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THE SPERMATOPHORES OF *DESMOGNATHUS* AND OTHER PLETHODONTID SALAMANDERS

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The Plethodontidæ form the dominant group of North American salamanders and yet very little is known of their spermatophores or method of impregnation. Bedriaga (1897) reported that *Hydromantes italicus* kept in a terrarium deposited spermatophores on the ground in October. Wilder (1913) found, in May, spermatophores in the cloacas of two females of *Desmognathus fuscus fuscus*. One of these females a moment previously was noted among wet leaves in the terrarium with its ventral surface pressed against that of a male. In a later paper Wilder (1923) reported an additional instance of a spermatophore being found within the cloaca of a female. The salamander was sexually immature and one of ten or twelve which had been "kept unassorted in shallow water in a covered glass crystallizing dish until May 20, when it was observed that in the water, which looked rather foul, there were several creamy white masses of irregular shape, measuring about one millimeter in diameter."

Microscopical examination revealed these masses to be spermatophores, but it was not possible to determine whether the spermatophore in the female's cloaca had been picked up in the water after deposition by the male. Wilder concludes that "if such be the case, it is probable that both the direct and the indirect method of impregnation of the female obtain in this species, the former operative when individuals find themselves under purely terrestrial conditions, the latter, which is of course the more primitive method, being employed under aquatic conditions." Very recently Blanchard (1928) has described the spermatophores of *Plethodon cinereus* as "nothing more than sacs or bags filled with sperm" which are "tucked closely into the posterior part of the cloaca" of the female from October 31 to December 9. During the past year we have made observations on the spermatophores of several plethodontid salamanders. We find ourselves in disagreement with various details given by Wilder and by Blanchard. Further, our observations include additional species and extend the account of the process of impregnation of the female plethodontid salamander as given by these investigators.

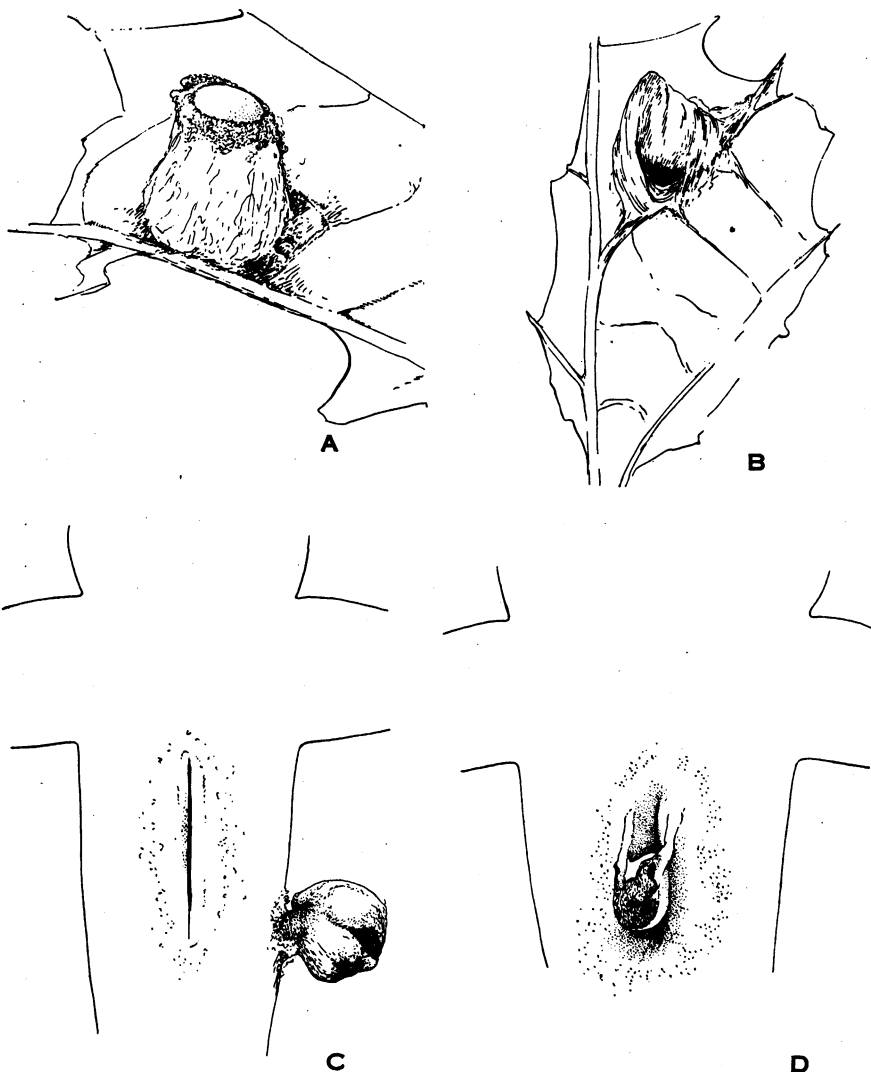


Fig. 1. The spermatophore of *Desmognathus fuscus*. $\times 15$.

