On Paternal Characters in Echinoid Hybrids.

By

Cresswell Shearer, Walter De Morgan, and H. M. Fuchs

With Plates 17 and 18 and 4 Text-figures.

(From the Laboratory of the Marine Biological Association, Plymouth).

I. Introduction.

Last autumn we published a paper (**12**) giving a brief summary up to date of some three years' work on the hybridisation of Echinoderms. The experiments were made with the three species of Echinus found at Plymouth. From a study of the late larval characters of the pure forms and of the hybrids we arrived at the conclusion that the inheritance of these characters was invariably maternal—that is to say, that the late larval hybrid always resembles its mother. Since the date of the publication of our preliminary paper we have continued our experiments, and have had occasion to repeat all our crosses. To our surprise, however, the behaviour of some of the hybrids has differed greatly this season from that of previous years. In late larval life some of the hybrid crosses have shown as strictly a paternal inheritance as in previous years they showed a maternal one. It may therefore be of interest to other investigators in this field of work if we publish a short account of these new facts before the completion of our full paper.

In our preliminary paper we have clearly pointed out that the very conflicting results which have been obtained by different investigators in Echinoderm hybridisation are in large part due to the study of insufficiently definite characters.

These characters are those developed in the early pluteus—in particular the larval skeleton. This is a structure which varies very readily with the state of health of the organism, and the variations in one form frequently tend to give it a shape resembling that of another. For instance, the skeleton of the antero-lateral arms of the Echinus pluteus is a simple rod, but in abnormal cases a lattice form is developed like that of Sphærechinus. For this reason it is evident that the skeleton is an unsatisfactory index of heredity, and it would seem to be doubtful if much of the previous work on inheritance in the very early pluteus is of substantial value.

In the forms which we have studied we have been forced to adopt larval characters later than those hitherto used as criteria of inheritance. This has been rendered possible by the elaboration of methods of rearing healthy larvæ in quantity up to and beyond metamorphosis. In the advanced plutei of Echinus we have found characters which do not vary in the parental forms and which are present in the one species and absent in the other.

Our experiments have been made with the three common English species of Echinus, namely E. esculentus, E. acutus and E. miliaris. The characters, of which we have investigated the inheritance, have been as follows: E. esculentus and E. acutus both develop posterior as well as anterior ciliated epaulettes (Pl. 17, figs. 2, 3). E. miliaris, on the other hand, has no posterior epaulettes, but two pairs of green pigment-masses are formed in the anterior epaulettes (Pl. 17, fig. 1). All the hybrid plutei have in previous years been purely maternal with respect to these characters. Thus in the crosses E. esculentus ♀ × E. miliaris ♂ (Pl. 17, fig. 5) and E. acutus ♀ × E. miliaris ♂ (Pl. 17, fig. 4) the posterior ciliated epaulettes are developed, although not to so great an extent as in E. esculentus or E. acutus, but the green pigment-masses are absent. In the crosses E. miliaris ♀ × E. esculentus ♂ (Pl. 18, fig. 8), and E. miliaris ♀ × E. acutus ♂ (Pl. 17, fig. 6), on the other hand, the posterior epaulettes are absent and the green pigment is present.

During 1909–1911 no exceptions were found to this rule of maternal inheritance, and in the present year the crosses E. esculentus ♀ × E. miliaris ♂ and E. acutus ♀ × E. miliaris ♂ have behaved as before. It is, however, in the hybrids with E. miliaris ♀ that we have obtained different results. All the cultures of E. miliaris ♀ × E. acutus ♂ have shown a paternal inheritance. Thus, in Pl. 18, fig. 7, the green pigment of E. miliaris is absent, while the posterior epaulettes of E. acutus are present. Again, in the cross E. miliaris ♀ × E. esculentus ♂, with an exception, all the fertilisations gave paternal larvæ (Pl. 18, fig. 9). It seems, then, that it is the E. miliaris eggs which are this year in general unable to transmit the characters of the species to the hybrids.

