On the STRUCTURE *and* ORIGIN *of the* SPERMATOPHORS, *or* SPERM-ROPES, *of two* SPECIES *of* TUBIFEX. By E. RAY LANKESTER.

IN the April number of this Journal last year I called attention to the fact that the so-called opalinoid parasite *Pachydermon* of M. Claparède, found in the copulatory pouches of certain Oligochœtous Annelids, is in reality a felted mass of spermatozoa, *i.e.* a spermatophor.

Such bodies have been described as occurring in Mollusca and Insects, sometimes presenting remarkable complexity of structure. Their occurrence in the Leeches was noticed, many years since, by Professor Max Schultze (also by Fr. Muller), who does not, however, ascribe to them, in this case, anything beyond a simple rope-like structure. The same authority makes the statement that spermatophors occur in the "Regenwürmer," but does not specify species nor form. Professor Leuckart, in his "Bericht," for the years 1848 to 1853, points out that the filamentous bodies found in *Stylaria* by d'Udekem, are spermatophors; a view which d'Udekem subsequently adopted. In Troschel's 'Archiv' for 1850, in a paper by Professor Budge, where the generative organs of a species of *Limnodrilus* and of a species of *Tubifex* are described as belonging to *T. rivulorum,* the spermatophors are figured, but their nature is not determined.[1] The very curious structure of these built-up masses of spermatophors, the fact that they are an example of *a kind of organisation* elsewhere without parallel—a secondary aggregation, not due to growth, as ordinarily presented by organized beings, but to accumulation of free independently-developed elements, gives them a claim on our attention, as well as the facts that they have been misunderstood by the ablest and latest writer (M. Claparède) on the animals which present them; and that they exhibit marked variations in form in the various genera and species of Oligochœt worms.[2]

In my former paper I figured, but roughly, the sperm-rope of a *Limnodrilus* and of *Nais serpentina.* In the plate accompanying this paper, more careful figures are given of

[1] I am indebted to Prof. Leuckart for reference to the papers in which spermatophors have been assigned to the Oligochæta. Prof. Gegenbauer in his 'Grundzüge der Vergleich Anat.,' second edition, p. 294, gives a brief but accurate notice of those of Tubifex—apparently from original observations—which has come under my eye whilst writing this.

[2] They do not occur in the earth-worm; perhaps this is correlated with its not inhabiting the water.

the sperm-ropes of two species of Tubifex—*T. rivulorum*, a worm abundant in nearly every muddy stream or river, and *T. umbellifer*, a remarkable form, living with the latter and *Limnodrilus Udekemianus*, in the Thames below London (see 'Annals and Magazine of Natural History,' February, 1871).

The sperm-ropes of *Tubifex rivulorum* I have found in the copulatory pouches both in summer and winter, but especially abundant and well-formed in the winter. They have a worm-like figure, with a curious conical head, and average from $\frac{1}{20}$th to $\frac{1}{15}$th of an inch in length, and from $\frac{1}{500}$th to $\frac{1}{200}$th of an inch in breadth, the narrowest part being that immediately succeeding the conical head, which has a breadth of about $\frac{3}{1000}$ths of an inch (Pl. X, fig. 1).

The general form of the sperm-rope is due to its being moulded in the long neck of the copulatory pouch. This is plainly seen from the manner in which the conical head corresponds with the shape of the orifice of the pouch (fig. 18). The sperm-rope may sometimes be seen lying in this position, in course of being moulded. The sperm-rope of *T. umbellifer* does not present the conical head which we find in *T. rivulorum*, and in accordance with this is the absence of the reduplication of the wall of the copulatory pouch at its orifice; the mouth is simple, and accordingly gives rise to a simple tapering extremity in the sperm-rope.

Not all the sperm-ropes, however, which are to be met with in the reservoirs of *T. rivulorum* have the conical head; some have been moulded lower down in the neck, and, consequently, exhibit a single blunt extremity, as fig. 9; others, again, from an insufficiency of the plastic material, are quite short, and consist of nothing but the conical head (fig. 8). All gradations are to be met with, parts of the conical heads short and long, according to the amount of plastic material introduced into the moulding reservoir-neck, and according to the part of the neck in which the moulding has taken effect.