- (a) The complete spermatophore attached to a leaf.
- (b) The base of a spermatophore after its head had been removed.
- (c) The head of a spermatophore adhering to the tail of a male *Desmognathus fuscus*.
- (d) The spermatophore head attached in its normal position to the cloacal lips of a female *Desmognathus fuscus*.

On June 16, 1928, several adult *Desmognathus fuscus fuscus* were collected in a very cold spring near Delaware Water Gap, Pa., and placed in a covered, glass dish half filled with water. Moss and well-leached leaves were added until they came just above the level of the water. On the morning of June 18 several freshly deposited spermatophores were found attached to the moist leaves which protruded above the water. It was assumed that the sudden change of temperature might have induced a premature deposition of spermatophores and as a result these might not be typical in form. But on the evening of June 28 two additional spermatophores were attached to leaves above water and these agreed in structure with the others, although the series exhibited some variation in form.

All spermatophores were attached to wet leaves out of water. The point of attachment was always near one of the leaf-ribs usually at the point of intersection of two such ribs. The fresh spermatophore had a glassy, transparent base surmounted by a small whitish cap (Fig. 1a). The spermatophores were not "creamy white masses of irregular shape" such as Wilder (1923) described in her aquatic *Desmognathus*. One spermatophore (Fig. 1b) lacked the white cap, due apparently to its having been removed by a female, although unfortunately at this date the cloacas of the females were not examined. It was apparent that *Desmognathus* agrees with *Ambystoma* and *Triturus* in producing stalked spermatophores, but these are deposited above the surface of the water on leaves. Each spermatophore has the same two parts as those of *Ambystoma* and *Triturus* but its shape is different. The base is wider than that of the newt (Jordan, 1893) and the head proportionately narrower than in spermatophores of either genus.

No further opportunity was had for studying spermatophores of *Desmognathus f. fuscus* until October 28, when Miss Gertrude Evans brought us a series of adults collected the previous day at Morristown, N. J. One of the females was found to have a whitish mass filling nearly the entire orifice of the cloaca. This was obviously homologous to the head of one of the spermatophores described above. It contained active spermatozoa and was sectioned to show the relation of the sperm to the whitish material. Before discussing this relationship some reference may be made to other spermatophores obtained.

On November 9, 1928, Mr. W. G. Hassler brought us a series of adult *D. f. fuscus* collected the previous day in Yonkers, New York. One large female as shown in figure 1d had a spermatophore protruding from the cloaca. The head inserted within the cloaca was white, smooth

and non-adhesive. Two semitransparent, gelatinous appendages extended forward from the spermatophore and securely held it in place. These appendages adhered readily to the forceps. They were evidently homologous to the gelatinous base of the first spermatophores described. One of the males collected the same day had a spermatophore adhering by a very short gelatinous base to the side of its tail (Fig. 1c) in the cloacal region. In view of our observations made the previous June it would seem that the female had rubbed a stalked spermatophore into her cloaca and that this had been held in place by parts of the stalk which adhered to the cloacal lips. Similarly, the male may have accidentally picked up a spermatophore with its tail after the spermatophore had been deposited. These November observations, if taken alone, would not exclude the possibility that spermatophores on very short stalks may be transmitted directly to the cloaca of the female. In either case it is important to emphasize that it is not the whitish material which adheres but some of the stalk substance. In addition to the above material we have available a number of preserved specimens. One female collected at Coytesville, N. J., March 31, 1928, has a large spermatophore head in the posterior part of her cloaca. As the spermatophore head was not soiled it would seem probable that it had been only recently deposited.

On January 6, 1928, a day following a week of cold weather, an adult female *Desmognathus phoca* was found moving over the wet rocks, in a terrarium in the American Museum, with a spermatophore held within the lips of the cloaca. The spermatophore almost filled the orifice and was held in place by shreds of gelatinous substance. The latter were not clearly marked off from the whitish material, some of which seemed to be adhesive, apparently due to a thin ventral covering of gelatinous substance. When the spermatophore was lifted from the cloaca the whole dorsal third was found to be a solid mass of spermatozoa, grayish white in color, and scarcely mixed at all with the underlying whiter material (Fig. 2c). In this character the spermatophore differed from that of *D. f. fuscus*. No other spermatophores were found in this terrarium although the species bred there successfully later in the season.

