

since 4 say 'sp. 4' and 4 say 'sp. 3'; I agree with the latter. In IVa, 7 say 1855, 8 say 1880, one says date when synonymy was first recognized: the answer depends on the meaning of the word 'removed'; if this be taken literally, the answer is 'either 1880 or any previous date when the synonymy may have been recognized'; but if we regard the spirit of the question, it will be obvious that when a genus is once established it includes all species congeneric with its genotype whether they have been 'removed' to it or no—therefore my answer was 1855. Ambiguity in this question may have been the cause of the equality of votes. In the case of question VI, the pronounced majority is perhaps due to ambiguity in the answer: what I say is that the reviser can not select as genotype of an early genus any species that is already genotype of a subsequent genus, so long as there remains any species free among the originally included species; therefore I wrote 'yes' to the first clause of the question, and 'no' to its second clause.

Adding my replies to those given, it appears that I agree with the majority, usually a large absolute majority, in twelve out of the thirteen cases, and that the thirteenth case, which is ambiguous, is a draw. After this Mr. Stone will probably admit that the method is understood by me, and he will perhaps accept my assurance that I am only an insignificant unit among a fairly large number of old-world writers of similar views and all provided with the small amount of intelligence required.

The considerable agreement attained by those who have answered his questions should prevent the wholesale condemnation of the elimination method; but it would add interest to the figures if we were told whether the minority was generally composed of the same writers. If so, they would probably yield only to *force majeure*; but if not, they might be brought into line by gentle argument.

Mr. Stone makes out a very strong case for the 'first species' method; but is he correct in saying that it 'can lead to but one result'? Would he kindly refer to *Annals and Mag. Nat. Hist.* (2), XVI., pp. 95, 96, and say

what, on that method, is the genotype of *Hemipedinina*?

F. A. BATHER.

LONDON, ENGLAND,

November 12, 1906.

#### SPECIAL ARTICLES.

##### POLYEMBRYONY AND THE FIXING OF SEX.

NATURALISTS have long been familiar with certain curious and unexplained phenomena connected with the life histories of certain parasitic hymenopterous insects of the family Chalcididae. DeGeer in 1752 figured a minute black species with dirty-white wings, which he reared from *minute cocoons* attached together side by side in the larva of one of the pear-leaf miners. Westwood, in the second volume of his Introduction, says of this insect: "The figure has somewhat the air of *Encyrtus*; but the pupæ are naked in that genus."

In the *American Naturalist* for February, 1882, in the second installment of an article entitled 'On some Curious Methods of Pupa-tion among the Chalcididae,' the writer described a precisely similar object found in the mines of an oak-leaf miner, *Lithocolletis fitchella*, at Washington and bred from it a number of specimens of an encyrtid of the genus *Copidosoma*. He further described somewhat similar cocoon-like formations within the larval skin of the pine-leaf miner, *Gelechia pini-foliella*; also in the skin of the larva of the twig borer, *Anarsia lineatella*, in the larval skin of the pine-leaf miner, *Gelechia pini-solidaginis*), and finally described at some length the strange habits of a congeneric parasite which attacks the larva of *Plusia brassicae*. The latter was described as follows:

The *Plusia* larva, up to the time of commencing to spin, appeared quite healthy, although perhaps a little sluggish. Then suddenly its torpor increased, and through the semitransparent skin were seen hundreds of small white parasitic larvæ. In two days at the most the host was dead, having perhaps partially finished its cocoon, while its entire body was completely packed with the parasitic larvæ or pupæ, each surrounded by a cocoon-like cell. A cross-section of the host at this stage showed a regular honeycombed structure. After remaining in the pupal state not longer than twenty days, the chalcidids com-

menced to emerge by the hundreds. My friend, Mr. Pergande, took the trouble to count the parasites which actually issued from one *Plusia* larva, and, to our utter astonishment, the number reached 2,528. An interesting problem now presents itself as to the nature of the cocoon-like cell surrounding each chalcidid pupa in all these different hosts from *Lithocolletis* up to *Plusia*. In the first place it is no silken cocoon, as is readily shown by the microscopic structure. Neither is it a membrane secreted in the general surface of the chalcidid's body, for but a single wall exists between two adjoining pupæ. For the same reason it is not the loosened last larval skin of the parasite. But one hypothesis remains, and that is that it is a morbid or adventitious tissue of the host. \* \* \*

