PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Relations between the Hybrid and Parent Forms of Echinoid Larvae

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VIII. *The Relations between the Hybrid and Parent Forms of Echinoid Larvæ.*

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Contents.

Introduction.

The study of the conditions under which hybrids between various species can be produced is of importance in relation to several problems of evolution, whilst the examination of the morphological relations between the parent forms and those of their hybrid progeny is able in many cases to throw additional light on questions of the transmission of parental characteristics, and of prepotency. Within the limits of the animal kingdom no more suitable species can be found than certain of the Echinoidea, and hence a number of observations have already been made upon members of this group. In the present research it is sought to extend these observations, more especially by determining systematically, over a period of several

months duration, the exact relationships of structure and size between certain hybrid and parent larval forms.

The plutei made use of in these observations were in all cases obtained from artificial fertilisations, carried out in the manner already fully described in a former paper.* Suffice it to mention here that the method consists in shaking pieces of the ovaries and testes in small jars of water, and then mixing portions of the two liquids, and at the same time carefully noting the temperature. After standing an hour, the now fertilised ova are poured into a large jar of water, holding as a rule from 2 to $3\frac{1}{2}$ litres. These large jars, covered with glass plates, were allowed to stand in a tank of running water, whereby the temperature of their contents was kept nearly constant, it varying less than a degree during twenty-four hours, and not more than about two degrees during the whole course of the experiment. As a rule the fertilised ova were allowed to proceed on their normal development for eight days, as it has been shown that the arm lengths of the artificially produced *Strongylocentrotus* larvæ reach their maximum length at this period. The plutei were then killed by adding sufficient saturated corrosive sublimate solution to the water to form a ·2 per cent. solution. After allowing them to settle, the supernatant water was gradually poured off, and the larvæ transferred first to 50 per cent. alcohol, and then to 80 per cent., in which latter fluid they were preserved until required. For microscopical examination, they were mounted in glycerin. No staining is necessary, as the calcareous skeleton, which was almost entirely relied on for diagnostic differences, is very obvious and sharply defined. These larvæ were then measured, in sets of fifty, by means of a micrometer eye-piece. Occasionally, in the case of certain hybrids which were obtained in but small numbers, less than fifty individuals were measured. The actual numbers are given in the Table at the end of the paper. In every instance, the position of each pluteus measured was read off on the mechanical stage and noted down, so that the same individual should not be measured twice.

In order to determine the proportion of ova which were actually fertilised, and also the number of plutei formed after eight days' development, the contents of each large jar of ova were vigorously stirred up, and a volume of 3 cub. centims. was drawn off with a pipette. This was run into a small glass cell, in which a drop of saturated corrosive sublimate had previously been placed. A cover-slip was slid over, and after giving the ova and blastulæ time to settle, the cell was fixed in a mechanical stage, and its whole area worked through carefully under a low power of the microscope. This counting was always made twenty-four hours after the time of fertilisation, so that the proportions of unfertilised or abnormally developing ova and of blastulæ present could be determined at one and the same time. Two separate determinations were always made, and if the numbers obtained were not in good agreement, the counting was repeated a third and sometimes a fourth time. In

* 'Phil. Trans.,' B, 1895, pp. 577–632.

order to obtain consistent results, it is essential that the contents of the jars should be most thoroughly stirred up. The number of plutei present after eight days' development was determined in a similar manner. In a few cases, when the proportion of plutei was but small, the number in 50 or 100 cub. centims. of the water was determined. When less than about 200 per litre were present the number was roughly estimated by observation of the whole contents of the jar. In the case of each experiment made, the number of ova present in 10 cub. centims., the percentage of these reaching the blastula stage after twenty-four hours' development, and the percentage of plutei surviving after eight days, are given in the Table at the end of the paper.

Altogether, eight different species of Echinoids were experimented with, but the great majority of observations were confined to three of them, viz., *Strongylocentrotus lividus*, *Sphærechinus granularis*, and *Echinus microtuberculatus*.

Crosses between Sphærechinus ♀ *and* Strongylocentrotus ♂.

That hybrid larvæ could be obtained between *Sphærechinus* and *Strongylocentrotus* appears to have first been demonstrated by Marion.* This observer obtained plutei on fertilisation of *Strongylocentrotus* ova with *Sphærechinus* sperm, but an attempt at the reciprocal cross only resulted in a few of the ova segmenting, and then ceasing development. R. Köhler† obtained somewhat similar results. The only detailed description of the form of the hybrid larvæ appears to be that given by Morgan,‡ who found the hybrids between *Sphærechinus* ♀ and *Strongylocentrotus* ♂ to be of a type somewhat similar to that of pure *Strongylocentrotus* larvæ, but with a variable number of skeletal rods to the anal arm.

My observations on these hybrid forms extended over a period of about nine months, during which artificial fertilisations were carried out at intervals of a few weeks. In the majority of cases plutei were obtained, though as a rule only in very small numbers. In making these cross-fertilisations, as has been pointed out by Pflüger,§ Born,‖ O. and R. Hertwig,¶ and by Morgan,** it is necessary to take great precautions to prevent any of the ova being directly fertilised by sperm of their own species. Thus in the first place the aquarium tank water in which the ova are shaken may itself contain some spermatozoa. A female Echinoid may have some sperm from another member of its own species clinging to its test; or lastly,

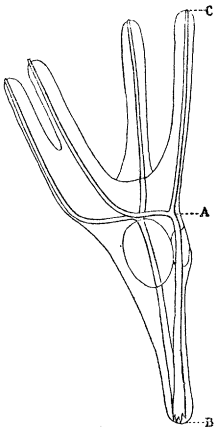
* 'Comptes Rendus,' vol. 73, p. 963, 1873.
† 'Comptes Rendus,' vol. 94, p. 1203, 1882.
‡ 'Arch. f. Entwicklungsmechanik,' vol. 2, p. 277.
§ Pflüger's 'Archiv,' vol. 29, p. 48.
‖ Pflüger's 'Archiv,' vol. 32, p. 453.
¶ 'Jenaische Zeitschrift f. Medicin,' vol. 19, p. 121, 1886.
** 'Anat. Anzeiger,' vol. 9, p. 141.

the jars and instruments used may be contaminated. In order to avoid these sources of error, the following precautions were taken. The water used in the fertilisations, or at least in the latter two-thirds of them, was allowed to stand in the jars for one and generally for two days before it was used. At the end of this time it was proved that any spermatozoa originally present no longer possessed sufficient vitality to fertilise ova, even of their own species. Again, a certain number of the ova used were allowed to remain in a jar of water to which no sperm had been added, and after twenty-four hours were microscopically examined to see if any of them had undergone segmentation. In one or two cases it was found that a few of them had, and hence some sperm must have effected an entry. The results obtained in the cross-fertilisations were in these instances accordingly rejected. Finally, great care was taken in the thorough cleansing of all the jars and instruments used, these being as a rule washed with fresh water first, and then with sea-water.

In order to obtain as average results as possible, the ova of several different sea-urchins were generally used in each experiment, and were likewise fertilised with the mixed sperm from several different individuals. Details on these points, and others connected with the experiments, such as the temperature of impregnation, and the mean temperature of the water during the progress of development, are given in the table at the end of the paper.

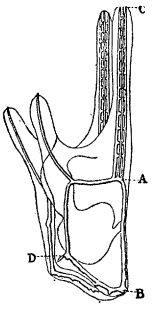
For the sake of comparison it is thought convenient to give figures of the normal pure *Strongylocentrotus* and *Sphærechinus* larvæ, the latter being represented from

Fig. 1.



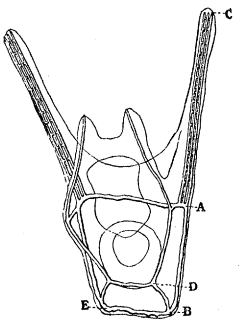
Strongylocentrotus.

Fig. 2.



Sphærechinus.

Fig. 3.



Sphærechinus

two points of view. These and the figures subsequently given were all drawn to the same scale by means of a camera lucida, and, as here reproduced, represent a magnification of about 120 diameters. Owing to their preservation in alcohol, the soft tissues of these larvæ have considerably shrunk, but the calcareous skeleton of course remains unaltered. In all cases the body length of the larvæ, AB, and the anal arm length, AC, were measured, and the latter calculated as a percentage on

the former. The length of the calcareous skeleton, and not of the soft tissues, was always measured, as it is so much better defined, and is moreover practically the same as the latter. The mean body and percentage arm length measurements are given in the table at the end of the paper, the values there given being in eye-piece scale units. If desired to reduce them to millimetres, they must be divided by 152·3.

It will be seen that the chief distinctions between the two parent larval forms, as far as mere measurement is concerned, lie in the fact that the *Strongylocentrotus* larva has a fairly long body (on an average about 33 scale units), and an arm length of about the same value, whilst the *Sphærechinus* larva has a short body (on an average about 17 units), and an arm length as a rule about double as great. Measurement alone therefore should be able in many cases to decide whether a hybrid larva approaches more closely to one type or the other.

In the accompanying table are given the results of all the experiments made on crossing *Sphærechinus* ♀ and *Strongylocentrotus* ♂. In the fourth column of the table are given the colours of the various female *Sphærechinus* individuals used, for, as will subsequently be discussed more fully, these sea-urchins present three fairly definite colour varieties, distinguished by their white, violet, and brown spines. In the next column are given the percentages of blastulæ formed twenty-four hours after fertilisation. It will be seen that as a rule less than 10 per cent. of the ova are fertilised, though occasionally the proportion rises to a third or half. The percentage of plutei surviving eight days after fertilisation is, however, much smaller, it being as a rule about 1 per cent. It is noticeable that the number of blastulæ and plutei formed is very much greater in the first half of the experiments, or those made between the months of May and November, than in those made in December and January. This difference would be still more marked if several of the experiments made in the latter two months, in which various artificial aids to bring about fertilisation were adopted, had been omitted. Thus in these experiments either about ten times as much sperm was added to the ova as had been used in the previous experiments, or else the ova were kept about nine hours in water previous to the addition of the sperm. The former method was found by Born* to increase the chances of cross-fertilisation in various Amphibia, but it has been denied by O. and R. Hertwig† to have any such efficacy in the case of Echinoids. The latter method was, however, found by these observers to be of very great assistance. The results given in the above table, and also those to be subsequently described, prove both methods to be of value, but especially the latter.

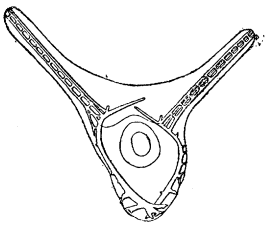
The rest of the table gives details as to the average size and relations of the hybrid larvæ obtained. In the first experiment cited, 64 per cent. of the larvæ measured were of a type approaching to that of the pure *Sphærechinus* larva, and 36 per cent. of an intermediate or *Strongylocentrotus* type. About half of the *Sphærechinus* type of hybrids bore no sign at all of their mixed ancestry, but the

* *Ibid.*

† *Ibid.*

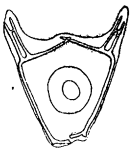
rest showed more or less obvious traces. Thus in a good many of them the lower horizontal part of the body skeleton (BE in fig. 3) was wanting, and less seldom, the part BD. In others, the body skeleton had irregular "prickles" projecting from it at various points. This is not, of course, a characteristic of either parental larval form, but it is one which was frequently found to show itself in these and other hybrid forms. Again, more rarely, the body skeleton was thickened and approached more nearly to that of the *Strongylocentrotus* pluteus. An instance of this is given in fig. 4. Still again, the anal arm skeleton may vary, the three rods composing it

Fig. 4.



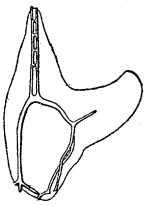
Sphærechinus ♀.
Strongylocentrotus ♂.

Fig. 5.



Sphærechinus ♀.
Strongylocentrotus ♂.

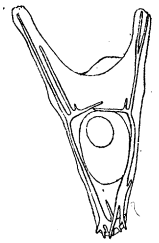
Fig. 6.



Sphærechinus ♀.
Strongylocentrotus ♂.

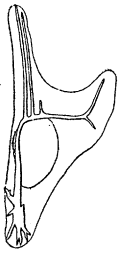
being spread out more widely than usual, and all lying in the same plane, or being considerably shortened. In exceptional cases there may be only two rods instead of three, and these may be joined by only a very few connecting rods. Examples of this type are given in figs. 5 and 6. It should be mentioned that not all these various types were found in one and the same cross-fertilisation, but this may have been due in most instances to only small numbers of the hybrids being obtained for

Fig. 7.



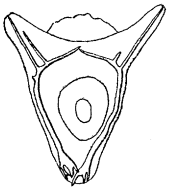
Sphærechinus ♀.
Strongylocentrotus ♂.

Fig. 8.



Sphærechinus ♀.
Strongylocentrotus ♂.

Fig. 9.



Sphærechinus ♀.
Strongylocentrotus ♂.

examination. It could not be determined definitely that one particular form of aberrant *Sphærechinus* type occurred more frequently in one cross-fertilisation, and another in another.

In the other group of hybrid larvæ the forms were even more variable than those already described. In figs. 7, 8, and 9 are represented three more or less typical forms. It will be seen that these larvæ approach much more closely to the *Strongylocentrotus* type than to the *Sphærechinus*. Their chief differences consist in the

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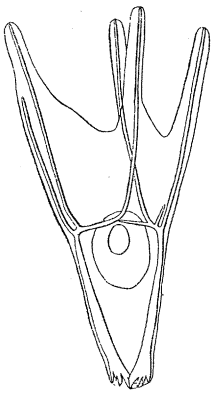
Number of experiment.	Date.	Number of larvæ measured.	Nature of ♀ *Sphærechinus* used.	Per cent. blastulæ formed.	Per cent. larvæ formed.	Mean body length.	Per cent. larvæ of *Sphærechinus* type.	Body length of these hybrids.	Arm length.	Body length of normal *Sphærechinus*.	Per cent. difference of hybrids from these.	Per cent. larvæ of *Strongylocentrotus* type.	Body length of these hybrids.	Arm length.	Body length of normal *Strongylocentrotus*.	Per cent. difference of hybrids from these.	Conditions.
21	24.v.	50	*Violet*-white .	2·0	0·1	19·31	64	17·42	272·4	17·21	+ 1·2	36	22·67	91·0	29·74	−23·8	
32	2.vi.	50	White . . .	36	17	25·49	0	..	..	..	..	100	25·49	85·3	31·25	−18·4	
35	,,	46	*Violet*-white .	9·1	1·0	19·40	87	18·23	317·0	18·14	+ 0·5	13	27·20	75·8	,,	−12·9	
53	1.vii.	33	White . . .	4·5	0·5	19·15	79	18·41	246·7	19·16	− 3·9	21	21·87	75·5	29·67	−26·3	
57	,,	50	*White*-violet .	50	34	18·05	96	17·93	255·4	18·05	− 0·7	4	22·70	67·3	,,	−23·5	
..	,,	0	*Violet*-white .	45	0·08												
..	20.viii.	0	*Violet*-white .	0													
75	11.xi.	50	White . . .	21	7·1	21·97	0	..	..	..	..	100	21·97	72·0	29·53	−25·6	
79	,,	25	*Violet*-white .	6·0	1·0	21·29	20	16·12	155·4	17·71	− 9·0	80	22·58	47·4	,,	−23·6	
..	,,	0	*White*-brown .	0													
82	,,	18	*Brown*-white .	3·0	3·0	18·19	28	14·94	120·3	17·24	−13·3	72	19·44	32·5	,,	−34·2	
..	24.xi.	0	*Violet*-white .	8·0	0												
116	13.xii.	28	Mixed . . .	1·0	0·43	20·08	0	..	..	..	..	100	20·08	38·9	30·72	−34·6	
117	,,	23	,, . . .	7·1	1·1	22·42	0	..	..	..	..	100	22·42	38·6	,,	−27·0	5 times more sperm
..	22.xii.	0	Mixed . . .	0													
..	,,	0	,, . . .	8·6	0	..	..	..	..	..	..	..	..	..	..	..	10 times more sperm
..	,,	0	,, . . .	6·7	0	..	..	..	..	..	..	..	..	..	..	..	Ova kept 9 hours
147	31.xii.	32	Mixed . . .	5·0	2·5	21·36	0	..	..	..	..	100	21·36	37·4	29·41	−27·4	10 times more sperm
148	,,	26	,, . . .	33	3·0	21·81	0	..	..	..	..	100	21·81	31·3	,,	−25·8	Ova kept 9 hours
168	7.i.	19	Mixed . . .	2·0	0·06	22·03	0	..	..	..	..	100	22·03	47·3	30·95	−28·8	10 times more sperm
..	,,	0	,, . . .	0·48	0	..	..	..	..	..	..	..	..	..	..	..	Ova kept 9 hours

anal arm skeleton being as a rule duplicated, whilst the body skeleton is of an irregular and branching form, and generally somewhat thinner. However, this almost always retains some resemblance to the club-shaped form of the pure *Strongylocentrotus* larva, and very rarely curves at its lower end like that of the pure *Sphærechinus*. The skeleton of the pluteus represented in fig. 7 shows, nevertheless, some tendency to do this. Some of the hybrid larvæ approach much more closely to the *Strongylocentrotus* type than those here represented, so that it would be practically impossible to differentiate them from these latter. For instance, some of them have only single anal arm skeletons, though even the duplication of this is no sure diagnostic sign of hybrid origin. Thus a certain proportion of pure *Strongylocentrotus* larvæ have a short double anal arm skeleton which, on rare occasions, becomes further developed. In fig. 10 is represented a pluteus of this kind. This larva was obtained in Exp. 61, in which instance no less than 35 per cent. of the larvæ had slight double anal arm skeletons.

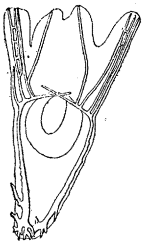
Again, a few of the larvæ, though still obviously of the *Strongylocentrotus* type, approach more nearly to that of *Sphærechinus*, and show connecting rods between

Fig. 10.



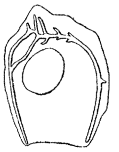
Strongylocentrotus.

Fig. 11.



Sphærechinus ♀.
Strongylocentrotus ♂.

Fig. 12.



Sphærechinus ♀.
Strongylocentrotus ♂.

double or triple anal arm skeletons. An instance of this is given in fig. 11. Absolutely intermediate hybrid forms seem to be practically never formed. One is very seldom at a loss to determine which of the parental types predominate. Occasionally, however, very aberrant forms are met with, such as that represented in fig. 12, which cannot be said to resemble one type more than another, but which, at the same time, cannot be said to be a mean between the two parental types.