During October and November, 1928, we collected large numbers of *Eurycea bislineata* at Leonia, N. J., and Yonkers, N. Y. On October 25, two females were found in Leonia with spermatophores within the cloacal lips. On October 28, two additional females with spermatophores were found in the same place. On November 11, two more females with spermatophores were found in Yonkers, and on November 25 another female with spermatophore in the cloaca was collected at

Englewood, N. J. All seven spermatophores were located in the posterior half or third of the cloacal orifice and in no case was the outlet of the alimentary tract materially obstructed by the spermatophore. The three spermatophores collected in November were distinctly smaller than the October material but the sperm they contained was found under the microscope to be active. The bulk of each spermatophore was composed of the whitish substance and the part which protruded slightly from the cloaca was stained, apparently due to dirt which adhered to the more gelatinous part of the spermatophore. The sperm-cap and the outwardly directed gelatinous material were found under the binocular to be not sharply demarcated one from the other. On December 15 the remnants of a spermatophore were found within the posterior part of the cloaca of a female collected in Yonkers. On January 20, 1929, the remnants of two additional spermatophores were found in two females collected at Closter, N. J. On February 17, 1929, four out of five adult females of *E. bislineata* collected at Leonia, N. J., contained remnants of spermatophores in their cloacas. The above series demonstrates that spermatophores are carried within the posterior part of the cloaca of *E. bislineata* from late October to at least mid-February, and further, that the spermatophores gradually dwindle away. How much time is required for a single spermatophore to be absorbed, if indeed they are absorbed, can not be determined from the present data.

Blanchard (1928), over a three-year period, found no less than ten females of *Plethodon cinereus* with spermatophores. As in the case of *Eurycea bislineata* these were in the posterior part of the cloaca but Blanchard's description of them as mere bags filled with sperm is completely at variance with the account given above of the spermatophores of other plethodontids. On November 4, 1928, we collected a female of *P. cinereus* at Yonkers, N. Y., with a spermatophore within its cloaca. This completely filled the space posterior to the dorsal prominence or pseudo-clitoris. Unlike the spermatophores of *Eurycea bislineata* it was completely enclosed by the cloacal lips and only visible when these were opened with the forceps. Nevertheless, a gelatinous base was attached to the spherical whitish mass and extended forward to attach to the anterior corner of the cloaca. Blanchard did not describe such a reduced stalk or base in his specimens and concluded "that the spermatophores are directly transferred." From our observations on the spermatophores of *Desmognathus fuscus* it would seem equally possible that *Plethodon*, as well as the other plethodontids discussed above, deposits stalked spermatophores on the ground. The head and part of the base of the

spermatophore is later squeezed off by the cloaca of the female which we may assume would move over it in much the same way as has been described for several other species of salamanders. This suggestion is rendered the more probable in view of the observations of Bedriaga (1897) who found that the terrestrial *Hydromantes* deposits spermatophores on the ground. We may conclude that although the process of impregnation is not completely known for any plethodontid the available evidence favors the view that all plethodontids deposit stalked spermatophores. Each of those which is favorably placed is later squeezed in two and the sperm bearing part engulfed by the cloaca of the female.

The Plethodontidæ have been derived from the Salamandridæ and presumably from mountain-brook species. One might expect that the process of impregnation in the family would agree with that of the mountain-brook salamandrids. Bedriaga (1897) pointed out that in *Euproctus* the spermatophore consists of a gelatinous base and a sperm-bearing head. The former, which adheres readily to objects, is described as being left behind when the female takes up the sperm-bearing part. Despax (1923) has presented evidence to show that in *E. asper* after the spermatophore has been deposited in the vicinity of the female's cloaca the spermatozoa are removed by the hind feet of the male and rubbed into her cloaca. But Dähne (1926) found that the spermatophore was placed in the cloaca of the female by the male with his hind feet, no intermediate stage of deposition occurring. This is more in agreement with the process in the other two species of the genus. *E. montanus*, according to Bedriaga (1897), transmits the spermatophore either directly from the cloaca of the male to that of the female or the spermatophore head is pushed into its final position by the upturned feet of the male. Bedriaga (1897) also reports direct transmission of the spermatophore in *E. platycephalus*. The direct method of impregnation characterizes at least some of these mountain-brook species and may be described as an adaptation to life in a current which would hinder the indirect process practiced by pond-dwelling salamandrids. Why, then, should we not expect the mountain-brook plethodontids to use the same methods of impregnation? There seem to be at least two reasons. First, brook plethodontids lack the prehensile tail of *Euproctus*. Secondly, to judge from our experiments in keeping *E. asper*, *E. platycephalus*, as well as several species of Plethodontidæ in similar tanks of loose stones and running water, most plethodontids (except the thoroughly aquatic *Leurognathus*, *Gyrinophilus danielsi*, *Pseudotriton rubra*, etc.) are more terrestrial during the breeding season than *Euproctus*. The most terrestrial salamandrids and pletho-

dontids practice the indirect method and there is no good evidence that the direct method once learned in streams was ever passed on to the more terrestrial derived stocks.