In former years cultures of E. miliaris have always shown a more rapid rate of growth in the laboratory than the other species. This, we have suggested in our preliminary paper, is possibly due to the fact that, E. miliaris being a shore form, laboratory conditions may be more suited to it than to E. esculentus or E. acutus, the habitat of which is in deeper water. Not only did E. miliaris develop rapidly, but any crosses into which it entered had their rates of growth accelerated. This year, however, this species can only be obtained in a late stage with great difficulty, and its rate of growth is slower than that of E. esculentus, E. acutus, or any of the hybrids.[1] With regard to the latter, when E. miliaris is used as the male parent, fertilisations can easily be made, and the inheritance is, as usual, maternal. When, however, E. miliaris eggs are used in the cross, only a very low percentage are fertilised, and the inheritance is, as stated above, paternal. A few exceptions only have been found to this. In one experiment only a high percentage of the E.

[1] Since the above was written this matter has been brought up at the Dundee meeting of the British Association. Prof. MacBride stated there that he had this year bred E. miliaris, obtained from Plymouth, at the Imperial College of Science in London, and that the larvæ could be raised as easily as in former years.

miliaris eggs fertilised with E. esculentus sperm, and some of the larvæ were paternal, others maternal.

From the above considerations it would seem that some condition in the environment has affected the E. miliaris, so that the eggs are unable to develop healthily when fertilised with sperm of the same species, and are usually incapable of transmitting their characters to hybrid offspring.

TEXT-FIG. 1.

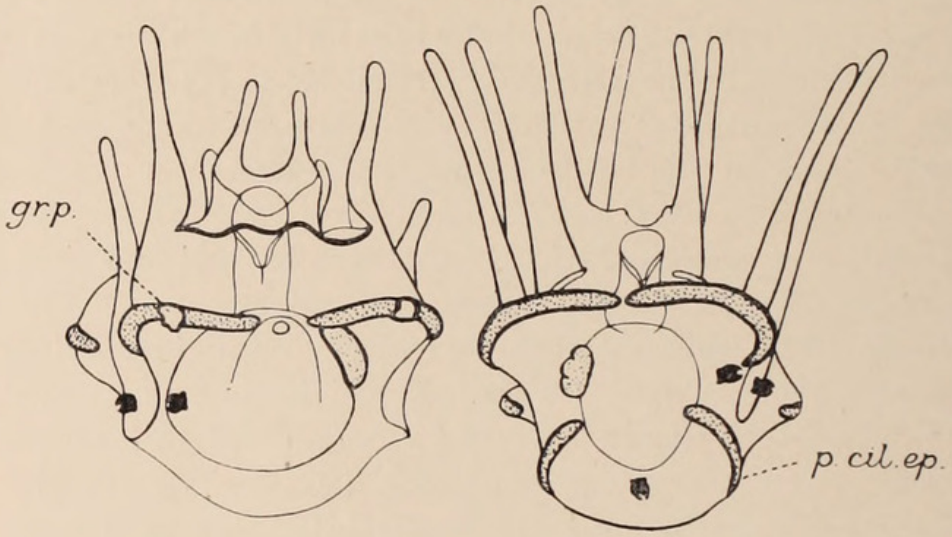


Diagram to show the characters of the late plutei of E. miliaris and E. esculentus. *gr. p.* Green pigment-mass. *p. cil. ep.* Posterior epaulette.

II. SUMMARY OF EXPERIMENTS.

In our preliminary paper we have sufficiently indicated the methods which have been adopted in carrying out this investigation. It only remains to add here that rigid precautions have always been taken against any possible error in the fertilisations. As in all our previous work proper controls have been kept. In addition, our results of this year are not based alone upon the examination of a few culture jars, but on a large number of experiments extending over several months.

The following is a brief description of the late larvæ of the three pure forms and of the hybrid crosses.

TEXT-FIG. 2.

