

**The Experimental Hybridisation of *Echinus miliaris*, *Echinus esculentus*, and *Echinus acutus*.**

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With Plate 16.

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THE few experiments on the hybridisation of the Echinoderms of which I am now giving the results were commenced on the advice of Professor MacBride, who himself had made similar experiments on other species. At his suggestion I chose *Echinus miliaris*, *Echinus esculentus* and *Echinus acutus*, in order to verify the interesting results obtained by Shearer, De Morgan and Fuchs ('11) in their experiments on these species. I should like in the first place to express my thanks to Professor Sedgwick, who so kindly received me at his institute, and to Professor MacBride, who has helped me so much by his valuable advice and has placed at my disposal material which he had himself collected.

The very numerous researches which have already been made in the hybridisation of the Echinoderm of various species have given results which appear to be very varied. Boveri ('89 and '95) in his crossings of *Sphærechinus* and *Echinus*, remarks that the characters of hybrids are mixed and inter-

mediate between the paternal and the maternal. These conclusions were soon considered doubtful by Seeliger ('94) and Morgan ('95), and very numerous experiments were again made on different material. In nearly all these experiments the student started with the idea of finding out if the hybrids had characters which were exclusively maternal, exclusively paternal, or intermediate between the characters of the two parents.

Driesch ('98) studied the crossings between *Sphærechinus*, *Strongylocentrotus*, *Echinus* and *Arbacia*, and he remarked that "Während alle anderen untersuchten Charaktere der Bastardlarven von Echiniden sich als rein mütterlich und damit sehr wahrscheinlich als von der Natur des Eiplasmas abhängig erweisen, treten bei der Skelettbildung zuerst die Charaktere der väterlichen species auf, indem sich dieselben als eine Mischung väterlicher und mütterlicher Eigenschaften, je nach der kombination mehr zum vater (*Sph.* ♀ × *Echinus* ♂, *Sph.* ♀ × *Str.* ♂) oder mehr nach der Mutter (*A.* ♀ × *E.* ♂, *E.* ♀ × *A.* ♂) hinneigend darstellt." The same author, in a more recent work ('03), does not notice the transmission of pigmentary characteristics by the father, but relying on Boveri's work, he considers that this transmission is sometimes possible, whereas Fischel's experiments and conclusions ('06) prove a certain paternal influence and the heredity of the paternal characters. Other material was used by Vernon ('98). He used eggs of *Echinocardium cordatum* and fertilised them by sperm of *Echinus*, *Strongylocentrotus*, *Sphærechinus* or *Arbacia*. The hybrids were all of the maternal type, but the aboral spike was considerably shorter in them than in the normal larvæ. In a case where the opposite crossing was successful, *Echinus* ♀ × *Echinocardium* ♂, the characters of the hybrids were exclusively maternal. The same material was again experimented on by MacBride ('11) and the results were quite different: whilst the crossing of *Echinus* ♀ × *Echinocardium* ♂ did not give larvæ, the hybrids *Echinocardium* ♀ × *Echinus* ♂ presented paternal and maternal characters.

In another set of experiments on *Sphærechinus* and *Strongylocentrotus*, Vernon ('00) notices considerable variations in the hybrids which he obtained.

At times they present characters which are almost exclusively maternal, at others almost exclusively paternal, and again, at times, characters more or less approaching one or the other. These results are confirmed by Steinbrück ('02), by Doncaster ('04), and by Herbst ('06 and '07), but whilst Vernon attributes these variations to the influence of the seasons, the last authors, above quoted, are more precise, and attribute them to the changes in the temperature.

Equally contradictory results were given by the crossings of other species. Hagedoorn ('09) studied only one characteristic of the skeleton of the hybrids of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. He discovered a purely maternal heredity; on the other hand, Loeb, King and Moore ('10) and Moore ('12), taking up the same study notice that the different characters of the skeleton of the hybrids are not of maternal origin, but always follow the law of dominance, that is to say that the same character is always present or absent in the hybrid, whether it is  $F. \text{♀} \times P. \text{♂}$  or  $P. \text{♀} \times F. \text{♂}$ .

Very similar conclusions were drawn by Tennent ('10) in his experiments in crossing *Hipponoë* and *Toxopneustes*, but he remarks that the characters which follow the law of dominance vary according to the alkalinity of the water which is used in the experiments.

At last we come to the experiments of Shearer, De Morgan, and Fuchs ('11). After examining the hybrids of *Echinus miliaris*, *esculentus* and *acutus*, the authors conclude:

A. "There is considerable evidence for the contention put forward by Loeb, King and Moore ('10) that the minor skeletal characters are inherited independently from either parent.

B. "In the presence or absence of the posterior ciliated epaulettes, of the green pigment masses, and of the posterior pedicellaria, we claim that we have found definite characters,

and we find them to be invariably inherited through the egg,"<sup>1</sup>

It is to verify the accuracy of this last opinion that I have again taken up the experiments of hybridisation of *Echinus miliaris*, *esculentus* and *acutus*. The animals which we used were collected at Plymouth and sent to London, where they always arrive perfectly fresh and in a fit state to give good fecundation. I used methods which were advised by Professor MacBride, and which he himself has described in two articles ('03, '11). I have only to add that the most minute precautions were taken to avoid errors of any description. The animals were carefully washed in fresh water before being dissected; all the instruments and utensils were sterilised before use; sea-water was never used unless it had been kept for a long time in the laboratory and filtered through a Berkefeld filter.

