CYST-FORMATION IN AEOLOSOMA HEMPRICHI (EHR)

HENRIETTE HERLANT-MEEWIS

Institute of Biologie of Brussells and University of Montreal

Cyst-formation has not often been observed in the Oligochaeta. It was observed for the first time by Beddard (1905a, 1905b) for the species *Aeolosoma hemprichi* Ehr. It was his opinion that cyst-formation appearing at the beginning of winter is due to the decrease of the temperature. According to Vejdowsky (1884), this phenomenon is a resting stage following a long period of asexual reproduction; while Dehorne (1916) considers it to be consecutive to sexual reproduction, which effectively occurs in the months of November and December, Beddard (1889) and Stolc (1889).

The opportunity to study this phenomenon occurred during my sojourn in Canada, at the University of Montreal. The author wishes to express her indebtedness to the Board of Trustees of this Institution; to Dr. Henri Prat, Director of the Institute of Biology and to Father O. Fournier, Assistant-Director, who helped us greatly in the accomplishment of this work. I wish to acknowledge the active collaboration of Miss Marthe Demers.

Scope of the Work

Our concern is cyst-formation in *Aelosoma hemprichi* Ehr, induced experimentally in order to follow the successive stages from the beginning of the phenomenon until the emergence of the encysted worm. These observations also permit a study of the causes that determine cyst-formation in natural surroundings, and the related factors and the importance of the phenomenon in the biological cycle of the worm.

Materials and Technique

The genus Aeolosoma

While studying the fresh-water fauna of some brooks in the region of Montreal, the three following species of Aeolosomatidae were encountered: *A. hemprichi, A. haedleyi* and *A. tenebrarum*. Their systematic position will be studied in another report. This paper will be restricted to the species *A. hemprichi*. The genus Aeolosoma is easily recognized by the ciliated prostomium and the lipoidic inclusions in the skin. The color of these inclusions varies with the species: in *Aeolosoma hemprichi* they are red. The presence of setae and of nephridia is the only means of recognizing metamerisation, since Aeolosomatidae possess neither intersegmentary septa, nor nerve cord with segmentary ganglia.

The transparency of the worm facilitates the study of the internal organs, especially the components of the digestive tract: the ciliated prostomium, the muscular pharynx, the narrow esophagus and the large stomach which gradually merges with the intestine.

This worm reproduces asexually very rapidly. The laws governing this scissiparity have been studied previously (Meewis, 1933). They may be summarized as follows:

(1). Every individual is composed of six seta-bearing segments, the pharyngeal, the esophageal and the four stomacho-intestinal segments. As soon as these have appeared, the pygidium becomes longer and divides into two parts: a small anterior one which will regenerate the pygidium and a longer posterior one which becomes a new zoid. This mode of scissiparity was previously termed "pygidial budding."

(2). When the pygidial bud is formed, it has no seta. However, the first groups of setae soon appear, and progressively, as the bud grows longer, the following groups are formed. When the bud reaches the five seta-bearing segment, all the setae are practically equidistant. Simultaneously, the cephalic zone develops and the pharyngeal setae appear at the anterior part of the bud. These are at first close to the esophageal setae, but as the esophagus grows longer they separate from each other. The zooid is then complete, and possesses a head, six seta-bearing segments and a pygidium capable of budding.

(3). The initial bud composed of six segments will now present a secondary bud.

(4). When cephalization is complete, the initial bud separates from the chain bearing the buds to which it gave rise. Thus, true scissiparity which divides an individual into two parts, more or less equal, and which characterizes fresh-water Oligochaeta, does not exist in this species. By means of an accelerated process, blastogenesis takes place exclusively by pygidial budding.

Aeolosoma hemprichi, represented in Plate I, Figure 1, is composed of an initial individual followed by the buds formed by its pygidium. It corresponds to the formula 6/0/3/6/0, which represents the number of seta-bearing segments of each successive zooid.

This pygidial budding forms chains comprising from two to six individuals or zooids, and of a mean length of 2 mm.