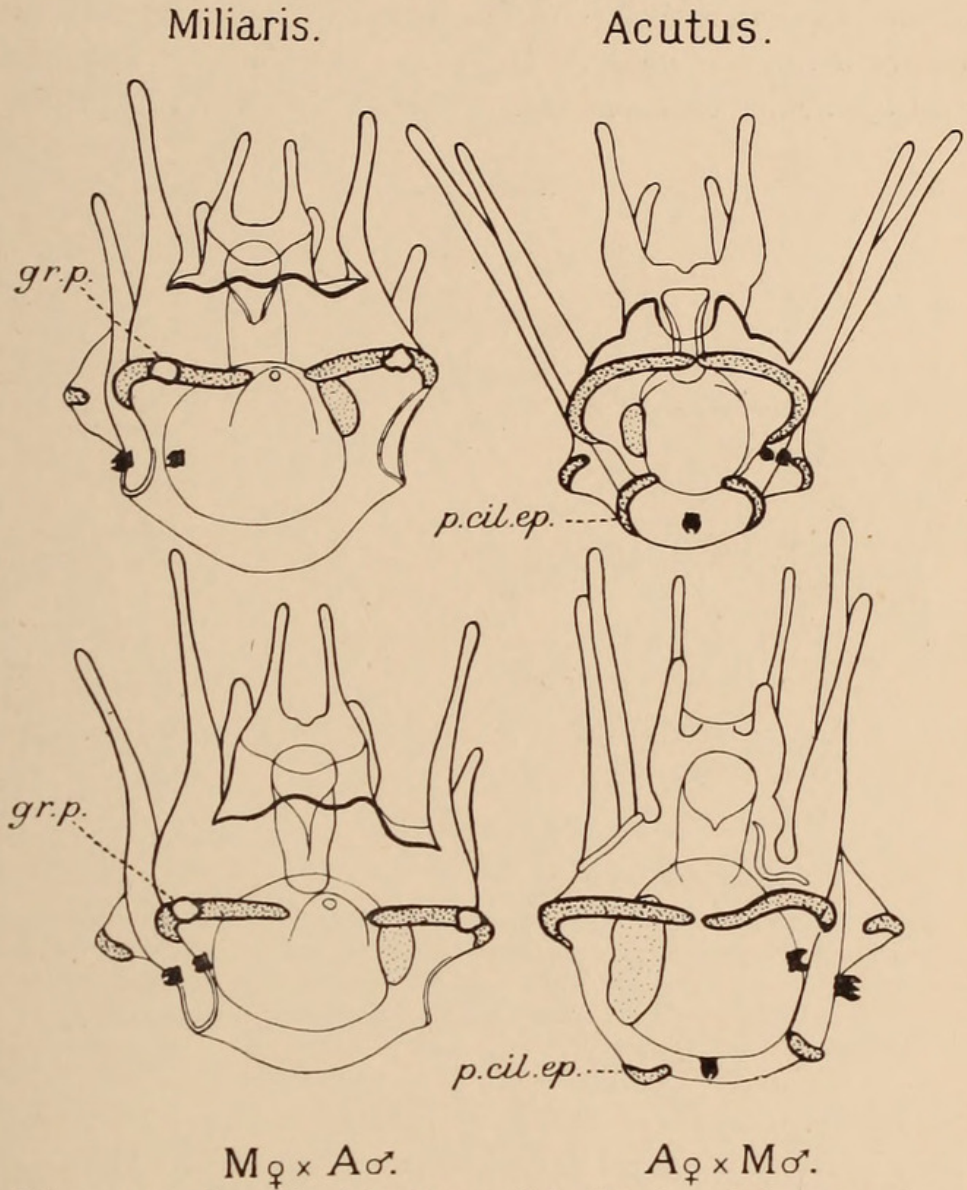
