The Probable Method of Fertilization in Terrestrial Hermit Crabs Based on a Comparative Study of Spermatophores[1]

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ALTHOUGH the physiological and mechanical phenomena of the vasa deferentia resulting in the elaboration of the spermatophores of marine hermit crabs (Pagurida) have been investigated (Mouchet, 1931; Matthews, 1953), these phenomena in terrestrial hermit crabs (*Coenobita* and *Birgus*) have been neglected. Spermatophores of marine hermits are made with precision; the so-called immutable form of their acuminate capsules is often distinctive of the species. These capsules, "elevated" on slender stalks, are admirably adapted for aquatic dissemination of the spermatozoa. Because marine hermit crabs are regarded as the progenitors of terrestrial hermit crabs, the present study was undertaken to reveal whether the form of the aquatic spermatophore has become modified in the change to a terrestrial environment, and if so, to consider, in lieu of actual observation, whether these modifications are of sufficient magnitude to make tenable the common assumption that in terrestrial hermits fertilization occurs on land.

METHODS AND TECHNIQUES

The method employed was to study the phenomena of spermatophore elaboration in (1) a truly aquatic hermit crab, (2) a "transitional" terrestrial hermit with gastropod shell, and (3) a terrestrial hermit, no longer protected by a gastropod shell. For the typical aquatic hermit, *Dardanus punctulatus* (*vide* Edmondson, 1946: 265) was selected. These were taken at Oahu, Territory of Hawaii, between June, 1953, and July, 1954. *Coenobita rugosus*, the selected "transitional" hermit, and *Birgus latro*, the selected terrestrial hermit, were taken at Eniwetok Atoll (Marshall Islands) between September 1, 1954, and September 15, 1954.

The abundant *C. rugosus* were easily collected but the scarce *B. latro* had to be dug from their burrows. These hermits were killed and dissected at the Marine Laboratory at Eniwetok. There, the male reproductive systems, for purposes of routine histological examination, were placed in Bouin's fixative, cleared in toluene, embedded in Tissuemat (54°–56° C.), sectioned at 10 microns, stained with standard alum-haematoxylin and counterstained with eosin (0.5 per cent solution in 90 per cent alcohol to which 04. cc. of 0.1 N HCl was added). Some testis sections of both aquatic and terrestrial hermits were stained with Heidenhain's iron-haematoxylin without a counterstain, while others were stained with safranin (Grubler's "Safranin O") and counterstained with light green. Because the large, proximal portions of the vasa deferentia of both aquatic and terrestrial hermits became extremely brittle after fixation, and were sectioned only with difficulty, sim-

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ilar unfixed portions were immersed for 10–30 minutes in an aqueous solution of toluidin blue (1–10,000), teased open in sea water, and their vitally stained contents studied.

OBSERVATIONS

Macroscopic Observation of Abdomens

When removed from the protective gastropod shell, the soft, vulnerable abdomen of *D. punctulatus* is seen to be twisted in accordance with the clockwise helical shell. Although five tergal plates are clearly discernable, they are weakly sclerotized. Pleopods are present only on the left side. The uropods are highly modified and serve primarily as hold-fasts.

Removal of the protective gastropod shell from *C. rugosus* discloses an abdomen which approximates that of *D. punctulatus*. Again the soft abdomen is twisted in compliance with the spiral shell and although five tergal plates are present they are poorly sclerotized. As in aquatic hermits, the pleopods are present only on the left side. Again the uropods are modified for attachment.

The unprotected abdomen of *B. latro* (*vide* Harms, 1932: figs. 1, 2, 3, 4) is straight, stubby, and its broad tergal plates are more heavily sclerotized than either those of *D. punctulatus* or *C. rugosus*. Pleopods are again restricted to the left side. The uropods are modified and reduced but obviously serve no longer as hold-fasts.

