BIFURCATION IN THE EMBRYOS OF TUBIFEX.[1]

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Introduction.

In the course of some experiments upon one of the common tubificids, *Tubifex tubifex* (Müll.), teratological phenomena were discovered in the early development which merit attention. Instances of bifurcation have been reported in collections of mature and semi-mature Oligochæta from natural environments, but always as great rarities and usually assumed to be the result of regeneration following some form of injury. A considerable number of bifid forms have also been produced in connection with regeneration experiments. Bifurcation as an embryonic phenomenon in Oligochæta has heretofore been known only from six records. According to Kleinenberg (1879, p. 219) and Vejdovsky (1888–1892, p. 250), Dugès (1828) observed and figured a double monster of "*Lumbricus trapezoides*," and Ratzel and Warschawsky (1868) described a similar abnormality in "*L. agricola*." Kleinenberg (1878; 1879, pp. 216–219) found bifurcating embryos not infrequent in the cocoons of "*Lumbricus trapezoides*." Vejdovsky (1888–1892, p. 252) found two such monsters in the cocoons of "*Lumbricus terrestris*," one instance in "*Allolobophora fœtida*," and a large number in "*Allolobophora trapezoides*." Korschelt (1904, pp. 257–258, Pl. 13, fig. 1) described and figured a bifid embryo taken from a cocoon of "*Allolobophora subrubicunda*." Weber (1917) studied the anatomy of twelve double embryos of "*Helodrilus caliginosus trapezoides*" although apparently others were found as indicated by the statement (p. 347) "Out of the 184 cocoons opened 57 contained only one individual, 101 two, and 25 eggs in various cleavage stages and 1 four embryos. Thirty-five of the 101 cases were in the form of monsters."

In order that the identity of the above-mentioned annelids be understood it is necessary to consider the present status of some

[1] Contribution from the Zoölogical Laboratory, University of Michigan.

of the old names just quoted. In connection with the following synonymy it should be noted that at present authorities on Lumbricidæ do not entirely agree as to whether *Helodrilus* or *Allolobophora* should stand as the name of that genus.

Helodrilus fœtidus (Savigny).

Allolobophora fœtida (Savigny).

Helodrilus caliginosus trapezoides (Dugès).

Lumbricus trapezoides Dugès.

Allolobophora trapezoides (Dugès).

Helodrilus subrubicundus (Eisen).

Allolobophora subrubicunda Eisen.

Lumbricus terrestris L., Müller.

Lumbricus agricola Hoffm.

It thus appears that several of these writers worked on the same species under different names. Failure to recognize this fact has evidently led Weber into the error (p. 339) of listing "*Lumbricus trapezoides*" and "*Allolobophora trapezoides*" as separate species. The same writer (p. 347), in discussing the difference of opinion expressed by Kleinenberg and Vejdovsky as to the origin of these monsters, suggests that such a difference "may be explained on the basis of the difference in the forms worked on by these two investigators," evidently regarding the species as different, although Vejdovsky (p. 11) states that the material was the same: "Aus meinen embryologischen Untersuchungen ergibt sich ferner, dass die genannte Art mit dem 'Lumbricus trapezoides,' deren Entwicklung *Kleinenberg* bearbeitete, identisch ist."

Only three of the above-mentioned reports were founded upon an appreciable number of bifid embryos, these three pertaining to the same species. The other records were based upon distinct rarities. All recorded instances fall within the Lumbricidæ, the highest family of the Oligochæta. The present paper presents the first account of embryonic bifurcation in one of the lower families (Tubificidæ) of the Oligochæta, these anomalies exhibiting remarkable frequency of occurrence and diversity of form.

Material.

The material on which this paper is based was identified by Professor Frank Smith as *Tubifex tubifex* (Müll.). Certain localities near Ann Arbor, Michigan, furnish an abundant supply of this annelid, and it has been possible, during the past three years, to obtain large quantities of sexually mature worms and cocoons from November until July either in the native environment or in cultures in the laboratory. Easy access to such an extensive supply of material has made possible the large number of examinations referred to in this paper.