We will now proceed to discuss the numerical results given in the above table. In the first experiment it will be seen that the body length of the *Sphærechinus* type of hybrids is 1·2 per cent. greater than that of the pure *Sphærechinus* larvæ obtained at the same time and under the same conditions, whilst that of the *Strongylocentrotus* intermediate type is 23·8 per cent. smaller than that of the pure *Strongylocentrotus* larvæ. As far as mere measurement can show, therefore, both types of larva appear

to have a tendency to approach a size intermediate between that of the parent forms. The arm lengths of the two types of hybrids vary considerably more than the body lengths, those of the *Sphærechinus* type having a value of 272·4, and those of the *Strongylocentrotus*, one of 91·0. The arm-lengths of the pure parental larvæ were respectively 305·6 and 80·3.

In the next two cross-fertilisations, made at the same time but with different colour varieties of *Sphærechinus*, there was a marked difference in the form of the hybrids obtained. Thus, in one case, when the white spined varieties were used, all the larvæ were of the *Strongylocentrotus* type, whilst in the other case, with *violet*-white varieties, no less than 87 per cent. were of the *Sphærechinus* type. There is some reason to suppose that this difference of result is directly connected with the parental varietal differences. Thus in Exp. 53, in which white specimens were used, a smaller proportion of the hybrid larvæ were of the *Sphærechinus* type than in Exp. 57, made with *white*-violet specimens. Again, in Exp. 75 no hybrids at all of the *Sphærechinus* type were obtained with the white specimens, whilst in Exp. 79, made at the same time, but with *violet*-white specimens, 20 per cent. were of this type.

As in the first experiment in the table, the *Sphærechinus* type of hybrid larvæ obtained in Exp. 35 were slightly larger than the pure *Sphærechinus* larvæ, and the *Strongylocentrotus* type considerably smaller than the pure *Strongylocentrotus* larvæ. Also the difference in the arm lengths of the two groups is still more marked. The *Strongylocentrotus* type of hybrids obtained in Exp. 32 were somewhat smaller than those obtained in Exp. 35, but this may be accidental, and be due to the insufficient number of larvæ obtained for measurement in the latter case.

In the next series of cross-fertilisations, almost all the hybrid larvæ were of the *Sphærechinus* type. For some unknown reason, practically none of the large proportion of blastulæ formed in one of the experiments made on July 1st arrived at the eight days' pluteus stage. About ten larvæ per litre survived, but these were thought too few to measure. In the next cross, made in the middle of August, the sea-urchins were in bad condition, owing to the very high temperature of the aquarium tank water. Perhaps this may have been partly the cause of no larvæ being obtained, and only very few of the ova undergoing segmentation. Thus even the normal direct *Sphærechinus* fertilisations failed to give any plutei in two out of the three experiments made. In the next series of experiments, made in November, hybrids of the *Strongylocentrotus*-intermediate type were largely in excess of these of the *Sphærechinus* type. Thus, on an average, only 16 per cent. of the larvæ were of this latter form, and they were, moreover, much smaller than those previously obtained, they being 11·2 per cent. smaller than the pure *Sphærechinus* larvæ. In all the cross-fertilisations subsequently carried out, during December and January, the *Sphærechinus* type of hybrid was entirely absent. We therefore observe the striking fact that *whilst in the summer months most of the hybrid larvæ are of the*

maternal type they in most cases become modified to the paternal type in the autumn, and in the winter the paternal type is the only one represented. As will be shown later on, this remarkable change of type probably depends on the relative degrees of maturity of the ova and sperm from which the hybrids are produced.

With regard to the *Strongylocentrotus* type of hybrids as a whole, we see that they are always considerably smaller than the pure *Stronyglocentrotus* larvæ, the difference in size being generally about 25 per cent. Also the dwarfing is rather more marked in the larvæ obtained in the latter half of the experiments than in the former half. Again the arm lengths of these hybrids dwindle down steadily in size with the progress of the season, those of the hybrids obtained in December being only about a third the value of those obtained in May, whilst at intermediate times intermediate values were found. Part of this diminution of arm length noticed in the winter months was due to the lower temperature of the water during the development of the plutei, but the larger part is a true dwarfing effect.

Crosses between Strongylocentrotus ♀ *and* Sphærechinus ♂.

In addition to the cross-fertilisations just discussed, the reciprocal crosses of *Strongylocentrotus* ♀ and *Sphærechinus* ♂ were attempted at the same time. The results obtained are given in the accompanying table.

Number of experiment.	Date.	Number of larvæ measured.	♂ *Sphærechinus* used.	Per cent. blastulæ.	Per cent. larvæ.	Mean body length.	Mean arm length.	Body length of pure *Strongylocentrotus*.	Per cent. difference.	Arm length of pure *Strongylocentrotus*.	Per cent. difference.
..	22.iv.	0	White.	97	0	..	..	..	..	..	..
..	24.v.	0	*Violet*-white. . .	12	0	..	..	..	..	..	..
..	2.vi.	0	*Violet*-white. . .	10	0	..	..	..	..	..	..
47	1.vii.	50	White.	87	19	28·69	126·0	29·67	— 3·3	99·6	+26·5
48	,,	50	*White*-violet . .	48	48	27·90	95·4	,,	— 6·0	,,	— 4·2
49	,,	50	*Violet*-white. . .	37	37	27·69	95·0	,,	— 6·7	,,	— 4·6
64	20.viii.	50	White.	67	51	30·23	171·7	31·09	— 2·8	118·0	+45·5
65	,,	50	*Violet*-white. . .	40	18	27·05	89·3	,,	—13·0	,,	—24·3
..	,,	0	*Brown*-white . .	0	..	..	..	..	..	..	..
73	11.xi.	10	White.	1·0	0·2	24·83	64·5	29·53	—15·9	151·1	—57·3
..	,,	0	*Violet*-white. . .	1·0	0	..	..	..	..	..	..
..	,,	0	*White*-brown . .	0	..	..	..	..	..	..	..
..	,,	0	*Brown*-white . .	0	..	..	..	..	..	..	..
..	3.xii.	0	Mixed.	2·2	0	..	..	..	..	..	..
..	13.xii.	0	White	0	..	..	..	..	..	..	..
..	22.xii.	0	*Violet*-white. . .	0	..	..	..	..	..	..	..
..	,,	0	,,	(4·0)	0	..	..	..	..	..	..
..	31.xii.	0	*Violet*-white. . .	0	..	..	..	..	..	..	..

Here it will be seen that whilst in the first three experiments made a fair proportion, or in one case nearly the whole, of the ova were cross-fertilised, and developed to the blastula stage, yet no plutei were obtained. In the three cross-fertilisations made in July, not only were a large number of the ova fertilised, but on an average 35 per cent. of them reached the full eight days' pluteus stage. Again, two out of the three experiments made in August gave a large percentage of plutei, but after this date, with the exception of a very small number obtained in November, no hybrid larvæ at all were produced. In most cases, also, not a single ovum was cross-fertilised, unless, as in one of the experiments made on December 22, the artificial method of assisting cross-fertilisation adopted by O. and R. Hertwig was made use of. Judged, therefore, by the present results, *Strongylocentrotus* ova are fairly amenable to cross-fertilisation by *Sphærechinus* sperm in the late spring, readily yield a large proportion of fully developed plutei in the summer months, but, as the winter is reached, pass to a state in which, under normal conditions, the capacity for cross-fertilisation seems to be entirely in abeyance.

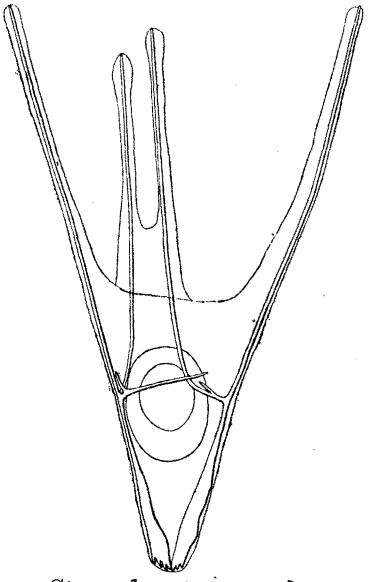
With respect to the colour varieties of *Sphærechinus* used, it would seem that the sperm of the white spined specimens is most favourable for the production of hybrids, both as regards the proportion and the size of the plutei produced, whilst that of the brown spined varieties is least favourable. Thus in the three experiments made with the latter, not a single ovum was cross-fertilised.

With regard to the hybrid larvæ themselves, it was found that in the majority of cases they showed no trace of their mixed ancestry. They were of the pure *Strongylocentrotus* or maternal type. They showed, however, a distinct diminution in size. Thus those obtained in July were on an average 5·3 per cent. smaller than the pure *Strongylocentrotus* larvæ, and those obtained in August, 7·9 per cent. smaller, whilst the few larvæ obtained in November were no less than 15·9 per cent. smaller. Of the variations from the normal type, those occurring in Exp. 64 were somewhat remarkable. In this instance the larvæ were of the pure *Strongylocentrotus* type as far as their structure and body lengths were concerned, but their arm lengths were very greatly increased. An instance of one of the most extreme members of the type is given in fig. 13. Thus the average arm length was found to be 171·7. Now of all the series of measurements made upon *Strongylocentrotus* larvæ in the present research, the maximum arm length is only 154·6 ; whilst of the very numerous series of measurements recorded in the previous paper, the maximum value for larvæ allowed to develop under normal conditions is 152·3, though on one occasion, with larvæ grown in diluted sea-water, a value of 163·5 was obtained. The hybrid larvæ obtained in Exp. 65, which was made at the same time as that giving these long-armed larvæ, have an arm length of only 89·3, whilst the pure *Strongylocentrotus* larvæ have one of 118·0. There can thus be little doubt that the long arms of these hybrids are an exceptional variation, of the nature of a sport. It may be that in this particular instance the male parent has been able to transmit to its hybrid

offspring the characteristic of a great arm length which it itself possesses, but one would expect in this case that the other characteristics, namely of a triple arm skeleton with cross rods, would also be transmitted. There was as a matter of fact a short anal arm skeleton in a few of these hybrids, though this was not so frequent as in the hybrids obtained in Exp. 65.

Of the other abnormal characteristics, perhaps the most obvious was a slight thickening of the calcareous skeleton, which was present in a considerable number of the hybrids. It would be quite impossible, however, to diagnose hybrids from pure *Strongylocentrotus* larvæ by means of this characteristic, as there is a considerable amount of variation in this respect even in these latter. Again, there was a tendency for the lower club-shaped end of the body skeleton to branch and split up, but this

Fig. 13.



Strongylocentrotus ♀. }
Sphærechinus ♂. }

was very much less frequent and less marked than in the reciprocally crossed hybrids. The skeleton itself was also at times rather irregular, short abnormal processes jutting out at various points. Again, a somewhat larger proportion of the hybrids exhibited a short second anal arm skeleton than is the case with the pure *Strongylocentrotus* larvæ, but this characteristic was not marked, or present in the majority of the larvæ, as with the reciprocal hybrids. These results differ from those of Morgan,* who found that the hybrids generally exhibited two or three skeletal anal arm rods. The difference may be due to his experiments having been carried out in the early spring, for we have seen how the hybrid structure may be modified according to the time of year. Again, he does not mention that he made more than one experiment, and hence it may have happened that the larvæ obtained on this occasion were of an aberrant and not normally occurring form.

* *Ibid.*

The Rate of Growth, and its Relation to Temperature.

It has been remarked that the characteristics peculiar to each parent do not seem to fuse at all readily in the hybrid larvæ, and that as a consequence one is hardly ever at a loss to decide whether a larva inclines to the paternal or maternal type. Is there any way of accounting for this want of fusion? The study of the rate of growth of these larvæ, and the relation of growth to temperature, appear to afford some explanation of the phenomenon.

In the former paper, measurements of the rate of growth of *Strongylocentrotus* larvæ between the third and sixteenth days after fertilisation were given. These values are reproduced here, together with two additional values for the relative size of the larvæ after respectively twenty-four and thirty-one days' growth. Measurements made with the plutei of *Sphærechinus* and of *Echinus microtuberculatus* are also included in the same table, so that the rate of growth of these three species may be more conveniently compared. It will be seen that both the body length and the arm length values of the eight days' plutei are taken as 100, and the measurements for other days calculated as percentages on them.

Strongylocentrotus.				*Sphærechinus.*				*Echinus microtuberculatus.*			
Numbers of experiments.	Days of development.	Body length.	Arm length.	Numbers of experiments.	Days of development.	Body length.	Arm length.	Numbers of experiments.	Days of development.	Body length.	Arm length.
	3	90·6	63·8					84	3	84·6	52·6
	4	95·8	..	18	4	102·0	50·4	5	4	95·9	105·3
	5	97·1	88·7								
	6	97·8	94·8					6	6	98·1	109·9
71, 107	8	100·0	100·0	19, 76	8	100·0	100·0	85, 7, 10, 119	8	100·0	100·0
	10	101·1	95·9								
	14	103·0	..	20	14	101·3	120·3	11	14	99·0	56·3
	16	104·7	71·2								
72	24	101·2	(40·2)	77	24	96·7	116·1				
108	31	100·3	55·0	78	30	99·6	81·1	120	31	99·8	24·8

The *Strongylocentrotus* pluteus shows a regular increase of body length from the third day, or period when the pluteus stage is first reached, up to the sixteenth day. From this point up to the thirty-first day it then appears to diminish slightly in size, but the apparent differences are only small, and almost within the limits of experimental error. In any case one may conclude, that whilst the body length increases appreciably between the third and eighth days, yet that from this date onwards the change is only very slight. The arm length, however, undergoes much greater variations, it rapidly increasing from the third day onwards to the eighth day,

when it reaches its maximum value. It then rapidly declines again, till after twenty-four or thirty-one days it is considerably shorter than at the beginning. The value obtained in one experiment after twenty-four days' development, is considerably smaller than that obtained in another after thirty-one days; but this is probably owing to the dwarfing effect of the large number of larvæ developing together in the former instance.

In the pluteus of *Sphærechinus* the rate of growth is quite different. It would appear that the body skeleton attains its full size the moment it is formed, and remains unchanged as long as the larvæ continue in their free swimming state. It was not found possible to measure the larvæ after three days' growth, the skeleton being at this time still in process of formation. The arm length also differs in its rate of growth from that of the *Strongylocentrotus* pluteus, as it does not attain its maximum value till the fourteenth day, and does not begin to undergo rapid absorption till after the twenty-fourth day.

In the right third of the table are given the values showing the rate of growth of the pluteus of *Echinus microtuberculatus*. Cross-fertilisations with this form will be subsequently described; but it may be pointed out here that the rate of growth is much more similar to that of *Strongylocentrotus* than is that of *Sphærechinus*. The body length increases gradually from the third till the eighth day, but from that date appears to remain constant. Again, the arm reaches its maximum length on the sixth and not on the eighth day, and then undergoes a much more rapid absorption than that of the *Strongylocentrotus* pluteus.

The absorption of the arms of these plutei is probably of a semi-pathological nature, and is due to the plutei feeding on their own tissues for want of natural food. Thus, A. Agassiz found* that in *Strongylocentrotus* larvæ collected from the open sea, the arms continued to increase in length until the young *Echinus* was formed. Other observers have also noticed a continued growth of the arms in other larval forms. The absorption occurring in the artificially developed larvæ was, however, a perfectly constant and well-marked phenomenon, which always showed itself at about the same time and to about the same extent.

In addition to differing so markedly in their rate of growth under constant conditions, these plutei differ no less strikingly in their reaction to changes in the temperature of development. Thus, in the former paper, it was shown that with *Strongylocentrotus* plutei the most favourable temperature for the growth of the body tissues was from about 17°·5 to 21°·5; but for that of the arm tissues, a temperature of 23° or more. In order to determine more exactly the relation of growth to temperature, parallel series of experiments were made with the plutei of *Sphærechinus* and *Echinus* as well as with those of *Strongylocentrotus*. The results obtained in the most complete experiment are given in the accompanying table.

* 'Memoirs of the American Academy,' vol. 9, p. 1, 1867.

Temperature of development.	*Strongylocentrotus.*			*Sphærechinus.*			*Echinus.*		
	Number of experiment.	Body length.	Arm length.	Number of experiment.	Body length.	Arm length.	Number of experiment.	Body length.	Arm length.
11°·4	132	100·0	100·0	143	100·0	100·0	149	100·0	100·0
15·9	133	113·5	143·4	144	109·4	287·0	150	113·4	116·3
20·4	134	120·6	156·8	145	104·6	327·2	151	124·5	106·6
23·7	135	122·5	149·1	146	100·6	386·7	152	123·9	113·7

In this experiment the fertilised ova of each species were divided into four portions. One of these was allowed to develop in jars placed in the aquarium tank water, which had an average temperature of 11°·4. Another was kept in a slightly warmed room, and the other portions were kept in jars placed upon the top of a water bath furnished with a gas regulator. By interposing various thicknesses of paper between the jars and the bath, the average temperature was kept at respectively 20°·4 and 23°·7.

In the case of the *Strongylocentrotus* plutei, the body length increases considerably with the temperature up to 20°·4, but is only slightly affected by a further rise. The arm length reacts markedly to the rise of temperature from 11°·4 to 15°·9, but only slightly to the further rise. *Sphærechinus* plutei on the other hand react quite differently to changes of temperature. Thus the body length reaches its maximum at 15°·9, and then falls off considerably with further rise of temperature. The arm length, however, increases steadily in value with the temperature. The effect of raising the temperature from 11°·4 to 15°·9, or an interval of only 4°·5, is most remarkable, as the arms are thereby nearly trebled in length. The *Echinus* plutei appear to react differently from either of these species, as in them the body length reaches a maximum at 20°·4, whilst the arm length is but slightly affected by the rise of temperature. Probably the body length is as a matter of fact affected to about the same extent as that of *Strongylocentrotus* plutei, the differences in the result at 20°·4 and 23°·7 in the two cases falling within the limits of experimental error.

This difference in the reaction of growth to temperature which is so marked in the case of the *Strongylocentrotus* and *Sphærechinus* plutei must obviously be another powerful check to the complete fusion of the parental characteristics in the crosses between the two forms, though it would probably not be so great a difficulty to overcome as that of the difference in rate of growth under constant conditions.

The Relation of the Maturity of the Sexual Products to Season.