The same phenomenon was referred to by the writer in *Insect Life*, Vol. IV., p. 193, with illustrations, and again in his paper on the 'Biology of the Hymenopterous Insects of the Family Chalcididæ,' in the *Proceedings of the U. S. National Museum*, Vol. XIV., p. 582 (1892). In the latter paper the statement was made that in no case had it been possible to count over 160 eggs in the ovaries of a single *Copidosoma*, and that the number of parasites issuing from a single *Plusia*, therefore, was puzzling and only to be explained on the ground that several females oviposited in a single larva at the same time, as all larvæ develop together, and transform together, and issue nearly together.

In the meantime E. Bugnion, in a most interesting and important paper entitled 'Recherches sur le Développement Postembryonnaire, l'Anatomie et les Mœurs de l'Encyrtus fuscicollis' (*Recueil Zool. Suisse*, Vol. V., pp. 435-536, 1891) had studied with care one of these interesting insects parasitic upon a little Tineid larva, *Hyponomeuta cognatella*, and he found that if one opens the little caterpillar at the end of April or the first half of May, almost always, or at least with some of them, the embryos of the *Encyrtus* (or *Copidosoma*) will be found associated together in the form of chains or strings. These chains are composed of from 50 to 100 or even 120 individuals. The sac which contains the parasites looks like a whitish tube, often bi- or trifurcate, flexuous, folded upon itself,

floating in the lymph of the caterpillar outside of the intestine. Formed of a cuticular membrane, it is clothed on the interior with a layer of epithelial-like cells and encloses a fatty-albuminous mass in which the embryos are enclosed. Later, according to the observations of Bugnion, when the larvæ have attained a certain size, say at the end of May or the beginning of June, the string or chain, which may be 3.5 cm. long, presents a series of swellings and constrictions. Each swelling contains an undeveloped larva in the nutritive substance. At the end of June, the parasites having passed their first molt, break the epithelial tube which envelops them, and find themselves free in the body of the caterpillar. This period (second larval stage) lasts about eight days. Finally the larvæ, having cleaned out the interior of the caterpillar, each one pupates by enclosing itself in an ovoid cocoon, and the caterpillar, whose skin molds itself exactly upon these cocoons, becomes only a rigid, bossy mass. The change from the larva to the nymph takes place by a new molt, and about twenty days afterwards the *Encyrtus* emerges.

In 1898 Alfred Giard (*Bul. Soc. Ent. France*, pp. 127-129) published a note on the development of *Litomastix* (*Copidosoma*) *truncatellus*, a parasite of *Plusia gamma*, in which he describes precisely the same phenomenon previously described by the writer with the same parasite infesting *Plusia brassicæ*, but he reared more than three thousand specimens of the parasite from a single caterpillar. He showed that a single female can lay not more than a hundred eggs and that, therefore, since all of the parasites emerge at the same time, it is almost necessary to suppose that several females (twenty-five to thirty) simultaneously attacked the caterpillar. This, however, Giard thought was most unlikely, and he believed, therefore, that the phenomenon with *Plusia* must be explained on the basis of Paul Marchal's preliminary note published the same year. This leads us to Marchal's observations.

Dr. Paul Marchal, entomologist of the agronomic station under the Ministry of Agriculture at Paris, a naturalist trained in

the very latest morphological methods, a skilled embryologist, and a man of broad culture, in 1897 began to study the development of *Encyrtus fuscicollis*, a parasite of several species of the genus *Hyponomeuta*; publishing his preliminary announcement of his first discovery in the same year, indicating that from a single egg of the parasite there develop many true embryos. His announced results were received with the greatest interest in France, as evidenced by the appreciative remarks of Giard in his own article just cited.