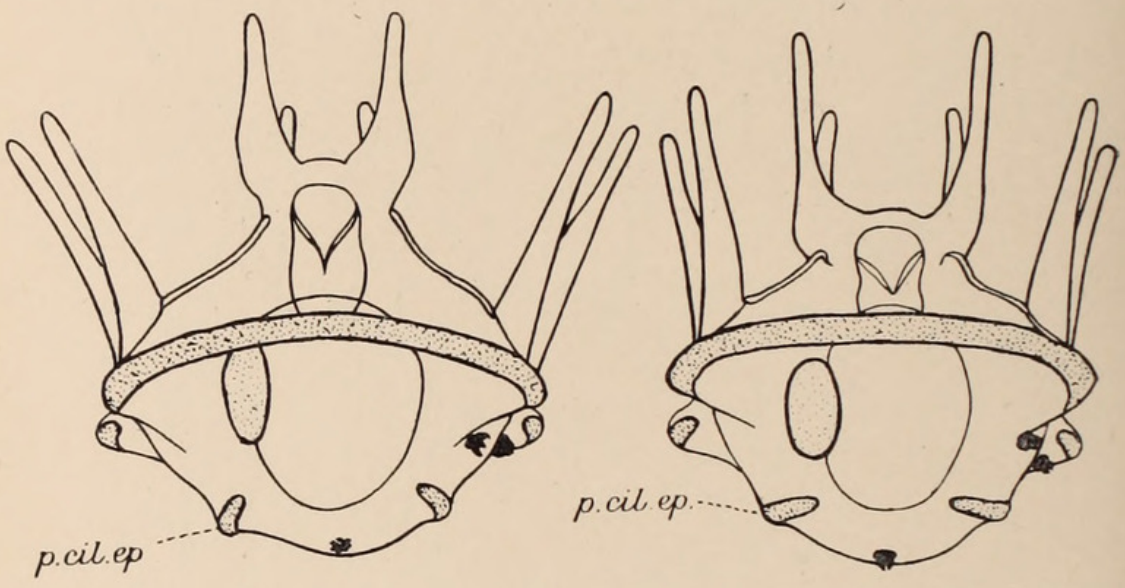