It appears that the material of which the sperm-ropes are formed, namely, spermatozoa and a cementing matrix, must be introduced in a viscid form from the male efferent duct, through the penis of one worm into the copulatory reservoir of another, and in the neck of that reservoir a "setting" occurs; for the sperm-ropes, when fully formed, are very firm and compact bodies, of high light-breaking power. The wall of the copulatory pouch is glandular, and undoubtedly furnishes a secretion which occupies part of its cavity, and in all probability also assists as a cementing material in the formation of the sperm-ropes. But the fact that the sperm-ropes are moulded in the mouth of the pouch makes it probable that the bulk of the cementing matrix is introduced into them with the spermatozoa from the male organs of another worm. A very large gland, adapted in every respect to this function, is situated on the male efferent duct in both *Tubifex* and *Limnodrilus*, and its function has hitherto been in doubt. Claparède regarded it as a seminal vesicle, though he admitted that he had never seen any spermatozoa in it, nor had he grounds for considering it vesicular. The gland may be called a "cement-gland," and its large size in *Tubifex* and *Limnodrilus* is in correlation with the abundant and complete character of their spermatophors. In *Clitellio*, which presents sperm-ropes, but has not this gland, the glandular portion of the male efferent duct itself is largely developed, and probably supplies its place. In *Nais*, where the sperm-ropes are very simple and small as compared with the Sænuridæ (see this Journal, April, 1870), there is no glandular development at all in connection with the male efferent duct, which is of a perfectly simple membranous character, and very short. In *Nais*, therefore, it is probable that the secretion of the copulatory pouch alone forms the cementing matrix of the spermatozoa.

It is not unusual to find (especially in the summer) very loose aggregations of spermatozoa in the copulatory pouches—such as that drawn in fig. 13—which are apparently ill-formed spermatophors. They are wanting in the compact, sharply-outlined character which the well-formed spermatophor presents, and this may be attributed to a deficiency in the secretion of the cement-gland, or to their not having been properly "set" in the neck of the copulatory pouch. They, and others less incomplete, are sometimes observed to adhere more or less to the wall of the copulatory pouch, which seems to show that a secretion from that wall enters into their formation. This adherence to the wall of the pouch is especially noticeable in *Nais*, where the spermatophors seem sometimes to be actually continuous with the lining membrane of the pouch. Those of the sperm-ropes of the Sænuridæ, which are well formed, do not exhibit this adherence.

A difference is noticeable among the sperm-ropes of *Tubifex rivulorum* in their refractive power, and their colour (some being of a brownish tint), which is in all probability due to the amount and quality of the secretion from the cement-gland, and perhaps, too, to their age.

In most of the sperm-ropes it is easy to observe a striated structure, which is clearer in some specimens than others. Further observation shows that this striated structure is due to the spermatozoa, which together with the cementing substance make up these bodies.

An optical longitudinal section of a well-formed spermatophor of *Tubifex rivulorum* exhibits the following structure (fig. 1, fig. 4):

Centrally an axial canal, or least refractive portion (*a* in figs. 4 and 7), probably more or less liquid, which is stained by carmine, the rest of the spermatophor being unstained, and contains granular matter and shrivelled epithelial cells; this canal runs from end to end, and varies much in width, enlarging in the broader posterior part of a large sperm-rope, and becoming finer towards the conical head, through which, however, it extends, expanding there in conformity with the outline. External to this, a dense, highly refringent layer, in which a dark line (*b*) is seen to run; following this, a less refringent striated layer (*c*), the striæ in which are directed, when thus seen in optical section, from without obliquely backwards (that is, away from the conical head), and towards the axial canal. External to the striated layer, we come again upon a bright, highly refringent layer (*d*), which in some cases, unless a very good glass is used, appears to bound the spermatophor, but there is externally to this a fringe (*e*) of excessively delicate filaments, the projecting vibratile portions of the spermatozoa. The extent to which these project varies, so that in some sperm-ropes it is difficult to make them out at all; in others, they are very obvious. In the sperm-rope of *T. umbellifer* they are longer (fig. 14) than in *T. rivulorum*, and in *Liminodrilus* and *Clitellio* they are even longer still. But the extent to which they are left free must depend very much on the completeness with which the spermatophor is developed, on the amount of cementing substance, and on its more or less complete condensation. This will vary much from time to time, and in different specimens.