With an admirable skill, with extreme powers of observation, and with an indomitable perseverance, Marchal continued these investigations during six or seven years, publishing four important papers, and finally, in 1904, his startling work entitled 'Recherches sur la Biologie et le Developpement des Hymenopteres Parasites'—La Polyembryonie Specifique ou Germinogonie, *Arch. Zool. Exp.* (4), Vol. II., pp. 257-335, pl. IX.-XIII.

The facts upon which he throws light may be summed up as follows, and in this summary the writer follows Bugnion:

1. The *Encyrtus* [*Litomastix* or *Copidosoma*] has, as well as its host the *Hyponomeuta*, a single annual generation.

2. The oviposition of the *Encyrtus* takes place after that of the *Hyponomeuta*, in July or in August, according to the species parasitized, and it is in the egg of the moth that the parasite introduces its own egg.

3. Each chain of embryos comes from a single egg, following the division of the germ into several distinct individuals in the morula phase.

4. A *Hyponomeuta* egg receives ordinarily only one *Encyrtus* egg. While it is possible that the *Hyponomeuta* egg may be pierced two or three times (perhaps by different individuals), in each case it forms in the caterpillar a corresponding number of chains of embryos.

5. The nutritive mass in which the embryos are encased results from the proliferation of the amniotic cells furnished by the germ of the *Encyrtus* (derived from the paranucleus).

6. The anhiste membrane, as well as the epithelial-like cellules which clothe the in-

terior, are formed at the expense of mesenchymatal elements furnished by the organism of the host. These formations can be assimilated to an adventitious cyst destined to isolate the parasites.

It is upon the eggs of *Hyponomeuta malinella* that the act of oviposition of the *Encyrtus* was for the first time observed (1897).

Marchal having enclosed a branch of apple in a covering of gauze, placed some cocoons of the moth within. The adult insects emerged during the latter part of June and the early part of July. On the fourth several pairs copulated. On the sixth several freshly deposited egg masses were seen on the branches. On the eighteenth, a large number of *Encyrtus* having issued from parasitized caterpillars placed in the cage, Marchal noticed at half past one in the afternoon (at the time when the rays of the sun were warmest) an *Encyrtus* which, poised upon an egg batch of the *Hyponomeuta*, seemed to be about ovipositing. Profiting by such a favorable opportunity, he was able, during the four succeeding hours, to follow with a lens the minute parasite which passed from one egg batch to another, piercing the eggs with its ovipositor. The operation lasted each time a little more than half a minute (two minutes toward the end of the day).

Other observations were carried on upon the parasites of *H. mahalebdeella*. As this insect issues later than the others, Marchal was able, thanks to this fact, to obtain new layings of the *Encyrtus* through a period extending from the twelfth to the twenty-second of August, and to complete at the same time the material which he needed for his work. He concludes from his latest observations that the *Encyrtus* does not live more than a dozen days in the imago state.

The search for the egg of the *Encyrtus* in the egg of the *Hyponomeuta* being extremely difficult if one is obliged to dissociate the vitellus, Marchal used the method of cross-section. Having collected on the tenth of September, 1901, the parasitized egg masses of *H. mahalebdeella*; having fixed them in Gilson's liquid, colored with carmine and having cut them into fine sections, he succeeded in

discovering the egg of *Encyrtus* enclosed in the general cavity of the embryo of the *Hyponomeuta* already voluminous and well advanced. The size of the egg is so small that it was not possible to make more than four or five serial sections of its substance. Its contour is ovoid, distinctly limited, and there is no trace of the shell and pedicel observed before laying. There are in the interior five nuclei in the as yet undivided protoplasmic mass, of which four are smaller, rounded, equal in size, and one more voluminous, placed excentrically, of an irregular lobate form, presenting a finer and denser reticulum. It may be stated that the four little nuclei are destined to engender by successive proliferation all the chain of embryos while the larger nucleus (paranucleus or amniotic nucleus) constitutes the first outline of the amnion.