As we will now endeavour to prove, the seasonal variation in the type of the hybrids between *Strongylocentrotus* and *Sphærechinus*, and the relative ease with which they may be obtained at various times of the year, depend chiefly, if not entirely, upon the comparative degrees of maturity of the sexual products. Now, as has already been shown in the former paper, the comparative variations in the maturity of the sexual products, in the case of *Strongylocentrotus* at least, can be more or less gauged simply by measurement of the size of the larvæ obtained. Thus it was found that in the spring these larvæ were of a maximum size, but that as the season progressed towards summer, they gradually dwindled, till in the middle of August they reached their minimum, they being then nearly 30 per cent. smaller than those obtained in the spring. From this point onwards they gradually regained their previous condition, and in the middle of September had nearly reached their full size again. It is therefore expedient for us to examine more closely this relation between the size of the larvæ and the season, in the case of *Sphærechinus* as well as of *Strongylocentrotus*.

We have just seen how very largely the larval growth is influenced by the temperature of the water during development. As therefore the normal temperature of the aquarium water varies considerably with the season, it is necessary to introduce corrections, in order to reduce the larval measurements to a constant temperature value. Probably this factor of temperature is the only one which varies much with the season. Thus the salinity of the water, and the amount of organic impurity it contained, were found to remain practically constant throughout the year.*

In order to determine the necessary correction for the temperature factor, other observations were made, in addition to those referred to above. As regards *Strongylocentrotus*, it was found in Exp. 109 that larvæ allowed to develop at 20°·5 were 15·7 per cent. larger than those grown at 12°·6, and in Exp. 125, that those grown at 24°·0 were 22 per cent. larger than those grown at 10°·0. Again, in the former paper it was found that larvæ grown at temperatures varying from 23°·1 to 23°·7 were, on an average of four experiments, 1·8 per cent. smaller than in the corresponding experiments, in which they were kept at from 19°·9 to 20°·7. This result is in contradiction to that given above, when larvæ grown at 23°·7 were found to be 1·6 per cent. larger than those grown at 20°·4. The difference is, however, within the limits of experimental error, and as in any case the correction would be a slight one, it may be ignored, and all temperatures above 17°·5 be considered as the optima for larval development, and therefore needing no correction. It is found, therefore, as a mean of the four experiments cited, that larvæ developing at from 20°·4 to 23°·7 are, on an average, 20·2 per cent. larger than those developing at from 10°·0 to 12°·6. Again, it was found in Exp. 133 that larvæ grown at 15°·9 were 13·5 per cent. larger than those grown at 11°·4, whilst in an experiment cited

* *Vide* 'Mittheilungen aux d. Zool. Stat. zu Neapel,' 1898, vol. 13, p. 347.

in the former paper, it was found that larvæ grown at 19°·2 were 4·6 per cent. larger than those grown at 15°·7. Upon these data the following corrections have been calculated :—

Temperature.	Correction.	Temperature.	Correction.	Temperature.	Correction.
°	per cent.	°	per cent.	°	per cent.
9·5–10·4	+ 24	12·5–13·4	13	15·5–16·4	4
10·5–11·4	20	13·5–14·4	10	16·5–17·4	2
11·5–12·4	16·5	14·5–15·4	7	17·5 and upwards	0

It is obvious that the data obtained for the intermediate temperatures are not sufficient to render the corrections particularly trustworthy, though those for the low temperatures are probably sufficient. If the importance of obtaining exact data had been earlier recognised, more experiments would have been made. As it is, some twenty series of experiments were made altogether in this connection. Thus in addition to those already cited with reference to *Sphærechinus*, it was found in Exps. 113 and 167 that larvæ grown at 12°·6 and 12°·2 differed by respectively + ·6 and — 5·2 per cent. from those grown at 20°·5 and 20°·2. On an average, therefore, *Sphærechinus* larvæ grown at from 20°·2 to 23°·7 are only ·2 per cent. larger than those grown at from 11°·4 to 12°·6. It would therefore seem inadvisable to make any temperature correction at all in the case of these larvæ, as, though in Exp. 144 the larvæ grown at 15°·9 were found to be 9·4 per cent. larger than those grown at 11°·4, it is nevertheless impossible to found corrections on this single result, which may perhaps be partly abnormal.

With reference to *Echinus*, in addition to the experiments cited, it was found in Exp. 170 that larvæ grown at 20°·2 were 10·1 per cent. larger than those grown at 12°·2. In the three experiments made, therefore, larvæ grown at from 20°·4 to 23°·7 were, on an average, 19·5 per cent. larger than those grown at from 11°·4 to 12°·6. This is about the same difference as was observed in the *Strongylocentrotus* larvæ. As the value obtained at 15°·9 is also very nearly the same as that for the corresponding *Strongylocentrotus* experiment, it would seem that the two species of larvæ are affected by temperature to about the same extent. The same corrections have therefore been applied.

It may have been noticed that no reference has been made to the temperature of impregnation in these experiments, though this condition was proved in the former paper to possess a very appreciable influence on the size of the subsequently developing larvæ. Thus in eight experiments in which the temperature of impregnation was, on an average, 8°, the larvæ were found to be 4·2 per cent. smaller than when the temperature was about 20°. It seemed best, however, not to make a second correction for this factor, as it would be only small in comparison with the correction for temperature of development and therefore practically negligible.

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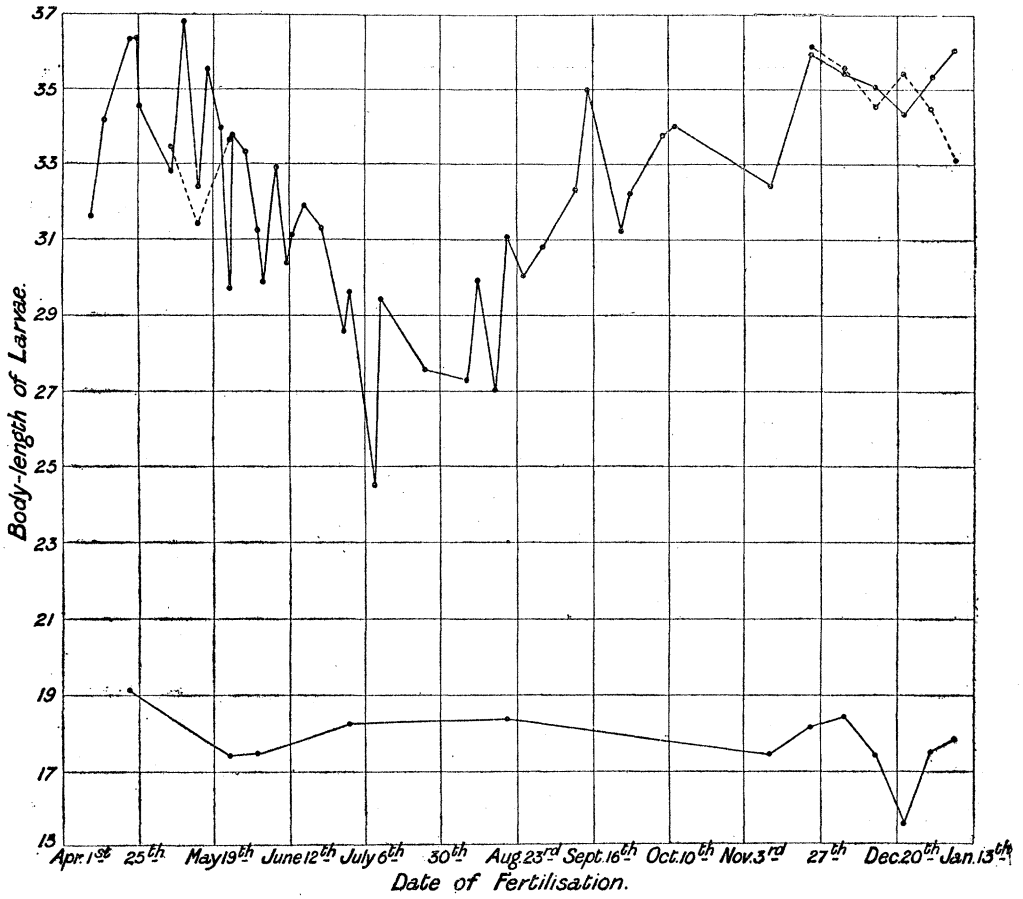
Number of experiment.	Date.	Temperature of development.	Body length of larvæ.	Corrected body length.	Number of experiment.	Date.	Temperature of development.	Body length of larvæ.	Corrected body length.
		°					°		
1	10.iv.	15·6	30·44	31·66	138*	25.vii.	23·7	27·61	..
12*	14 ,,	18·0	34·20	..	147*	7.viii.	23·1	27·34	..
2	22 ,,	17·5	36·30	..	61	11 ,,	23·5	29·97	..
21*	24 ,,	16·0	34·95	36·35	157*	16 ,,	22·5	27·07	..
26*	25 ,,	16·0	33·24	34·57	63	20 ,,	23·2	31·09	..
4	5.v.	17·4	32·18	32·82	164*	25 ,,	23·3	30·09	..
39*	9 ,,	17·2	36·08	36·80	67	31 ,,	22·9	30·80	..
9	14 ,,	17·2	31·77	32·41	68	10.ix.	23·2	32·36	..
48*	17 ,,	19·3	35·53	..	176*	14 ,,	22·2	34·99	..
52*	21 ,,	19·2	33·95	..	188*	25 ,,	20·7	31·24	..
14	24 ,,	19·0	29·74	..	69	28 ,,	20·0	32·25	..
58*	25 ,,	18·2	33·78	..	198*	8.x.	17·8	33·79	..
67*	29 ,,	18·7	33·34	..	70	12 ,,	17·3	33·38	34·05
29	2.vi.	21·0	31·25	..	71	11.xi.	13·9	29·53	32·48
75*	4 ,,	19·5	29·91	..	83	24 ,,	12·3	30·86	35·95
87*	8 ,,	19·1	32·93	..	96, 98, 99	3.xii.	13·5	32·24	35·46
36–44	11 ,,	20·8	30·37	..	107	13 ,,	12·6	30·72	34·71
97	13 ,,	18·7	31·15	..	109	,, ,,	20·5	35·55	..
110*	17 ,,	19·5	31·95	..	124	22 ,,	10·0	27·94	34·64
120*	22 ,,	20·8	33·07	..	125	,, ,,	24·0	34·10	..
45	22 ,,	21·5	29·55	..	132	31 ,,	11·4	29·41	35·30
130*	29 ,,	21·8	28·61	..	133	,, ,,	15·9	33·39	34·73
46	1.vii.	23·2	29·67	..	134	,, ,,	20·4	35·47	..
60	9 ,,	24·1	24·49	..	135	,, ,,	23·7	36·04	..
135*	11 ,,	23·3	29·45	..	159	7.i.	12·2	30·95	36·05

In this table are given the values obtained for the body lengths. The results given in the former paper are also included, the "number of experiment" being in this case marked with an asterisk. Altogether fifty different determinations were made, extending over a period of nine months. It will be noticed that only a small proportion of the measurements have been corrected for temperature, as from the middle of May to the middle of October the temperature of development was above 17°·4, or within the limits of the optimum temperature. In the upper curve, in fig. 14, these measurements are represented graphically. One can thereby see at a glance how the average size of the larvæ gradually falls from April onwards, till by the middle of July it has reached its minimum value. Thus the larvæ obtained on July 9 were no less than 33·4 per cent. smaller than those obtained on May 9. Till the middle of August the larvæ continue to be very small, but from this point onwards they rise more or less steadily, till at the end of November they have again attained their maximum size. They remained at this level till the beginning of January, when the observations ceased.

It may be remarked that this gradual waning and subsequent waxing in the size of the larvæ does not necessarily prove that there is any variation in the maturity of the sexual products, and that the phenomena may be attributable to some entirely different cause. This is of course possible, but it seems highly improbable. Thus

this view of diminished maturity is confirmed by direct evidence. Of the specimens of *Strongylocentrotus* obtained in the winter months, nearly every individual contains ripe sexual products in large quantities, whilst of those obtained in the summer months, not more than about one in four yields any ova or sperm on shaking the ovaries or testes in water. For instance, on August 11, only one individual out of the twenty opened contained any ripe sperm. Again, the best of the specimens obtained in the summer months do not contain a fifth as much of the ripe sexual products as they do in the winter. Still again, from the numbers given in the table

Fig. 14.



at the end of the paper, it may be seen that, on an average, distinctly more of the fertilised ova reached the eight days' pluteus stage in the spring and winter months than in the summer ones.

In the lower curve of the above figure are represented the *Sphærechinus* body length measurements. These are much fewer in number, only twelve series being made altogether. For reasons given above, these values were not corrected for the temperature factor. It will be seen that in the first place variations in maturity, as evidenced by variations in the size of the larvæ, are much smaller than in the case of

Strongylocentrotus. One may, in fact, reasonably doubt whether they exist at all, and whether the existing variations may not be referred to changes in the environmental conditions, and to experimental error. Also the maturity of the echinoids themselves was not noticed to undergo any definite and well-marked variations with the season. Sometimes, it is true, they were in better condition than at others, but this seemed to be more or less a matter of chance. The values representing the percentages of ova reaching the pluteus stage seem to show that the maturity is least in the summer months, but that after the middle of November it reaches its maximum and then remains fairly constant. Probably, however, the low values obtained in the summer are due in part to the high temperature of the water in which the larvæ developed.

Numbers of experiments.	Date.	Per cent. larvæ formed.	Body length.	Numbers of experiments.	Date.	Per cent. larvæ formed.	Body length.
3	22.iv.	32	19·13	90	24.xi.	95	18·17
19	24.v.	2·0	17·43	101	3.xii.	82	18·39
30, 31, 33, 34	2.vi.	44	17·49	112, 114, 115	13.xii.	88	17·42
50, 51, 52, 54, 55, 56, 58, 59	1.vii.	42	18·24	127	22.xii.	47	15·63
				143, 144, 145, 146	31.xii.	81	17·49
66	20.viii.	1·0	18·37	166, 167	7.i.	96	17·82
74, 76, 80, 81	11.xi.	86	17·47				

It now remains for us to endeavour to interpret the results obtained on crossing *Sphærechinus* and *Strongylocentrotus* in the light of these conclusions as to variation in the maturity of the sexual products. As regards the hybrids between *Sphærechinus* ♀ and *Strongylocentrotus* ♂, we have seen that in the summer months they approach in structure towards the paternal type. In the middle of November, however, the majority of them are of the maternal type, and in December they are entirely of this type. Now, we have just seen that in the summer months the maturity of *Strongylocentrotus* reaches its minimum, and that it then gradually returns, so that by the end of November it has again reached its maximum. That is to say, *as the maturity of the* Strongylocentrotus *sperm increases, it is able to transmute first a portion and then the whole of the hybrid larvæ from the* Sphærechinus *to its own type. In other words, the characteristics of the hybrid offspring depend directly on the relative degrees of maturity of the sexual products.*

With reference to the hybrids between *Strongylocentrotus* ♀ and *Sphærechinus* ♂, we have seen that no larvæ were obtained in the spring and early summer, but that from July 1 till August 20 a considerable percentage of the ova developed to eight days' plutei. Subsequently, except in one experiment on November 11, it was not only found impossible to obtain plutei, but, under normal conditions, few, if any, of

the ova were fertilised at all. That is to say, *the hybrid larvæ can be obtained only when the* Strongylocentrotus *ova are at their minimum of maturity.* Ripe ova obtained in the normal breeding season are totally unaffected by *Sphærechinus* sperm.

To return again to the reciprocal cross, it has been pointed out that these *Sphærechinus* ♀–*Strongylocentrotus* ♂ hybrids are much more readily obtainable in the summer months than in the winter ones. One would accordingly expect that the maturity of the *Sphærechinus* ova was also less in the summer months. As we have seen above, this view is favoured by the fact that very much smaller numbers of the summer ova developed to plutei than of the winter ones, but disfavoured by the absence of any diminution in the size of the summer larvæ. The balance of evidence may perhaps be held to point to a slight diminution of maturity during the summer. In any case the variations in the maturity of *Sphærechinus* are very much less than in the case of *Strongylocentrotus*, and similarly the variations in capacity for cross-fertilisation of the ova with season are also much less marked.

These results serve as a confirmation and extension of those obtained by O. and R. Hertwig, who found that the ova of *Sphærechinus* could be easily cross-fertilised by *Strongylocentrotus* sperm, if their vitality and condition were first diminished by keeping them some hours in sea-water. When freshly shed, however, but very few of them were so fertilised. It was accordingly concluded that the most favourable conditions for the production of hybrids are a diminished vitality of the ova, but a maximum of vitality and condition of the sperm. This view is in contradiction to those of Pflüger and of Born, who think that both the sexual products should be in good condition. The former observer, however, attaches special importance to the condition of the ova, and the latter, to that of the sperm. The present experiments would seem to show that the relative maturity of the sperm is of but secondary importance, so far as the amount of cross-fertilisation is concerned: for in one case certainly, and in the other case possibly, the period of maximum cross-fertilisation more or less coincided with that of minimum maturity of the sperm.

Crosses between Echinus ♀ *and* Strongylocentrotus ♂.

It was pointed out by O. and R. Hertwig that the ova of *Echinus microtuberculatus* could be almost invariably fertilised by *Strongylocentrotus* sperm, though the reciprocal cross was much more difficult to bring about. This conclusion was confirmed in the present experiments.

In the accompanying table are given the results obtained with *Echinus* ♀ and *Strongylocentrotus* ♂.