I principally concentrated my attention on three characters which are very characteristic of the species and easy of observation.

(i) The posterior epaulettes which are present in *Echinus esculentus* and *acutus*, absent in *Echinus miliaris*.

(ii) The posterior pedicellaria which is present in *Echinus esculentus* and *acutus*, absent in *Echinus miliaris*.

(iii) The green pigment which is absent in *Echinus esculentus* and *acutus*, present in *Echinus miliaris*.

It is noticeable that, starting with these three characters, it is impossible to distinguish the larvæ of *Echinus esculentus* and of *Echinus acutus*. Besides, no differential character has been recorded in these larvæ. The hypothesis that they are two varieties of the same specie becomes more and more probable. Besides, the results which I obtained were identical whether I used *E. esculentus* or *E. acutus*.

<sup>1</sup> A letter from Shearer, De Morgan and Fuchs, which appeared in a recent number of 'Nature,' June 27th, 1912, states that this year's experiments give results which are quite different from those of other years—results which are doubtless in a great measure analogous to those which we have obtained. I shall discuss their opinion regarding this variation farther on.

I obtained the pure larvæ for each type (figs. 1, 2, 3) and the four hybrids which are possible :

M. ♀ × E. ♂ (fig. 4) ; E. ♀ × M. ♂ (figs. 5, 6) ; M. ♀ × A. ♂ (figs. 7, 8) ; A. ♀ × M. ♂ (figs. 9, 10).

I was able to rear all the hybrids of *E. acutus* through the metamorphosis until they reached the stage of the adult echinus. The hybrids of *E. esculentus* did not go beyond the metamorphosis.

The heredity of the three characters which I studied was as follows :

(1) The posterior epaulettes were present in all the larvæ of the four kinds of hybrids.

This character, therefore, was transmitted to the bastard larvæ in each case, in some through the father, in others through the mother. This character, however, is not wholly transmitted to hybrids. In the larvæ of pure *E. acutus* or *esculentus* the epaulettes are formed at the expense of the loop of the ciliated band which intervenes between the post-oral arm and the postero-dorsal arm, and then the epaulettes entirely separate themselves from it. Now in hybrids we have observed that the posterior epaulettes remain attached to this commissure, and do not separate themselves from it. Consequently the epaulettes are not so perfectly formed in hybrid larvæ as in pure larvæ.

(2) The posterior pedicellaria is to be found in the following hybrids: M. ♀ × A. ♂, A. ♀ × M. ♂, E. ♀ × M. ♂, and probably also in M. ♀ × E. ♂. But the posterior pedicellaria is sometimes absent in certain individual larvæ. One must notice that in the pure larvæ of *E. miliaris*, one does not find a pedicellaria at the posterior pole, but a cellular proliferation in which a calcareous plate is formed which often bears a spine. Now in hybrids one sometimes finds one spine, sometimes two, and sometimes two spines and a pedicellaria.

(3) The green pigment is never transmitted to the larvæ in any of the four kinds of hybrids. This green pigment is present in the larvæ of *Echinus miliaris* in four

little masses which are in the concavity of the four anterior epaulettes, and it is scattered in little grains in the arms and along the ciliated band. In the hybrid larvæ it is not to be found in any of these places. One might suppose that the disappearance of the green pigment is due to degeneration or to a weakness of the hybrids. In fact it is noticeable that the pigment diminishes considerably in the larvæ of *E. miliaris* when in a weak state, as, for instance, when they are hungry. This supposition cannot be maintained if one considers (1) that the green pigment never completely disappears in the pure larvæ, (2) that this diminution is accompanied by the diminution of the red pigment. Now in hybrids green pigment is always entirely absent and the quantity of red pigment is not lessened; rather the contrary.

To sum up and to explain these results it seems necessary to look at the question from another point of view than that which is customary.

In these experiments of crossing amongst *Echinus miliaris*, *esculentus*, and *acutus*, there is not exclusively maternal or paternal heredity; one might say that the hybrids inherit simultaneously from the paternal and the maternal influence; but this formula does not express all the results obtained, for there is no mention of the fact that certain characters are always absent or always present in all hybrids, and that the paternal or maternal origin of the characters does not influence their transmission to descendants.

But if we view the question as Tennent ('10) has done, and especially as Loeb, King and Moore ('10) have done, the solution seems to be much more adequate. Certain characters are dominant, others are recessive. In the experiments which we are discussing, the dominant characters are the presence of posterior epaulettes and of posterior pedicellaria. They are always transmitted to the descendants, either through the father or through the mother; the green pigment is a recessive character. Its appearance is always prevented, either by the maternal, or by the paternal influence.

