edges of the colonies become indented at intervals by more or less prominent peripheral folds, the oscules of the sponge opening outward at the apex of the arches. The appearance of such an association is shown in Fig. 1. It seems likely that the upturned folds along the edge of the coral are formed in response to the strong currents of exhalant water flowing out through the oscules of the sponge.

The arches tend to be relatively uniformly spaced along the edge of the coral, a distance of 9 to 12 cm being a common interval as measured along a line following the coral's undulating edge (Table 1). Since each arch in the coral's edge bears an oscule of the sponge, the relative uniformity of spacing of the arches suggests that there is an optimum volume of sponge tissue which can be drained through any one exhalant canal system. Arches and oscules tend to be smaller when closer together and larger when more widely spaced.

The association appears to have advantages for both animals. The underside of the coral offers the sponge a substrate that progressively increases in surface area, the more so because of the folding of the coral's growing edge induced by the sponge. If *Mycale laevis* becomes established on a young, clean coral, by keeping up with the growth of the coral the sponge is provided with a continually enlarging substrate that is free from competitive sessile organisms. The coral may benefit in two ways. The outwardly directed, exhalant water currents issuing from the oscules of the sponge probably increase water circulation over the adjacent coral surface and thus lead to a localized increase of the coral host's feeding efficiency and growth rate. This effect may facilitate

Table 1. Arch intervals (in centimeters) along the edges of coral colonies (*Montastrea annularis*) overgrown by the sponge *Mycale laevis*. Diameters of colonies: specimen 1, 23.0 and 16.5 cm; specimen 2, 16.5 and 11.5 cm; specimen 3, 12.0 and 10.5 cm.

Specimen 1	Specimen 2	Specimen 3
9.5	9.5	11.5
8.0	9.0	8.0
11.0	11.0	8.0
11.0	11.5	4.0
12.0	9.0	5.5
20.0	3.0	5.0
12.0	2.5	2.5
14.0	4.0	6.0
11.5		7.0
11.0		5.0