At this stage the egg of the *Encyrtus* is not surrounded by any membrane. One observes only in its neighborhood the presence of certain mesenchymatous cellules belonging to the host. It is a little later, when the number of embryonic nuclei has risen to eight or ten, that an adventitious cyst commences to form by the drawing together of the mesenchymatous elements which apply themselves against the egg and form a clothing of even cellules. As to the amniotic cellules derived from the paranucleus, their rôle is to form the albumino-fatty mass which surrounds the embryos and which serves indeed as food for the young larvæ.

At the end of September the little larvæ of the *Hyponomeuta* hatch, but they feed only upon the débris of the eggs and remain until springtime protected by the covering of the egg mass. In opening these larvæ under the microscope it is noted with certain ones that there are sometimes two or three little rounded bodies still difficult to distinguish floating among the viscera. These little bodies are the eggs of the *Encyrtus*. Examined by transmitted light at the end of autumn, the egg shows a globular or ovoid mass of protoplasm in which are situated, first, a mass of embryonic nuclei pressed together to the number of from fifteen to twenty; second, a large

excentric paranucleus frequently divided into two segments.

This condition just described persists almost without modification through the winter. Meanwhile in a considerable number of eggs may be found, in the month of March and even in February, a grouping of the embryonic nuclei which already announces the division of the germ into several embryos. The formative vitellus (characterized by its clear tint) is divided into several rounded masses isolated from each other and each surrounding a group of nuclei. These last, which formerly had two nucleoli, now show multiple nuclei often placed in two rows.

But the phenomenon of polyembryony reaches its greatest intensity at the period when the young larvæ of the *Hyponomeuta* leave their winter shelter and commence to eat the leaves.

The egg, at first spherical, grows with an extraordinary rapidity and takes upon itself little by little an elongated ellipsoidal form. It is of this shape and with a considerably increased diameter that it is found in the interior of the larvæ of *H. cognatella* about the twentieth of April. The same condition is found in *H. mahalebdeella* toward the tenth of May.

Studied at this time in a fine cross-section, the germ of *Encyrtus* is found to be composed of small, rounded masses, which have already in certain instances commenced to shape themselves at the end of winter.

Having become more numerous, these are formed of small collections of protoplasm surrounding the nuclei (to the number of eight to twelve to each mass) and offering already quite distinct cellular limits. Each one of these masses is lodged in a round and well-differentiated cavity hollowed out of the common nutritive granular protoplasm. These bodies, which may be likened to gemmules and which may be called hereafter muriform, increase by the multiplication of their elements until reaching a certain size—each one comprising then twelve to fifteen cellules—they divide by cleavage.

In the latter days of April, when the complex polygerm of *Encyrtus* has reached a half

millimeter in length and has taken the form of a sausage, there are about forty muriform bodies in the interior, all distinct from each other and surrounded by the common granular mass. The number of cellules which composes them is always somewhat reduced.

Toward the middle of May the complex polygerm has become a string of from three to four millimeters in length; the gemmules have multiplied until they are often more than a hundred. They have on an average twenty to forty cellules which, by reciprocal pressure, seem polygonal. From this period the embryonic buds begin to issue and the form of the body to become fixed. The embryo, abandoning its spherical form, becomes more discoidal and takes on a reniform aspect. This very characteristic form is generally found about the twenty-fifth of May with *H. cognatella*. Finally toward the tenth of June, the embryo having passed to the larval condition, the chains of the *Encyrtus* reach their definite length and show the typical form described at the beginning of this article.

The most striking fact in the development of the *Encyrtus* is then that a single egg placed in the egg of the moth proliferates by the division of the nucleus in such a way as to form a certain number of plurinuclear masses, and that these, dividing in their turn, give rise to as many morules as there will be embryos in each of the chains.

Polyembryony being, as appears from what precedes, the ordinary method of development of *Encyrtus fuscicollis*, one can predict that the study of the Chalcididae, especially of the Encyrtinae, will show other analogous cases.

Marchal cites already *Ageniaspis testaceipes* Ratz., parasite of *Lithocolletis cramerella*, a miner of oak leaves. He has been able to see, it is true, only the advanced stages of the evolution of this species, his observation having been made in the month of October. The larvæ to the number of twelve or fifteen to each caterpillar had for the most part already formed their cocoons. But in some caterpillars the parasites were grouped in an epithelial tube similar to that of *E. fuscicollis*. The structure of the tube being absolutely

the same, it is probable that the development goes on in the same way.