Owing to the abundance of material it was often possible to secure cocoons easily by merely bringing masses of the mud into the laboratory, spreading it out in large shallow dishes, covering it with a small amount of water, allowing the finely divided matter to settle, and gently working over the material with a pipette. Fortunately the mud does not adhere to these cocoons and they will usually appear clean and distinct when uncovered. Furthermore, their color, which is usually of a very light orange, is such as to offer distinct contrast to the mud in which they occur and except for the rough resemblance to the small sand grains sometimes mingled with the mud, the recognition of the cocoons offers no particular difficulty. For securing large numbers of cocoons quickly the writer has employed the method of running quantities of pond and river margin materials through a set of graded sieves, the finest one having a mesh just small enough to retain the cocoons. The residue was then thoroughly washed by a gentle stream of water and examined under good light conditions. Undue handling of materials sometimes results in the loss by the cocoons of their ability to maintain perfectly clean external surfaces, fine particles tending to adhere and thus render cocoons difficult to detect.

Since this work was largely concerned with certain abnormalities in development the cocoons were handled as carefully as possible. That the sieve method of collection was not responsible for the production of abnormalities was demonstrated by comparing the materials so collected with those taken simultaneously from the same habitat by other methods.

Ordinarily, cocoons were carried through their development in the laboratory by merely transferring them to shallow dishes containing river or pond water. For convenience of examination such water was sometimes filtered, although this procedure is not always necessary since the finely divided matter will usually settle and has the advantage of serving as food material for the young worms after they emerge. Occasionally mould attacked the cocoons but its appearance was not frequent enough to offer serious difficulty.

The Cocoon.

Dixon (1915, pp. 85–86) has described briefly the cocoon of "*Tubifex rivulorum*," a form now regarded as identical with *Tubifex tubifex*. In practically all respects this description has been found correct. However, the color of the cocoons demands comment. Dixon describes them as "Whitish or greyish in colour and semi-transparent, but when viewed with the naked eye they appear opaque, but this is due to the eggs which they contain." In this work it has been found that the external appearance depends somewhat upon the degree of development of the eggs within. When first deposited they may have a whitish or creamy tinge but as development proceeds there is an increasing amount of yellow or orange, the maximum intensity being reached at the time when the young worms are ready to escape. At all stages the cocoon proper (the surrounding capsule) is colorless and transparent.

Excluding occasional malformed cocoons, the shape varies from ovoid to sub-spherical with two symmetrically formed, cylindrical, projecting necks, one at each end of the major axis. These necks bear an important relation to certain matters considered in this paper since they are the exclusive avenues of escape for the young worms. At the time of deposition these necks are completely filled by plugs of material somewhat similar in appearance to the remainder of the cocoon but distinctly less resistant since they are easily penetrated at the proper time, while in the hundreds of cocoons examined not a single one had been punctured or ruptured elsewhere although they were often subjected to considerable pressure by developing worms

within. The presence of the plugs indicates that no emergence has yet occurred. These plugs vary in form in different cocoons but are usually cone-shaped at both ends, the outer end extending beyond the distal margin of the neck and the inner end extending into the cavity of the cocoon.

The number of eggs included in a cocoon is subject to considerable variation. In more than five hundred cocoons studied the maximum was found to be seventeen and the minimum one. Cocoons containing only one egg were rare, but numbers above ten were rather common, although the general average was somewhat lower. Dixon (1915, p. 85) found a maximum of only thirteen or fourteen. The size of the cocoon is somewhat dependent upon the number of eggs present.

Development of the Eggs.

Owing to the transparency of the cocoon, development of the eggs can be followed with some ease. Two distinct steps occur in the progress from the newly formed egg to the free living condition of the resulting worm, namely, escape from the egg membrane, and subsequent emergence from the cocoon. Ordinarily, eggs "hatch" in a very few days by breaking the delicate, transparent egg membrane but escape from the cocoon is subject to surprising variation and since this phenomenon is directly concerned with the problems in hand, special attention was given to it. This variation occurs not only among different cocoons but also among eggs of the same cocoon. Emergence ordinarily occurs during the tenth to the twentieth day but not infrequently the sojourn within is much longer. Delayed emergences are usually, if not entirely, due to some inability to pass through the neck of the cocoon and usually results in the death of the worm, although occasionally escape is finally accomplished.

Dixon (1915, p. 86) was unable to find more than nine young developing from one cocoon and thought it probable that in those with an unusually large number of eggs certain eggs only develop, the others ultimately deteriorating. Attention was given to this matter and the occasional failure of certain eggs to develop was confirmed. However, more than nine worms

emerging from one cocoon were often observed, the maximum number noted being thirteen. Futhermore, there seems to be no way of predicting where the sterile eggs will occur. Apparently they do appear more frequently in the larger cocoons but are not exclusively confined to them. After the development has progressed for two or three days it is usually possible to distinguish eggs which will develop and those which will not since the latter retain for a time their initial appearance thus offering distinct contrast with the changing color and shape of the former.