Bedriaga (1897) stressed the functional differences in the two parts of the spermatophores which he described. The structure of the spermatophore of *Desmognathus* may be readily understood by examining the cloaca of the male. Squeezing the pelvic region will induce a flow from the three sets of glands within the cloaca. The pelvic glands which occupy the roof and part of the upper portion of the sides of the cloaca produce a creamy white secretion which will be readily recognized as the material which forms the bulk of the head of the spermatophore. The cloacal glands which have the greater number of their outlets perforating the villousities on either side of the cloaca produce a viscid transparent mucus which forms the stalk or area of attachment of the spermatophore. The abdominal glands which lie in the posterior angle of the male cloaca do not seem to take part in the formation of the spermatophore, or at least their secretion which is less viscid can not be recognized in the completed structure. The spermatozoa on reaching the cloaca mix with the pelvic gland material and this is ejected with a sticky base of cloacal gland secretion. All plethodontids have cloacal glands and hence we assume that their spermatophores are equipped with gelatinous stalks whether or not these be long or short.

We noticed from our binocular examination that the degree of mixing of the sperm and pelvic gland secretion varied from species to species. This variation is well shown in our sections of the spermatophores, figure 2 (b, c, e). It will be noticed that the spermatozoa form a cap and a central core to the spermatophore head found in the cloaca of the female *Desmognathus phoca* (Fig. 2c). In *Desmognathus fuscus* (Fig. 2b) and particularly in *Plethodon cinereus* (Fig. 2e) the sperm are more evenly distributed through the pelvic gland material. The spermatophore of the latter species is in no sense a bag as Blanchard concluded from a study of the external appearance, but, as shown in the sections, consists of a mass of sperm and pelvic gland secretion well mixed.

Wilder (1913) has made a study in *Desmognathus f. fuscus* of the spermatozoa from both the spermatophore and from the vas deferens. She states:

I have invariably noted a certain appearance of which I have as yet found no mention in the literature upon the subject. [When the spermatozoa uncoil] there is gradually revealed an elongated or oval mass (*cor*), apparently of semifluid consistency and of translucent material which seems to form the core about which the coiling