Another case of polyembryony has been observed by Marchal: *Polygnotus minutus* Lindeman, a minute prototrypid, .5 mm. long, parasite of the Hessian fly. The embryos, which are found to the number of ten to twelve in the gastric sac of the larva of the Hessian fly, are grouped in such a manner as to form a single ovoid mass.

The author, it is true, has not observed the *Polygnotus* in the act of oviposition, but, having found freshly-laid eggs in the gastric cavity, he has succeeded in following the multiplication of the nuclei, then the grouping of the cellules in several individuals as distinctly as with the *Encyrtus*. Polyembryony is then well established for this species. The only differences from *Encyrtus fuscicollis* are, first, that to the morula stage succeeds a true blastula with a central cavity before the formation of the embryo; second, that the proliferation of the germ being much less active, the number of individuals issuing from the egg does not appear to exceed twelve.

Following the publication of this remarkable paper, the subject of polyembryony was taken up by Dr. Filippo Silvestri, of the Royale Scuola Superiore di Agricoltura, at Portici, Italy, who published in 1905 a paper entitled 'Uno Nuovo Interessantissimo Caso di Germinogonia,' etc. (*Rendiconti della R. Accademia dei Lincei*, Vol. XIV., 2d sen., Serie 5<sup>a</sup>, fac. 10°), which consisted of a preliminary note on the study of polyembryony with *Litomastix truncatellus*, the same species which had been observed by the writer and by Giard.

In 1906, in a paper entitled 'Contribuzioni alla Conoscenza Biologica degli Imenotteri Parassiti,'<sup>1</sup> the same writer, Silvestri, goes into detail, with text figures and plates, regarding a most interesting series of observations, in which he sums up practically as follows:

*Litomastix truncatellus* lays its egg in the egg of *Plusia gamma*.

<sup>1</sup>1. Biologia del *Litomastix truncatellus* (Dalm.); 2d. Nota Preliminare, Portici, 1906, pp. 1-45, pl. I.-V.

The larva of *Plusia* parasitized by the *Litomastix* lives in summer three or four days longer than the healthy larva and reaches a greater size.

Each generation of the *Plusia* corresponds to a generation of the *Litomastix*.

The maturation of the egg is identical with fertilized and unfertilized (parthenogenetic) individuals. In the development of the egg of the *Litomastix* we have a process of germ-inogony or specific polyembryony, quite different from that found by Marchal in *Encyrtus fuscicollis* and *Polygnotus minutus*.

From one egg of *Litomastix* there originate about a thousand sexual larvæ and some hundred or more asexual larvæ. The first transform into adults, while the second are destroyed, serving probably as aids to the sexual larvæ in lacerating the internal organs of the host larvæ.

Asexual larvæ are notable from their form, in the structure of the exoskeleton, and by the lack of a circulatory system, of a respiratory system, of the malpighian tubules, and, above all, of the reproductive system.

Each embryo of the sexual or asexual larva is surrounded by two involucres, of which the external one is derived from the ooplasm and the polar nucleus; the internal from a layer of cellules derived by delamination from the embryonal morule.

The fecundation of the egg with *Litomastix* determines the female sex.

And now, what are the broad bearings of this interesting work?

Giard had already in 1898, in his note cited above, in discussing the value of Marchal's discovery as announced in his preliminary note, stated that if one wishes to seek in other classes of animals embryonic peculiarities comparable to those revealed by Marchal, it is perhaps in the degraded platyhelminths of the families Orthonectidæ and Dicyemidæ that something analogous may be found. The sporocysts of *Rhopalura* are in effect, he stated, filled with embryos by a process of ovular multiplication which is not unlike that which takes place in the embryonal tubes of the *Encyrtus*.

