

sehr kurz, nicht halb so lang wie das dritte; bei *Weismannella* dagegen ist es länger. Auch das fünfte Beinpaar weicht erheblich von dem der drei *Weismannella*-Arten ab. — Beim Männchen ist das vorletzte Glied der Greifantenne von *Schmackeria* kurz und dick, von *Weismannella* schlank. Am linken Beine des fünften Paares ist bei *Schmackeria* das Grundglied außerordentlich stark und als Greiforgan entwickelt. Bei den Männchen der *Weismannella*-Arten ist dieses Glied das kleinste und nicht umgebildet.

Trotz dieser Verschiedenheiten ist die Ähnlichkeit der beiden Genera sehr groß, und man kann sie deshalb als einander vollkommen entsprechende Gattungen Asiens und Südamerikas bezeichnen. Ich habe *Weismannella* eine Brackwassergattung genannt. Nun wird allerdings von *Schmackeria* angegeben, daß sie im süßen Wasser vorkomme, aber genau ebenso wie *Weismannella* in der Nähe der Küste. Meine Ansicht wird also nur bestärkt. Interessant wäre es zu erfahren ob es auch in Asien mehrere Arten gebe und wie sich Afrika und Australien in Bezug auf seine Brackwässer verhalte. — Wenn man sich der Ansicht anschließt, daß die Cyclopiden mit zwei Eiersäcken sich aus calanidenartigen Thieren mit einem Eiersack entwickelt haben, so kann man die asiatische Gattung *Schmackeria* gerade in Bezug auf die Eiersäcke als um eine Stufe in der Umbildung weiter vorgeschritten bezeichnen. Die beiden Eiersäcke sind hier schon vollkommen symmetrisch, während bei *Weismannella* die Bildung erst eingeleitet ist, indem erst eine Art *W. Richardi* zwei Eiersäcke und zwar zwei ungleiche Eiersäcke besitzt, die beiden andern Arten dagegen noch einen. — Eine wichtige und interessante Frage würde es sein, ob sich die beiden so ähnlichen Genera unabhängig von einander gebildet haben und welches Genus als Ausgangspunkt anzusehen ist.

Kiel, den 6. Januar 1894.

3. Artificial Modification of the Segmentation and Blastoderm of *Limulus Polyphemus*.

By William Patten, Hanover, N.H.

eingeg. 9. Januar 1894.

During the last two or three years I have collected a large number of abnormal embryos of *Limulus Polyphemus* which I hope to describe in detail at some future time. At present I shall consider certain modifications of the segmentation and of the blastoderm only, caused apparently by artificial conditions prevailing during development. The observations were made last summer while enjoying the privilege of the U. S. Fish Commission Laboratory at Woods Holl.

Limulus deposits its eggs near high water mark in clean, fine sand. In the few instances I have observed the discharge of the sexual products, the pair — the male firmly attached to the abdomen of the female — was half buried in sand, covered by three or four inches of water. In one instance that I observed a milky cloud of spermatozoa suddenly appeared around the male, and at about the same time the eggs were probably discharged into a space filled with water under the abdomen of the female. Such a space certainly occurs, for one could see by the currents of water escaping from the apex of the slit between the thorax and abdomen, that the gills of the female were moving freely. When the cloud of spermatozoa appeared the abdominal appendages of the female worked with much greater vigor, stirring up the eggs, sand and spermatozoa.

During this process none of the eggs escape from beneath the carapace of the female. On turning the pair over I found the eggs thoroughly mixed with sand beneath the abdomen of the female. If undisturbed the pair ploughs forward to a new spot to repeat the operation. Meantime the sides of the excavation fall in covering the eggs with from three to six inches of sand.

In some cases an excess of a gelatinous secretion, probably derived from the walls of the oviducts, is deposited with the eggs binding them together in large compact masses an inch or more in diameter. Such eggs are usually surrounded by blackened sand that smells strongly of sulphuretted hydrogen. Some of these eggs do not develop at all and many of the remaining ones are abnormal. These conditions are probably due to lack of fertilization of the eggs in the first place, and also to the lack of circulation of pure water through the dense egg masses.