These sterile eggs have at least two possible fates: (1) They seem to be consumed as food by the developing worms in the same cocoon whenever one or more have been delayed in their escape. Often every trace of such eggs is eventually obliterated. (2) Sometimes all of the developing worms escape early, leaving sterile eggs behind. Such cocoons soon swarm with microscopic organisms which facilitate the dissolution and disappearance of the contents.

Possibly these sterile eggs have something to do with enabling worms to live for long periods within the cocoon after completing their embryonic development. However, instances were observed where worms continued to live in that confinement long after all the granular egg contents had disappeared.

As mentioned before, the only avenues of escape from the cocoons are the two necks. After the plugs are removed the diameter of these necks is just sufficient to permit the average, normally formed individual to squeeze through. Departures from the normal body form, especially swellings or bifurcations, constitute very effective barriers and since, as will be shown later, abnormalities are common in *Tubifex tubifex*, many an individual never reaches the outside world. At the proper time for emergence, exploring movements, especially with the anterior end, are exhibited and when the aperture is finally discovered, efforts leading to escape are quickly exerted. Complete exit may require several minutes, even the greater part of an hour under some conditions. Exit seems to be more easily and more quickly made when the cocoon is surrounded by mud or debris than when deposited in water on the botton of a glass aquarium, this

advantage evidently being due, in part at least, to the anchorage which the emerging portion of the worm secures against the surrounding materials with its setæ.

Abnormalities.

In the course of this work several different types of abnormalities were found, all of which appeared early in development and were almost exclusively confined within cocoons since their form was such as to prevent their escape. The following forms were noted: (1) Whole body swollen and shorter than normal; (2) anterior region swollen, posterior region normal; (3) local swellings along the body; (4) bifurcations; and (5) combinations of bifurcations with 1, 2, and 3. Since this paper is concerned only with the fourth type, the other forms of abnormality will receive no further consideration.

Frequency of Bifurcation.

During the past three years a large number of these bifurcated worms have been found. While it is possible to examine a cocoon and detect bifid individuals previous to any emergence, they can be secured easily and in some quantity by allowing cocoons to develop under as favorable laboratory conditions as possible until most or all of the normal individuals have escaped. Since the abnormalities only very rarely escape they are left behind in the cocoons and are thus easily collected at a later time. Records based upon more than five hundred cocoons indicate that about twenty percent yielded bifurcated individuals.

The maximum number of bifid individuals found in a cocoon was three, one or two being more common. Thus far no relations between the number of eggs per cocoon and the number of bifurcations has been detected, the latter being about as frequent in cocoons with few eggs as in those containing the larger numbers.

Forms of Bifurcation.

The diversity of form of these bifurcations is striking and demands some notice here. While an occasional irregularity was difficult to interpret, most individuals could be classified

according to the following analysis, all of the types specified being represented. It should be stated that this analysis is based upon observations on the activities and external features of the living anomalies.

A. Either anterior or posterior extremity bifid—
  1. Bifurcation simple—
    a. Branches equal.
    b. Branches unequal.
  2. Bifurcation compound—
    a. Plane of bifurcation—
      (1) Secondary bifurcation in same plane as primary.
      (2) Secondary bifurcation at right angles to primary.
    b. Equality of bifurcation—
      (1) Parts of primary bifurcation equal; secondary equal or unequal.
      (2) Parts of primary bifurcation unequal, secondary equal or unequal.

B. Both anterior and posterior extremities bifid—
  1. Primary bifurcations in same plane.
  2. Primary bifurcations in different planes.
  3. Either or both bifurcations compound.

In the writer's records anterior bifurcation with normal posterior end occurs about twice as frequently as a posterior bifurcation with normal anterior end. Furthermore, individuals with both ends bifid are almost as frequent as anterior ones. One series of one hundred and ten records taken at random shows the following proportions: forty-five anterior, twenty-four posterior, and forty-one combined anterior and posterior bifurcations. Other records yield somewhat similar results.

In most instances the recognition of compound bifurcations involved no difficulty. Occasionally, however, a specimen appeared which seemed at first sight to be some form of trifurcation, but careful examination demonstrated that some at least were really compound bifurcations; others, however, could not be definitely classified by external examination. Compound bifurcations are not uncommon and a considerable number were observed during the progress of this work.