Marchal himself publishes an important

section entitled 'Relations existing between Specific Polyembryony of the Hymenoptera and Other Modes of Agamic Reproduction.' These instances are well summed up by Bugnion in a paper entitled 'La Polyembryonie et le Déterminisme Sexuel' (*Bulletin de la Société Vaudoise des Sci. Nat.*, XLII., No. 153, March, 1906), in which he also includes a consideration of certain additional observations, and we may adopt in a very free translation Bugnion's summary:

Other examples taken from the whole range of the animal kingdom somewhat approach the polyembryony of insects.

With the cyclostomes (Bryozoa) one finds a budding which takes place in the egg at the beginning of development. In the genus *Lichenopora* this budding is replaced by the dissociation of the primitive embryo into a great number of secondary embryos. We have then here a phenomenon comparable to that which we have seen with the parasitic Hymenoptera. It is necessary to note, however, that the secondary embryos thus formed offer already an indication of embryonic buds, while the morules of *Encyrtus* or the blastules of *Polygnotus* present no apparent differentiation. With other Bryozoa (*Lophopus*, *Cristatella*) there is also to be seen a budding in the egg, but this takes place at a later period.

With the worms, Kleinenberg announced in 1879 the curious case of *Lumbricus trapezoides*, in which the egg develops into two embryos; here the multiplication takes place by a sort of internal budding intervening in the gastrula stage, at which time differentiation of the buds is already effected.

With the tunicates, the species of *Diplosoma* offer a curious case of precocious budding which gives the appearance of the simultaneous formation of two embryos in the same egg, but in reality this proceeds from the internal budding of an embryo already differentiated (Salensky, Caullery, Pizon, Perrier). With *Pyrosoma* the budding also takes place in the egg, but by a slower method, and it is only when the embryo is organized that it pushes out a ventral stolon immediately cleaving transversely into four buds which each

develop into a new individual (according to Huxley, Kovalevsky, Seelicer, etc.).

From the cases mentioned, where the budding takes place in the egg, one passes insensibly to more frequent and better-known phenomena in which agamic reproduction takes place after the individual has already issued from the egg (as in the Cœlenterates, Orthonectidæ, Dicyémidæ, Platyhelminths, Tunicates). The preceding observations seem then to establish a continuous series connecting the polyembryony of Hymenoptera with the cases of agamogenesis occurring in advanced stages of development.

In general the facts of polyembryony may be also said to approach the cases of experimental blastotomy recently observed by various authors.

Dreisch (1892), passing a temperature of 31° over the eggs of echinids, obtained a separation of the blastomeres into two or more groups; and Loeb (1893), by mixing distilled water in equal parts with the sea-water in which the eggs were found, produced the same result.

Another experiment of Loeb, 1894, upon the eggs of *Echinus*, and by Bataillon, 1900, upon the eggs of *Petromyzon* and of teleosts, consists in dissociating the egg into several groups of blastomeres by means of a heated needle. Both obtained complete larvæ, each blastomere or group of blastomeres forming an embryo.

Ryder in 1893 obtained double monsters by the shaking of the eggs of trout. The vitellus forming on both sides of the egg made two distinct individuals.

One can even make two complete larvæ of *Triton* united only by the skin of the abdomen, by constricting the egg with a silken thread (Endres 1895, Speman 1900 and 1901).

These facts favor, it may be seen, what is called the isotropic condition of the egg, each blastomere or group of blastomeres isolated by one of the methods indicated being capable of forming a complete individual.

Marchal expresses this very well in saying that both in spontaneous polyembryony and in experimental blastotomy each part of the egg contains the complete hereditary patrimony

capable of ending in the formation of an individual conforming to the specific type.

In the papers of Marchal and Bugnion no reference is made to the recent very important work of Professor Conklin in which he shows that the eggs of the Ascidians, *Cynthia partita* and *Ciona intestinalis*, are not isotropic and that the cytoplasm of the egg is not equipotential. Dr. Conklin concludes that "Experiments which demonstrate the totipotence of blastomeres or regions of the egg prove nothing with regard to the presence or absence of differentiation in these parts. Some eggs with a high degree of differentiation have at the same time great capacity for regulation." Workers in this field must reckon with these important results.