The two bright borders *d* and *b* are due to a peripheral hardening of the cementing substance, a more complete condensation at the inner surface where the axial canal is excavated, and at the outer free surface. The dark line *b*, which is sometimes seen very clearly traversing the inner bright layer, is interesting as an optical phenomenon, in connection with the dark line traversing the intermediate substance placed between the doubly refracting discs in striped muscular tissue, and like it, as explained by Heppner, is due to total internal reflexion.

Whilst a longitudinal section may be easily obtained through the focussing of the microscope, to obtain a satisfactory transverse section the spermatophor must be imbedded in wax and oil, and cut with the razor. A section so obtained is drawn in fig. 7. The same layers are seen as in fig. 4, but it is observed that the filaments (spermatozoa) visible in the striated layer have an imbricated, curved arrangement. This is important, as it helps to explain the way in which the spermatozoa have been felted together. The section represents the proximal portion, that is, the portion nearer the conical head, and it indicates that the sperm-rope has been twisted from right to left (on its own axis), by which means the curved, imbricated radiation has been produced.

If we now examine the structure of the sperm-rope from the surface downwards, we find the following structure. In a living specimen from *T. rivulorum* the surface is often, but not always, seen to be in a state of active vibration. With a high power (No. 10 à immersion, Hartnack) the vibratory surface presents the appearance drawn in fig. 2, when in active movement. The moving bodies are the vibratile filaments of the spermatozoa, three of which, isolated and greatly enlarged, are seen in fig. 6. The reflected portion forming a loop is the filament of the spermatozoa. In *T. rivulorum*, as before observed, the projecting portion of the filament is short, but in *T. umbellifer*, and other Sænuridæ, it is of considerable length, and gives rise to most active *locomotion* of a very graceful kind, and strongly resembling that of the ciliated Infusoria. This is a very remarkable phenomenon, and calculated to throw some light on the nature of ciliary movement, when we remember that each of the vibrating elements here cemented together, has had an independent development, and is in no kind of organic connection with its neighbour, and yet the vibration of the whole surface proceeds with as much regularity and results in as definite a locomotion as in the case of Infusoria whose cilia are presumably in organic connection, and, therefore, possibly under some central control. We might have expected in these spermatophors irregular spasmodic vibrations of the component spermatozoa, the one acting so as to neutralise the other, but instead of this we obtain a perfect and regular "wave" of vibration, which resembles that seen on any ciliated membrane. This fact proves, firstly, an actual identity between cilia and the filaments of spermatozoa, and secondly, that the co-ordination of ciliary movement is independent of any common organic connection of the cilia.

Leaving, for the present, the physiological questions here raised, we pass below the vibrating surface of the sperm-rope, and find a striated structure indicating the spiral arrangement of the heads or motionless portions of the spermatozoa embedded in the cement. The striation of the most superficial layer invariably presents an oblique direction passing from left to right posteriorly, as seen in fig. 3. Focussing more deeply, we come upon the optical long-section already described (fig. 4); and then, more deeply still, we obtain the converse of fig. 3, the striation running from right to left obliquely and posteriorly. These appearances may be explained if we imagine a cylinder of a soft viscid material, to be stuck full of small bristles, each a little longer than the radius of the cylinder's cross section, each placed at right angles to the cylinder's surface, and passing nearly to its centre, in fact, arranged somewhat like the bristles of a rotatory hair-brush, but more deeply set. Then we must imagine the viscid cylinder, with its embedded bristles, to be pushed into a closely fitting sheath, and slowly rotated on its own axis from right to left, whilst it is, at the same time, undergoing the longitudinal movement. In this way all the bristles would become directed along the lines of a series of spirals, running from above downwards, from left to right of the observer, and the transverse and longitudinal sections would give the same appearance which we observe in the spermatophor of Tubifex. Hence we may suppose that some such process has taken place in the building-up of these bodies.

I have already mentioned that considerable difference is exhibited in the intensity with which the spermatozoa make themselves apparent as a spiral fibrous structure. Some spermatophors are quite brown and strongly marked in this respect, others are much paler, and some present actually no trace at all of spermatozoa; so that I am inclined to regard them as blank "cement-forms," which have assumed their appropriate shape without enclosing any spermatozoa (fig. 12).