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Number of experiment.	Date.	Hybrids.				*Echinus.*			*Strongylocentrotus.*			Per cent. difference of hybrids from—	
		Per cent. larvæ.	Body length.	Arm length.	Type of hybrid.	Per cent. larvæ.	Body length.	Arm length.	Per cent. larvæ.	Body length.	Arm length.	*Echinus.*	*Strongylocentrotus.*
8	5.v.	77	33·20	123·1	*Echinus*	86	31·48	114·3	100	32·18	154·6	+ 5·5	+ 3·2
12	14.v.	78	35·24	90 8	Intermediate	70	30·83	81·8	39	31·77	97·4	+14·3	+10·9
17	24.v.	100	32·50	116·8	Intermediate: irregular . . .	54	33·64	86·2	35	29·74	80·3	− 3·4	+ 9·3
86	24.xi.	87	31·53	119·1	*Echinus*-intermediate	53	31·06	133·4	85	30·86	105·3	+ 1·5	+ 2·2
103	3.xii.	93	31·87	159·3	*Echinus*	69	32·35	149·0	92	32·10	103·0	− 1·5	− 0·7
104	,,	85	32·44	156·2	*Echinus*	,,	,,	,,	100	33·39	113·3	+ 0·3	− 2·8
105	,,	81	30·98	156·9	*Echinus*	,,	,,	,,	34	31·23	98·5	− 4·2	− 0·8
121	13.xii.	89	36·92	118·9	*Strongylocentrotus*-intermediate .	88	30·58	176·0	80	30·72	118·3	+20·7	+20·2
129	22.xii.	39	31·46	97·9	*Strongylocentrotus*: prickles . .	46	28·58	100·0	41	27·94	66·1	+10·1	+12·6
153	31.xii.	70	33·38	102·9	*Strongylocentrotus*-intermediate .	96	28·38	147·8	40	29·41	74·7	+17·6	+13·5

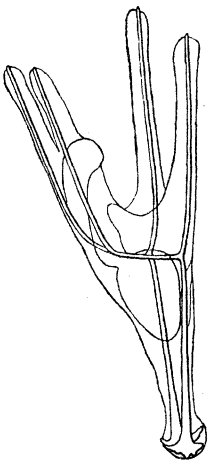
Altogether, ten different series of observations were made. The sizes of the pure *Echinus* and the pure *Strongylocentrotus* larvæ were determined at the same time, and in the last two columns of the table are given the percentage differences of the hybrid larvæ from them. Here it will be seen that *in the majority of cases the hybrid larvæ are considerably larger than either of the parent forms*, this increase of size amounting in one case to over 20 per cent. If the three observations made on December 3 be counted as one—for in this case the same *Echinus* ova were used, but different varieties of sperm—then on an average the hybrids are found to be 8·1 per cent. larger than the pure *Echinus* larvæ, and 8·8 per cent. larger than the pure *Strongylocentrotus*. Again, judging by the percentage numbers of ova arriving at the blastula and pluteus stages, it would seem that the cross-fertilisations take place even more readily than the direct. Thus of the *Echinus* ova cross-fertilised by *Strongylocentrotus* sperm, on an average 89 per cent. arrived at the blastula stage, and 78 per cent. at the pluteus stage, whilst of those directly fertilised, respectively 89 per cent. and 70 per cent. so developed. The corresponding numbers for the pure *Strongylocentrotus* fertilisations are respectively 98 per cent. and 62 per cent.

As regards individual values, it happened in most instances that the pure parental larval forms were of about the same size, and that accordingly the hybrid forms differed from them by more or less similar amounts. In Exp. 17, however, the pure *Strongylocentrotus* larvæ were some 11·6 per cent. smaller than the pure *Echinus*, and in this case the hybrids were 3·4 per cent. smaller than the *Echinus* larvæ, but 9·3 per cent. larger than the *Strongylocentrotus*. The sperm which produced the dwarfed *Strongylocentrotus* larvæ had thus exerted an obvious influence on the size of the hybrids. In Exps. 103, 104 and 105, the sperm from respectively violet, green, and black spined varieties of *Strongylocentrotus* was used to cross-fertilise portions of the same *Echinus* ova. Though the differences in the size of the hybrids are not great, yet they are sufficient to show that the nature of the *Strongylocentrotus* sperm has produced a distinct effect on their size. Thus the largest hybrids were obtained with the same sperm that gave the largest pure *Strongylocentrotus* larvæ, and the smallest with the one giving the smallest.

With respect to the morphological structure of the hybrids, it is difficult to draw very definite conclusions, as the parental larvæ show such similarity of type. Thus, if figs. 15 and 16, representing pure *Echinus* larvæ, be compared with the previous figures of *Strongylocentrotus* larvæ, it will be seen that the only appreciable difference in the form of the skeleton consists in the "base" of the body skeleton being rather spread out or fan shaped, instead of club shaped. Even this distinction is not a constant one, though it was present in the great majority of larvæ obtained at Naples. In spite of these difficulties of diagnosis, however, it was quite obvious that the larvæ obtained at different times were not always of the same type. Thus in the above table the average type of the various hybrids has been roughly designated. Those obtained in Exp. 8 were mostly of the pure *Echinus* type, but contained a few larvæ

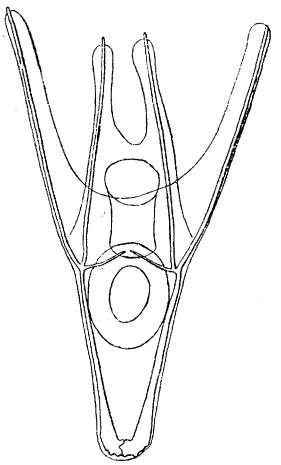
of an intermediate and of a *Strongylocentrotus* type. In Exp. 12, on the other hand, the larvæ were of an intermediate but somewhat irregular type. There were no larvæ of the pure *Echinus* type, but a few of the pure *Strongylocentrotus*. In Exp. 17 the larvæ were also intermediate in character, but more irregular than before. Thus irregular processes jutted out here and there from the body skeleton, but especially near the base, in somewhat the same way as that represented in figs. 7, 8, 11 and 17. There were also a few larvæ of the pure *Strongylocentrotus* and *Echinus* types. In Exp. 86 the larvæ were mostly of the pure *Echinus* type, but there were a few of the intermediate type. In Exps. 103–105 the larvæ appeared to be practically all of the pure *Echinus* type. In Exp. 121, on the other hand, they approached the *Strongylocentrotus* type, but showed some signs of their

Fig. 15.



Echinus microtuberculatus.

Fig. 16.



Echinus microtuberculatus.

mixed ancestry. They are accordingly designated "*Strongylocentrotus*-intermediate." In Exp. 129 the larvæ, as far as the base of the body skeleton was concerned, were of the pure *Strongylocentrotus* type, but in almost every instance there were numerous small "prickles" projecting from the whole of the body skeleton somewhat after the manner represented in fig. 27. Finally, in Exp. 153, the larvæ were again of the *Strongylocentrotus*-intermediate type.

Is it possible to account for these variations of type, and do they depend on the relative maturity of the sexual products, as was the case with the *Sphærechinus-Strongylocentrotus* hybrids? In the first place, there would seem to be a distinct correlation between the size of the hybrids and their structure. Thus in those experiments in which hybrids of the *Echinus* type were obtained, the size varied from the mean size of the parent forms by respectively + 4·4, + 1·9, — 1·1, — 1·3, and — 2·5 per cent. When the hybrids were of the intermediate or *Strongylocentrotus* type, however, they differed by respectively + 12·6, + 3·0, + 20·5, + 11·4, and + 15·6 per cent. from the mean, *i.e.*, were on an average some 12·6 per cent. larger, instead of ·4 per cent smaller.

With regard to the relative maturity of *Echinus* at different seasons of the year, not much was elucidated. Thus the dotted line curve in fig. 14 indicates the *Echinus* body-length measurements corrected for temperature. In the three observations made during May, the larvæ had about the same body-length as the *Strongylocentrotus* larvæ. Unfortunately, no further observations were made until July 9, when the sexual products were found to be absolutely immature. This was also the case on August 31. When the next attempt at fertilisation was made, on November 24, larvæ of a maximal size were obtained, and again, therefore, the critical intermediate period, when one might hope to have obtained dwarfed larvæ, was missed. From November 24 onwards till January 7 five more series of fertilisations were made. The larvæ appeared to gradually diminish somewhat in size, but this was probably only accidental. In every case, however, the larvæ were distinctly larger than those obtained in the spring; hence, it seems probable that the sexual products were in a more mature condition than then. In any case, from the few data available, it is not possible to trace any general relation between maturity and the structure of the hybrids.

In addition to the body-length measurements of the hybrid larvæ, there remain those of the arm-length, and by comparing these one is able to derive some additional information concerning the relations of form. In the previous paper it was shown that the arm-lengths of *Strongylocentrotus* larvæ were considerably affected by the number of larvæ developing together in a given volume of water. This was presumably owing to the adverse effect of the increased products of metabolism, and possibly also to the want of food material. It was shown also that this variable environmental factor could be more or less eliminated by introducing a correction, the amount of which depended on the "concentration" of the developing larvæ. This same correction has accordingly been introduced in the present series of arm-length measurements, and the corrected values, as well as the uncorrected, are given in the table at the end of the paper. The data obtained with reference to the other larval forms were insufficient to prove with any certainty to what extent they were affected, but as far as one could judge, it was somewhat similar to that occurring in *Strongylocentrotus*. Accordingly, a similar correction was applied, both in the case of the pure and the hybrid larval forms.

As regards the hybrids at present under discussion, it would appear that as a rule the arm-lengths are more or less a mean between those of the pure parental forms. Thus from the above table one may see that of the ten series of observations made, the hybrid arm-lengths fall between those of the parent forms in six cases, they being practically midway between them in four of the six. In the other four observations the hybrid arm-length is greater, in most cases considerably greater, than that of either parent form. If the three experiments made on December 3 be counted as one, for, as above mentioned, the same *Echinus* ova were used, then one may say that in six out of the eight observations made the hybrid arm-length fell between those of the parent forms.

Crosses between Strongylocentrotus ♀ *and* Echinus ♂.

The reciprocal cross of *Strongylocentrotus* ova with *Echinus* was always attempted at the same time as the above-described crosses. The results obtained are given in the accompanying table.

Number of experiment.	Number of larvæ measured.	Date.	Per cent. blastulæ formed.	Per cent. larvæ formed.	Body-length.	Arm-length.	Per cent. difference from *Strongylocentrtus.*		Type of hybrids.	Conditions of fertilisation.
							Body-length.	Arm-length.		
..	..	5.v.	0	..	..	..	..	..		
13	50	14.v.	2·0	1·6	26·15	123·1	−17·7	+26·4	Intermediate	
..	..	24.v.	0	..	..	..	..	..		
97	50	3.xii.	16	12	25·79	101·2	−19·6	−1·7	*Strongylocentrotus*-intermediate	
..	..	„	14	0	..	..	..	..		
100	21	„	68	2·2	25·24	88·0	−19·2	−10·7	*Strongylocentrotus*-intermediate	
..	..	13.xii	0	..	..	..	..	..		
..	..	„	0·6	0	..	..	..	..	.. ..	Ova kept 18 hours
..	..	22.xii.	0	..	..	..	.	..		
..	..	„	1·0	0	..	..	..	..	.. ..	Ten times more sperm
126	50	„	36	9·1	25·59	69·6	− 8·4	+ 5·3	*Strongylocentrotus:* prickles	Ova kept 9 hours
140	13	31.xii.	0·8	0·5	23·53	95·9	−20·0	+28·4	*Strongylocentrotus*	
141	36	„	1·8	0·8	25·94	64·3	−11·8	−13·9	*Strongylocentrotus:* prickles	Ten times more sperm
142	50	„	55	1·0	25·91	67·9	−11·9	− 9·1	*Strongylocentrotus:* irregular	Ova kept 9 hours
163	8	7.i.	2·1	0·9	27·59	61·8	−10·9	−43·8	*Strongylocentrotus*-intermediate	
164	25	„	2·1	0·5	29·15	65·4	− 5·8	−40·5	*Strongylocentrotus*-intermediate	Ten times more sperm
165	45	„	5·9	3·0	28·85	67·8	− 6·8	−38·4	*Strongylocentrotus*	Ova kept 9 hours

Here, it will be seen that on several occasions, not only were no plutei obtained, but none whatever of the ova developed to the blastula stage, or were, in fact, cross-fertilised at all. In the most favourable experiment 12 per cent. of the ova developed to eight days' plutei, but in most cases only about 1 per cent. developed. As regards the hybrid larvæ themselves, they were found in all cases to be considerably smaller than the pure parent forms, in striking contrast to the reciprocally crossed larvæ. The difference in size from the pure *Strongylocentrotus* larvæ amounted in one instance

to 20 per cent., and on an average was 13·2 per cent. The arm-lengths were also, as a rule, somewhat shortened, they being on an average 9·8 per cent. less than those of the pure *Strongylocentrotus* larvæ, and still shorter as compared with pure *Echinus* larvæ.

From the last column of the table it will be seen that in several instances the methods of Born and of O. and R. Hertwig were made use of to increase the amount of cross-fertilisation. In the experiments on December 22 no ova whatever were fertilised under normal conditions. When about ten times the usual amount of sperm was added, a few of the ova underwent segmentation, but gave no plutei; when in addition the ova were allowed to stand nine hours in a jar of water, and were then fertilised by fresh *Echinus* sperm, a third of them were cross-fertilised, and 9·1 per cent. reached the pluteus stage. In the experiments on December 31 a somewhat similar, though not so striking result, was obtained. A still larger proportion of the ova kept before fertilisation reached the blastula stage, but not many more of them developed to plutei than in the other two experiments. In the experiments on January 7 a rather greater number of plutei were obtained from the normal cross-fertilisation than from that in which more sperm had been added. Hence it would seem that though the increase in the sperm as a rule somewhat increases the amount of cross-fertilisation, yet it has not nearly so potent an influence as that of keeping the ova some hours before adding the fresh sperm. As regards the larvæ themselves, in both the fertilisations made under normal conditions the size was somewhat smaller than in those obtained under the artificial conditions, but this may, perhaps, have been due to an error arising from the smallness of the numbers of larvæ measured. The larvæ obtained from the kept ova were of practically the same size as those obtained with an increased amount of sperm.

In structure the hybrids were, as a rule, distinctly inclined to the maternal or *Strongylocentrotus* type. In Exp. 13 they were of an intermediate type, and practically undistinguishable from the hybrid larvæ obtained in the corresponding experiment with *Echinus* ♀ and *Strongylocentrotus* ♂. In the next two experiments the larvæ were of a distinctly *Strongylocentrotus* type, but still showed some traces of their mixed parentage. Also they were rather irregular in size and form, a few of the larvæ having a short second skeleton in the anal arm, and others of them being of a dwarfed form. In Exps. 126 and 141 the larvæ were of the *Strongylocentrotus* type, but had prickles projecting from the body skeleton. They were, in fact, of the same type as the reciprocally crossed hybrids in Exp. 129. In Exp. 142 the larvæ were again irregular in size and form, double anal arm skeletons being fairly common. In the next two experiments the larvæ were chiefly of the *Strongylocentrotus* type, but some of them approached the intermediate form. In the last experiment they seemed to be of an almost pure *Strongylocentrotus* type. On the whole, therefore, these hybrids appeared to be more inclined to the *Strongylocentrotus* type than were the reciprocally crossed hybrids to the *Echinus* type. As in the case of the *Sphærechinus-Strongylo-*

centrotus hybrids, *Strongylocentrotus* seemed as a rule to be prepotent over the other parent in transmitting its own specific characteristics.

We have seen that hybrids between *Strongylocentrotus* and *Echinus* are obtainable much more readily than those between *Strongylocentrotus* and *Sphærechinus*. This is not to be wondered at, for it was shown that both the rate of growth and its relation to temperature are about the same with the former pair of larvæ, but entirely different with the latter. One may conclude, therefore, that in the former case there is a greater physiological compatibility, as shown both by the reaction of the tissues to environment, and interaction of the sexual products.

Crosses between Sphærechinus *and* Echinus.

Crosses between *Sphærechinus* ♀ and *Echinus* ♂ have a peculiar interest in consequence of the recent discussion between Boveri on the one hand, and Seeliger and Morgan on the other. Thus Boveri stated* that he had succeeded in fertilising non-nucleated fragments of *Sphærechinus* ova with *Echinus* sperm, and had obtained therefrom larvæ of the purely paternal type. Nucleated fragments on the other hand, when so cross-fertilised, always gave plutei of an intermediate type. This experiment, if valid, would therefore show that the transmission of the female specific characters depended entirely on the nucleus, and not at all on the cytoplasm. Seeliger then showed† that the hybrid larvæ do not invariably possess a structure intermediate between those of the parent forms, but that a certain proportion of the larvæ may be of the pure paternal type. Morgan confirmed this conclusion,‡ and found that sometimes 10–20 per cent. of the hybrids were of the paternal type. Boveri then repeated his experiments,§ and still persisted in his statement that all the hybrids from nucleated eggs or egg fragments possessed an intermediate type. He thought that the difference of Seeliger's results from his might be due to their being obtained at Trieste, whilst his own were obtained at Naples. Seeliger then made further observations,‖ confirming and extending his previous results. He also found that Naples larvæ did not differ from those obtained at Trieste.

We see therefore that the chief point in dispute is as to whether or not the hybrid larvæ are invariably of an intermediate type. The observations of Seeliger and Morgan prove that they are not, and hence, though Boveri obtained only intermediate larvæ, it is no proof that the hybrids are always of this character. Thus, as in the case of the *Sphærechinus-Strongylocentrotus* hybrids, it is probable that the type varies with the relative degrees of maturity of the sexual products.

In the present research very few results were obtained as regards the particular

* 'Berichte d. Gesellsch. f. Morph. u. Phys. zu München,' 1889.
† 'Arch. f. Entwicklungsmechanik,' vol. 1, p. 203.
‡ *Ibid.*, vol. 2, p. 268.
§ *Ibid.*, vol. 2, p. 412.
‖ *Ibid.*, vol. 3, p. 477.

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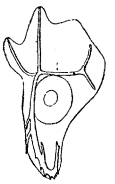
Date of experiment.	*Sphærechinus* ♀ : *Echinus* ♂.						*Echinus* ♀ : *Sphærechinus* ♂.					Pure *Echinus* larvæ.	
	Number of experiment.	Per cent. blastulæ.	Per cent. larvæ.	Body-length.	Arm-length.	Conditions of fertilisation.	Number of experiment.	Per cent. blastulæ.	Per cent. larvæ.	Body-length.	Arm-length.	Body-length.	Arm-length.
24.v.	..	0·23	0	..	..	..	..	0·12	0	..	..	..	..
24.xi.	..	0	..	..	..	..	87	62	59	29·80	122·8	31·06	133·4
3.xii.	..	..	..	..	..	..	106	84	84	30·22	127·2	32·35	149·0
13.xii.	118	0·20	0·05	21·45	37·5	..	..	1·5	0	..	..	30·58	176·0
„	..	0	..	..	..	Ova kept 6 and 18 hours	..	..	..	..	..	..	..
22.xii.	..	0	..	..	..	..	..	0·2	0	..	..	..	..
„	..	0	..	..	..	Ten times more sperm	..	(5·4)	0	..	..	..	..
„	..	8·3	0	..	..	Ova kept 9 hours . .	..	..	..	..	..	..	..
31.xii.	..	0	..	..	..	Ten times more sperm	..	0	..	..	..	..	..
„	..	0	..	..	..	Ova kept 9 hours . .	..	..	..	..	..	..	..
7.i.	171	0·67	0·03	23·17	27·1	Ten times more sperm	..	..	..	..	..	29·24	166·7

cross-fertilisation under discussion. Apparently all the above-cited experiments on the Naples larvæ were made in the early spring, which is the most favourable period of the year for obtaining them. Unfortunately this was just the period which the present experiments did not extend over.

In the table are given the results obtained, both with the cross under discussion, and with the reciprocal cross of *Echinus* ♀ and *Sphærechinus* ♂.