Another question which presents itself is that of knowing whether among insects polyembryony ought to be considered as having preceded or followed phylogenetically the other methods of agamic reproduction such as pædogenesis among the Cecidomyiidæ or cyclical parthenogenesis among the Aphididæ and the Cynipidæ. Harmer, for the Bryozoa, arrived at the conclusion that embryonic scission is a consequence of the blastogenetic faculty of the adults. Perrier extends the same point of view to all budding animals.

Considered from this point of view, the polyembryony of the Chalcididæ appears not as an initial phenomenon, but as a secondary adaptation due to an acceleration of embryogenetic process (*tachygenesis* of Perrier, 1902). The result of this adaptation is, considering the short and precarious existence of the adult *Encyrtus*, to assist in the preservation of the species by pushing its multiplication to the highest possible degree.

As to the determining cause of the division of germ, Marchal thinks that it is from the sudden surrounding with more dilute liquids in the interior of the nourishing mass and in a concomitant modification of the osmotic exchanges in the interior of the cellules. One sees, in fact, with *Encyrtus* that polyembryony reaches its greatest intensity at the moment when the larva of the *Hyponomeuta* commences to feed (in the early days of April), and for the *Polygnotus* at the period

when the young larva of the Hessian fly engorges itself with sap. Now, the production of the rapid changes bringing about osmotic pressure constitutes precisely the procedure employed to bring about the separation of the blastomeres and their evolution into several distinct individuals, as has been shown by the experiments already mentioned of Loeb and Bataillon.

Polyembryony is connected with the question of the fixation of sex, and offers from this point of view an especial interest.

Bugnion observed already in the course of his studies upon *Encyrtus* (1891) that all of the individuals coming from a single caterpillar most often belonged to a single sex. A total of twenty-one observations carefully controlled gave the following result: five times of males exclusively; nine times of females exclusively; three times a great majority of males; once a great majority of females; three times males and females in nearly equal numbers.

Marchal has stated similarly that with *Polygnotus*, those coming from a single larva of the Hessian fly almost always belong to the same sex.

These facts, which Bugnion thought should be attributed to an occasional parthenogenesis (the caterpillars giving birth exclusively to males having been, according to his supposition, those which had been pierced by a non-fertilized *Encyrtus*), are now to be explained in a much more rational manner.

With man, true twins enclosed in the same chorion probably come from a single egg. While different hypotheses have been suggested, especially lately (Rosner, 1901), it is natural to suppose that twins develop by the separation of the egg into two parts (spontaneous blastotomy). Then it is established that true twins are always of the same sex. Exceptions to this rule are explained by the fact that certain unusual twins are formed by the joining of two eggs.

Another case presents itself with the mammals, which seems much more comparable to those of *Encyrtus* and *Polygnotus*, namely, that of the armadillo (*Dasypus* or *Tatusia*). These animals give birth, according to the

species, to a litter of from four to eleven young which are all and always of the same sex. It has been noted by Ihering (1886) that all of the fetuses are enveloped in a common chorion and belong, therefore, to the type of true twins. Rosner (1901) explains this fact by the habitual presence of several ovules in a single graafian follicle, and has even concluded that all of the cases of monochorial multiple birth can be explained in the same way. But Cuenot (1903), reviewing the question, has found that with the species studied by Rosner (*Tatusia novemcincta* Linnaeus) the monovular follicles are twenty times more numerous than the pluriovular follicles. It is then impossible to admit that the latter only furnish the fertilizable eggs, and the author concludes that, according to all probability, the multiple births of armadillos come from a single egg.

The discovery of Marchal, therefore, comes extremely apropos to throw new light upon this interesting and greatly discussed question. In the cases where *Encyrtus* and *Polygnotus* issuing from the same larva are almost all males or all females, it must be admitted that this is a natural consequence of polyembryony, and that one would expect the sexes to be separated in this way wherever the embryos come from the division of a single egg.

The fundamental fact coming from this study is that every caterpillar or larva which contains a single chain of embryos gives birth to imagos of the parasite belonging to a single sex, but as the same caterpillar frequently contains two or three chains it will not be astonishing to find males and females given out in quite equal number. The cases in which we find individuals of both sexes, but in unequal numbers, are to be explained by the partial aborting of one of the chains and the survival of only a few individuals, while the other chain develops normally.