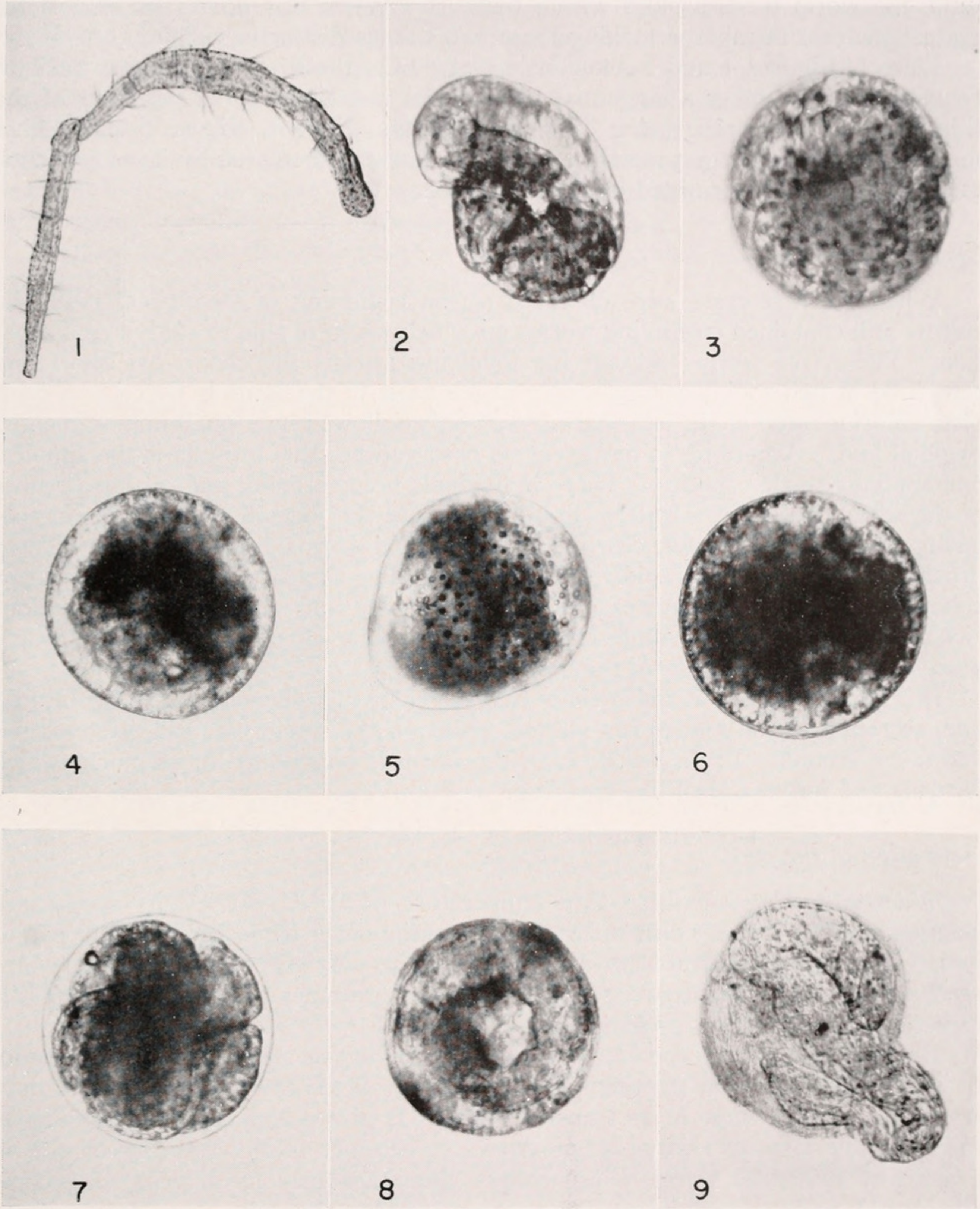
When environmental conditions are stable, pygidial budding occurs regularly and rapidly. The rate of growth varies with the amount of food available and the temperature.

In order to study the influence of environmental conditions on the laws of scissiparity, some cultures were kept at different temperatures ranging from 11° C. to 29° C., whereas the stock culture was kept in front of the laboratory window. Because of a sudden cold spell in the middle of the winter this stock culture was submitted to a temperature of 6° C.–7° C. for about 10 days and it was at this time that the first cyst-formation was noticed.

Description of the cyst

The cysts are generally spherical and of variable size, measuring from 175 to 250 microns in diameter; some may have irregular forms. The worm is easily visible through the thin and completely transparent membrane. The skin of the worm is in close contact with the cyst-membrane, the latter appearing as though spotted with red. The digestive tract, which is very opaque, is joined to the skin by means of mesenteric filaments.