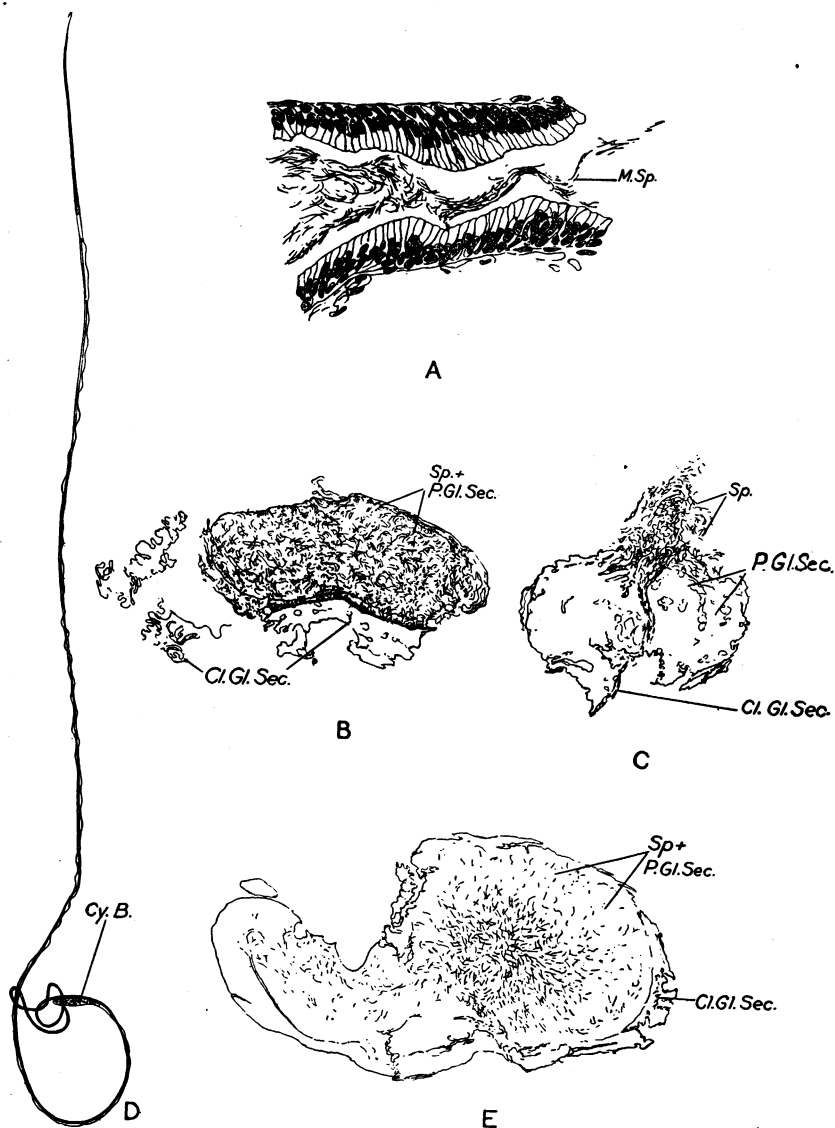


Fig. 2. Plethodontid spermatophores, spermatozoa and spermatheca.
 (a) A portion of the common duct of a spermatheca of *Eurycea bislineata*, showing the hypertrophy of the cells forming the epithelium. The cloaca is on the right. $\times 250$.
 (b) Spermatophore of *Desmognathus fuscus*, cross-section. $\times 58$.
 (c) Spermatophore of *Desmognathus phoca*, cross-section. $\times 58$.
 (d) Spermatozoan of *Desmognathus phoca*. $\times 848$.
 (e) Spermatophore of *Plethodon cinereus*, cross-section. $\times 58$.
 Cl. Gl. Sec. = Secretion product of cloacal glands.
 Cy. B. = Cytoplasmic body.
 M. Sp. = Migrating spermatozoa.
 P. Gl. Sec. = Secretion product of pelvic glands.
 Sp. = Spermatozoa.

takes place and which is enclosed in the last final loop of the tail as it uncoils. As spermatozoa within the tubules of the testis do not possess this structure, it is apparently acquired in the passage from the testis into the duct and is very likely a secretion of mucous nature derived from certain specialized cells. Its nature and source, however, as well as its function, require further investigation. If it is peculiar to *Desmognathus* it may well be an adaptation to the prolonged period which must often elapse between the introduction of the spermatozoa into the spermathecae of the female and the actual fertilization of the eggs, and is one of the many devices which it is necessary for a terrestrial form to develop to resist the drying action of the air.

We have examined sperm from the vas deferens of *Desmognathus fuscus fuscus*, *D. f. carolinensis*, *D. phoca*, *D. quadramaculatus*, *Leurognathus marmorata intermedia*, *Eurycea bislineata* and *Plethodon cinereus*. Our material was in the form of smears, some fixed in Zenker's, others in Bouin's, and others in osmic vapor. In all species the cytoplasmic body described by Wilder was plainly visible although it was not present in all spermatozoa and obviously because it may be easily detached. Thus, the body is not characteristic of terrestrial species, for it is equally well developed in aquatic forms. The structure is no doubt the same as that described some years previously by Ballowitz (1906) in the European plethodontid, *Hydromantes italicus*. He noted that the body was easily destroyed by salt solutions. The cytoplasmic body, therefore, is a characteristic of the Plethodontidae (so far as known) and not restricted to certain species having special habits.

The cytoplasmic body seems to us to be merely a part of the cytoplasm which in most Amphibia is thrown off at the time the tail membrane is formed. Such temporary globules of cytoplasm have been regularly found in the immature sperm of Salientia (Broman, 1900, 1907; Ballowitz, 1905; Retzius, 1906). Retzius (1906) described them in the young sperm of *Salamandra maculosa* and McGregor (1899) records the persistence of such a cytoplasmic body on the tail of the mature sperm of *Amphiuma*. McGregor finds that this body is broken off during the active movements of the spermatozoan. Only in the Plethodontidae does the cytoplasmic body remain as an apparently useless vestige of a previous stage of development carried by most sperm until at least the time of impregnation.

The spermatozoa of the Plethodontidae mature in the testes and Wilder was mistaken in claiming that the cytoplasmic body does not occur in sperm obtained from these organs. We have found the cytoplasmic body fully formed and attached to the tail of spermatozoa from the testes of *Desmognathus fuscus fuscus*, *D. f. carolinensis*, *D. quadramaculatus* and *Plethodon cinereus*. Many spermatozoa from the testes

lack cytoplasmic bodies but this is also true of spermatozoa from the vas deferens. The cytoplasmic bodies are easily broken off, for many detached bodies are seen in the smear preparations.