Diagram to show the inheritance of the late larval characters in hybrids between E. miliaris and E. acutus during 1909–11. *gr p.* Green pigment-mass. *p.cil.ep.* Posterior ciliated epaulette. The two upper figures represent the unhybridised larvæ.

The pluteus of E. esculentus reaches the 8-armed stage at about a fortnight after fertilisation. The age of a pluteus is, however, seldom a good indication of its stage of development, as the rate of growth in different cultures

varies within wide limits. The larva (Pl. 17, fig. 2) has a body which is deeper than wide, the posterior pole being flattened. As soon as the eight arms are developed the ciliated band around the anterior edge of the larva thickens, four crescentic bands being abstricted. These are the anterior epaulettes, and by means of their strong cilia they form the principal

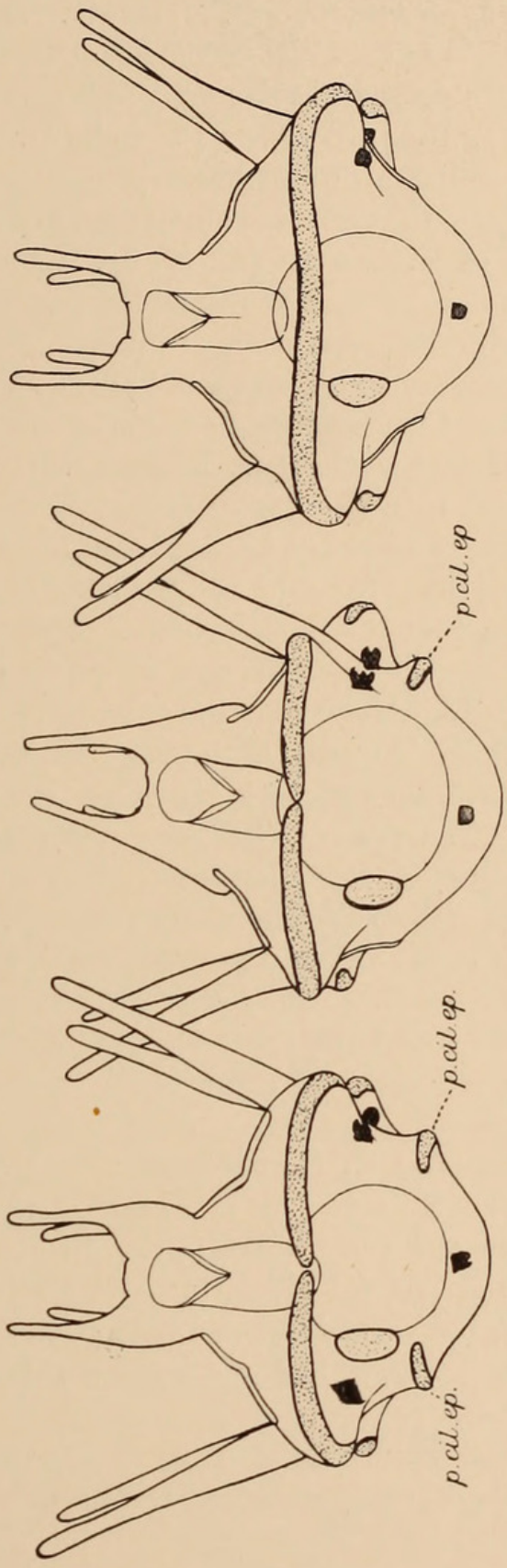
TEXT-FIG. 3.



M ♀ × A ♂ A ♀ × M ♂.

Diagram to show the inheritance of the late larval characters in hybrids between E. miliaris and E. acutus during 1912 (cf. Text-fig. 2) and absence of the green pigment-masses in the hybrids when E. miliaris was maternal. *p. cil. ep.* Posterior ciliated epaulette.

locomotory organ of the fully formed pluteus. Eventually they coalesce to form one continuous band. At the end of about three weeks a pair of posterior ciliated epaulettes is formed. The presence of these structures in this species is one of the characters of which we have investigated the inheritance (Text-fig. 1). A pair of pedicellariæ appear dorsally and ventrally on the right side of the larva, opposite to the Echinus-rudiment, which is now well advanced. A posterior pedicellaria is usually present as well. It is also developed in E.



TEXT-FIG. 4.

Diagram to show an exceptional case of the inheritance of the late larval characters in the cross E. esculentus ♀ and E. miliaris ♂, in 1912. *p. cil. ep.* Posterior ciliated epaulette.

acutus, but is never present in E. miliaris. The inheritance of this pedicellaria follows that of the epaulettes, but it is not a very reliable feature, as it occasionally fails to develop in pure E. esculentus, E. acutus, or the hybrids. The larva is studded with reddish-brown pigment spots, which are concentrated at the tips of the arms and along the ciliated epaulettes.

The pluteus of E. acutus (Pl. 17, fig. 3, and right upper figure in Text-fig. 2) closely resembles that of E. esculentus, and develops posterior epaulettes. It has, however, a rather smaller body, with more slender and divergent arms. Owing to the similarity in essential features between this species and E. esculentus, hybrids between them give no information of hereditary value.

The E. miliaris larva (Pl. 17, fig. 1, and Text-fig. 1) is of a different general shape from the two described above, the body being wider and having a domed posterior end. The arms are comparatively short. A dorsal and a ventral pair of green pigment-masses are developed in the anterior epaulettes. This pigment is completely absent in the plutei of E. esculentus and E. acutus. No posterior epaulettes are developed in E. miliaris. The presence of the green pigment and the absence of the posterior epaulettes are the important features of this larva for the study of heredity (Text-fig. 1).

This year (Text-fig. 3), as in previous seasons (Text-fig. 2), hybrids of the cross E. acutus ♀ × E. miliaris ♂ (Pl. 17, fig. 4) have developed maternal characters. The posterior epaulettes are present, and there is no trace of the green pigment-masses.