From the cross-fertilisations of *Sphærechinus* ova, eight days' plutei resulted on only two occasions, and then in each case only four specimens suitable for measurement were obtained. On two other occasions a few blastulæ were formed, but these died off before developing to plutei. In one instance, in which the *Sphærechinus* ova had been kept nine hours before the addition of the sperm, 8·3 per cent. of them were cross-fertilised, but in the experiments on December 13 blastulæ and larvæ were obtained from the ova cross-fertilised normally, but not from those which had been kept respectively six and eighteen hours. As regards the hybrid plutei, those obtained in Exp. 118 were all of different, but more or less intermediate, types. One of them, which is represented in fig. 17, might be described as of an intermediate type,

Fig. 17.



Sphærechinus ♀
Echinus ♂

inclining, in respect of body skeleton, to the *Sphærechinus* form, whilst the other three inclined more to the *Strongylocentrotus* form. They were in fact very similar to the hybrids represented in figs. 7, 8, and 9. For further illustrations of the hybrid larvæ reference should be made to the above cited papers of Seeliger and Boveri. The larvæ obtained in Exp. 171 were even more irregular, one of them being somewhat similar to that represented in fig. 12, and the other three of irregular *Strongylocentrotus*-intermediate type. As regards actual size, the hybrids were on an average respectively 29·8 and 20·8 per cent. smaller than the pure *Echinus* larvæ, but respectively 23·2 and 25·3 per cent. larger than the pure *Sphærechinus*. The arm-lengths also were very greatly shortened. These results therefore, as far as they go, are in accord with those of Seeliger's, for they prove the great variability of the hybrid larvæ.

The experiments on the reciprocal cross of *Echinus* ova by *Sphærechinus* sperm gave rather more positive results. Plutei were obtained on only two occasions, but in every experiment but one some of the ova were cross-fertilised and developed to the blastula stage. On the two occasions in question the results obtained were rather

remarkable, as considerably over half the ova developed to eight days' plutei. It does not seem possible that this result could have been due to experimental error, as some of the unfertilised *Echinus* ova, after being kept twenty-four hours, failed to show any segmented ova or blastulæ. The hybrid larvæ were all of the *Echinus* type, the chief abnormality noticed being a tendency of the basal part of the body skeleton to spread out into irregular projections. This was especially the case in the larvæ of Exp. 106. Again, a small proportion of the larvæ was dwarfed in size, especially in respect of their body length. A few also had rather twisted skeletons, and in a few there were projections and abnormalities in other parts of the skeleton besides the base. The average size of the hybrids was slightly less than that of the pure *Echinus* larvæ, the body-length being in the two experiments respectively 4·1 and 6·6 per cent. smaller, and the arm-length respectively 8·7 and 14·6 per cent.

The remarkable variations in capacity for cross-fertilisation seem to bear some relation to the relative degrees of maturity of the sexual products. Thus we have seen that the most favourable time was found to be towards the end of November and beginning of December. This was shortly after the period when the ovaries and testes of *Echinus* had again become ripe. It is true that the pure *Echinus* larvæ obtained at this time were of a maximal size, but nevertheless it would seem that the sexual products were still, as a rule, in a state of only partial maturity. Thus at first only about a third of the Echinoids opened were ripe, whilst a month later nearly all of them were in this condition. Again, the few data available show that the capacity for cross-fertilisation more or less gradually declined from December 3 onwards. Thus on December 13 1·5 per cent. of the ova were cross-fertilised, but on December 22 only ·2 per cent. of them, the larger proportion of 5·4 per cent., which is given in the above table, being due to the addition of ten times more sperm than usual. Finally on December 31 none of the ova were cross-fertilised.

The Effect of Staleness of the Sexual Products on Larval Growth.

We have seen that in several instances the method adopted by O. and R. Hertwig to effect an increased amount of cross-fertilisation proved of value. We have also seen that in the one or two experiments thus far mentioned this plan of keeping the ova in water for some hours before cross-fertilisation did not appear to produce any adverse influence on the size of the larvæ obtained. It seemed of interest, however, to examine this question somewhat more closely, and to determine what changes, if any, are produced by the staleness of the ova on the one hand, and of the sperm on the other. Thus this is obviously a very important factor in nature, for in the case of Echinoderms and other of the lower members of the animal kingdom it is probable that in many instances the ova are not fertilised for some time after they are extruded, and even then by spermatozoa which are frequently not fresh.

The results obtained are collected in the accompanying table;—

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Number of experiment.	Abnormal larvæ.					Normal larvæ.				Per cent. difference from normal larvæ of—	
	Conditions of fertilisation.	Per cent. blastulæ.	Per cent. larvæ.	Body-length.	Arm-length.	Per cent. blastulæ.	Per cent. larvæ.	Body-length.	Arm-length.	Body-length.	Arm-length.
110	*Strongylocentrotus* ♀ kept 18 hours *Strongylocentrotus* ♂ fresh	34	29	25·31	100·5	94	80	30·72	118·3	−17·6	−15·0
111	*Strongylocentrotus* ♀ fresh *Strongylocentrotus* ♂ kept 18 hours	21	12	34·10	79·3	,,	,,	,,	,,	+11·0	−32·8
123	*Echinus* ♀ fresh *Strongylocentrotus* ♂ kept 18 hours	6·7	0·67	34·98	97·6	89	89	36·92	118·9	− 5·3	−17·9
136	*Strongylocentrotus* ♀ fresh *Strongylocentrotus* ♂ kept 9 hours	87	85	32·24	115·0	99	40	29·41	74·7	+ 9·6	+53·9
138	*Strongylocentrotus* ♀ fresh *Strongylocentrotus* ♂ kept 22 hours	93	33	30·43	47·8	,,	,,	,,	,,	+ 3·5	−36·0
137	*Strongylocentrotus* ♀ kept 22 hours *Strongylocentrotus* ♂ fresh	95	81	26·09	52·3	,,	,,	,,	,,	−11·3	−30·0
139	*Strongylocentrotus* ♀ kept 22 hours *Strongylocentrotus* ♂ kept 22 hours	91	36	30·01	67·0	,,	,,	,,	,,	+ 2·0	−10·3
154	*Echinus* ♀ kept 22 hours *Strongylocentrotus* ♂ kept 22 hours	89	66	32·79	97·2	92	70	33·38	102·9	− 1·8	− 5·5
161	*Strongylocentrotus* ♀ fresh *Strongylocentrotus* ♂ kept 9 hours	97	95	28·00	133·1	84	65	30·95	110·0	− 9·5	+21·0
160	*Strongylocentrotus* ♀ kept 9 hours *Strongylocentrotus* ♂ fresh	81	63	30·38	107·1	,,	,,	,,	,,	− 1·8	− 2·6
162	*Strongylocentrotus* ♀ kept 9 hours *Strongylocentrotus* ♂ kept 9 hours	88	90	29·91	108·9	,,	,,	,,	,,	− 3·4	− 1·9

Here it will be seen that all but two of the experiments were made with *Strongylocentrotus* ova. In the two experiments Nos. 110 and 137, in which the ova were kept for respectively eighteen and twenty-two hours before fertilisation, the larvæ were diminished in body-length by respectively 17·6 and 11·3 per cent., and in arm length by 15·0 and 30·0 per cent. In the eighteen hours' experiment, also, only a third of the ova were fertilised at all, but almost all of this third developed to plutei. This result may have been more or less accidental, as in the twenty-two hours experiment considerably more plutei were obtained than in the corresponding normal experiment. A highly adverse effect seems to be produced, therefore, by keeping the ova for all these hours; but with shorter periods, such as nine hours, the effect is slight or absent. Thus there is a decrease of 1·8 per cent. in Exp. 160, and in the previously mentioned Exps. 142 and 165, in which *Strongylocentrotus* ova were fertilised after nine hours by *Echinus* sperm, the variations are respectively —·1 and —1·0 per cent. In the reciprocal experiments with fresh ova and stale sperm the larvæ proved to be if anything larger than those arising from fresh sexual products. Thus, in Exps. 111, 123, and 138, in which the sperm had been shaken in water and kept respectively eighteen, eighteen, and twenty-two hours, the resulting plutei differed by respectively +11·0, —5·3, and +3·5 per cent., or on an average by +3·1 per cent., from the normal. In Exps. 136 and 161, in which the sperm had been kept only nine hours, the larvæ varied by respectively +9·6 and —9·5 per cent. from the normal. Although the larvæ thus seem to be slightly increased in size, there is a considerable reduction in the number of ova fertilised and of plutei formed. Thus, in the first three of the experiments just cited, on an average only 40 per cent. of the ova reached the blastula stage, and 15 per cent. of them the larval stage, instead of the respective 94 per cent. and 70 per cent. of the normally fertilised ova. However, in the other two experiments, with nine hours' stale sperm, there was no diminution either of the blastulæ or the plutei.

The somewhat variable results obtained are easily to be accounted for. Thus it must be remembered that the sexual products were not from the same individual sea urchins as those from which the normal larvæ were obtained. The stale ova and stale sperm were, of course, the same, but the fresh sperm and fresh ova with which they were respectively mixed, were from freshly opened, and therefore different, individuals.

As staleness of the ova gives rise to larvæ of diminished size, and of sperm to ones of, if anything, increased size, one might be inclined to expect that when both the products were stale, the larvæ would within certain limits remain practically unaltered. This was in fact the case. Thus in Exps. 139, 154, and 162, in which both ova and sperm had been kept respectively twenty-two, twenty-two, and nine hours before fertilisation, the larvæ varied by respectively + 2·0, — 1·8, and — 3·4 per cent. from those developing from the same sexual products when fresh. The arm lengths were diminished by respectively 10·3, 5·5, and 1·9 per cent., or somewhat more

than the body-lengths. What at first sight seemed rather curious was the fact that there was practically no diminution in the proportion of ova reaching the blastula and pluteus stages. This result is, however, more or less explicable in the light of the experiments of O. and R. Hertwig, if one may suppose that the conclusions they drew for cross-fertilisations are more or less true also for direct fertilisations. Thus they concluded that cross-fertilisation took place most readily when ova of diminished vitality were mingled with sperm in the best condition possible. Conversely there would be the least tendency to cross-fertilisation when the ova were in good condition, and the sperm in bad. This was the case in the previously cited series of experiments, in which not half of the ova were fertilised at all, and only 15 per cent. of them, instead of 70 per cent., developed to plutei. When both the ova and sperm are stale, however, we have a condition in which the ova are more ready than usual to undergo fertilisation, and the spermatozoa less ready than usual to effect it. As the sum total, therefore, the fertilisation takes place about as readily as with fresh sexual products. With stale ova and fresh sperm the above experiments ought of course to have shown an increased degree of fertilisation. This was actually the case in three of the five experiments, whilst in one of the remaining two the proportions were about the same.

We have seen that in all the above-described experiments, in which the sexual products were kept in water for at most twenty-two hours, fertilisation and development took place fairly readily. Probably, however, after keeping a few more hours the vitality of the ova and sperm becomes so diminished that normal fertilisation can no longer take place. Thus in some observations made both with *Strongylocentrotus* and with *Sphærechinus*, it was found that after keeping either the ova, or the sperm, or both, for two days, there was no sign whatever of normal development.

Crosses with Arbacia.

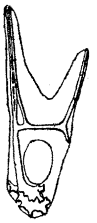
Next to the three species already dwelt upon, *Arbacia pustulosa* seems to have been most frequently employed in cross-fertilisation experiments. The results obtained with this species in the present research will, therefore, be next described, though they are but few in number. Of previous observers, Stassano* found that ova of *Arbacia* could be cross-fertilised by sperm of *Echinocardium*, of *Sphærechinus*, and of *Echinus*, whilst *Arbacia* sperm would fertilise *Echinocardium* ova. The ova were, however, examined for only the first few hours after fertilisation. Also no details are given as to any precautions being taken against accidental direct fertilisation of the ova. O. and R. Hertwig found that *Arbacia* ova with *Strongylocentrotus* sperm gave a small number of morulæ, but no result at all was obtained in several attempts at the reciprocal cross. Similarly also the combination *Arbacia* ♀-*Sphære-*

* 'Zool. Azueiger,' 1883, p. 373.

chinus ♂ was found to give a few segmenting ova, but there was no result at all with the reciprocal cross.

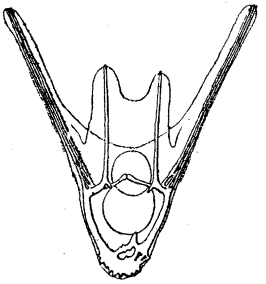
In figs. 18 and 19 are given two examples of normal *Arbacia pustulosa* plutei after eight days' development. It will be seen that these larvæ are quite different in structure from those previously figured; but they bear more resemblance to *Sphærechinus* plutei than to those of *Strongylocentrotus*, in that their arm-length is very much greater than their body-length. The body skeleton in actual measurement is about 15 per cent. shorter than that of the *Sphærechinus* pluteus. The anal arm skeleton also consists of three rods, joined by a small number of cross-bars at the lower end, but apparently none at the upper. The rods are also thicker and closer together, and indeed the whole body skeleton is more massive than in other larvæ.

Fig. 18.



Arbacia.

Fig. 19.



Arbacia.

As the following figures show, the rate of growth of *Arbacia* larvæ seems to be more similar to that of *Strongylocentrotus* than of *Sphærechinus* larvæ.

Number of experiment.	Day of development.	Body-length.	Arm-length.
23	4	94·0	77·4
24	8	100·0	100·0
25	10	97·4	91·7
26	14	98·8	64·0

Thus the body-length increases between the fourth and eighth day, and then remains more or less constant, whilst the arm-length reaches its maximum on the eighth day, and then rapidly shortens.

Most of the experiments on *Arbacia* larvæ were made on a single occasion, viz., May 24. Crosses were then attempted between this and the three species of sea-urchins already discussed. Only in the instance of *Strongylocentrotus* were both cross-fertilisations successful. With *Arbacia* ova, and *Strongylocentrotus* sperm, 1·7 per cent. of the ova reached the blastula stage, but only ·006 per cent. of them developed to eight days' plutei. Thus only four larvæ suitable for measurement were

obtained. These proved to be of the pure *Arbacia* type, their body-length being on an average 5·5 per cent. shorter than the normal. In the reciprocal cross, 3·2 per cent. of the ova were cross-fertilised and reached the blastula stage, but only ·1 per cent. the eight days' pluteus stage. The plutei themselves were of the *Strongylocentrotus* type, though they showed traces of their mixed origin. Thus in most of them there was a short second anal arm skeleton, and occasionally a third one. The skeleton was sometimes rather irregular, and showed a few abnormal projections at various points. The larvæ were also 3·9 per cent. smaller in body-length than the pure *Strongylocentrotus* larvæ, but 43·7 per cent. greater in arm-length. In another attempt at a cross between *Arbacia* and *Strongylocentrotus*, made on November 24, 9·2 per cent. of the *Arbacia* ova were cross-fertilised, but no plutei were obtained. After three days, ·17 per cent. of the ova were still surviving, but they had then arrived only at the gastrula stage. As, however, on this same occasion none of the directly-fertilised *Arbacia* ova survived more than four days, this result was in comparison more favourable than the previous one. Cross-fertilisations were again attempted on December 22, but were entirely unsuccessful, not a single ovum of either species being cross-fertilised. It was possible that a few of the *Arbacia* ova may have been fertilised and escaped notice, as, by reason of their opaqueness, it is very difficult to determine if they are undergoing segmentation. In any case none of them arrived at the free-swimming blastula stage, though most of the ova directly fertilised by *Arbacia* sperm did so. There seems to be some difficulty in obtaining even the normal *Arbacia* plutei in the winter months, as all the gastrulæ obtained on this occasion also died off on the third or fourth day, without developing to the pluteus stage.

In the case of *Echinus*, a cross was effected between *Arbacia* ova and *Echinus* sperm, 3·3 per cent. of the ova used on May 24 becoming blastulæ, and ·05 per cent. of them eight days' plutei. These larvæ also proved to be of the pure *Arbacia* type, the only difference noticed being a slight increase in the irregularity of the calcareous skeleton at the base of the body. They were also of practically the same size, the body length being ·4 per cent. less than that of the normal larvæ. The reciprocal cross of *Echinus* ova with *Arbacia* sperm was on this occasion quite unsuccessful, not a single ovum being fertilised. However, in another attempt, made on November 24, the condition of things was reversed. None of the *Arbacia* ova were cross-fertilised by *Echinus* sperm, but in the reciprocal cross no less than 45 per cent. of the ova were fertilised, and all of these developed to eight days' plutei. These plutei appeared to be of the pure *Echinus* type, but they were 4·0 per cent. smaller than the normal in body length, and 9 per cent. in arm length. In the experiments made on December 22, none of the ova of either species were cross-fertilised.

In the case of *Sphærechinus*, a very small proportion, viz., ·24 per cent. of the ova used were, on May 24th, successfully cross-fertilised by *Arbacia* sperm, and a third of these developed to eight days' plutei. These plutei were of the *Sphærechinus*

type, but about a third of them bore obvious traces of their hybrid origin. Thus the anal arm skeletons were very similar to those of *Arbacia* plutei. The three rods were somewhat closer together, and were connected by only one or two cross-bars at the body end, instead of numerous ones throughout the whole length. In actual size they were 3·0 per cent. smaller than the normal in respect of body length, and 11·8 shorter in arm length. The reciprocal cross of *Arbacia* ova with *Sphærechinus* sperm, which was made at the same time, did not yield any plutei, but 4·8 per cent. of the ova developed to the blastula stage. In the experiments made on November 24, ·09 per. cent. of the *Sphærechinus* ova survived as free-swimming gastrulæ till the third day, but no plutei were obtained. Also 6·8 per cent. of the *Arbacia* ova appeared to have been cross-fertilised, and ·26 per cent. of them survived till the third day. No plutei were obtained, however. In the experiments made on December 22, no cross-fertilisation on either side was observed.

On the whole, therefore, we have seen that the cross-fertilisations made in the spring were fairly successful. Those made in November were more or less a failure, and those made in December absolutely so. No data were obtained during the present research as to the relation of the maturity of the sexual products to season, but the best time for artificial fertilisations is stated to be from November to January.* This was not found to be the case in the present experiments, but it is possible that the low temperature or some other factor was the cause of the developing ova so soon dying off. The absence of all tendency to cross-fertilisation of the *Arbacia* ova which was observed in the December experiments is in support of the view that they had then reached their maximum maturity, whilst the absence of any reciprocal crossing may have been due to the fact, already demonstrated, that the sexual products of the other species are then fully mature.

Crosses with Echinocardium.