This preliminary statement is necessary in order to understand the difference between the natural conditions and the artificial ones now to be described.

The eggs to be fertilized are scooped out from the sides of the carapace, washed in sea water to remove clots of blood and then transferred to a perfectly clean shallow glass dish. The latter is slowly tilted from side to side, and as the eggs come in contact with the clean surface they stick there firmly until the whole dish is beautifully lined with a very compact single layer of eggs. The eggs are now washed again to remove any remaining clots of blood and immature eggs, and then covered for two or three hours with water containing plenty of spermatozoa. After fertilization the eggs require very little attention.

As the eggs are either spherical or slightly oval and apparently of equal density throughout, it is obvious that if they have any prede-

terminated axes or poles, pure accident will determine the relation of those poles in the attached eggs to the direction of gravity.

Segmentation was first seen about twenty-four hours after fertilization. After twenty-four hours more the upper half of all the eggs was divided in to from fifteen to twenty segmentation spheres, well rounded and distinct in the middle of the upper surface, and becoming flatter and less sharply defined on the sides. The under half of the eggs showed no trace whatever of segmentation.

On stripping off a sheet of one hundred or more eggs, one can compare the two surfaces and note the contrast between them. The under surface of each egg may be identified by the presence of a distinct scar on the chorion showing where it was attached to the glass. Now when such a sheet of eggs, about forty-eight hours old, was turned over, the unsegmented hemisphere, now uppermost, begins to segment rapidly, and in from ten to fifteen minutes it is broken into spheres of approximately the same size and number as those on the original upper surface of the egg. It should be observed that the original under surface on being turned upwards, does not segment in the regular sequence, two, four, etc., but breaks up at once into as many spheres as are found on the original upper side for that age.

The original upper side, after reversing the egg, becomes flattened against the chorion by the weight of the overlying yolk and conforms perfectly to it so that the furrows become fainter and fainter. However they do not disappear altogether but are still visible after the opposite side has begun to segment. This shows conclusively that the egg has not rotated inside the chorion, and indeed one can watch the process closely enough to feel satisfied that such a possibility is entirely excluded.

These fixed eggs then, externally, appear to be meroblastic, while the eggs taken from the sand are holoblastic. However if the artificially fixed eggs are removed from the glass and rolled about, they soon show equal segmentation of all surfaces and appear holoblastic. The eggs naturally deposited in the sand are no doubt fixed and if examined »in situ« would probably appear to be meroblastic and with the segmented side uppermost. It is no doubt the rolling about incident upon freeing the eggs from the sand that causes them to segment equally over the whole surface, as described by most authors.

It is well known that under normal conditions the segmentation nuclei are pretty uniformly distributed throughout the yolk, and it is therefore highly probable that in the »fixed«, apparently meroblastic eggs nuclei are already present in the unsegmented under side. It would appear therefore that the weight of overlying material has pre-

vented the segmentation of the yolk around each nucleus. Whether these conditions would influence the division of the nuclei themselves is not apparent from these experiments, but that the nuclear activities are influenced by them to a certain extent seems very probable in view of some facts to be mentioned presently.

The character of the segmentation is determined therefore by the interaction of two forces: namely, first, the force determining the form of the protoplasmic masses, and second, the amount, size and specific gravity of the enclosed inert substances. If we knew these mechanical conditions of weight, pressure and surface tension, etc., we could predict the resulting type of segmentation as well as the form of the future organs of the body.

We can readily see how conditions such as these can influence the resulting type of segmentation. Only a slight exaggeration of the conditions in *Limulus* would be necessary to produce a typical meroblastic egg such as we find in vertebrates. Of course it has been generally recognized that the amount of yolk present in an egg would have a great influence on its segmentation; but just how it acted has not heretofore been clearly and experimentally demonstrated.

