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Vision has a role in *Limulus* mating behaviour

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In 1928 Hartline noted that the lateral eye of the horseshoe crab, *Limulus polyphemus*, was an admirable preparation for research in visual physiology. Since that time, extensive studies of *Limulus* have yielded fundamental understandings of visual processes and of sensory processes in general[1,2]. However, except for demonstrations of primitive phototaxis[3-5], studies have not yet revealed a possible role of vision in the animal's behaviour. We have further investigated the problem by studying mating behaviour, the only known behaviour exhibited by *Limulus* in its natural habitat. We report here that horseshoe crabs discriminate form and contrast during mating: males are attracted to painted cement castings of the female carapace and other forms, the degree of attraction depending on the form and contrast of the castings, and on the time of day. The discrimination of form may result from information transmitted by several sensory systems but the discrimination of contrast requires vision.

Along the eastern coast of North America, *Limuli* move in from deep water in the spring and build nests on protected beaches near the water's edge at high tide[6]. Numerous female–male pairs arrive, together with an excess of males. We observed the activity of males in the vicinity of cement castings placed in the water at a nesting beach.

The cement castings were made from an adult female carapace (27 cm wide), a hemisphere (29.5 cm diameter) and a cube (1,640 cm^2). The exposed surface areas of the hemisphere and cube were each equivalent to that of the female carapace (1,365 cm^2). Each casting was painted black, grey or white, yielding relative contrasts against sand underwater of −0.70, +0.1 and +0.75, respectively. The average relative contrast of the *Limulus* carapace against sand was -0.33 ± 0.03 ($n = 12$). All contrast values were determined by reflection densitometry, using a reflection density guide (Kodak 146 5947) and panchromatic black and white film (Kodak Plus-X). The three different castings, together with three shades of paint, yielded nine experimental objects. Two of each object, giving a total of 18 castings, were placed in random order in a row 4 m below the high-water line on the south side of Mashnee Dike, Cape Cod, Massachusetts. We observed the behaviour of males in the vicinity of each casting during eight high tides, four at night and four during the day.

Figure 1*a* shows a male *Limulus* mounted on a black cement casting of the female carapace. The male has mounted the dorsal part of the casting in the usual position for egg fertilization. Some males maintained this position for several hours. Figure 1*b* shows a single male soon after it approached and made contact with the prosomal region of a grey casting. Figure 1*c* shows a male mounted on a white casting; four other males hover nearby, apparently searching for an opportunity to mount. The behaviour in Fig. 1 is indistinguishable from that of normal mating. Similar behaviour was observed in the vicinity of every cement casting, but the number of males around a specific casting depended on its form and contrast.

Figure 2*a* shows that the number of males was greatest around the female models and lowest around the cubes. In each of eight observation periods, males selected female models in preference to hemispheres and hemispheres in preference to cubes. The probability of these results occurring by chance is 3^{-8}; $p < 0.001$. The degree of selection was not significantly influenced by time of day. We conclude that male *Limuli* can discriminate form. Discrimination of form may be based on tactile or visual inputs, or both.