Within the cyst, in cold surroundings, the worm is motionless and its contours difficult to see, though the peristaltic movements of the intestine make orientation



EXPLANATION OF PLATE I

1. *Aeolosoma hemprichi:* Photograph of a live normal worm; 2. Pre-cystic stage; 3. Beginning of cyst-formation; 4. Quiescent cyst: equatorial view; 5. Quiescent cyst: tangential view; 6. Beginning of activation; 7. Activating cyst; 8. Pre-emergence stage; and 9. Emergence stage.

possible. When seen through the microscope, because of the heat emitted by the lamp, the worm turns around within the cyst. When this occurs its skin slides against the cyst membrane making these two tissues distinctly visible.

Plate I, Figures 4 and 5 show a cyst at 5° C.; the first one being a medium (equatorial); the other a tangential view (polar), shows the red pigments of the skin, the cyst membrane being quite transparent. Plate I, Figure 6 shows that the space between the cyst membrane and the worm is increased by its movements, as a result of the heat emitted by the lamp.

Cyst formation

When the first cysts were observed at the beginning of December, 1949, the culture still contained free living worms on which we were able to study cyst formation. These free worms present the following aspect: the chains are short and composed of two zooids corresponding at the most to the formula 6/1, *i.e.*, a complete anterior zooid of six seta-bearing segments, followed by a small non-segmented pygidial bud. According to our previous observations, this formula is the simplest one applying to this species. These individuals become bulky and opaque because of the accumulation of refractive granules around the digestive tract. Simultaneously, their movements slow down; whereas normal worms, because of their ciliated prostomium, move about rapidly in a way resembling that of rhabdocoela. Worms submitted to low temperatures crawl, change form, and evidently prefer regions rich in organic debris. While crawling and turning about they secrete a heavy mucus which hardens to form the cyst membrane.

In Plate I, Figures 2 and 3 show two stages of this phenomenon. In the first one, secretion of the mucus has started, although the worm can still stretch itself and move around. In the second case, the worm is imprisoned in its mucus which hardens and forms a shell-like covering.

Activation of the cyst

When a cyst is submitted to a temperature of 18° C.–20° C., its appearance changes rapidly. The worm turns around continuously while its head and pygidium, which were indistinct, become visible again. At the same time, the worm recovers its initial transparency; the refractive granules which surrounded the digestive tract disappear progressively.

Nevertheless, during the first few days, the worm (as shown on Plate I, Figure 6) is still as bulky as at the time of the formation of the cyst. After 8 to 10 days it reaches another stage in its transformation. It grows and bears new segments, following the laws of normal scissiparity. It becomes totally transparent and in every way resembles a free-living worm. Simultaneously its movements become very rapid, permitting it to turn over very quickly within its cyst. The growth can be followed by the study of the seta and this is rendered possible by the transparency of the cyst membrane. After twelve days, generally, at this temperature (18° C.–20° C.) the worm endeavors to free itself. In certain cases, emergence from the cyst seems easy enough: the membrane is first slightly ruptured, the worm then pushes its pygidium out through this opening and the remainder of its body follows. This phenomenon can take place within half an hour or sometimes necessitates several hours. It may happen that the cyst is covered with foreign matter or with a heavy coating of bacteria. When this occurs the worm cannot easily break through its cyst membrane and it may remain imprisoned for several days. In this case, its growth progresses as if it were free, producing new zooids, and thus there may be several chains within the cyst. This would explain the exceptional finding of three worms within a single cyst. But when the worm emerges under normal conditions it is generally composed of two zooids answering to the formula 6/3, *i.e.*, the chain is composed of a complete anterior individual with six seta-bearing segments and a three-segment pygidial bud.

It does not seem that emergence is caused by the action of a corrosive substance secreted by the worm, but is purely mechanical.

As soon as the worm is free it grows normally, its growth being regulated by the environmental temperature and the quality of the food present. It may live normally without eating for several days, but if the fasting persists, once the reserve substances have been used up, the worm grows thin and its coloring disappears. The effect of fasting on these animals has been previously described.

Plate I, Figures 7, 8, and 9 show three successive stages under heat activation. The worm appearing in Figure 5 is still bulky but its outline is quite distinct. In Figure 8 the worm has reached the pre-emergence stage and in Figure 9 it is emerging. We can observe, in the emerged part, the limit existing between the two zooids which make up the chain.

We can summarize the successive stages of cyst formation and emergence in *Aeolosoma hemprichi* as follows:

(1). Normal worm: (N.W.) the chains are composed of 2 to 6 individuals, the animal is transparent and moves rapidly.

(2). Depressed worm: (D.W.) the chains are of normal length but the movements are slower.

(3). Pre-cystic stage: (P.C.) the chains are made up of 2 individuals, at the most. The worm is bulky and opaque, its movements are very slow.