Plutei of the cross E. esculentus ♀ × E. miliaris ♂ (Pl. 17, fig. 5) have similarly always been purely maternal with respect to the same characters. In one experiment, however, all the larvæ had the usual material absence of green pigment, but some developed both posterior epaulettes, some neither and others had one on one side of the body only (Text-fig. 4).

With the cross E. miliaris ♀ × E. acutus ♂ we have, however, obtained totally different results this season from

those of former years. In 1909–1911 the hybrid larvæ were always maternal (Pl. 17, fig. 6, and Text-fig. 2), having no posterior ciliated epaulettes, but developing the green pigment. In all the experiments of this year only a very low percentage of the eggs would fertilise with the foreign sperm. Perfectly healthy larvæ were obtained from these fertilisations, but in all the cultures they showed an inheritance which was the exact reverse of the previous years. Posterior epaulettes were developed, but no green pigment, showing that the larvæ inherited their characters through the sperm (Text-fig. 3).

From the cross E. miliaris ♀ × E. esculentus ♂ we have also obtained results which conflict with those of other years. Formerly the characters of the hybrid plutei were always maternal (Pl. 18, fig. 8). Posterior epaulettes were absent, and green pigment was present. This year we have had as great a difficulty to fertilise the E. miliaris eggs with E. esculentus sperm as with E. acutus sperm. In one case only did we get a good fertilisation, about 80 per cent. of the eggs segmenting. In cultures made from this cross some of the hybrid larvæ were maternal, others paternal. In all other experiments the percentage of eggs which fertilised was twenty or less. Nevertheless, these

	1909, 1910 and 1911.		1912.	
	Posterior epaulettes.	Green pigment-masses.	Posterior epaulettes.	Green pigment-masses.
E. esculentus, ♂ / E. esculentus, ♀	+	0	+	0
E. miliaris, ♂ / E. esculentus, ♀	+	0	+	0
E. miliaris, ♂ / E. miliaris, ♀	0	+	0	+
E. esculentus, ♂ / E. miliaris, ♀		+	+	0

larvæ developed quite healthily, but their characters were paternal (Pl. 18, fig. 9). The posterior ciliated epaulettes were developed, but the green pigment was totally absent.

On p. 345 is given in tabular form a summary of the inheritance of the late larval characters investigated. The difference between the inheritance this year and that in previous years can be seen at a glance. The crosses between E. miliaris and E. acutus gave the same results as those between E. miliaris and E. esculentus.

III. Discussion.

In a comparison of the general results of hybridisation among Echinoderms with that in other divisions of the animal and vegetable kingdoms it is striking that the products of reciprocal crosses are, in the former case, so frequently unlike one another. In the species of Echinus with which we have experimented, the characters investigated have, until this year, always been inherited through the maternal parent. Such behaviour seems to be of quite a different order from that of pairs of characters which follow the usual Mendelian law, in which cases the hybrids of one cross resemble those of its reciprocal, irrespective of the sex of the parent forms. Prof. Punnett has pointed out to us a case among plant hybrids which would seem to be parallel with this parental inheritance in Echinoderms. In crossing two species of Œnothera, de Vries (**15**) found that the hybrids were always strongly paternal. Hybrids of the cross Œ. biennis ♀ × Œ. muricata ♂ resembled Œ. muricata while those of the reciprocal resembled Œ. biennis; so that in this case the hybrids showed an inheritance through the male germ-cells. It is interesting to note here that he kept each of the hybrid strains for four generations without observing any alteration in their characters.