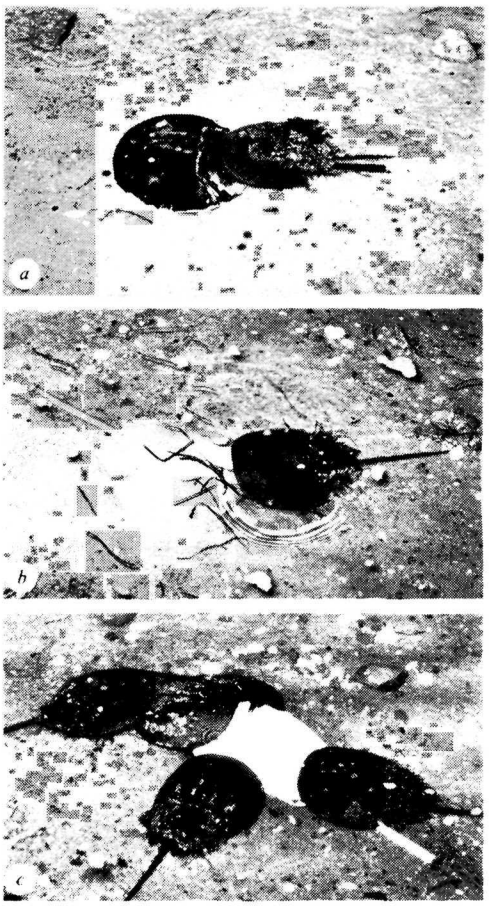

Fig. 1 Male horseshoe crabs in the vicinity of submerged, painted castings of the female carapace. *a*, A male *Limulus* mounted on the posterior portion of a black casting in the normal position for egg fertilization. *b*, A male in contact with a grey casting soon after approaching the model. *c*, One male mounted on a white casting with four others hovering nearby apparently searching for the appropriate mating position. All photographs were taken during the same night-time observation period with Kodak Plus-X film and flash. The animals and models are under water in each photograph. Note in *b* the ripples generated on the water surface by the male. The overall length of the black casting in *a* is 55 cm.

Figure 2*b* shows that the contrast of a casting also influences male mating behaviour. The data on male contacts with castings were pooled according to the shade of paint used (black, grey or white). Black castings yielded the highest percentage (40%) of male contacts, regardless of time of day. The attraction of males to the grey castings was also high and almost equal to that of black castings during the day but not at night. Black and grey castings may have attracted most males during the day because they are similar in shade to the female carapace and are clearly visible. Note that the relative contrast of the female carapace against sand is −0.33, which falls within the contrast values of the black and grey castings (−0.7 and +0.1). At night, the low relative contrast of the grey castings against sand may have made them less visible. Indeed, the grey *Limulus* casting in Fig. 1*c* is difficult to distinguish from its underwater background. We could not see the grey castings at night but had no difficulty in detecting the white and black castings by illumination from either a new or full moon. The results in Fig. 2*b* suggest that, when visibility is not a limiting factor, male *Limuli* are attracted to objects that appear similar in shade to the female carapace.

Taken together, the data in Fig. 2*a* and *b* show that males were attracted most to the black *Limulus* casting and least to the white cube: in each of the eight observation periods, black *Limulus* castings yielded the highest number of male contacts and white cubes the lowest number.

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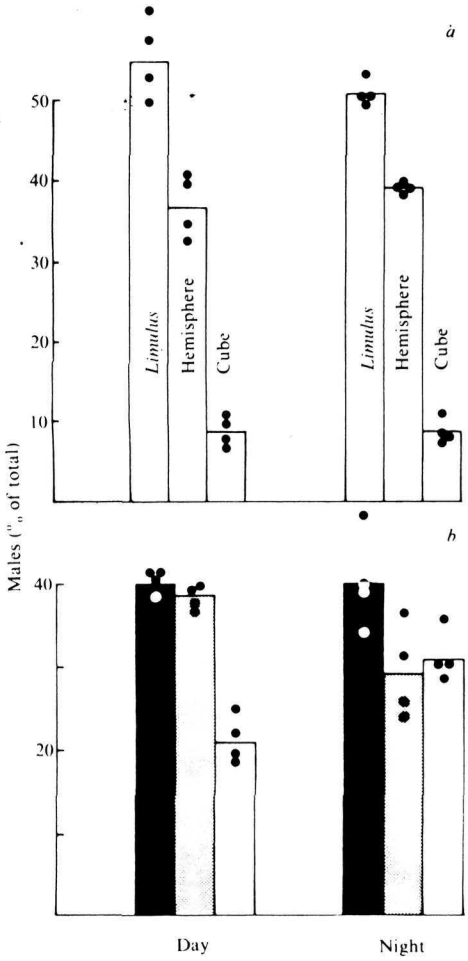

Fig. 2 Percentage of males observed at the cement castings during the day and at night. *a*, Filled circles indicate the percentage of males observed at each cement form during four daytime and four night-time observation periods. The daytime percentages did not differ significantly from the night-time values. Columns represent the average values. *b*, Filled and unfilled circles indicate the percentage of males in contact with the black (solid columns), grey (stippled columns) and white (open columns) castings during the same eight observation periods as in *a*. Columns represent the average values. The black castings attracted the greatest percentage of male *Limuli* regardless of time of day. The attraction of the grey castings was greater during the day, that of the white castings lower. Results are based on a total count of 6,988 males in contact with the set of 18 castings described in the text. The data were collected during eight high tides as males, in their search for females along the water's edge, approached and explored the submerged castings. Two observers separately surveyed the castings every 5 min during the 3-hr period that the tide covered the set of castings. The day- and night-time data were then summed and normalized.

