specific information through existing cells. In any event, a clonal distribution of antibody-forming cells is not necessarily a confirmation of the clonal theory of antibody formation (*5*) since the events reflected in the present tests are probably only the response of individual cells to information produced within, and transmitted from, a different cell; the latter may even be of an entirely different type (*6*).

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3. In fact, the variances are so low that a word about this is in order. It should be noted that the replicate samples from a single piece are derived from a thoroughly homogenized cell suspension and that only a very minor proportion of the many cells plated are scored. A Poisson distribution, therefore, cannot be expected for these samples.
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Pheromone: Evidence in a Decapod Crustacean

Abstract. *Males of the species* Portunus sanguinolentus *display a behavioral response to the presence of premolt females which is the same as their behavior when they are exposed to water in which premolt females have been kept. Release of a sex-attractant pheromone is indicated. When females are prevented from releasing urine, there is no evidence of the attractant.*

Copulation in many crustaceans is associated with the molting period (*1*). In the Brachyura, or true crabs, copulation in some species occurs immediately after molt of the female. For several days, the male carries the premolt female, and the male is in attendance at ecdysis. Copulation occurs 2 to 5 minutes after the female extracts herself from the exuvia (*2*). The way the male crab detects the premolt condition of the female is unknown. Certain workers conclude from their experiments that only the submissive behavior of the female indicates her condition to the male (*3*), whereas others suggest that detection of the premolt condition is chemosensory in crabs and other crustaceans (*4*). Occurrence of sex attractants in insects, in contrast with crustaceans, is well documented (*5*).

My experiments demonstrate release of a sex-attractant pheromone in *Portunus sanguinolentus* (Herbst), a large edible crab of the Indo-West Pacific, which closely resembles the blue crab of the United States. Evidence is also presented that suggests where the locus of release of the substance is in the female.

In 114 mating pairs of this species from Kaneohe Bay, Hawaii, males carried females up to 6 days before molt of the female, which occurred in any month. As Agassiz noticed (*6*), whenever a premolt female was placed in a holding cage containing several males, the latter began a peculiar display and search behavior. Each male became active, walked about the cage on the tips of its dactyls with its body elevated at maximum height above the substratum, extended its oxblood red chelae (female chelae are white), and attempted to pull whatever crab it came in contact with, male or female, into the precopulatory holding position. Crabs other than premolt females usually escaped.

During a 4-year period, 850 molting crabs were kept with adult males in holding cages which held up to 40 crabs at one time. In absence of premolt females, adult males never exhibited the search behavior or attempted to grasp premolt males or premolt juvenile females. The molting crabs had to be removed at the time of ecdysis to prevent them from being devoured. Adult males of *P. sanguinolentus* displayed no search behavior nor did they copulate with premolt or soft females of two other species of Portunidae, *Thalamita crenata* and *Podophthalmus vigil*, which also copulate when they molt. Nor did males of these other species make any attempt to copulate with *Portunus sanguinolentus* females which molted in their presence.

Evidence that a pheromone was present was sought by siphoning water in which females had been kept into aquariums containing adult males. Subject males were in intermolt and had been in captivity without exposure to premolt females for at least 48 hours prior to the experiments. Each female to be tested was kept in an 8-liter polyethylene bucket of clean sea water for 2 hours and then removed. The premolt females had been tested during the premolt stage before either of the two sexually mature instars (*2*). Hard, intermolt females served as controls. A positive response to the introduction of water was display and search behavior.

Table 1. Release of sex attractant by *Portunus* females as indicated by the response of males to water in which either premolt or intermolt females had been kept. Positive response (+) is search behavior of one male within 5 minutes after the siphon flow ended; negative response (−) is no reaction in 5 minutes by one male.

Trial	Crabs (No.)			Response	
	Premolt ♀(A)	Intermolt ♀(B)	♂	A	B
1	2	1	6	++++	−−
2	2	1	6	++−−	−−
3	2	1	6	++++	−−

Males were tested with water from the premolt and control females in an adjacent pair of wooden tanks. Dimensions of the tanks varied in different replicates, but identical conditions were maintained in the paired tanks in each replicate. Two males were placed in each tank, with running sea water, 1 hour before testing. The sea water supply was turned off, and the water level was lowered to allow test water to be added. Equal flow and pressure were maintained by placing each of the identical 8-liter buckets of water at the same height above the water level of the aquariums. Siphon tubes were of

Table 2. Release of sex attractant by *Portunus* females as indicated by the response of males to water in which premolt females had been kept before (C) and after (D) the excretory pores had been capped and after cap removal (E). Positive response (+) is search behavior of one male 5 minutes after the siphon flow ended; negative response (−) is no reaction in 5 minutes for one male.

Trial	Crabs (No.)		Response		
	♀	♂	C	D	E
1	1	4	++	−−	Not done
2	1	6	+−	−−	++
3	1	6	++	−−	++
4	1	6	++	−−	++

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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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,