Wilder (1913) has figured the spermatozoan of *Desmognathus f. fuscus* but has discussed very little of its structure other than that of the cytoplasmic body. Wilder does not indicate an acrosome which is clearly differentiated in all the plethodontids described above. Under the highest powers it may be seen that the tip is bent back at an acute angle forming a barb such as Retzius (1906) figured in *Hydromantes italicus*. The barb is clearly visible only when seen in profile. We have figured a spermatozoan of *Desmognathus phoca* (Fig. 2d). It differs slightly in size and proportions from the spermatozoa of other species of *Desmognathus*. In *D. f. fuscus* as well as in the other species of the genus the middle piece is proportionately longer than Wilder figured. The spermatozoa of the urodeles unlike those of the Salientia are singularly uniform. It is possible that the differences in relative length of acrosome, head and middle piece found in the Plethodontidæ may have some systematic value but our series is not sufficiently extensive to draw any conclusions at this time.

There are several questions concerning the impregnation of the female salamander which have not been adequately considered in any species. How do the spermatozoa find their way into the spermatheca? What happens to the remnant of the spermatophore after the spermatozoa have left it? We have attempted to answer these questions by studying sagittal sections of the cloacas of two females of *Eurycea bislineata* with the spermatophore *in situ* and sections of one other with only a remnant of the spermatophore remaining. In regard to the first question Wilder (1923) implies that in *Desmognathus* the spermatophores in the air would undergo drying "and thus become sufficiently compact to be stored in the spermatheca." There is no evidence from our *Desmognathus* material that the spermatophores do undergo drying, while our sections of the cloacas of *Eurycea* reveal that the process of filling the spermatheca is one of migration of individual spermatozoa. This is clearly shown in one of the females collected October 25. Spermatozoa are distributed either singly or in small groups extending from the disintegrating spermatophore (Fig. 3) to the common duct of the spermatheca. It is remarkable that although spermatozoa are abundant posterior to the opening of the common duct into the cloaca not a single one is found in our sections anterior to this point. No spermatozoa are found in the large intestine or oviducts although their openings into the cloaca

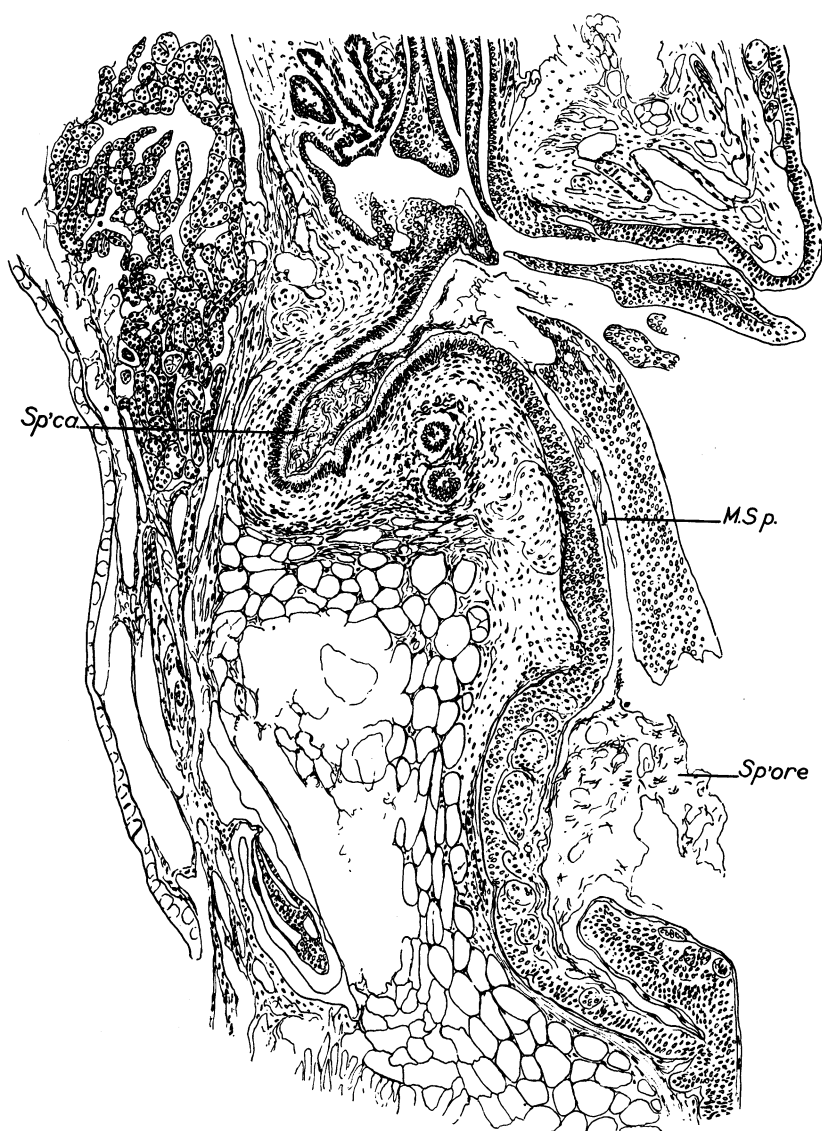


Fig. 3. Sagittal section of the cloacal region of the female *Eurycea bislineata*, showing the spermatophore within the cloacal lips and the migration of spermatozoa to the spermatheca. $\times 44$.