Blinded animals were not attracted to the cement castings. In one night-time experiment we covered the median and lateral eyes of 75 male *Limuli* with black acrylic paint. The density of males around the castings fell by a factor of 8, and the distribution of the few animals found near the castings did not match that shown in Fig. 2. This result cannot be considered conclusive because some of the blinded animals buried themselves in the sand after being released at the water's edge. Nevertheless, the results are consistent with the notion that vision has a role in mating behaviour.

Visually guided behaviour is also evident from films taken during the day, which show that male *Limuli* passing within 2 m of a casting of a female carapace often change orientation and travel directly towards the casting, then explore and attempt to mount it.

Vision thus appears to play a part in *Limulus* mating behaviour, but other sense modalities may also be involved. Our observations suggest that male animals are attracted to objects by form and contrast. Maintained contact with objects may be based on tactile cues transmitted by a well developed mechanoreceptor system[7,8]. Chemical cues may also be involved[9], but as yet no information on chemical attractants is available.

Circadian rhythms in the *Limulus* visual system[10] may be an integral part of the mechanisms underlying the behaviour reported here. At night a circadian clock in the *Limulus* brain transmits efferent neural activity to the lateral eyes, increasing the response and decreasing the noise of single photoreceptor cells[11]. Circadian changes in retinal physiology and anatomy increase the sensitivity of a dark-adapted lateral eye by as much as 30–100 times at night[12]. High visual sensitivity may adapt the animal for specific nocturnal behaviour such as mating.

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Dr. Robert B. Barlow
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Dear Dr. Barlow:

I have read with interest your recent paper on the mating behavior of Limulus (Nature, Vol. 296, pp. 65-66) and would appreciate have a reprint if available. Upon reading it I went back to my dissertation to see if I recorded a similar situtation (males attracted to a round stone at the water's edge). A copy of the pertinent pages are enclosed for your use as well as two of my recent Limulidae articles.

If your earlier papers on Limulus are available, I would like to have them also.

Sincerely,

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encl.

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ON MORPHOMETRIC AND SEROLOGICAL RELATIONSHIPS WITHIN THE LIMULIDAE, WITH PARTICULAR REFERENCE TO LIMULUS POLYPHEMUS (L.)

Carl Nathaniel Shuster, Jr., Ph.D.
New York University, 1955

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ON MORPHOMETRIC AND SEROLOGICAL RELATIONSHIPS WITHIN THE LIMULIDAE, WITH PARTICULAR REFERENCE TO LIMULUS POLYPHEMUS (L.)

(Publication No. 24,890)

Carl Nathaniel Shuster, Jr., Ph.D.
New York University, 1955

Adviser: Harry A. Charipper

This is a study of horseshoe "crabs" (Limulidae). Throughout, the compelling desire was to find out more about the animals. Two distinct phases of research were pursued: (1) a description of the Limulidae based upon morphometric and serological data, and (2) observations on the natural history of Limulus.

The investigation was carried out under the co-sponsorship of Professor Harry A. Charipper, New York University, and Professor Thurlow C. Nelson, Rutgers University, The State University of New Jersey.

The thesis was largely supported by a grant from the Research Council, Rutgers University, The State University of New Jersey (July, 1953, to June, 1955). A preliminary study was sponsored by the Woods Hole Oceanographic Institution during the summers of 1949 and 1950.

Over 20,000 specimens of Limulus polyphemus, both adults and immature specimens, were collected from fifteen localities along the eastern coastline of the United States, from Maine to Florida, and varying amounts of morphometric data obtained. The number of specimens studied was sufficiently large to permit statistical treatment of the data. Adults of the Indo-Pacific species in the United States National Museum collection, Tachypleus tridentatus, 5 specimens, and Carcinoscorpius rotundicauda, 2 specimens, were studied.

The text of the thesis, 119 pages, includes 23 tables of data and a bibliography of 131 references. In addition, there are 36 plates with 101 figures and an appendix of 30 pages.