To this assertion I must add two remarks :

(1) The dominant character is not necessarily transmitted in its entirety ; it may be lessened by a contrary influence. An example of this is to be found here in the fact that the posterior epaulettes are less perfectly developed in hybrids than in pure larvæ. This statement does not contradict Mendel's law of dominance. It is necessary to remark that one is not working on adult individuals with fully developed characters, but on larvæ. Now Lang ('08) has observed in his crossings of *Helix* that in young hybrids the rate of development of certain characters is less than in pure individuals. Moore ('12) remarks and explains the same fact in Echinoderm hybrids.

(2) It would seem necessary to admit that the dominant characters are variable, that is to say, that if they are normally transmitted to hybrids, this may not be the case if certain factors vary. The present experiments perhaps give us an example of this. The posterior pedicellaria is, in fact not always present in the hybrids. Too much importance must not, however, be attached to this example, because one cannot absolutely determine what relationship exists between the posterior pedicellaria of *Echinus esculentus* or *acutus* and the posterior spine of *E. miliaris* ; and we do not know enough of the changes which occur in these elements in the pure larvæ. Better examples of the variability of the dominant characters are to be found in the researches on echinoderm hybrids, which have been already published. According to Tennent ('10) the heredity of the characters varies with the alkalinity of the water. According to Vernon ('00), Steinbrück ('02), Doncaster ('04), and Herbst ('06, '07), this heredity varies with the seasons or more probably with the temperature. It seems, therefore, that one may take it that a normally dominant character is weakened under certain conditions and that a recessive character may, under certain conditions, be strengthened and be transmitted as a dominant character.

We should like to compare our results and conclusions with

those obtained by other experimentalists, but in most of the experiments the question of dominant characters was not considered, and the examination of their works would go beyond the limits of this paper. I will simply say that my conclusions agree with those of Loeb, King and Moore ('10), of Moore ('12), and of Tennent ('10). They would also probably agree with those of Vernon ('00), Doncaster (04), and Herbst ('06, '07).

It only remains for me to speak of the difference which exists between the results of my experiments and of the earlier experiments of Shearer, De Morgan, and Fuchs ('11). This year these authors obtained results which are partly opposed to the results they had obtained in former years, and which, as far as I can judge from their preliminary notes ('Nature,' June 27th, 1912) are fairly analogous to the results which I am now describing. They believe that this difference is due to the fact that certain external circumstances affect the sexual cells of *Echinus miliaris*.

It seems difficult to admit this hypothesis because, contrary to their observations, I have noticed that the eggs of *E. miliaris* × *miliaris* which developed were relatively more numerous than the eggs of *E. acutus* × *acutus*; and that the larvæ of *E. miliaris* × *miliaris* were very easy to rear; in the same way the larvæ of *miliaris* ♀ × *acutus* ♂ were relatively more numerous than those of *acutus* ♀ × *miliaris* ♂. If one studies the question with regard to the dominant characters, one comes to a conclusion which is quite opposed to the one given by these authors.

In their account of this year's experiments one notices that these authors deal with two dominant characters (posterior epaulettes and posterior pedicellaria) belonging to *E. acutus* and one recessive character (green pigment) belonging to *E. miliaris*. In the experiments of former years these dominant characters were not transmitted by the sperm of *E. acutus*, nor was the recessive character crushed by the action of the sperm of *E. acutus*. It appears, therefore,



that some influence weakened the force of the sexual cell of *E. acutus* or strengthened the force of the sexual cells of *E. miliaris*.

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#### EXPLANATION OF PLATE 16,

Illustrating the paper by Dr. G. Debaisieux on "The Experimental Hybridisation of *Echinus miliaris*, *Echinus esculentus*, and *Echinus acutus*."

#### LIST OF ABBREVIATIONS EMPLOYED.

*ant. ep.* Anterior ciliated epaulette. *ech.* "Echinus rudiment."  
*gr. pig.* Green pigment. *l. ped.* Lateral pedicellana. *post. ep.* Posterior ciliated epaulette. *p. ped.* Posterior pedicellaria. *sp.* Adult spine.

[N.B.—Only the green pigment is indicated in the drawings; the red pigment, which is present in all the larvæ figured, is left out.]

Figs. 1-2.—Pluteus of *Echinus miliaris* × *E. miliaris* viewed from the ventral surface. *Gr. pig.* The green pigment.

Fig. 3.—Pluteus of *E. esculentus* × *E. esculentus* viewed from the right side. After the drawings of Mr. MacBride.

Fig. 4.—Hybrid pluteus of *E. miliaris* ♀ × *E. esculentus* ♂, from

the ventral surface. *p. ped.*<sup>1</sup> Knob-like outgrowth probable rudiment of posterior pedicellaria.

Figs. 5-6.—Hybrid plutei of *E. esculentus* ♀ × *E. miliaris* ♂, viewed, the first from the ventral, the second from the right side.

Figs. 7-8.—Hybrid plutei of *E. miliaris* ♀ × *E. acutus* ♂, viewed, the first from the ventral, the second from the right side.

Figs. 9-10.—Hybrid plutei of *E. acutus* ♀ × *E. miliaris* ♂, viewed, the first from the ventral, the second from the right side.