In the Gulf of Naples two forms of *Echinocardium* are commonly met with, viz., *E. cordatum* and *E. mediterraneum*. These two forms are considered by A. Agassiz† to be distinct species, but other observers seem inclined to regard them merely as varieties. The present experiments, so far as they go, appear to favour the view of specific difference.

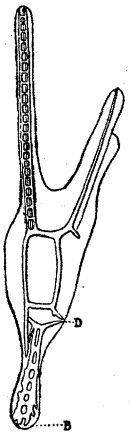
Even the pure *Echinocardium* larvæ were as a rule obtained in but small numbers, as the ovaries are very small, and only a slight proportion of the ova obtained from them develop to the pluteus stage. In figs. 20 and 21, two examples of the eight days' plutei are given. It will be seen that the skeleton is much more complicated in structure than in the forms previously described. The most important

* Lo Bianco, 'Mittheilungen aus d. Zool. Stat. zu Neapel,' vol. 8, p. 385.

† 'Memoirs of the Museum of Comp. Zool. at Harvard Coll.,' vol. 5, p. 580, 1877.

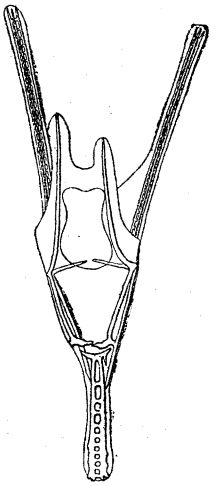
difference consists in the basal part of the body skeleton, BD, which seems to be an addition unrepresented in the other larval forms. Thus, it is not directly connected with the rest of the body skeleton, and is, moreover, of later growth. In fig. 22 a five days' pluteus is represented, and here it will be seen that this basal part is still very small and does not yet project beyond the other parts of the body skeleton. The anal arm skeletons of these plutei consist of three rods, joined by numerous cross-bars, as in *Sphærechinus* larvæ. The oral arm skeleton is thick and well-developed in the eight days' plutei, but is short or almost wanting after only five days' development.

Fig. 20.



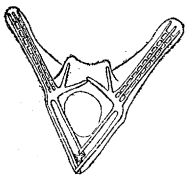
Echinocardium.

Fig. 21.



Echinocardium.

Fig. 22.



Echinocardium (5 days).

The attempts at cross-fertilisation were fairly successful. Thus on November 24 the following results were obtained with *Echinocardium cordatum* ova :—

Number of experiment.	Ova fertilised with sperm of—	Number of larvæ measured.	Per cent. blastulæ.	Per cent. larvæ.	Body length.	Length of "handle."	Difference.	Arm length.
91	*Echinocardium* . .	10	78	7·0	31·16	17·42	13·74	139·2
92	*Strongylocentrotus*. .	20	67	2·5	29·86	15·70	14·16	128·5
93	*Echinus*	9	6	3·5	27·33	14·15	13·18	130·5
94	*Sphærechinus* . . .	6	50	4·0	23·62	8·42	15·80	142·2
95	*Arbacia*	22	74	3·0	26·80	12·27	14·53	132·8

In every case the cross-fertilisation was successful, the proportion of plutei obtained being, as a rule, about half as great as that in the direct fertilisation experiment. The larvæ in each case appeared to be of the pure maternal type, but their size was distinctly influenced by the nature of the sperm. Thus the body length of the *Strongylocentrotus* hybrids was 4·2 per cent. less, and that of the *Echinus* 12·3 per cent. less. Those of the *Sphærechinus* and *Arbacia* hybrids, on

the other hand, were respectively 24·2 and 14·0 per cent. less. There seemed, therefore, to be a direct relation between the body-lengths of the hybrids and those of the pure paternal larvæ, for, as we have already seen, the body-lengths of the pure larvæ of the first two species are about twice as great as those of the second two. This distinction is rendered still more obvious if the lengths of the calcareous "handle" of the larvæ be considered. Thus in the *Strongylocentrotus* and *Echinus* hybrids it is respectively only 9·9 and 18·8 per cent. shorter than in the pure *Echinocardium* larvæ, but in the *Sphærechinus* and *Arbacia* hybrids respectively 51·7 and 29·6 per cent. shorter. In the last column but one of the above table are given the differences between the total body-lengths and the "handle" lengths. It will be seen that a practically constant number is obtained. It follows, therefore, that the shortening of the total body-length measurements was due entirely to a shortening of the "handle" portion, the rest of the skeleton remaining unaffected. The somewhat large difference value obtained with *Sphærechinus* was probably only accidental, for in this case only six larvæ were obtained for measurement, and in two of these the "handle" had not begun to develop at all. On consideration of the fact that the "handle" portion of the skeleton is formed almost entirely between the fifth and eighth days of larval growth, it will, however, be seen that the view that the sperm was able to partly impress its own larval type upon that of the hybrid has but little justification. It can only be concluded that with *Sphærechinus* and *Arbacia* hybrids there is a somewhat greater delay in the development than with those of *Strongylocentrotus* and *Echinus*. Supposing, on the other hand, the hybrids had been allowed to develop 12 or 16 days, and were still found to present the same considerable shortening of the "handle" portion, then the former view would be more justified. Unfortunately the numbers of larvæ obtained were so small that it was not possible to carry out this further experiment.

The next series of experiments was not nearly so successful. Thus a few pure *Echinocardium* larvæ were obtained, but these only lived five days. The measurements made are given under Exp. 130. No hybrid plutei were obtained, but of the ova cross-fertilised with *Strongylocentrotus* sperm 30 per cent. reached the blastula stage; of those with *Echinus*, 8·3 per cent., and of those with *Sphærechinus*, 25 per cent. In the third series, made on January 7, 1·5 per cent. of the ova in the direct fertilisation experiment survived to the eight days' pluteus stage, and 1·4 per cent. of those cross-fertilised by *Echinus*. One or two eight days' plutei were also obtained from the cross-fertilisation by *Strongylocentrotus* sperm. As before, the hybrid larvæ were of the maternal type, but the body-length of the *Echinus* hybrids was found to be 6·2 per cent. greater than that of the pure form. As, however, the numbers of larvæ obtained for measurement were so small, this result may have been accidental.

On December 31, cross-fertilisations were made with *Echinocardium mediterraneum*. The sea-urchins used on this occasion contained large numbers of ripe

ova, and of these, when directly fertilised, 54 per cent reached the blastula stage. A day or two later, however, almost all the gastrulæ died off, and only 0·4 per cent. survived till the fifth day. These were preserved and measured, and proved to be 11·2 per cent. smaller in size than the five days' plutei obtained in Exp. 130. When cross-fertilised by *Strongylocentrotus*, 16·7 per cent. of the ova reached the blastula stage, but only ·06 per cent. were free-swimming on the fourth day. On the fifth day there were no free-swimming larvæ, so those settled at the bottom of

Fig. 23.

E. mediterraneum ♀.
Strongylocentrotus ♂.

Fig. 24.

E. mediterraneum ♀.
Strongylocentrotus ♂.

Fig. 25.

E. mediterraneum ♀.
Echinus ♂.

Fig. 26.

E. mediterraneum ♀.
Echinus ♂.

the jar of water were preserved and examined. Only two out of the two hundred or more obtained had reached the pluteus stage, but these larvæ were not of the maternal type, but of an aberrant intermediate form. They are represented in figs. 23 and 24. The cross-fertilisation with *Echinus* sperm gave a somewhat similar result. Thus no less than 46·4 per cent. of the ova reached the blastula stage, but these rapidly died off a day or two later, and in the deposit of larvæ and gastrulæ which was preserved on the fifth day, again only two definite plutei were obtained. These are given in figs. 25 and 26, and it will be seen that they are also of an aberrant intermediate type. There were in each case a number of larvæ in course of formation, but only small portions of the skeleton had been formed, and hence they were not measureable, nor their special characteristics recognisable.

With *Sphærechinus* sperm some cross-fertilisation also took place, 8·2 per cent. of the ova reaching the blastula stage. However, the gastrulæ died off even sooner than in the two previous instances, and no plutei whatever were obtained.

The evidence, so far as it goes, would therefore seem distinctly to favour the view that *E. cordatum* and *E. mediterraneum* are two distinct species. Thus in the one case only a small proportion of ova were obtained from the ovaries, and of these only a small number developed to plutei. The hybrid plutei also were of a purely maternal type. In the other case a large number of ripe ova were obtained, more than half of which reached the blastula stage. The very few hybrid larvæ obtained were again of an aberrant intermediate, and not of the maternal type.

As regards the reciprocal crosses of *Echinocardium*, the results were almost entirely negative. In only a single instance were plutei obtained, viz., in Exp. 89, made on November 24. In this instance, 52 per cent. of the *Echinus* ova used reached the blastula stage, and 35 per cent. the pluteus stage. The plutei themselves appeared to be of the purely maternal type, but they were 1·1 per cent.

smaller in body-length, and 7·0 per cent. in arm-length, than the normal *Echinus* plutei. This was the only occasion on which any *Echinus* ova at all were cross-fertilised by *Echinocardium*. This was probably due to their being only semi-mature at this time, but fully ripe by December 22, when the next attempt at cross-fertilisation was made. Attempts at cross-fertilisation of *Strongylocentrotus* and of *Sphærechinus* ova were made on both these occasions, and also on December 31, but in no case was a single ovum fertilised, even when the vitality had been diminished by keeping the ova nine hours in water. *Arbacia* ova also seemed entirely unaffected. On January 7, however, in 300 *Strongylocentrotus* ova, a single blastula was found, and in 500 *Sphærechinus* ova a single segmented ovum, which had apparently ceased further development. As most careful precautions had been taken to prevent the entrance of all foreign sperm, it is probable that in these instances cross-fertilisation with *Echinocardium* had taken place. Thus STASSANO* succeeded in cross-fertilising *Echinus*, *Sphærechinus* and *Arbacia* ova with *Echinocardium* sperm, but his observations, made at Naples on March 17, were continued for only the first few hours of development.

Crosses with Dorocidaris *and with* Echinus acutus.

Upon these species but very few observations were made. Thus at Naples *Dorocidaris papillata* seems to be absolutely immature through the greater part of the year. On July 9, however, some apparently mature individuals were obtained. Of the directly fertilised *Dorocidaris* ova, 17·0 per cent. reached the blastula stage, but they all died off a day or two later. When cross-fertilised with *Strongylocentrotus* sperm, only 1·5 per cent. of the ova became blastulæ, and none of them reached the free-swimming stage. On August 11 the maturity of the sexual products appeared to be at a maximum, as 85 per cent. of the ova, when directly fertilised, developed to blastulæ. They all died off a day or two later, however, but this may partly have been due to the high temperature (23°·5) of the water in which they were developing. Of the *Dorocidaris* ova cross-fertilised by *Strongylocentrotus* sperm, 25 per cent. developed to blastulæ, but these died off a day or two later. In the reciprocal cross of *Strongylocentrotus* ova with *Dorocidaris* sperm, 19·0 per cent. of the ova developed to the blastula stage, and 2·9 per cent. to eight days' plutei. These plutei were of the *Strongylocentrotus* type, though they perhaps showed slightly more tendency to the formation of irregular processes jutting out from the calcareous skeleton, and also to a short second anal arm skeleton. In size they were nearly identical with the pure *Strongylocentrotus* larvæ, their body-length being ·3 per cent. greater, and their arm-length 13·1 per cent. greater. On August 31 these observations were repeated, but the maturity of the sexual products seemed already waning. Thus only 43 per cent. of the ova were directly fertilised. On the

* *Ibid.*

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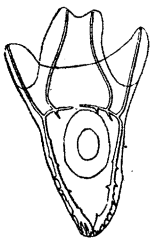
fifth day 1·4 per cent. of these were still surviving as free-swimming larvæ. They had all died off by the eighth day, but on preserving and examining under the microscope the deposit in the jar, a few macerated plutei were noticed. They were of a somewhat similar type to *Sphærechinus* larvæ, but they were too broken up for it to be possible to measure them. The actual structure of these plutei has been described by Prouho,* who succeeded in keeping a few of the larvæ obtained from artificial fertilisations for as long as three months. His observations were made at Banyuls, during the spring.

On attempting the cross-fertilisations, on this same occasion, not a single *Strongylocentrotus* ovum was fertilised, but in the reciprocal cross 20 per cent. of the *Dorocidaris* ova reached the blastula stage. They died off a day or two later however, and no plutei were obtained. When the next attempt at cross-fertilisation was made, some two months later, the sexual products were found to be quite immature.

As regards *Echinus acutus*, the results obtained are also very meagre. This

Fig. 27.



E. microtuberculatus ♀.
E. acutus ♂.

splendid species o sea-urchin is found but seldom at Naples, and the only female specimen I was able to obtain proved to be immature. This was on November 24, probably somewhat before the usual breeding season. Mature male specimens were, however, obtained on two occasions. On December 24 cross-fertilisations were attempted with the ova of *Echinus microtuberculatus* (the common species which has hitherto been referred to simply as *Echinus*) *Strongylocentrotus*, *Sphærechinus* and *Arbacia*. Only in the case of the first named did any cross-fertilisation take place, but then no less than 98 per cent. of the ova reached the blastula stage, and 12·8 per cent. the eight days' pluteus stage. The hybrids themselves were by no means of the pure *E. microtuberculatus* type. Thus the skeletons were considerably thinner, and showed numerous irregularities. The fan-shaped base to the body skeleton had entirely disappeared, and was replaced by a more or less club-shaped form, like that found in *Strongylocentrotus*. It was much more irregular, however, and there were, in most larvæ, numerous spikes and irregular projections arising from the body skeleton. A typical instance of these hybrids is given in fig. 27. In

* 'Arch. d. Zool. exp. et. gén.,' II sér., tom. 5, 1887.

size also the larvæ were much diminished. No direct fertilisation of the *E. microtuberculatus* ova was attempted on the same day as they were crossed, but a direct fertilisation had been made two days previously. Compared with these larvæ, the hybrids were no less than 22·2 per cent. smaller in respect of body-length, and 15·2 per cent. smaller in arm-length. Whether these hybrids were of a form midway between those of the pure parental larvæ, it is impossible to say. Thus, as already mentioned, no pure *E. acutus* larvæ were obtained in the present investigation, and I have been unable to find any references to them by other observers.

In the next attempt at cross-fertilisation, made on December 31, none of the *Strongylocentrotus* ova were fertilised, but 3·8 per cent. of the *Sphærechinus* ova reached the blastula stage, and ·2 per cent. of them were free swimming after three days, after which they died off. Again, 2·7 per cent. of the *Echinocardium mediterraneum* ova developed to blastulæ, but these also died off a day or two later. Of the *E. microtuberculatus* ova, 91 per cent. developed to blastulæ, and 22 per cent. to eight days' plutei. These hybrid larvæ were similar to those already described, though not quite so irregular, and not showing so many projecting spikes. In respect of size they also differed less, the body-length being 13·9 per cent. smaller, and the arm-length 18·1 per cent. smaller than that of the pure *E. microtuberculatus* larvæ obtained at the same time.

As far as the few results available can show, the sperm of *E. acutus*, therefore, appears to have more power of cross-fertilisation than that of *E. microtuberculatus*. Thus, in this second series of experiments, when three out of the four species tried were successfully cross-fertilised by *E. acutus*, only *Strongylocentrotus* ova were cross-fertilised by *E. microtuberculatus*.

We have now completed the description of the cross-fertilisation experiments undertaken. They are admittedly in many instances incomplete, and so, as a rule, not much value can be attached to a negative result. Very probably if more favourable conditions had been chanced upon, the apparent absence of capacity for cross-fertilisation would have been reversed. For the sake of comparison, the results obtained are collected together in the accompanying table.

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Female parent.	Male parent.							
	Strongylocentrotus.	*Sphærechinus.*	*E. microtuberculatus.*	*E. acutus.*	*Arbacia.*	*E. cordatum.*	*E. mediterraneum.*	*Dorocidaris.*
Strongylocentrotus	plutei	maternal plutei	maternal-intermediate plutei	nil	maternal plutei	blastulæ (?)	nil	maternal plutei
Sphærechinus	intermediate plutei	plutei	intermediate plutei	gastrulæ	maternal-intermediate plutei	segmented ova (?)	nil	
Echinus microtuberculatus . . .	intermediate plutei	maternal plutei	plutei	intermediate (?) plutei	maternal plutei	maternal plutei	nil	
Echinus acutus								
Arbacia pustulosa	maternal plutei	gastrulæ	maternal plutei	nil	plutei	nil		
Echinocardium cordatum . . .	maternal plutei	maternal plutei	maternal plutei	..	maternal plutei	plutei		
Echinocardium mediterraneum .	intermediate plutei	gastrulæ	intermediate plutei	gastrulæ	..	..	plutei	
Dorocidaris	gastrulæ	..	..	..	..	..	..	plutei

Here it will be seen that of the 64 possible direct and cross-fertilisations, 40 were attempted. Of these 6 gave no result at all; in 6 cases blastulæ or gastrulæ, but no plutei, were obtained, whilst in 27 cases plutei, in almost every instance of eight days growth, were obtained. As regards the hybrid plutei themselves, they were of the almost pure maternal type in 12 out of the 20 instances in which they were obtained. In the remaining instances they were generally of the intermediate or intermediate-maternal type. In no case were all the hybrids of the pure paternal type, though in the case of the *Sphærechinus* ♀-*Strongylocentrotus* ♂ cross a certain proportion of the hybrids appeared to be of this nature. Of the 13 instances in which reciprocal crosses were attempted, a positive result was obtained in 9 of them. Out of these 9 instances, plutei were obtained with both crosses in 7. The capacity for reciprocal crossing seems, therefore, to be the rule rather than the exception. This is somewhat contrary to the results of Pflüger and of Born, who, in the case of amphibia, found it to be the exception rather than the rule.

Crosses between the Colour Varieties of Sphærechinus.