Macroscopic Observations of Dissected Abdomens

Typical of the dissected abdomens of aquatic hermits is (Matthews, *op. cit.*, fig. 2–b, p. 257) that of *D. punctulatus* in which a large, paired, hepatopancreas almost fills the entire abdominal cavity. The hepatopancreas, because of connecting mesenteries, carries the testes and vasa deferentia as it follows the turns of the abdomen and is largely responsible for the asymmetrical visceral arrangement. As in other aquatic hermits which inhabit right-handed shells, the right testis and right vas deferens are crowded and somewhat reduced.

The dissected abdomen of *C. rugosus* is almost identical with that of *D. punctulatus*. Again the large hepatopancreas almost fills the abdomen and carries with it the crowded testes and vasa deferentia and again this results in the reduction of the right testis and right vas deferens.

The dissected abdomen of *B. latro* reveals a large hepatopancreas joined to the other viscera by mesenteries. The testes and vasa deferentia are neatly fitted between the right and left portions of the hepatopancreas. Slight differences in size of testis and vas deferens are observed, but the smaller occurs on the right or left side indiscriminately.

The living vasa deferentia of *D. punctulatus*, *C. rugosus*, and *B. latro* exhibit spasmodic, muscular contractions (*vide* Matthews, 1953: 258). However, the opacity of the vasa deferentia prevents the actual observation of the effects of these contractions on the pliable sperm mass. But, as will be shown subsequently, these contractions serve both to move the sperm mass along and to mold it in compliance with gradual changes in the conformation of the vasa deferentia.

Microscopic Examination of Sectioned Testes

The sectioned testis of *D. punctulatus* is a highly coiled, continuous, thin-walled tube into which numerous sacculi open (*vide* Matthews, 1953: 258, fig. 3). The sacculi in cross section are observed in all stages of maturity. The immature sacculus is filled with large primary spermatocytes; others, more mature, are filled with spermatids in all stages of metamorphosis. These are expelled from the sacculus into a minute collecting tubule of the testis by the proliferation of new primary spermatocytes from the germinal epithelium of each sacculus. In other words, the old metamorphosing spermatids are crowded or pushed out of the sacculus by the development of new primary spermatocytes. The study of many sacculi reveals this to be a rhythmical process. This process produces a continuous sperm mass. This undifferentiated sperm mass enters the small, proximal vas deferens (*vide* Matthews, 1953: 259, fig. 6).

Fundamentally, the sectioned testis of *C. rugosus* resembles that of *D. punctulatus*. The sacculi open into a minute, highly coiled collecting tubule. Sacculi are seen in all stages of maturity. Except for slight differences of detail, the process of filling the sacculi with primary spermatocytes and the expulsion of metamorphosing spermatids is identical with that of *D. punctulatus*. Again the combined, rhythmical, saccular activity provides a continuous, undifferentiated sperm mass which enters the proximal portion of the vas deferens.

The sectioned testis of *B. latro* resembles those of *D. punctulatus* and *C. rugosus*. Sacculi in all stages of maturity are observed and again their rhythmical activity fills the minute collecting tubule with metamorphosing spermatids. The differences observed are primarily of size, the sacculi of *D. punctulatus* and *C. rugosus* being generally smaller than those of *B. latro*. Also observed were slight differences in the number and arrangement of sustentacular cells (*vide* Matthews, 1954: 116, fig. 2b). Although these were present in all sacculi whose spermatids were metamorphosing, their fate still remains obscure.

Microscopic Examination of Sectioned Vasa Deferentia

Cross sections through the proximal portion of the vas deferens of *D. punctulatus* reveal a minute cylindrical tube with a thin wall of contractile tissue and cuboidal epithelium, the almost circular lumen of which is completely occupied by a discoidal portion of the continuous, rod-shaped sperm mass (*vide* Matthews, 1953: 259, fig. 6). Except for slight differences in size, comparable portions of the proximal vasa deferentia of *C. rugosus* and *B. latro* appear identical with those of *D. punctulatus*.