The egg acts like a number of vesicles enclosed in a hollow sphere. Fill the vesicles with some light expansive matter and in any position they will remain nearly alike in shape, resembling the segmentation spheres of a holoblastic egg; but if in addition they contain an inert substance whose weight exceeds the expansive force acting within, we would have a condition like that just described in the egg of *Limulus*, and like what probably prevails in most meroblastic eggs: that is, in whatever position we place the sphere, the contained masses will always be clearly outlined and more or less protuberant on the upper surface; but these characters will become fainter on the sides and finally disappear altogether beneath, owing to the pressure of the overlying masses, etc.

It is therefore obvious that the character of the segmentation is not influenced so much by the amount of yolk, as is usually stated, as by its specific gravity. This fact probably explains why some eggs with a comparatively large amount of yolk are holoblastic, while others with comparatively little yolk are meroblastic.

If the yolk and protoplasm were of the same specific gravity, both would probably be uniformly distributed throughout the ovum. When the difference in specific gravity between the two substances increases, a time comes when one must occupy one side of the ovum to the exclusion of the other. As the yolk becomes heavier, it seeks the lower pole of the egg, and it becomes increasingly difficult for the

protoplasm to penetrate it, and finally a typical meroblastic egg is the result. When this condition is reached, some provision must be made to fix the egg in a constant position during development, or else to allow the quick adjustment of the ovum within its envelope to a state of stable equilibrium before the relative distribution of yolk and protoplasm within the egg itself can be disturbed. If such a disturbance occurred frequently, it would be impossible for any two eggs to develop alike because in every egg the tension within that determines form would be different at corresponding times and places.

Most meroblastic eggs rotate freely within the egg membranes on albuminoid cushions that equalize the pressure very beautifully. The relative position of yolk and protoplasm to each other and to the direction of gravity is fixed with great precision, although free to move under unusual stresses.

The size of the yolk particles has a curious effect on the embryonic tissues, that to my knowledge has not before been commented upon. When they are large, the individual tissue cells are unable to surround them and consequently the embryos of both Insects and Crustacea, where these conditions prevail, are characterized by their clear, protoplasmic composition. On the other hand in the Arachnids, Gannoids and Amphibia, the yolk particles are relatively small and not apparently markedly different in specific gravity from the protoplasm. Here each cell takes in large numbers of yolk particles giving the embryonic tissues of these forms their characteristic coarsely granular appearance.

It is very desirable that we possess reliable data concerning the specific gravity of yolk and protoplasm in various types of eggs.

The effect of gravity in cell division is well shown by the following facts:

Eggs that have been fastened to the glass in the manner described above are allowed to develop undisturbed for from five to ten days. At the beginning of this period a well formed blastoderm is developed over the upper surface of the egg. This surface then has a different naked eye character from what it had before. It is whiter, smooth and shining and under a low power one can distinguish the polygonal outlines of the blastoderm cells, with a clear spot in the centre marking the position of the nucleus. On turning the egg over no nuclei or cell outlines can be seen on the under side. As the egg develops the margin of the blastoderm forms in some cases a more and more distinct equatorial welt, with infolded edges, which slowly grows over the under side of the egg, leaving a gradually diminishing »yolk-plug«, in exactly the same way as in Amphibia, *Acipenser*, etc. The whole

process is on such a large scale that it can be easily followed with the naked eye, such eggs presenting a striking contrast with the normal forms, when the blastoderm is formed nearly simultaneously over the whole surface of the egg. As the lips of the »blastopore« contract, (I use the term to suggest what seems to me to be the same thing in vertebrates, but without for a moment assuming that it is in any way associated with a »gastrula«) they often turn inward and unite a little below the surface, thus pinching off a goodly portion of the yolk from the rest of the egg. In hardened eggs this isolated mass of yolk, if not already completely free from the rest of the egg, may be loosened with needles and removed. It leaves a great hemispherical depression on the under side of the egg and lined with a layer of blastoderm cells continuous with those on the upper surface.