In addition to the observations on the crossing of various species of Echinoids, it was thought to be of interest to make parallel experiments between the varieties of one and the same species, and to thereby determine how far, as judged by a purely physiological standard, the varieties and species are separated from one another. Thus, it has been regarded as a general rule, with exceedingly few exceptions, that the varieties of a species are perfectly fertile *inter se*, but that on the other hand the species themselves are generally infertile, and their hybrid offspring almost invariably so. In connection with this subject, I have recently suggested a theory,* which I have termed "Reproductive Divergence." This theory was enunciated as follows: "Supposing that amongst the members of any species, those individuals more alike in respect of any characteristic such as colour, form, or size, are slightly more fertile, *inter se*, than less similar individuals, then it necessarily follows that in the course of succeeding generations the members of this species will diverge more and more in respect of the characteristic in question, whereby ultimately the original species may be split up into two or more fresh species." This theory I considered to afford a hitherto unrecognised factor in organic evolution, but one of which the relative importance could only be determined by experiment. Thus this theory asserts that whenever there is a differential fertility, no matter how small, between the varieties of a species, divergence will inevitably ensue. It is therefore necessary to determine, in as many cases as possible, whether such differential fertility exists. Hitherto no systematic observations whatever appear to have been made upon members of the animal kingdom, though upon those of the vegetable kingdom they are fairly numerous. These Echinoids seemed to offer a

* 'Nat. Science,' vol. 9, p. 181 and p. 404, 1897.

fitting opportunity of testing the point, especially as one of them, viz., *Sphærechinus*, occurs in such well-marked varieties. These varieties fall into three main groups, namely, the white, the violet, and the brown spined individuals. In the members of the first group, the spines are quite white, with occasionally a faint pinkish tinge about them. In those of the second group, the spines vary from white, with a faint violet tinge at their base, to a deep violet colour throughout. Sea-urchins with all their spines violet throughout are practically never met with, the extreme tips of some of them always remaining unpigmented. In the third group, the spines vary through a similar range of pigmentation, only the violet colour is replaced by a reddish-brown. Finally, there is a fourth group, in which the colour of the spines lies between the pure violet and brown, and in which, of course, the degree of pigmentation also varies.

In order to determine the relative frequency with which these varieties are met with, 182 individuals, all of which were obtained in November and the beginning of December, were roughly tabulated according to their colour. The following represent the percentage numbers in each group :—

White		19·8 per cent.
White-violet	9·3	
White-violet	7·1	38·9 „
Violet-white	22·5	
White-brown	3·3	
White-brown	3·8	21·4 „
Brown-white	14·8	
Violet-brown-white	3·8	
Violet-brown-white	8·8	
Brown-violet-white	6·6	19·8 „
White-brown-violet	0·6	

Here we see that about a fifth of the sea-urchins had pure white spines, about two-fifths violet and white, a fifth brown and white, and the remaining fifth mixed colours. There is thus a very distinct tendency to the formation of well-marked varieties. This is emphasised by the fact that, of the violet-white individuals, considerably over half belong to the *violet*-white class, *i.e.*, had the larger portion of each of their spines pigmented, and that more than two-thirds of the brown-white individuals belonged to the *brown*-white class. Again, the group of individuals in which the brown and violet pigments were mingled was the smallest of all, instead of being the largest, as would be the case if there were no tendency to the formation of varieties. Some of the individuals in the violet and brown groups had, it is true, a tinge of brown or violet, but it was not thought sufficient to relegate them to the mixed colour group.

It is not to be concluded, however, that, because of this tendency to separate into varieties, there is necessarily any segregating agency at work. Thus it is a well-

known phenomenon, as in the case of the cat and the rabbit, that the offspring of two varieties may resemble either parent, and yet but seldom show the mixed characters of both.

In the first experiment, made on June 2, white and *violet*-white spined individuals were crossed. Cross-fertilisations with *Strongylocentrotus* were also made at the same time. The results obtained were as follow :—

Number of experiment.	Colour of *Sphærechinus* ♀.	Colour of *Sphærechinus* ♂.	Per cent. blastulæ.	Per cent. larvæ.	Body-length.	Per cent. difference from mean.	Arm-length.	Per cent. difference from mean.
30	White	White	97	76	17·64	} mean	242·6	} mean
33	*Violet*-white . .	*Violet*-white . .	100	70	18·14	} 17·89	278·4	} 260·5
31	White	*Violet*-white . .	99	22	17·30	−3·3	246·2	− 5·5
34	*Violet*-white . .	White	37	9·2	16·89	−5·6	350·0	+34·3
32	White	*Strongylocentrotus*	36	17	25·49	..	85·3	
35	*Violet*-white . .	*Strongylocentrotus*	9·1	1·0	19·40	..	285·6	

Here we see that, when the ova were fertilised by sperm of similar colour varieties, on an average 98·5 per cent. of them reached the blastula stage, and 73 per cent. the eight days' pluteus stage. On the other hand, with sperm of dissimilar colour varieties, on an average only 68 per cent. developed to blastulæ, and 15·6 per cent. to plutei. The number given for the percentage of blastulæ in Exp. 34 is, however, somewhat misleading. Thus, in this experiment, the segmentation appeared to have been considerably delayed, so that, whilst only 37 per cent. of the ova had reached the blastula stage after twenty-four hours' development, yet 59 per cent. of them were actually fertilised and developing normally. The size of the plutei formed was also diminished, the dissimilar colour variety plutei being respectively 3·3 and 5·6 per cent. smaller than the similar ones. The arm-lengths of the dissimilar were, however, on an average somewhat longer than those of the similar. With reference to cross-fertilisation with *Strongylocentrotus* sperm, the two varieties also reacted differently. Thus the ova of white *Sphærechinus* yielded a fair percentage of hybrid larvæ of an intermediate type, whilst those of the violet variety gave only a very small percentage of hybrids, most of which were of the *Sphærechinus* type.

In the next experiment, made on July 1, the varieties were separated into three groups, white, *white*-violet, and *violet*-white, and the following results were obtained :—

Number of experiment.	Female Echinoids.	Male Echinoids.	Per cent. blastulæ.	Per cent. larvæ after—		Body-length.	Per cent. difference from mean.	Arm-length.	Per cent. difference from mean.
				8 days.	12 days.				
50	White	White	98	55	0·07	19·16	..	257·6	..
51	„	*White*-violet . .	95	40	0·05	17·90	−3·8	323·0	+13·2
52	„	*Violet*-white . .	96	47	0	18·41	−0·7	280·0	+12·3
54	*White*-violet . .	*White*-violet . .	92	67	1·4	18·05	..	291·5	..
55	„ „ . .	White	94	25	1·1	18·34	−1·4	263·8	− 7·5
56	„ „ . .	*Violet*-white . .	79	34	0·93	17·40	−3·3	290·0	+ 8·9
58	*Violet*-white . .	„ „ . .	87	8·1	1·2	17·93	..	241·0	..
59	„ „ . .	White	97	56	0·05	18·75	+1·1	339·8	+36·3
..	„ „ . .	*White*-violet . .	0	..	..	..	..	..	..
53	White	*Strongylocentrotus*	4·5	0·5	..	19·15	..	210·4	..
57	*White*-violet . .	„	50	34	0·17	18·05	..	247·7	..
..	*Violet*-white . .	„	45	0·08	..	..	..	..	..
46	*Strongylocentrotus*	„	98	69	14	29·67	..	99 6	..
47	„	White	88	19	..	28·69	..	126·0	..
48	„	*White*-violet . .	48	48	6·2	27·90	..	95·4	..
49	„	*Violet*-white . .	40	40	32	27·69	..	95·0	..

In these observations, on an average 92·3 per cent. of the ova fertilised by sperm of a similar colour variety reached the blastula stage, and 43·4 per cent. the eight days' pluteus stage. Owing to the high temperature of the water (viz., 23°·2), the plutei then began to die off rapidly, so that a fresh enumeration four days later showed that, on an average, only ·89 per cent. were still surviving. Of the ova fertilised by sperm of dissimilar colour varieties, on an average only 76·8 per cent. arrived at the blastula stage, 33·7 per cent. at the eight days' pluteus stage, and ·36 per cent. at the twelve days stage. The differences of fertility, though still quite obvious, are therefore not so marked as in the previous series of experiments. The differences in the size of the larvæ are also smaller. In Experiments 51 and 52, in which the ova of white individuals were fertilised by the sperm of *white*-violet and *violet*-white ones, the larvæ were respectively 3·8 and ·7 per cent. smaller than the means between the respective pure parental larvæ. In Exps. 55 and 56, again, they were 1·4 and 3·3 per cent. smaller, but in Exp. 59, with the ova of *violet*-white individuals, and the sperm of white ones, they were 1·1 per cent. larger. In the cross of *violet*-white ♀ with *white*-violet ♂, not only were no larvæ obtained, but not a single ovum was fertilised. This result must be regarded as exceptional, but it may at least be considered to weigh in the balance against the result obtained in Exp. 59. Thus, in this case, in which the ova were fertilised by dissimilar sperm, considerably more plutei were obtained than in Exp. 58, when similar sperm was used. These and the other observations therefore seem to show that *violet*-white spined sea-urchins are less fertile with *white*-violet individuals than they are with white ones,

which is, of course, contrary to expectation. As, however, the differences of fertility are in any case somewhat small, and the errors of experiment and causes of chance variation considerable, such an abnormal result is not surprising. Exact numerical results could only be obtained by repeating the experiments a large number of times, and taking means.

As an average of the five sets of experiments made, the dissimilar varietal larvæ were found to be 1·6 per cent. smaller than the similar ones. In every experiment but one, however, their arm-lengths were greater, and hence the measurements do not speak nearly so much in favour of a differential fertility as do the relative percentages of blastulæ and larvæ. Of course, it is not necessary that they should do so, a diminution in the percentage of larvæ formed being all that is required to support the theory above mentioned.

In the second half of the above table are given the results obtained on crossing the various colour varieties with *Strongylocentrotus*. Of the *Sphærechinus* ova, on an average 33·2 per cent. reached the blastula stage, and 11·5 per cent. the pluteus stage. In the reciprocal cross of *Strongylocentrotus* ova, the numbers were respectively 55·3 and 35·7 per cent. In this latter case, therefore, more plutei were formed than in the direct cross of dissimilar colour varieties of *Sphærechinus*, and, also, considerably more of them survived to the twelve days' stage. The hybrid larvæ were, however, 3·0 to 6·7 per cent. smaller than the pure *Strongylocentrotus* larvæ. The nature of the *Sphærechinus* sperm appeared to have a distinct influence on the size of the hybrids. Thus, in the present case the ova cross-fertilised by the sperm of the white-spined individuals gave plutei about 3 per cent. larger than those of the other colour varieties. Now, in the direct fertilisations those larvæ arising from white sperm are on an average 4·5 per cent. larger than the mean of all the other larvæ.

In the next series of experiments, made on November 11, it was thought unnecessary to make measurements of all the larvæ obtained, but the percentages of plutei surviving were determined on five different occasions. As the values obtained after twelve and eighteen days' development were practically the same as those after eight days, a mean of the three sets of measurements has alone been given. It will be seen that a considerable number of plutei survived even for thirty days. This must have largely been due to the low temperature of the water (viz., 13°·0).

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Female Echinoids.	Male Echinoids.	Per cent. blastulæ.	Per cent. plutei after—		
			8–18 days.	24 days.	30 days.
White	White	100	86	100	54
,, ...	*Violet*-white . . .	98	79	97	77
,, ...	*White*-brown . . .	100	67	64	52
,, ...	*Brown*-white . . .	100	87	88	48
Violet-white . . .	*Violet*-white . . .	100	97	80	35
,, . . .	White	100	61	54	69
,, . . .	*White*-brown . . .	100	80	97	90
,, . . .	*Brown*-white . . .	100	83	81	60
White-brown . . .	*White*-brown . . .	99	92	92	57
,, . . .	White	97	67	49	41
,, . . .	*Violet*-white . . .	96	83	81	25
,, . . .	*Brown*-white . . .	95	67	76	53
Brown-white . . .	*Brown*-white . . .	100	59	24	5
,, . . .	White	82	54	11·4	..
,, . . .	*Violet*-white . . .	94	65	7·7	..
,, . . .	*White*-brown . . .	98	63	21	..
White	*Strongylocentrotus* .	21	7·1	..	..
Violet-white . . .	,, .	6·0	1·0	..	..
White-brown . . .	,, .	0	..	..	..
Brown-white . . .	,, .	3·0	3·0	..	..

The sea-urchins were divided up differently in this series, the brown and not the violet-coloured specimens being sorted into two classes. It will doubtless be noticed that the percentage numbers are occasionally rather variable and contradictory. This is due to the difficulty of getting the plutei evenly distributed through the water, previous to the withdrawal of a sample for their enumeration. Probably, however, the average error of these numbers is not more than 5 per cent. On taking means of the four experiments with similar colour varieties on the one hand, and of the twelve with dissimilar colour varieties on the other, the following numbers are obtained :—

	Blastulæ.	8 days plutei.	24 days.	30 days.
Similar colours	100	84	74	49
Dissimilar colours . . .	97	71	61	57

That is to say, the dissimilar colour-fertilisations yield a smaller number of blastulæ, and distinctly smaller numbers of eight and twenty-four days' plutei, but on the other hand a distinctly larger number of thirty days' plutei. The crossed larvæ, though fewer in number, thus appear to be more hardy and capable of surviving. In calculating these mean numbers, the percentage of pure *brown*-white larvæ surviving to thirty days was not included, as the corresponding determinations with the dissimilar colour variety larvæ were not made. This was due to the fact that all of the small number of larvæ surviving till twenty-four days were then needed for enumeration.

As in the previous instance, these numbers fail to show that the *brown*-white varieties are less fertile with the white varieties than are the *white*-brown ones; but as the differences of fertility are in any case so small, one would scarcely expect this graduated relationship to show itself. When crossed with *Strongylocentrotus* sperm, the ova of the white-spined *Sphærechinus* were the only ones to yield a fair proportion of blastulæ and larvæ. The percentages of plutei given in the eight to eighteen days column of the table are for eight days only in the case of these hybrids.

In the next and last series of observations, made on December 13, the colour varieties were split up into only three groups. The following are the results:—

Sphærechinus ♀.	*Sphærechinus* ♂.	Per cent. blastulæ.	Per cent. plutei after—	
			8 days.	31 days.
White	White	100	85	66
„	*Violet*-white . . .	99	97	86
„	*Brown*-white . . .	99	100	62
Violet-white . . .	*Violet*-white . . .	100	78	77
„ . . .	White	95	44	9
„ . . .	*Brown*-white . . .	99	90	84
Brown-white . . .	*Brown*-white . . .	100	100	100
„ . . .	White	89	78	68
„ . . .	*Violet*-white . . .	93	78	68

As a mean of the three experiments with similar colour varieties, it was found that respectively 100 per cent. of blastulæ, 88 per cent. of eight days' plutei, and 81 per cent. of thirty-one days' plutei resulted. In the six experiments with dissimilar colour varieties, 96 per cent. of the blastulæ, 81 per cent. of the eight days' plutei, and 63 per cent. of the thirty-one days' plutei resulted. The difference of fertility is, therefore, about the same as in the preceding case, as far as the number of blastulæ and eight days' plutei are concerned, but departs from it in respect of the thirty-one days' plutei, which are in this instance considerably fewer in number as regards the dissimilar colour varieties.

In these last two series of experiments the difference of fertility is distinctly less than in the second series, whilst in this again it is very much less than in the first series made. The degree of mutual infertility would, therefore, appear to be largely dependent on the time of the year at which the fertilisations are made. It may therefore be bound up either with the variations in the maturity of the sexual products, or, less probably, depend on the temperature of the water in which the sea-urchins had been kept, and in which the ova were fertilised and allowed to develop.

Judging from these four series of experiments made, there can thus be little doubt that the physiological attribute of sexual compatibility is in some way bound up

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with the morphological, or rather chemical, attribute of pigmentation. Whether the one depends directly on the other, or whether they are only indirectly connected by some third and unknown characteristic with which both are correlated, it is immaterial for our purpose to know. Provided only that the two attributes are found to occur, as a rule, in the same individuals, then it necessarily follows that the species will gradually diverge more and more in respect of the colour variations, till at length varieties so distinct both in colour and physiological relationships may arise, as to entitle them to be regarded as distinct species.

A further proof of the physiological differences of these colour varieties was afforded some pages back, when it was pointed out that the hybrids of white-spined *Sphærechinus* ♀ and *Strongylocentrotus* ♂ tended to be of the maternal type, whilst those of violet-white *Sphærechinus* tended to be of the paternal type. Again, with reference to the reciprocal cross, it appeared that the sperm of white-spined *Sphærechinus* had the greatest capacity for cross-fertilising *Strongylocentrotus* ova, whilst that of the brown-spined individuals had little or none.

Crosses between the Colour Varieties of Strongylocentrotus.

The sea-urchins of this species do not show nearly such marked variations in colour as those of the species just discussed, chiefly because the colours are almost always mixed with varying degrees of darker pigment, which render them less distinct. The actual colour-range they embrace is, however, greater. Thus one may distinguish individuals with spines of light red, violet, brownish-green, yellowish-brown with a tinge of green or red, and black with a tinge of red, green, brown, or violet. These varieties occur in more or less even numbers, except that the "blackish" group predominate.

In the first series of experiments, made on June 11, three groups were worked with. The results were as follow :—

Number of experiment.	Nature of *Strongylocentrotus* ♀.	Nature of *Strongylocentrotus* ♂.	Per cent. blastulæ.	Per cent. larvæ.	Body-length.	Per cent. difference from mean body length.	Arm-length.	Per cent. difference from mean arm length.
36	Black . .	Black . .	100	52	29·37	..	133·5	..
37	,, . .	Brown . .	53	23	28·82	− 3·2	122·6	− 7·0
38	,, . .	Red . . .	1·9	0·9	28·24	−11·7	127·6	− 6·4
39	Brown . .	Brown . .	100	73	30·18	..	130·1	..
40	,, . .	Black . .	89	76	29·85	+ 0·2	138·9	+ 5·4
41	,, . .	Red . . .	31	31	32·12	− 0·8	148·2	+10·1
42	Red . . .	,, . . .	11	5	34·58	..	139·0	..
43	,, . . .	Black . .	96	50	30·56	− 4·4	124·0	− 9·0
44	,, . . .	Brown . .	98	71	29·58	− 9·8	129·0	− 4·2

On an average in the three experiments made with similar colour varieties, 70 per cent. of the ova reached the blastula stage, and 43 per cent. the eight days' pluteus stage. In the six fertilisations made with dissimilar colour varieties, the numbers were respectively 61 per cent. and 42 per cent. The differences in fertility are therefore not nearly so striking as in the case of the *Sphærechinus* colour varieties. In fact, they almost fall within the limits of experimental error. As regards actual size, however, the differences are more marked. Thus, on an average, the dissimilar larvæ were 4·9 per cent. smaller in body-length than the similar, an amount much too large to be due only to experimental error. As regards individual experiments, we see that in five out of the six experiments made with dissimilar varieties, the larvæ were appreciably smaller than the mean between the parental larval forms, whilst in the sixth experiment they were of about the same size. In one case the difference in size amounted to no less than 11·7 per cent. The importance of the nature and quality of the sperm is clearly evidenced by the experiments with the red-spined variety. Thus, of the ova fertilised by this sperm, on an average only 15 per cent. became blastulæ and 12 per cent. plutei, whilst with sperm of the black and brown-spined varieties, the numbers of blastulæ were respectively 95 per cent. and 84 per cent., and of the larvæ 59 per cent. and 56 per cent. Despite the slight power of fertilisation, the larvæ actually produced proved to be considerably larger than the average in two out of the three fertilisations made (viz., Exps. 41 and 42).