(4). Cyst-formation: (C.F.) the worm turns around in the secreting cyst.

(5). Quiescent cyst: (C.) the worm is motionless and its contours are indistinct.

(6). Activating cyst: (A.C.) the worm moves around and becomes transparent.

(7). Pre-emergence stage: (P.E.) the very active worm lengthens and tries to rupture the cyst.

(8). Emergence: (E.) the worm ruptures the cyst.

Experimental Study of the Factors Determining Cyst-Formation

Effect of low temperatures

No cyst is formed in cultures which are not subjected to low temperature from June to December: the worms undergo asexual reproduction.

Three groups of two hundred and fifty worms each were taken from a culture maintained at 18° C. and placed in an infusion rich in microorganisms. These cultures were then kept at three temperatures: 3° C., 6° C. and 11° C. The results are summarized in Table I.

These results show that:

1. At 3° C., the movements of normal worms are retarded and only a certain number of them attain the cystic stage. But these individuals seem less bulky and

less opaque than individuals at normal pre-cystic stages. The majority of them resemble fasting worms, as described earlier.

2. At 6° C., slowing of movements is followed by the formation of a normal pre-cystic stage. The first cysts appear after the worms have been kept about 15 days at this temperature. About 50 per cent of them, however, do not go through cyst-formation and remain in a pre-cystic stage for two months.

TABLE I

Duration: days	0	3	7	15	30	60
Exp. at 3° C.	N.W.	D.W.	A few P.C.	A few P.C.	A few P.C.	A few P.C.
Exp. at 6° C.	N.W.	D.W.	P.C.	First C.	Many C.; remainder P.C.	Many C.; remainder P.C.
Exp. at 11° C.	N.W.	D.W.	D.W.	D.W.	D.W.	D.W.

N.W.—normal worm; D.W.—depressed worm; P.C.—pre-cystic stage; C.—cyst.

3. At 11° C., activity is reduced and asexual reproduction is slower; the worms do not reach the pre-cystic stage, the long chains persist and the growth is similar to the one observed during summer months at the same temperature.

We can conclude as follows: (1) The optimum temperature for cyst-formation appears to be about 6° C. (2) The cysts are formed after about 15 days at a low temperature, but this value differs with each individual of the culture. (3) A low temperature is not alone responsible for cyst-formation, since this phenomenon takes place in part of the population at 6° C., and does not affect worms kept at 3° C.

Effect of food

To study those conditions besides temperature which must operate in order to produce cyst-formation, some worms were taken from a culture kept at 18° C. and rich in nutritive substances, and were placed in pure water at 3° C. and 6° C. At both temperatures the movements of the animals were slowed down but pre-cystic stages did not appear; after fifteen days the worms were either dying or dead.

Effect of the combined factors of temperature and food

Different combinations of these two factors were studied so as to determine precisely the role of the nutritive medium during the different stages of cyst-formation.

Two hundred and fifty normal worms were placed at 6° C. in a rich nutritive medium; when they reached the pre-cystic stage, they were divided into two groups: one was kept in the same conditions of temperature and food, while the other was placed in pure water at the same temperature. After seven days, the worms from the first group were encysting while those from the second group were nearly all dead, none having reached the cyst stage. So that besides being necessary for cyst-formation, food is also necessary after the pre-cystic stage.

The final experiments help to explain the results first obtained.

1. At 3° C., the microorganisms on which the worms feed no longer multiply actively and the medium becomes poorer in nutritive substances up to a stage where no cyst-formation can take place. Therefore, if this medium is renewed periodically, to supply sufficient food, normal pre-cystic stages will appear after 15 days and the first cysts within a month. Cyst-formation can take place at 3° C. provided the culture medium is constantly replenished; however, this phenomenon proceeds more slowly than at 6° C.

2. We have seen previously that if cultures which are kept at 6° C. (where cyst-formation is possible) are not maintained at a high nutritive level, a certain number of the worms will remain in a pre-cystic stage; these individuals thrive because enough food is present, but it is impossible for them to transform into cysts. Such pre-cystic worms, taken from a culture where no cyst-formation had resulted after one month at a low temperature, were placed in a rich nutritive medium: three days later, cysts appeared.

The influence of food is therefore obvious; the worm must be able to accumulate reserves during the stages preceding cyst-formation. The elaboration and the nature of these reserves will be studied in a future paper. The worm is especially in need of these reserves during its encysted and emergence stages.