Morphometric considerations included a study of the external anatomy of Limulus with twenty-two figures, a terminology to describe the topographical characteristics, and a list of the synonyms in terminology. Thirty-four photographs of Limulus show sixteen representative adult males and females and eighteen examples of variations and abnormalities. Aspect views of the Indo-Pacific Limulidae are shown by thirty-three pictures.

The morphometric data revealed that the American species of horseshoe "crab," Limulus polyphemus (L.), is comprised of morphometrically distinguishable races which are for the most part geographically isolated. Although the geographical range of this species is from Yucatan, Mexico, to Maine, the optimum portion of this range, judged by the body size of the adults, is from Georgia to Cape Cod. The animals are smaller in the southern and northern portions of the range.

Body proportions, relative to the size of the animal, the sex, and the geographical location have been studied and significant results have been obtained.

The morphometric data show as great or greater agreement between Limulus polyphemus (subfamily Limulinae) and Tachypleus tridentatus (subfamily Tachypleinae) than between Tachypleus and Carcinoscorpius rotundicauda (subfamily Tachypleinae).

Precipitin reaction tests were conducted on the serum of ~~the~~ three species of Limulidae, ~~listed above~~. Serologically the Indo-Pacific species are far removed from Limulus indicating that the three species, on the basis of their serum proteins, are not cogeneric.

Observations on the natural history of Limulus were on the season of spawning and spawning behavior, growth stages and increments of growth. The one-year age class in one population was determined. Data suggesting that adult males do not molt were given. A field test for distinguishing between immature and adult females was explained. The unusual distribution of Limulus within the Gulf of Mexico, where it abounds at the "entrance" to the Gulf, along the shores of Florida, and Yucatan and Campeche, was discussed. The enemies of Limulus were listed and certain abnormalities of the exoskeleton, particularly the telson, described.

This thesis increases our knowledge of the horseshoe "crab": the taxonomy of the extant species and the natural history of Limulus polyphemus. It contains information that should be of interest to paleontologists, physiologists, and to zoologists in general.

287 pages. $3.70. Mic 58-4034

Microfilm copy of complete manuscript of 287 pages available from University Microfilms, Inc., Ann Arbor, Michigan, $3.70. Enlargements 5-1/2 x 8-1/2 inches, .04¢ per page.

* *Limulus polyphemus*
Tachypleus gigas
Carcinoscorpius rotundicauda

that the males approach the shoreline earlier than the females. It has been commonly observed that many more adult males are found along the spawning beaches during a spring following a "warm" winter than after a "cold" winter, when the ratio of males to females is more nearly one-to-one.

Spawning males generally precede the females to the beaches where they move about in the shoal water seemingly anticipating the arrival of the females (Shuster, 1953). At high water time the males approach the beach and "patrol" along the beach in the shallow water. Their general path of motion carries them in a zig-zag path, "zigging" up to the water's edge and then "zagging" back into the shallow water. The behavior of the females as they approach the beaches is different. They generally proceed directly to the beach, up to the water's edge, crossing the area of the "along-shore" paths of the males. If an unattended female passes through this area of the patrolling males which is parallel to the beach, she will usually be "observed" and then accompanied by one or more males. It is the general direction of motion and perhaps the larger size of the females that appears to stimulate the patrolling males. Even a male moving up to the water's edge will be clasped by another male. But in most cases the continued "searching" activity of the

first male along the beach "discourages" the attached male which then leaves.

Another interesting note on the behavior of these spawning animals was recorded by Noble (1945) who wrote that "a pair of horseshoe crabs about to spawn will be hastened in their duty if confronted by another pair already in action." But this amount of "perception" or "instinct" is not always so keen. On the night of May 5, 1951, between the hours of 2150 and 2245, Dr. Haskin and the writer found four males attempting to mate with a large smooth rock at the water's edge. This rock was roughly circular in shape, about one foot in diameter and four inches high at its middle, sloping toward its edges. The shape of the rock, its presence at the water's edge, and its lack of motion apparently misled the males which were jousting each other about to obtain a "more favorable" position beside the rock.

Cape May shore of Delaware Bay near Pierce Point

Pomerat (1933) has analyzed mating in Limulus, based upon one hundred spawning pairs. We can add to his observations in the following respects: (1) There are three principal areas of contact between the male and female in amplexus. Two points of contact are formed by the coupling action of the male pedipalps upon the lateral projections of the female opisthosoma; the third by the contact of the arched male prosoma upon the