The length of the duplicated parts is a matter of considerable variation and the only general relation discovered is that the longer ones occur on the posterior end. Up to date, the writer has not found an anterior bifurcation to exceed six somites, while posterior ones may include seventeen somites. Instances were observed in which the posterior branches constituted more than one half the total length of the body. Many anterior bifurcations are only one somite in extent, two and three being also common. Posterior bifurcations may be short, however, comprising but one somite. A trunk portion of several somites extent is always present, no instances having been seen of such narrow connection of duplicated parts as described by Weber (1917) for *Helodrilus caliginosus trapezoides*.

Emergence of Bifurcate Individuals.

Among approximately four thousand young worms just emerged from cocoons the writer found only ten which exhibited bifurcations. These were all alive and active. Three were slightly bifid both anteriorly and posteriorly; six had slight anterior bifurcations and normal posterior extremities; and one had a very slight anterior bifurcation and a very deep bifurcation of the posterior end.

The discovery of these ten free individuals indicates clearly that while emergence is possible it is really a rarity. Since nine had completely emerged before they were detected, their method of escape is unknown. The remaining one, caught in the act, was emerging backward, the normal posterior end going in advance. This form of escape appeared to be effective, although the bifurcated anterior end gave some trouble in traversing the cocoon neck. This observation suggests the possibility that the above-mentioned individuals with normal posterior ends emerged backwards. Added weight is given to this suggestion by the repeated observation that in the escape efforts of normal individuals the posterior ends were often projected through the cocoon neck. It seems that the escape of the specimen with shallow anterior bifurcation and very deep posterior one of necessity must have been with the anterior end in advance

since as will be shown later deep bifurcations present difficulties which are practically insuperable.

The difficulty of escape from the cocoon by a bifurcate individual is due to one or both of two features, namely, the increased bulk of the double end thus exceeding, ordinarily, the diameter of the neck passage, and the split nature of the end causing the frequent situation of one branch projecting through the neck and the other turned up inside the wall—a situation which is prohibitive of escape. Escape by working the way out backward has already been mentioned but that likewise appears impossible when the posterior end is deeply bifurcate. Even the most striking inequality of diameter in the arms of a bifurcation observed constitutes a serious obstacle to emergence. Both anterior and posterior extremities execute searching movements and instances have been found in which a worm with a deep posterior bifurcation had one posterior branch projected through one neck of the cocoon and the other through the opposite neck. Every kind of bifurcate individual manifests movements which in the normal animal would result in emergence and there seems to be no doubt whatever that failure to escape is due entirely to the abnormal body structure.

Viability in Cocoons.

Mention has already been made of the remarkable viability of some of these worms in cocoons and it should be stated in this connection that both bifurcate and non-bifurcate anomalies exhibit this phenomenon. The writer has records of sojourns within the cocoon for varying periods with ninety-eight days as a maximum and while in almost every case the individual untimately died without having escaped, a few of them (even in the record for ninety-eight days) finally emerged. Why escape could occur after so long a period is not known since the cocoon undergoes no observable change, but it is conceivable that such a form living for days after all the available food material apparently has been exhausted might, because of the continued state of inanition, become reduced somewhat in body size, this reduction permitting the animal to pass out through

the cocoon opening. Such body diminution is said to occur in certain other animals when subjected to a continued absence of food. Failure to emerge by non-bifurcate individuals was apparently due to abnormal proportions, this condition being attained before the individual was ready to leave the cocoon.

Rearing of Bifurcate Individuals.

Attempts were made to determine whether it is possible for bifurcate individuals to live and develop if assisted in escaping from their cocoons. In order to insure that they had reached a degree of development at least comparable with that of their normal fellows which were escaping and successfully assuming the free-living life, cocoons containing bifurcations were isolated and bifurcate individuals allowed to remain in the cocoons until most or all normal individuals had emerged. Then the former were dissected out with as great care as possible and introduced into a culture identical in composition and operation with the one in which the normal, newly emerged worms were thriving. Normal worms were used in this way as controls. Up to date the writer has been unable to rear a single bifurcation, although the controls were easily maintained. The reasons for this failure are not understood at present.

Absence in Natural Conditions.

The almost complete, if not wholly complete, absence of bifurcate individuals of *Tubifex tubifex* in nature is shown by the fact that in an examination of over 1,000 worms representing various degrees of maturity and immaturity and collected both from cultures and from native environments not a bifid form was found. The literature seems to contain only one record (Abel, 1902) of a bifid tubificid found in nature, but since similar results have been produced artificially in post-embryonic stages, there is no certainty that Abel's record has any significance in this connection since it might have resulted from an accidental injury. Evidently the cocoon constitutes a very efficient device which automatically eliminates the vast majority of abnormalities.