M. Sp. = Migrating spermatozoa.

Sp'ca. = Spermatheca filled with spermatozoa.

Sp'ore = Spermatophore.

would seem available to spermatozoa migrating along the dorsal surfaces of the latter structure. It is apparent that the spermatheca does not act as a catch-basin trapping the spermatozoa which happen to enter its common duct. Rather it would seem to function as a source of attraction, for otherwise spermatozoa would be found widely distributed about the voluminous cloaca.

An examination of the structure of the epithelium of the cloacal region strongly suggests that the source of attraction is a secretion released from the epithelium of the spermatheca and particularly from its common duct. The epithelium of this duct is hypertrophied, its cells tall and columnar (Fig. 2a). In slides fixed in Zenker's and stained in Mallory's, the distal half of the cells stain pale blue similar to the mucus of the mucous glands in the integument. The same region stains feebly in eosin. Aside from the glands, only the spermatheca and the cloacal epithelium immediately surrounding its common duct exhibit this hypertrophied form. No cilia are found in the common duct nor on the cloacal epithelium. We have made a study of the epithelium directly underlying migrating spermatozoa and have found no cilia which could be propelling them. Nevertheless, numbers of spermatozoa may be seen directed toward the small opening (Fig. 3) of the common duct. It thus seems that the response must be chemotropic to secretions released by the spermatheca.

Unfortunately, we have not been able to examine sections of the cloaca of the female *E. bislineata* taken at different times of the year. But the cloaca of a *Desmognathus fuscus* killed in March exhibits no hypertrophy of the epithelium of the common duct of the spermatheca, although the spermathecal tubules are filled with sperm.

It will be noted from figure 3 that the spermatozoa migrate a considerable distance from the spermatophore before finding the small entrance to the spermatheca. How are they released from the spermatophore head? Does the mucous surface alone permit them to free themselves from the pelvic gland secretion which binds them together? An examination of the sections of the posterior part of the cloaca of the specimen of *Eurycea bislineata* discussed above has revealed a remarkable modification of the epithelium not found anywhere else in the cloaca. The epithelium is vacuolated by clusters of polymorphonuclear leucocytes (Fig. 4). Some of these vacuoles break through to the outside, thus forming crypts. At several points the leucocytes may be noted in the act of leaving the crypt and entering the spermatophore. Although numbers of mononuclear and polymorphonuclear leucocytes cover the dorsal and