Before the blastopore closes the exposed yolk may be coarsely segmented as in the normal egg and the same is true of the isolated yolk-plug. In most cases the lips of the blastopore contract slowly and finally close at the surface without cutting off any yolk.

The »blastopore« is often so irregular in outline that the gradually closing lips may nip off some of these irregularities, thus leaving one large blastopore with one or two smaller ones near its periphery.

I have had thousands of artificially fixed eggs under observation and have never seen one with a blastopore on its upper surface. On the other hand, a very large percentage of the eggs that developed at all, certainly something over fifty per cent., show traces of this remarkable blastopore on the under side. On stripping off a layer of eggs and reversing them one can easily see how, as the blastopore narrows, that it coincides more and more accurately with the small scar of attachment on the chorion. There seems to be no doubt that the blastopore occurring as it does at the point where the egg is fixed, is caused either by constant pressure of the overlying mass of yolk, or by the lack of aeration. The latter supposition does not seem very probable, when we consider how little the eggs of *Limulus* are influenced by the condition of the water. On the other hand I do not recall having seen these »blastopores« on eggs that had been raised under normal conditions. This is somewhat surprising, for, if it is the constant pressure of the overlying yolk alone which prevents the formation of the blastoderm on the under surface, we ought to find these blastopores in eggs deposited naturally in the sand, for there is no reason to suppose that they shift their position so as to equalize the pressure on all sides.

While gravity thus seems to determine the position of the »blastopore« it has no influence whatever on the position of the embryo or on its planes of symmetry in reference to the blastopore. In eggs

kept in a constant position the embryo may appear on any side of the egg and with its axes pointed in any direction. I have seen cases in which parts of the embryo were involved in the enfolded lips of the blastopore. Such parts then apparently failed to develop, while the parts lying freely on the surface appeared to be nearly normal.

Whatever may be the cause for this abnormal method of development, it is important to recognize that apparently insignificant changes in external conditions are capable of producing profound modifications of the normal methods of growth.

The facts here described are, to my mind, very suggestive. I see in them not retrogressive variations indicating conditions in ancestral types. They indicate rather the direction of flexibility of the organism under the stress of a new environment. They may be regarded as prophetic of the variations to occur in the descendants of Limuloid Arthropods that might culminate finally in the normal meroblastic egg and »yolk blastopores« of vertebrates.

These facts may be regarded as constituting a part of the indirect, or circumstantial evidence of the relation ship of *Limulus* to the vertebrates. If, in other words, the eggs of *Limulus* under abnormal conditions segment meroblastically and develop »yolk blastopores« like those of Ganoids and Amphibia, it is almost certain that if these unusual conditions become constant then the result of these conditions, i. e., meroblastic segmentation and a yolk blastopore, would also become constant.

Dartmouth College, Dec. 26th 1893.

4. Beiträge zur Herpetologie Transkaukasiens und Armeniens.

Von L. v. Méhely, Brassó (Ungarn).

eingeg. 11. Januar 1894.

Der Freundlichkeit des Herrn Dr. G. von Horváth verdanke ich eine kleine Collection Batrachier und Reptilien aus Transkaukasien, die er im Monate Juni 1893 theils in der Gegend von Tiflis, Eriwan und Kvirili, theils in Aralich, am Fuße des Ararat gesammelt hat. Trotzdem, daß die Zahl der zusammengebrachten Arten kaum ein Fünftel der von Keßler in seiner Arbeit von 1878 aufgezählten Arten Transkaukasiens ausmacht, dürften diese Zeilen doch manchen Beitrag zur eingehenderen Kenntniss der hier behandelten Thiere enthalten.

I. Batrachier.

1) *Rana esculenta* L. var. *ridibunda* Pall.

Boettger in Radde's Fauna und Flora d. s.-w. Caspigebiets, Leipzig 1886, p. 76. Boettger, Wissensch. Erg. d. Reise Dr. J.

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Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Zoologischer Anzeiger](#)

Jahr/Year: 1894

Band/Volume: [17](#)

Autor(en)/Author(s): Patten William

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