If data could be obtained of inheritance through more than one generation in Echinoderms, it might be found that the facts could be interpreted on Mendelian lines. As, however,

up to the present no hybrids have been raised to sexual maturity, any suggested Mendelian explanation must remain unconfirmed. Nevertheless we would suggest that the following hypothesis might be put forward to express the purely maternal inheritance. Suppose that the presence of a pair of posterior ciliated epaulettes (P) and the absence of the same (p) are a pair of allelomorphic characters. Then the germ-cells of E. acutus will all have P and those of E. miliaris will all have p. Suppose, moreover, that in hybrids when P comes in through the male it is recessive to p, but when through the female it is dominant,

then E. acutus ♀ × E. miliaris ♂ receives	recessive p through the ♂	the hybrid having posterior epaulettes;
	dominant P through the ♀	
and E. miliaris ♀ × E. acutus ♂ receives	recessive P through the ♂	the hybrid having no posterior epaulettes.
	dominant p through the ♀	

If this hypothesis were true and segregation took place, the F_2 generations from both reciprocal crosses would have to be composed of larvæ in equal numbers with and without the posterior epaulettes. If, on the other hand, there were no segregation, all the F_2 individuals would be alike and similar to the F_1 hybrid from which they were bred.

We have explained above that two of our crosses have this year shown a complete reversal in the inheritance of the parental characters. While in hybrids between E. esculentus or E. acutus and E. miliaris, in which the latter was used as the male parent, the characters were maternal as formerly, when E. miliaris was used as the female the hybrids this season usually resembled the father. Formerly they had been invariably maternal. This complete change in heredity from one year to another seems to be without parallel in hybrids other than those among Echinoderms. Using the same notation as above we should have to suppose that this year P behaves as a simple dominant over p.

As we have emphasised above, previous investigators in Echinoderm hybridisation have used characters which were not definite enough to give unquestionable results. Never-

theless the frequent inconsistencies between the conclusions of different experimenters may in part be of the same nature as our change in inheritance. In 1889 Boveri (**2**), working at Naples, found that hybrids of the cross Sphærechinus ♀ × Echinus ♂ were all intermediate in their characters. Seeliger (**11**) made the same cross at Trieste in 1894, but found that in every culture some of the larvæ were paternal. Morgan (**10**) repeated the work at Naples in 1895 and substantiated Seeliger's conclusions. It is possible that the inheritance of parental characters in this cross does not remain the same during a series of years. Similarly the opposite results obtained with hybrids between Strongylocentrotus franciscanus and S. purpuratus by Hagedroon (**7**) and Loeb, King and Moore (**9**), who worked at Pacific Grove, Cal., in two consecutive years, may possibly both be correct. In any case our results serve to emphasise the fact that it is necessary to repeat the same experiments many times and to extend them over a considerable period. If the investigation had been made at Plymouth during this year alone, the conclusion would probably have been arrived at that the characters of E. esculentus and E. acutus are dominant over those of E. miliaris. We know, however, that this has not been the case in preceding years.

Vernon (**14**), working at Naples in 1900, found that the inheritance in hybrids between Strongylocentrotus and Sphærechinus was different according to the time of year at which the crosses were made. In spring the hybrid larvæ resembled Strongylocentrotus, while in summer they were like Sphærechinus. Strongylocentrotus was found to be much riper in the spring than in the summer, and accordingly Vernon concluded that the dominance of the one species over the other was controlled by the relative ripeness of the sex cells used to make the cross. Doncaster (**3**), however, found that the cause of the change in inheritance was the difference in temperature at the two seasons. We cannot compare our results strictly with those described above. The breeding period of the three species of

Echinus found at Plymouth is relatively short so that the crosses can be made at one season of the year only. It appears to be plain, however, that the relative ripeness of the germ-cells used in our experiments is of no account. For, during the four seasons of this investigation, hybrids made at the commencement or end of the breeding season have not differed from those made at the period of maximum maturity.

Since it was in crosses in which E. miliaris was used as the female parent that the inheritance was different this year from that in previous seasons, there would appear to be some alteration in the eggs of this species. This is borne out, not only by the fact that the normally fertilised eggs of E. miliaris develop slowly and irregularly, but also that in the only hybrid cross in which the percentage of fertilisation was high, some of the larvæ showed the usual maternal inheritance. Since this species has failed to develop healthily it might seem that the eggs were immature and that the breeding period had for some reason been postponed. But even if this were so, previous experience has shown us that immature eggs, although they may give unhealthy larvæ, do not alter the inheritance in hybrids. On the whole it seems most probable that some factor in the environment has affected the metabolism of E. miliaris in its habitat this year, so that the condition of the female germ-cells is changed.