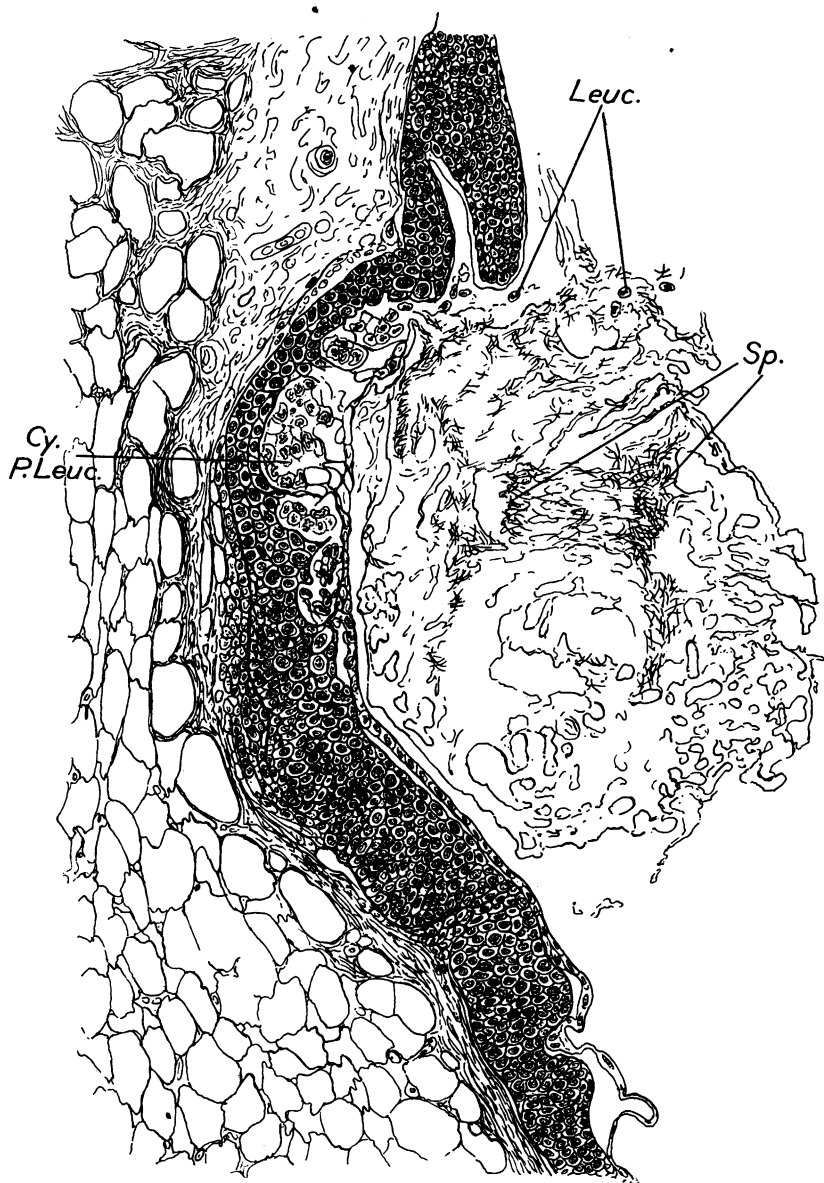


Fig. 4. Spermatophore of *Eurycea bislineata*, *in situ*, within the cloacal lips of the female. Polymorphonuclear leucocytes have formed cysts within the epithelium of the cloacal lips and other leucocytes are phagocytosing the spermatophore. $\times 122$.

Cy. P. Leuc. = Cysts of polymorphonuclear leucocytes.

Leuc. = Leucocytes.

Sp. = Spermatozoa.

anterior portions of the spermatophore, they have not penetrated to any depth into the mass. Many of the polymorphonuclear leucocytes show eosinophilic or basophilic granules. No capillaries are found in the cloacal epithelium. It would seem that the leucocytes had invaded the epithelium to phagocytose the overlying spermatophore, and that disintegration of the spermatophore is hastened by this action.

This interpretation is supported by the structure of the cloacas of the other two spermatophore-bearing females of *Eurycea bislineata* sectioned. Cysts of polymorphonuclear leucocytes are found only in the cloacal epithelium immediately underlying the spermatophores. That they have nothing to do with the shedding of the surface layers of this epithelium is obvious from the specimen collected October 28, which shows that the superficial layer of the epithelium alone is shed. Both specimens have the spermathecas crammed with spermatozoa and apparently represent the last stages in the process of migration. In the December specimen there is a small residue of spermatozoa and cell debris in the posterior part of the cloaca. Only a few leucocyte vacuoles are present and only one of these is open to the outside. There are a few spermatozoa in the cloaca immediately anterior to the opening of the spermathecal duct but none elsewhere in the cloaca. The specimen collected October 28 has a quantity of pelvic gland secretion and of cell debris in the posterior part of the cloaca. Polymorphonuclear leucocyte vacuoles are smaller and less numerous than in the other October specimen. No spermatozoa are in the cloaca but a few are migrating singly up the common duct of the spermatheca. It is interesting that these two specimens should differ so greatly in amount of spermatozoa and pelvic gland secretion remaining after the spermatheca was filled. Perhaps the proportions of these two ingredients of the spermatophore head differed originally in the two individuals. It is further difficult to explain why the leucocytic infiltration of the cloacal epithelium should be so much greater in the specimen with the migration of the spermatozoa at its height. It would be interesting to know whether a similar migration of spermatozoa and a phagocytosis of the spermatophore head occurs in other families of salamanders.

SUMMARY

(1) *Desmognathus f. fuscus* deposited stalked spermatophores on leaves out of water in June.

(2) The head and part of the stalk of the spermatophore were found in the cloaca of the female *Desmognathus f. fuscus* in October and November, and also in late March.

(3) The same parts of a spermatophore were found in the cloaca of a female *Desmognathus phoca* confined in a terrarium in January.

(4) The same parts were found in the cloacas of a series of female *Eurycea b. bislineata* collected from October 25 to February 17.

(5) It is suggested that all plethodontids deposit stalked spermatophores and that the females remove the head and part of the stalk with their cloacal lips.

(6) The spermatophore of *Plethodon cinereus* is not a sac of sperm but agrees so far as known with that of other plethodontids.

(7) The cytoplasmic body of the spermatozoan is a characteristic of the Plethodontidae and is not a terrestrial adaptation.

(8) The spermatheca is filled by a migration of individual sperm from the disintegrating head of the spermatophore while lodged in the posterior angle of the cloaca of the female.

(9) A secretion from the spermatheca, and especially from its common duct, guides the spermatozoa to the spermathecal tubules.

(10) The spermatophore head is phagocytosed by leucocytes which escape in numbers through that part of the cloacal epithelium underlying the spermatophore.

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