In *D. punctulatus*, as the vas deferens gradually increases in diameter the once circular lumen becomes ellipsoidal (*vide* Matthews, 1953: 260, fig. 7). This change in shape of the lumen is the result of the bounding epithelial cells which have become columnar except at the more pointed extremities of the lumen where cuboidal epithelium still persists. An acidophylic secretion from these cuboidal cells lines the lumen but later surrounds and adheres to the sperm mass to form the thin, sperm mass sheath (*vide* Matthews, 1953: 261, fig. 9). In compliance with the change in shape of the lumen, the sheathed sperm mass becomes ellipsoidal. In *C. rugosus* and *B. latro* cross sections through comparable regions of the vas deferens also reveal ellipsoidal lumina and sheathed sperm masses.

In *D. punctulatus*, cross sections of the vas deferens reveal that the ellipsoidal lumen gradually becomes pear-shaped and that the sheathed sperm mass becomes folded into arches (*vide* Matthews, 1953: 262, fig. 10). A new secretion arises from the epithelial cells at the more pointed end of the lumen; this secretion fills the spaces between the closing arches and forms the "upright" stalks (*vide* Matthews, 1953: 262–263, figs. 11, 12, 13). As this secretion accumulates, the stalks lengthen and the ampullae of sperm are carried "aloft."

In *C. rugosus* and *B. latro* a similar change from an ellipsoidal to a pear-shaped lumen is observed and, accompanying this change, the contained, sheathed, sperm mass is folded into arches. In like manner a secretion, arising from epithelial cells of the pointed end of the lumen, fills the spaces between the closing arches and forms the "upright" stalks. Again, as this secretion accumulates, the stalks lengthen and "elevate" the closing arches to form the ampullae of sperm.

In *D. punctulatus*, *C. rugosus*, and *B. latro* (Fig. 1*a–c*) still another secretion is given off by the epithelial cells (*ep.*) bordering the lumen which surrounds the "upright" stalks (*st.*) and ampullae (*am.*) and forms the veil (*v.*). In *D. punctulatus* the groove of the lumen is deep and results in the formation of tall, thin spermatophores (Fig. 1*a*). In *C. rugosus* (Fig. 1*b*) and *B. latro* (Fig. 1*c*), the groove is shallow and results in the formation of short, broad spermatophores.

DISCUSSION

The spermatophores of *D. punctulatus*, *C. rugosus*, and *B. latro* are formed with great precision. The spermatophores of any one *D. punctulatus* are similar to those of any other of its species. In like manner, the spermatophores of *C. rugosus* and *B. latro* do not vary within the species. However, only in this restricted sense is it permissible to refer to the spermatophores of any one species as being immutable. Because immutable precludes change, its use in connection with spermatophores is untenable. What is probably meant by the use of this term is that, for any one species at the time of observation, spermatophores appear identical. Surely, the complicated mechanical and physiological phenomena associated with spermatophoric development have evolved throughout the ages and these changes have been accompanied by changes in spermatophores.

Although in *D. punctulatus* the actual process of copulation has not been observed, there is little doubt that it occurs in water. This species does not frequent the shallow reef waters and, so far as is known, never comes ashore even at night. Specimens taken in fishermen's nets frequently show spermatophores attached to both body and gastropod shell. This same attachment of spermatophores is also observed in this species at the Honolulu Aquarium where, prior to spermatophoric deposition, males are frequently seen dragging females about. However, since copulation probably takes place at night, it has not been possible to observe the process in these captive specimens. Moreover, as these specimens are afforded no opportunity to leave the tanks, copulation and the subsequent process of fertilization must occur in water. Therefore, the possibility of terrestrial copulation and fertilization in free living specimens remains but seems rather unlikely.