It is seen, therefore, that the discovery of polyembryony confirms a fact already suspected but until now incompletely demonstrated, and that is that *the determination of the sex in the fecundated egg is definitely brought about before the first segmentation*



of its nucleus. If then the facts drawn from the observation of parasitic Hymenoptera apply equally to the higher animals, it will be inexact to speak, as has sometimes been done, of an embryonic period which is indifferent from the sexual point of view. The indifference is probably apparent rather than real, and it appears probable that once fecundation is effected the sex is irrevocably fixed.

It is strange that Marchal's work and that of Silvestri following it have received so little attention from English-speaking naturalists.

The extraordinary nature of the discoveries and their wide bearing upon profound biological problems render them among the most important discoveries in biology of recent years. Recently published volumes on insects contain no mention of them; no competent reviews have been published in American or English journals, so far as I am aware, and it is for the sole purpose of directing the attention of American workers to this extremely important field that I have written this lengthy account. After reviewing one of Marchal's preliminary papers in *SCIENCE* in 1898, I endeavored to induce several university teachers, possessing well-equipped laboratory facilities, to take up the subject of this investigation, but without success. It is a fertile field. In the parasitic Hymenoptera there are many thousands of species, and an unlimited material exists at our very doors. The most promising fields of investigation have recently been pointed out by the writer in a paper read before the Entomological Society of Washington. Marchal has studied two or three species; Silvestri has studied another; and both workers have found radical and interesting differences in all. There is, therefore, a vast and unexplored field whose richness can well be predicted from the results of Marchal's work.

L. O. HOWARD.

LE FONDULE (*FUNDULA CYPRINODONTA*) OF  
CARBONNIER AN UMBRA.

I HAVE been several times asked what the Fondule of Carbonnier (1874) was. The breeding habits of this American fish were noticed in considerable detail by P. Carbonnier in the *Bulletin Mensuel de la Société*

*d'Acclimatation* for November, 1874 (pp. 665-671), but under a strange name which has evaded and even prevented identification. The article in question is entitled 'Le Fondule (*Fundula cyprinodonta* Cuv.)' and it is especially claimed: "Ce poisson américain a été désigné par Cuvier sous le nom de *Fundula cyprinodonta*." But Cuvier never gave such a name to a fish, neither in the first or second edition of the 'Règne Animal,' nor (with Valenciennes) in the 'Histoire Naturelle des Poissons.' Carbonnier was probably told by some one who looked casually at his fish that it was a *Fundulus*, a cyprinodont, but the slight notice given of it by Carbonnier does not agree with any cyprinodont. The only means he has given to determine what it was are meager data respecting size, color, sexual differences and habits. The size was small—12 to 15 centimeters at most; there were numerous longitudinal parallel lines; there was no constant difference in color between the sexes, but the females were twice as large (bulky) as the males; they were noticeable for immobility<sup>1</sup> and also for apparent power to turn the head.<sup>2</sup> Here we have a combination of characteristics which is not true of any cyprinodont but which is on the whole realized by an Umbra or mudfish (*U. pygmaea*), and doubtless specimens of that mudfish (to be found abundantly about New York) were the fishes sent to Carbonnier. The sender was a 'M. Godillot,' a Frenchman doing business in New York, as appears from a previous notice by Carbonnier in the *Bulletin* (1871, p. 650).

Interesting details are given of the play of the sexes, the change in color during the nuptial season, the mode of oviposition, the care of the female for her eggs<sup>3</sup> and the char-

<sup>1</sup> L'immobilité qui est un caractère de cette espèce (p. 666).

<sup>2</sup> J'ai dit elle tourne la tête, et avec intention, car cet organe chez le Fondule paraît ne pas être invariablement soudé à la charpente du tronc, et jouit, au contraire, d'une certaine mobilité (p. 669).

<sup>3</sup> Pendant tout le temps que dure l'incubation, qui est de treize à quatorze jours, la femelle veille avec une tendre sollicitude sur ses œufs (p. 669).