The spermatophors of *T. rivulorum* will bear considerable pressure without breaking, and are of a tough leathery consistency. When they do break, they tear, along spiral lines, and become teazed out into fibres, as seen in fig. 11. Strong acetic acid facilitates this tearing, but does not otherwise alter them. I have not made a thorough micro-chemical examination of the spermatophors. They may be preserved excellently, apparently without any change, in glycerine jelly.

As already mentioned, the free vibracula of the component spermatozoa are sometimes to be seen moving with great regularity, whilst the sperm-ropes are lying within the copulatory pouches. A solution of from one to two per cent. of common salt served to bring them into action when they were previously quiescent.

Sperm-ropes of Tubifex umbellifer.—The sperm-ropes of this species, the characteristic setæ of which are drawn in figs. 16 and 17, are somewhat smaller and of a more elegant and tapering form than those of *T. rivulorum.* In this interesting worm—recorded as yet only from the neighbourhood of Lake Onega and the Thames—the penis is not preceded by a short pyriform glandular enlargement of the vas deferens, on to which the cement-gland is grafted, as in *T. rivulorum,* but a long tortuous canal, resembling the preceding portion of the vas, comes between the penis and the enlargement on to which the cement-gland is grafted. This may possibly have some connection with the difference in the form of spermatophor. The chief difference is in the absence of the conical head, which is due to the fact that the neck of the copulatory sac in *T. umbellifer* is of a different shape to that of *T. rivulorum,* and consequently moulds the mixture of cement and spermatozoa to another form. This form is seen in fig. 14. Both extremities are acute and tapering, but that which corresponds to the "head" of *T. rivulorum's* sperm-rope is broadened out for some length. This general form —but more blunt and rounded than here seen—occurs in *Limnodrilus* and *Clitellio* (see Claparède's figures of Pachydermon from *Clitellio,* 'Recherches sur les Oligochêtes'). The same constituent parts are noticeable, as in *T. rivulorum,* but I have not seen sperm-ropes of so compact and dense an appearance in *T. umbellifer* as in the former species. The fringe of vibratile filaments is always deeper and more obvious than in *T. rivulorum.* The axial canal widens out to a correspondingly large cavity in the broad anterior extremity of this spermatophor, and there are granular or sometimes broken-down cellular contents in it.

Three of these sperm-ropes extruded from their containing copulatory pouch into a two per cent. solution of salt, exhibited very graceful movements of a definite character. The whole body, assuming a double curve of a sigmoid character, moved with great rapidity, in such a way as to describe a figure of eight passing and repassing on the same track, or very nearly the same. Fig. 15 is intended to represent one of these bodies in motion; all three presented the same graceful sigmoid movement. It was impossible in watching this regular, rapid and graceful gliding movement, in which the whole body seems to take part like that of a coiling snake, to persuade oneself that one was not looking at an organized being, but at an agglutination of seminal filaments. The beat of the cilia was so regular and exact—the longer and more delicate extremity so gracefully arched as it flowed after the broader head and again straightened out and again bent round to form the second loop of the figure of eight, and this motion was so continuously kept up—that it was difficult to look upon it as due to a mere mechanical fusion of uneducated (*i.e.* not originally developed in connection with this condition) independent vibratile elements.

The spermatozoa do not appear to fuse in the way in which the amœboid "Schwärmer" of Didymium fuse to form a plasmodium, which is the nearest approach to be found to such a building up of an organism as the spermatophors present. It is probably more correct to suppose that each spermatozoon remains perfectly distinct from its fellows, and that the unity of their action is due to their uniform condition and properties and to the uniformly diffused character of the stimulus (a chemical one) in response to which they act. If this view be correct we may assume similar independence for the cilia of all ciliated membranes—a fact already inferred from experiment. Did we know of a number of free unicellular organisms after complete development becoming fixed together by a cement to form a secondary organism capable of locomotion and possibly of nutrition, we should have a parallel to the spermatophors; as it is, they are, I believe, the only examples[1] of the building up of an organ or quasi-organism by agglomeration instead of histogenesis.

[1] Such social organisms as *Conochilus* may be regarded as in a measure parallel. But the union in that case is less intimate, and moreover is a union of secondary aggregates.