The spermatophores of *C. rugosus* (Fig. 2*b*) resemble closely the spermatophores of *D. punctulatus* (Fig. 2*a*). There are obvious differences in size of ampullae, upright stalks, veil, etc., but the fundamental plan is the same. This fact, coupled with similarities of spermatophoric development, strongly suggests that for this species the rate and extent of adaptive changes in the reproductive system did not parallel the rate and extent of other terrestrial adaptations. The similarity of these spermatophores with those of *D. punctulatus* should not be interpreted as due to the immutability of *C. rugosus* spermatophores, but rather, that at the particular time of observation, the spermatophores of *C. rugosus* had not "progressed" beyond the aquatic spermatophore level attained by *D. punctulatus*.

Again, copulation and the subsequent process of fertilization in *C. rugosus* have not, to the author's knowledge, been recorded. Even though *C. rugosus* in certain of its organ systems is admirably adapted for a terrestrial habitat, caution should be exercised in assuming that all organ systems are equally so adapted. The literature is replete with reference to the female's dependence on the sea. Harms (1932: 260 [translated]) states:

> The East Indian "Coenobites" seem to spawn all year long, but they seem to reach a climax in the months of January to March. During those months I observed how flocks of *Coenobita cavipes* and [C.] *clupeatus* wandered during the night toward the coast of Perbaoengan (East coast of Sumatra) in order to deposit their zoëa into the water. At the end of May till the beginning of July I observed how the beach swarmed with "Coenobites" in all phases of metamorphosis. . . . On the the island of Siberoet I saw the same thing between May 31 to June 5, 1929, but here *Coenobita rugosus* were involved. The young "Coenobites" emerge from the water with tiny snail-shells, and live at first amphibiously.

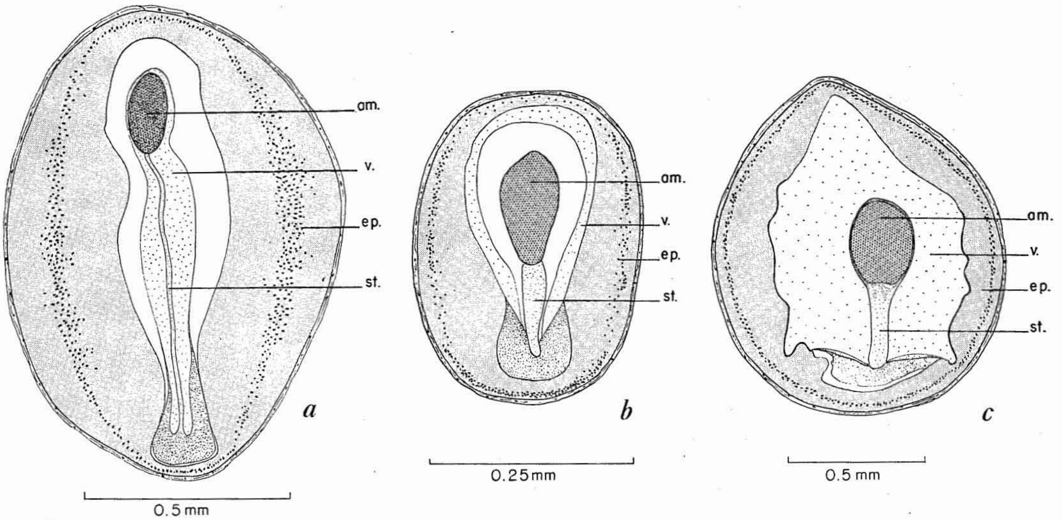

FIG. 1. Diagrammatic cross section of the vas deferens of: *a*, *Dardanus punctulatus; b*, *Coenobita rugosus; c*, *Birgus latro* showing: *am.*, ampulla; *v.*, veil; *ep.*, veil-producing epithelium; *st.*, stalk.

Again in reference to "Coenobites" dependence on the sea, Dr. Yoshio Kondo, terrestrial malacologist of the Bernice P. Bishop Museum, informs me (*in litt.*) that on Saipan swarms of these land crabs make monthly, full-moon migrations to the sea. At this time they are gathered by the bushels for food and, so far as is known, both males and females are taken. The possibility therefore exists that these nocturnal migrations may serve purposes other than the moistening of the gills or the deposition of zoëa.