Discussion and Conclusions

The phenomenon of cyst-formation cannot be considered solely as a resting stage occurring after a long period of asexual reproduction as proposed by Vejdowsky, for no cysts were formed in cultures kept in winter at temperatures above 11° C. Neither can it be considered as a quiescent period following sexual reproduction, as thought by Dehorne, for that type of reproduction did not occur at all in our cultures. But cyst-formation is certainly affected by low temperatures as stated by Beddard, and this phenomenon takes place only when sufficient food is present so that reserves can be accumulated.

There exist several groups of fresh-water animals which hibernate during the course of their biological cycles; for example, the Bryozoa survive the winter period as statoblasts or hibernacula (Brien, 1936) and the sponges of the Spongillidae family, as gemmules (Brien, 1932; Leveaux, 1939). In both instances, the organisms could not otherwise withstand the winter cold. They form buds which consist of a mass of undifferentiated cells containing large quantities of nutritive reserves and surrounded by a protective covering. In the following spring, these cells multiply and differentiate, the shell splits open and a new individual escapes.

The same cannot be said of Aeolosoma. Here, the whole animal is enclosed within the cyst and its life processes are slowed down. Transformations occurring in the reserve tissues are not as pronounced as in Bryozoa or sponges.

In natural surroundings, cyst-formation may be explained in the following manner: in autumn, the water becomes colder and richer in products of decomposition from vegetable matter: Aeolosoma then stores its reserves. When the water is sufficiently cold, the worm becomes encysted and falls to the bottom of the creek into some hollow spot which does not freeze. In this way the worm can resist a temperature of a few degrees above 0° C. during winter. When the water warms up in spring, the worm emerges and asexual reproduction is resumed. The biological cycle can thus take place without the occurrence of sexual reproduction.

However, this last type of reproduction was observed in a few cases in *Aeolosoma hemprichi* and *Aeolosoma quaternarum* but it did not occur in any of our cultures though submitted to various environmental conditions. Here, as in other limicolous Oligochaeta, for example *Chaetogaster diaphanus,* where asexual reproduction is very intense, sexuality is a secondary phenomenon which appears only in certain definite circumstances. Scissiparity alone can definitively assure the survival of the species, and in places where the winter months are rather cold, the individuals survive by encystment.

Summary

(1). The fresh-water oligochaete, *Aeolosoma hemprichi,* hibernates in the form of a cyst.

(2). When the worms are placed in a rich nutritive medium at 6° C., their movements are slowed down, and their growth stops. At this period, chains are formed of two zooids, at the most. Meanwhile, the worm accumulates food reserves, becomes opaque and secretes a mucus which will harden and form a transparent cyst.

(3). The worm stays motionless inside the cyst during the entire cold period. When the temperature increases, it becomes active again, uses up its food reserve and becomes transparent. After about fifteen days, it emerges and resumes its growth according to certain definite laws of scissiparity particular to this species.

References

Beddard, F. E., 1905. Oligochaeta. Oxford.

Beddard, F. E., 1905. Note upon certain species of Aeolosoma. *Ann. Mag. Nat. Hist.,* **6**: 262–265.

Beddard, F. E., 1889. Note upon the germ cells in the integument of Aeolosoma. *Proc. Zool. Soc.,* **16**: 51–56.

Brien, P., 1932. Regénération naturelle chez les Spongillidae. *Arch. Zool. Exp. Gen.,* **74**: 461–506.

Brien, P., 1936. Contribution à l'étude de la reproduction asexuée chez les Phylactolémates. *Mem. Mus. Hist. Nat. Bruxelles,* 569–626.

Dehorne, L., 1916. Les Naidimorphes et leur reproduction asexuée. *Arch. Zool. Exp. Gen.,* **56**: 88.

Herlant-Meewis, H., 1950. Les lois de la scissiparité chez *Aeolosoma hemprichi.* *Bull. Soc. Canad. Biol.,* **2**.

Herlant-Meewis, H. and Boulanger, J. P., 1949. Le genre Aeolosoma. *Compte-rendu A.C.F.A.S.*

Leveaux, M., 1939. La formation des gemmules chez les Spongillidae. *Ann. Soc. R. Zool. Belgique,* **70**: 53–96.

Meewis, H., 1933. Remarques au sujet de la scissiparité chez les Oligochétes. *Ann. Soc. R. Zool. Belgique,* **63**: 117–140.

Stephenson, J., 1930. Oligochaeta. Oxford.

Stolc, A., 1889. On the structure of Aeolosoma. (In Bohemian.) *Sitz. Ber. Bohm. Ges.,* 183–194.

Vejdowsky, F., 1884. System und Morphologie der Oligochaeten. Prague.