In 1906 Kupelwieser (8) fertilised Echinus eggs with Mytilus sperm and obtained hybrid larvæ which were purely maternal. He found, however, that the chromatin of the sperm had not fused with that of the eggs, so that there had been no true fertilisation. In the earlier stages of our investigation this suggested to us that the invariable maternal dominance which we found might be a case of virtual parthenogenesis similar to that of Kupelwieser. Godlewski (6) fertilised Echinus eggs with Antedon sperm but obtained a result which differed from that of Kupelwieser. The larvæ were maternal, but he showed that there had been a true fusion of the nuclei, the male chromatin taking an active part in the segmentation mitoses. This result showed

that the sperm chromatin has not necessarily any influence on the structure of the hybrid. Other investigators in Echinoderm hybridisation who have found a purely maternal inheritance (Driesch (**5**) and Hagedoorn (**7**)) have not investigated the cytology of their crosses. In order to decide whether or not there was a true fertilisation in our experiments we handed over hybrid eggs fixed in the early segmentation stages to Doncaster and Gray. A preliminary account of the results of their investigation was published last autumn (**4**). At that date they had only examined one cross into which E. miliaris entered, namely E. acutus ♀ × E. miliaris ♂. In these hybrids there was a true fusion of the male and female pronuclei, so that this seemed to be a case parallel with that of Godlewski.

Baltzer (**1**), working at Naples in 1908, found that in his crosses, although there was a true fusion of the pronuclei, a varying number of chromosomes were thrown out in the early segmentation divisions. He also claimed that chromatin was eliminated as late as the blastula stage. Tennant (**13**) has has also found elimination of chromosomes in the early stages. It is possible that such a rejection of chromatin may take place at an early or a late stage in our Echinus crosses, and that sometimes paternal chromatin may be thrown out while at others maternal. A change in elimination of this nature might be correlated with the change in inheritance which we have described above.

A full account of the investigation of Doncaster and Gray, which was made on identical material from which some of our crosses were raised, will appear shortly in this journal.

During this summer we have investigated the inheritance of characters in the young hybrid sea-urchins after metamorphosis. An account of this work will be given in a later paper. A method has been found of feeding and raising the urchins in the laboratory and a number of these have now reach a considerable size. It is hoped that we may obtain from them a second generation.

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EXPLANATION OF PLATES 17 AND 18,

Illustrating the paper by Messrs. Cresswell Shearer, Walter De Morgan and H. M. Fuchs on "Paternal Characters in Echinoid Hybrids."

LETTERING.

a. cil. ep. Anterior ciliated epaulette. *ech. r.* Echinus-rudiment. *gr. p.* Green pigment. *p. cil. ep.* Posterior ciliated epaulette. *ped.* Pedicellaria.

PLATE 17.

Fig. 1.—Pluteus of Echinus miliaris. Dorsal view. Note green pigment-masses in anterior epaulettes and absence of posterior epaulettes.

Fig. 2.—E. esculentus. Dorsal view. Green pigment absent. Posterior epaulettes present.

Fig. 3.—E. acutus. Dorsal view. Green pigment absent. Posterior epaulettes present.

Fig. 4.—E. acutus ♀ × E. miliaris ♂. Ventral view. Maternal characters.

Fig. 5.—E. esculentus ♀ × E. miliaris ♂. Dorsal view. Maternal characters.

Fig. 6.—E. miliaris ♀ × E. acutus ♂. Dorsal view. Maternal characters.

PLATE 18.

Fig. 7.—E. miliaris ♀ × E. acutus ♂. Ventral view. Paternal characters

Fig. 8.—E. miliaris ♀ × E. esculentus ♂. Dorsal view. Maternal characters.

Fig. 9.—E. miliaris ♀ × E. esculentus ♂. Dorsal view. Paternal characters.