At Eniwetok I observed both male and female *C. rugosus* on the beach at night although I did not observe any in the water or in the act of copulation. Because females are observed with spermatophores both on their bodies and on their gastropod shells, and because the tubelike extensions on the coxopodite of the fifth pereiopods are too large to serve as penes, it appears quite unlikely that an internal deposition of spermatophores takes place. Both the origin and the aquatic form of *C. rugosus* spermatophores suggest that copulation and fertilization occur in water.

The spermatophores of *B. latro* (Fig. 2*c*) resemble closely the spermatophores of *D. punctulatus* (Fig. 2*a*) and *C. rugosus* (Fig. 2*b*). There are obvious differences in size of ampullae, upright stalks, veils, etc., but again, the fundamental plan is the paguridan aquatic spermatophore. As in *C. rugosus*, this fact, coupled with the similarity of their spermatophoric development, strongly suggests that in *B. latro* also the rate and extent of reproductive adaptations did not parallel the rate and extent of other adaptations. Again, this is not an example of the immutability of spermatophores but rather, at the time of observation, the spermatophores of *B. latro* had not "progressed" beyond the level of the aquatic spermatophore attained by *D. punctulatus*.

Harms (*op. cit.*: 262 [translated]) in reporting on the reproductive activity in *B. latro* says:

> The process of copulation is at this time still unknown. The males do not have special organs for copulation. Yet copulation must take place, and seems to do so on land, since females are found at great distance from the coast. The sperms are probably transmitted by means of spermatophores. In response to excitation I got the male to emit masses of sperm [spermatophores]. These hardened subsequently. . . .

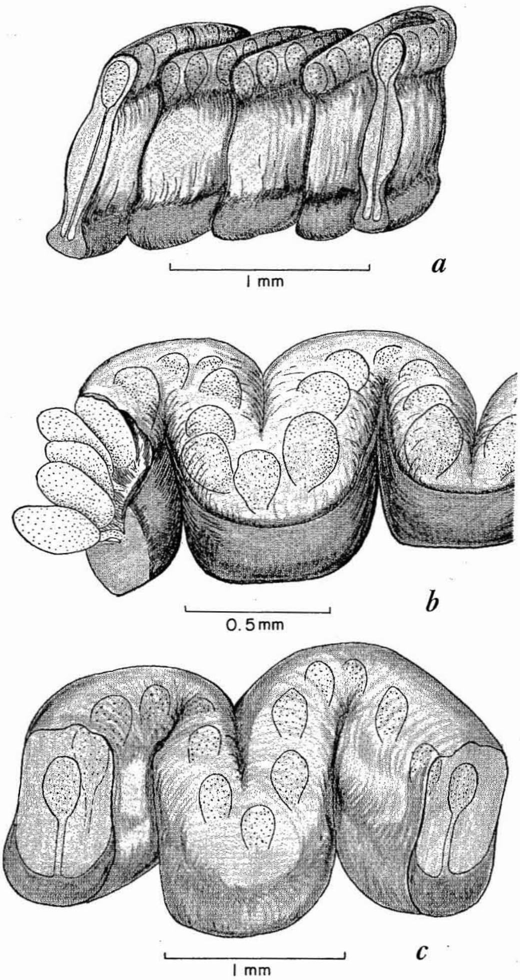

FIG. 2. Portion of vitally stained spermatophore of: *a*, *Dardanus punctulatus;* *b*, *Coenobita rugosus;* *c*, *Birgus latro*.

> The possibility exists that these gummed up masses of sperm are transferred to the female . . . the development inside the egg goes as far as the zoëa, which has been described by Borradaile [1900]. . . . Birgus has a typical paguride zoëa, which resembles on the whole the zoëas of *Spiropagurus* and of *Eupagurus*. . . . The peculiarity of the zoëas of Birgus lies in a certain simplification of its over-all organization, illustrated especially by the absence of otherwise typical bristles and teeth. Perhaps it can be assumed with certain confidence that further development up to the metamorphosis takes place exactly as with the rest of the pagurids, especially the "Coenobites."