How universal this high frequency of bifurcation is in the development of *Tubifex tubifex* in all of the varied conditions under which it lives remains yet to be determined. Furthermore, no statement can be made at this time concerning the relation of bifid embryos to those extremely rare bifid oligochætes found in nature, although the available evidence leads one to suspect that the former do not survive and that the latter are the result of regeneration following injury.

Morphology.

Only a preliminary account of the internal structure of these anomalies, based upon serial sections of a few individuals, will be given at this time. All specimens studied thus far show the trunk portion of each worm to be a double region whose construction would be simulated by opposing intimately the cut surfaces of two similar ventral halves of normal embryos of the same degree of development. The branches of the bifurcate portion, however, lack this doubleness, being constructed on the plan of a normal individual. Evidence of the double character of the trunk portion is most readily exhibited in the setæ bundles, the nervous system, the nephridia and the principal blood vessels.

Sections through bifurcate portions of specimens show in each branch four setæ bundles arranged in the normal fashion with the component setæ so set and curved as to conform with those of normal specimens provided the nerve cord be interpreted as corresponding to the normal ventral one. In the trunk portion of the body eight bundles of setæ occur in sets of four, each group being the reverse of the other in arrangement and each being properly related to one of the two ventral nerve cords. Furthermore, the distribution of uncinate and capilliform setæ is as this interpretation would require.

Throughout the trunk portion two nerve cords, 180 degrees apart, extend without change to points where bifurcation appears. If the anterior end be unbranched, a mass of nervous tissue encircles the pharynx; if on the other hand it be branched each branch bears a cerebral mass and esophageal commissures.

If the bifurcation plane is frontal in position each nerve chain passes unchanged into each of the branches. Certain modifications of this arrangement occur if the plane of division be sagittal, chief among which is the swinging to one side or the other of each nerve cord.

Nephridia are sufficiently developed in these forms to be detected and one pair is normally related to each nerve cord in each somite, that is, two pairs per somite in the trunk portion. In the branches there is only one pair per somite.

In the bifurcate portions, the main vessels of the circulatory system occur in the normal position, but at the origin of the branches, the two dorsal vessels approach each other and are then laterally displaced by the union of the alimentary canals, one occupying a position to the right, the other to the left. A ventral vessel occurs just above each nerve cord.

It thus appears that at least some of these double embryos are of the type representing dorsal union, the type which Vejdovsky (1888–1892, p. 257) evidently referred to as "Die Doppelmissbindung, deren Individuen längs der Rückenseiten verwachsen," and which Weber (1917, p. 342) has also discussed. While none of the other types found by these authors in double embryos of certain Lumbricidæ have thus far been detected in *Tubifex tubifex* it is possible that some or all of them will appear when a more extensive study involving a much larger quantity of material is completed. Such a study is in progress and results will be reported in a subsequent paper. No attempt is made at present to account for the origin of these anomalies.

Summary

1. *Tubifex tubifex* (Müll.) deposits from one to seventeen eggs per cocoon, a few of which are often sterile.

2. Emergence of young worms from the cocoon is exclusively through two oppositely located apertures.

3. Various forms of abnormality commonly appear among the developing worms, chief among which are bifurcations of the body. Approximately twenty percent of the cocoons examined contained bifurcate embryos. This is the first report of bifur-

cation as an embryonic phenomenon in any of the lower famiies of the Oligochæta.

4. Diverse forms of bifurcation involving both extremities of the body occur in either simple or compound form.

5. Of more than four thousand recently emerged worms examined only ten bifid individuals were found. Any departure from normal body form involving increase in diameter constitutes an extremely effective barrier to escape from the cocoon and practically all of the numerous abnormalities are automatically eliminated.

6. While bifid worms may live for long periods of time imprisoned within the cocoons, emergence is a rarity.

7. Attempts to rear bifid individuals have thus far been unsuccessful.

8. An examination of more than one thousand worms from natural habitats and representing various stages of maturity yielded no bifid forms.

9. A preliminary morphological study, based principally on the setæ, the nervous system, the nephridia, and the circulatory system, shows the body region of these monsters to be double in composition while the bifurcate portions simulate the normal tubificid structure.

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