Harms (1937: 14) further states that the eggs are fertilized at the place of deposit and become attached immediately. He does not say however, how or where this process takes place.

According to the observations of Andrews (1900) on Christmas Island, *B. latro*, too, like the "Coenobites" wanders in great flocks to the sea in the months of January to March. Presumably both males and females take part in these migrations and the possibility exists that in this case, as with *C. rugosus*, the purpose might be for copulation as well as the deposition of zoëa.

At Bikini in July 1947 Dr. Robert Hiatt observed numerous zoëa in the water in which a berried female had been contained overnight. He also observed berried females with their abdomens immersed in intertidal pools liberating zoëa.

Because this part of the reproductive cycle of *B. latro* requires an aquatic environment it appears likely that the liberation of sperm from the spermatophore and fertilization are aquatic. This would not obviate the possibility of a terrestrial attachment of the spermatophore. However, the similarity in the development of spermatophores in the "terrestrial" *C. rugosus* and *B. latro* and in the truly aquatic *D. punctulatus*, seems to argue for the assumption that copulation and the subsequent process of fertilization are also aquatic in these terrestrial species. Surely, this assumption is more valid than the assumption that for these species fertilization takes place on land, "since females are found at great distance from the coast." Without attempting to explain what has brought about modifications for a terrestrial existence, modifications of the reproductive system are not as urgent as, for example, those of the respiratory system which must be used continuously once emergence has occurred. Reproduction, although obviously important to maintain the species, is not a continuous process, therefore, not of primary urgency for the survival of the individual. When necessary, the crabs can return to their old habitat, water, as do the Amphibians.

In lieu of actual observation to the contrary the modifications of the spermatophores of *C. rugosus* and *B. latro* are not of sufficient magnitude to make tenable the common assumption that terrestrial hermit crabs are fertilized on land. Stationed at a likely spot, a patient observer, equipped with an infra-red light, may provide the ultimate solution to this problem by direct observation of copulation and fertilization in terrestrial hermit crabs.

REFERENCES

ANDREWS, C. W. 1900. *A monograph of Christmas Island.* xv + 337. British Museum (Natural History), London.

BORRADAILE, L. A. 1900. On the young of the robber crab (*Birgus latro* L.). In *Zoological results based on material from New Britain, New Guinea, Loyalty Islands and elsewhere, collected during the years 1895, 1896, and 1897 by Arthur Willey.* Part 5, 585–590. University Press, Cambridge.

EDMONDSON, C. H. 1946. *Reef and shore fauna of Hawaii.* [Rev. ed.] iii + 381 pp., 223 figs. Bernice P. Bishop Museum, Honolulu.

HARMS, J. W. 1932. Die Realisation von Genen und die consecutive adaption. II. *Birgus latro* Linné als Landkrebs und seine Beziehungen zu den Coenobiten. *Ztschr. f. Wiss. Zool.* 140: 167–290.

——— 1937. Lebensablauf und Stammergeschichte des *Birgus latro* L. von der Weihnachtsinsel. *Jenaische Ztschr. f. Naturw.* 71: 1–34.

MATTHEWS, D. C. 1953. The development of the pedunculate spermatophore of a hermit crab. *Dardanus asper* (De Haan). *Pacific Sci.* 7(3): 255–266.

——— 1954. The origin and development of the spermatophoric mass of a nephropsid lobster, *Enoplometopus occidentalis* (Randall). *Pacific Sci.* 8(2): 115–120.

MOUCHET, S. 1931. Spermatophores des crustacés décapodes, anomures et brachymes et castration parasitaire chez quelques pagures. *Sta. Océanogr. de Salammbô, Ann.* 6: 1–203.