In the second series of experiments, made on December 3, the varieties used were the violet, black, and brownish-green ones. On this occasion the size of the larvæ was as a rule not measured, but the surviving plutei were counted after twenty-one and thirty-one days, as well as after eight days.

Strongylocentrotus ♀.	*Strongylocentrotus* ♂.	Per cent. blastulæ.	Per cent. plutei after—		
			8 days.	21 days.	31 days.
Green	Green	100	100	75	87
,,	Violet	100	91	89	81
,,	Black	100	100	100	100
Violet	Violet	100	92	92	100
,,	Green	97	84	83	73
,,	Black	97	83	0·7	0
Black	,,	93	34	0·6	0
,,	Green	87	..	..	..
,,	Violet	94	82	2·6	0

On taking means of the three similar colour variety fertilisations on the one hand, and of the six dissimilar ones on the other, the following values are obtained :—

	Per cent. blastulæ.	8 days' plutei.	21 days.	31 days.
Similar colours . . .	98	75	56	62
Dissimilar colours . .	96	88	55	51

These figures prove that in this series of experiments at least, the dissimilar colour varieties were just as mutually fertile as the similar, or, if anything, more so. It will be noticed that in one experiment no values are given for the percentages of plutei formed. This was owing to some additional water getting into the jar in which the larvæ were developing, and so altering the concentration of the larvæ.

The results obtained with the colour varieties of *Strongylocentrotus* on the one hand, and those of *Sphærechinus* on the other, thus afford a somewhat instructive comparison. In the one case, in which the colour varieties are not very distinctly marked off, the differential fertility is so slight that it cannot with certainty be said to have been demonstrated at all; whilst in the other case, where the varieties themselves are much more distinct, and where the individuals of intermediate character are much less numerous, well-marked variations of physiological compatibility were in every case shown to be present.

Attention has already been drawn to the fact that the nature of the sperm is of considerable influence on the size of the larvæ. It was, of course, taken for granted that the nature of the ovum had also an influence, but upon what does this influence depend? Is it upon some intrinsic properties of the protoplasm, or chiefly upon the actual amount of food material available in the ovum? In order to get information on this question, some measurements of the unfertilised ova themselves were made. These were measured in the same manner as the larvæ, the diameter of the yolk substance, and not of the vitelline membrane in addition, being determined. In the fresh unfertilised ova there is, of course, no visible membrane, but one is almost invariably formed in the course of preservation. At first, fifty of the ova were measured in each case, but subsequently, in that the range of variation in the size of the ova of each group was found to be but slight, only twenty-five. The results obtained were the following :—

Number of experiment.	Date.	Nature of ova.	Mean diameter.	Body-length of larvæ (corrected).	Number of experiment.	Date.	Nature of ova.	Mean diameter.	Body-length of larvæ (uncorrected).
99	3.xii.	*Strongylocentrotus*	11·00	34·35	101	3.xii.	*Sphærechinus* . .	11·52	18·39
96	,,	,,	11·15	35·31	112	13.xii.	,, . .	10·93	16·11
98	,,	,,	11·15	36·72	115	,,	,, . .	11·71	17·52
107	13.xii.	,,	11·29	35·13	114	,,	,, . .	11·82	18·62
124	22.xii.	,,	11·18	34·37	127	22.xii.	,, . .	11·52	15·63
132	31.xii.	,,	11·96	35·38	143	31.xii.	,, . .	11·64	16·87
159	7.i.	,,	11·32	36·05	166	7.i.	,, . .	11·36	18·29
102	3.xii.	*Echinus*. . . .	13·79	35·59	130	22.xii.	*Echinocardium cordatum*	13·47	(20·12)
119	13.xii.	,, ...	12·56	34·55	156	31.xii.	*Echinocardium mediterraneum*	12·03	(17·87)
128	22.xii.	,, ...	13·23	35·44	..	22.xii.	*Arbacia* . . .	9·47	..
149	31.xii.	,, ...	13·84	34·51	..	..	..	..	..
169	7.i.	,, ...	13·04	33·13	..	..	..	..	..

In the case of *Strongylocentrotus* and *Echinus*, the body-length values given have been corrected for temperature of development, but those of the other larvæ, owing to lack of sufficient data, have been left unaltered. As regards *Strongylocentrotus*, we see that the mean diameter of the ova varies from 11·00 to 11·96, or as 1·00 to 1·09. As the variations both of this diameter and of the mean body-length are so slight, it is not possible to gather much as to the dependence of the one on the other; but on dividing all the values into two halves, it is found that ova with an average diameter of 11·12 give rise to larvæ with an average body-length of 35·19, whilst with those having a diameter of 11·52, the body-length is 35·52. Thus the one measurement is evidently related to the other. On similarly dividing up the *Echinus* values, it is found that ova with a diameter of 12·94 produce larvæ with a body-length of 34·37, and those with one of 13·82, larvæ with one of 35·05. The variation is thus more marked, and the interdependence of the two values also more evident. In the case of *Sphærechinus*, the relation also shows itself. Thus, in one of the three experiments made on December 13, the ova were about 7 per cent. smaller than in the other two, whilst the larvæ arising therefrom were about 12 per cent. smaller. Also on dividing up the values into two groups, it is found that ova with an average diameter of 11·33, produce larvæ averaging 17·10 in body-length, and ova of 11·72 larvæ of 17·67. We see, therefore, that in every case the average values for the size of ova and of larvæ more or less correspond; but as the individual values show considerable variations, there are obviously other factors at work as well.

With reference to the two values for *Echinocardium* ova, it will be noticed that that of *E. mediterraneum* is considerably less (10·7 per cent. in fact) than that of *E. cordatum*, the five days' plutei being also 11·2 per cent. less in body-length. Now

the extreme limit of variation in any of the other measurements of ova was only 8·9 per cent. Hence an additional argument is afforded in favour of the specific difference of these two Echinoids.

General Conclusions.

These experiments on hybrids, though somewhat incomplete, are yet sufficient to justify one in making one or two generalisations and deductions. In the first place, in the case of these Echinoids at least, it is obvious that there is present a very general capacity for cross-fertilisation. The various species are not separated from each other by any rigidily-defined physiological barrier. In the case of one hybrid at least (viz., *Echinus* ♀—*Strongylocentrotus* ♂), we have seen that the cross-fertilisation takes place with greater ease, and produces larvæ of greater size, than does the direct fertilisation. On the other hand, we have seen that between the different colour varieties of *Sphærechinus* there is a distinctly diminished fertility. As far as one can judge by this physiological test, therefore, the varieties in this latter case are separated by a wider interval than are the species in the former. These results thus serve to confirm the reiterated contention of Darwin and of Wallace as to the absence of any fundamental physiological difference between species and varieties.

Upon the question of prepotency, the results obtained also afford some information. We have seen that as a rule the hybrid larvæ are of the maternal type, there frequently being little or no indication of the mixed parentage. On the other hand, it is evident that some species have much greater potency than others in the transmission of their characteristics. Thus, in the crosses with *Strongylocentrotus* ova, the hybrids were in every instance but one of the pure maternal type, and in that one case, *i.e.*, with *Echinus*, the larvæ inclined more to the maternal than to the paternal type. In the crosses with *Sphærechinus* ova, on the other hand, the hybrids on the whole approached more closely to the paternal than to the maternal type. The results obtained with these and the other species show a distinct amount of uniformity, and prove that the degree of prepotency, though apparently somewhat capricious, is yet by no means entirely so.

From a practical and more generalised point of view, it is possible that the results obtained may be of some value. Thus it seems highly probable that similar conditions for the production of hybrids may obtain amongst other classes of the animal kingdom to those found to be true in some cases for Echinoids. Even amongst Mammals, it seems probable that experiments on crossing might have more chance of being brought to a successful conclusion if the fertilisation of the ova could be brought about, by natural or artificial means, some little time after they had been shed from the ovary, and when they had thereby become less resistant to the attacks of the foreign spermatozoa.

Summary.

The following are the chief conclusions arrived at in this paper :—

On crossing *Sphærechinus* ♀ with *Strongylocentrotus* ♂, as a rule only about 10 per cent. of the ova are cross-fertilised, and only about 1 per cent. of them develop to the eight days' pluteus stage. The hybrids are most easily obtained in the summer months, few or none being obtained under normal conditions in the winter. The majority of the hybrids obtained in May, June, and July were of an almost pure *Sphærechinus* type, only a third or less being of an intermediate or *Strongylocentrotus* type. In November, on the other hand, only about a sixth were of the maternal, and five-sixths of the paternal type. Finally, in December and January, all the hybrid larvæ were of the paternal type.

In the reciprocal cross of *Strongylocentrotus* ♀ and *Sphærechinus* ♂, no plutei were obtained in April, May and June, but a fair number of the ova were cross-fertilised. In July and August some 47 per cent. of the ova were fertilised and 29 per cent. of them survived to the eight days' pluteus stage. In November and December on the other hand, with one exception, not only were no plutei obtained, but as a rule not a single ovum was cross-fertilised. These variations in the capacity for cross-fertilisation seem to depend on the relative maturity of the sexual products. Thus, from the gradual dwindling down in the size of the larvæ, and in the amount of ripe sexual products obtainable from the sea-urchins, it appeared that in *Strongylocentrotus* the maturity was at its maximum in April and the beginning of May : that it then diminished and reached a minimum at the beginning of July, and that it remained at this point till the middle of August. It then gradually rose, and by the end of November had again reached its maximum. It follows therefore that *the* Strongylocentrotus ♀–Sphærechinus ♂ *hybrid is only formed at the time when the* Strongylocentrotus *ova have reached their minimum of maturity ; whilst in the case of the reciprocal hybrid, it follows that as the maturity of the* Strongylocentrotus *sperm increases, it is able to transmute first a portion and then the whole of the hybrid larvæ from the* Sphærechinus *to its own type. In other words, the characteristics of the hybrid offspring depend directly on the relative degrees of maturity of the sexual products.*

The hybrid larvæ obtained on crossing *Echinus* ♀ and *Strongylocentrotus* ♂ were 8 per cent. *larger* than the pure parental larval forms, and moreover even more of the cross-fertilised ova developed to plutei than of the directly-fertilised ones. In the reciprocal cross, on the other hand, only a few of the ova were fertilised, and only about 1 per cent. of them reached the pluteus stage. These plutei were 13·2 per cent. *smaller* than the pure parental larvæ.

Reciprocal crosses were effected between *Sphærechinus* and *Echinus* ; between

Arbacia and *Strongylocentrotus*, *Sphærechinus* and *Echinus*; also between *Echinocardium* and *Echinus*; whilst various other single crosses were effected with *Echinocardium*, *Dorocidaris* and *Echinus acutus*. As a rule the hybrid plutei were of the maternal type, but sometimes of the intermediate type. Judged by physiological standards, it appeared that the two doubtful species, *Echinocardium cordatum* and *E. mediterraneum* were true species.

On crossing the various colour varieties of *Sphærechinus*, a distinct degree of infertility was found to exist between them. Between the colour varieties of *Strongylocentrotus*, on the other hand, there were little or no differences of fertility.

In conclusion, I wish to take this opportunity of expressing my warmest thanks to the authorities at the Naples Zoological Station, where this research was carried out, for the invariable kindness and assistance they showed me during the progress of my work.

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TABLE.

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
1	10.iv.	1 *Strongylocentrotus*	1 *Strongylocentrotus*	50	13·7	15·6	8	110	100	77	30·44	108·98	127·5
2	22.iv.	1 *Strongylocentrotus*	1 *Strongylocentrotus*	”	15·8	17·5	8	189	80	42	36·30	116·25	134·9
3	”	5 *Sphærechinus*	1 *Sphærechinus*	”	16·5	”	8	170	92	32	19·13	215·35	236·8
4	5.v.	1 *Strongylocentrotus*	1 *Strongylocentrotus*	”	17·9	17·4	8	60	100	100	32·18	134·09	154·6
5	”	5 *Echinus*	5 *Echinus*	”	17·9	”	4	56	86	86	31·48	109·86	. .
6	”	”	”	”	”	”	6	”	”	”	32·19	114·71	. .
7	”	”	”	”	”	”	8	”	”	”	32·82	104·34	114·3
8	”	”	1 *Strongylocentrotus*	”	18·3	”	8	47	77	77	33·20	114·91	123·1
9	14.v.	1 *Strongylocentrotus*	1 *Strongylocentrotus*	”	17·9	17·2	8	167	100	39	31·77	86·16	97·4
10	”	5 *Echinus*	5 *Echinus*	”	17·5	”	8	220	100	70	30·83	62·59	81·8
11	”	”	”	”	”	”	14	”	”	”	30·53	35·22	. .
12	”	”	1 *Strongylocentrotus*	”	17·7	”	8	172	98	78	35·24	71·65	90·8
13	”	1 *Strongylocentrotus*	2 *Echinus*	”	17·7	”	8	96	2·0	1·6	26·15	122·66	123·1
14	24.v.	1 *Strongylocentrotus*	6 *Strongylocentrotus*	”	19·0	19·0	8	170	100	35	29·74	71·82	80·3
15	”	”	1 *Arbacia*	”	19·2	”	8	222	3·2	0·1	28·57	115·36	115·4
16	”	4 *Echinus*	5 *Echinus*	”	18·9	”	8	100	92	54	33·64	77·79	86·2
17	”	”	6 *Strongylocentrotus*	”	18·9	”	8	59	100	100	32·50	104·45	116·8
18	”	1 *Sphærechinus*	1 *Sphærechinus*	”	19·2	”	4	404	2·5	2·0	17·56	154·12	. .
19	”	”	”	”	”	”	8	”	”	”	17·21	300·58	305·6
20	”	”	”	”	”	”	14	”	”	”	17·44	368·00	. .
21	”	”	6 *Strongylocentrotus*	”	18·7	”	8	600	2·0	0·1	19·31	202·90	203·1
22	”	”	1 *Arbacia*	”	19·2	”	8	610	0·24	0·08	16·90	269·10	269·4
23	”	2 *Arbacia*	”	”	19·0	”	4	664	97	34	14·00	124·10	. .
24	”	”	”	”	”	”	8	”	”	”	14·90	160·42	233·3
25	”	”	”	”	”	”	10	”	”	”	14·51	147·15	. .
26	”	”	”	”	”	”	14	”	”	”	14·72	102·63	. .

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TABLE (continued).

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
27	24.v.	2 *Arbacia*	6 *Strongylocentrotus*	4	19·0	19·0	8	862	1·7	0·006	14·08	227·15	227·2
28	,,	,,	5 *Echinus*	50	19·4	,,	8	913	3·3	0·05	14·84	167·89	168·1
29	2.vi.	2 *Strongylocentrotus*	2 *Strongylocentrotus*	,,	20·1	21·0	8	85	96	64	31·25	104·61	116·6
30	,,	2 white *Sphærechinus*	1 white *Sphærechinus*	,,	20·7	,,	8	168	97	76	17·64	193·20	242·6
31	,,	,, ,,	3 *violet*-white *Sphærechinus*	,,	20·7	,,	8	174	99	22	17·30	228·96	246·2
32	,,	,, ,,	2 *Strongylocentrotus*	,,	20·4	,,	8	220	36	17	25·49	79·38	85·3
33	,,	1 *violet*-white *Sphærechinus*	3 *violet*-white *Sphærechinus*	,,	21·3	,,	8	72	100	70	18·14	250·33	278·4
34	,,	,, ,,	1 white *Sphærechinus*	,,	21·3	,,	8	87	37	9·2	16·89	344·26	350·0
35	,,	,, ,,	2 *Strongylocentrotus*	46	20·8	,,	8	77	9·1	1·0	19·40	285·13	285·6
36	11.vi.	2 black *Strongylocentrotus*	4 black *Strongylocentrotus*	50	20·5	20·8	8	63	100	52	29·37	125·36	133·5
37	,,	,, ,,	3 brown ,,	,,	20·9	,,	8	57	53	23	28·82	119·43	122·6
38	,,	,, ,,	1 red ,,	30	20·8	,,	8	80	1·9	0·9	28·24	127·44	127·6
39	,,	1 brown ,,	3 brown ,,	50	20·5	,,	8	182	100	73	30·18	102·95	130·1
40	,,	,, ,,	4 black ,,	,,	20·9	,,	8	70	89	76	29·85	125·67	138·9
41	,,	,, ,,	1 red ,,	,,	20·8	,,	8	114	31	31	32·12	137·97	148·2
42	,,	1 red ,,	1 red ,,	,,	20·8	,,	8	65	11	5	34·58	138·10	139·0
43	,,	,, ,,	4 black ,,	,,	20·9	,,	8	112	96	50	30·56	111·41	124·0
44	,,	,, ,,	3 brown ,,	,,	20·9	,,	8	92	98	71	29·58	114·11	129·0
45	22.vi.	2 *Strongylocentrotus*	2 *Strongylocentrotus*	,,	20·6	21·5	8	142	93	49	29·55	107·25	122·2
46	1.vii.	4 *Strongylocentrotus*	2 *Strongylocentrotus*	,,	24·4	23·2	8	58	98	69	29·67	92·18	99·6
47	,,	,,	1 white *Sphærechinus*	,,	24·9	,,	8	8	88	19	28·69	125·55	126·0
48	,,	,,	1 *white*-violet *Sphærechinus*	,,	24·8	,,	8	48	48	48	27·90	91·31	95·4
49	,,	,,	1 *violet*-white ,,	,,	24·6	,,	8	38	40	40	27·69	92·28	95·0
50	,,	3 white *Sphærechinus*	1 white ,,	,,	24·5	,,	8	146	98	55	19·16	222·02	257·6
51	,,	,, ,,	1 *white*-violet ,,	,,	24·6	,,	8	213	95	40	17·90	276·08	323·0
52	,,	,, ,,	1 *violet*-white ,,	,,	24·6	,,	8	203	96	47	18·41	235·44	280·0

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TABLE (continued).

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
53	1.vii.	3 white *Sphærechinus* . .	2 *Strongylocentrotus* . . .	33	25·0	23·2	8	67	4·5	0·5	19·15	210·25	210·4
54	,,	1 *white*-violet *Sphærechinus*	1 *white*-violet *Sphærechinus*	50	24·8	,,	8	107	92	67	18·05	254·44	291·5
55	,,	,, ,,	1 white ,,	,,	24·8	,,	8	133	94	25	18·34	247·81	263·8
56	,,	,, ,,	1 *violet*-white ,,	,,	24·8	,,	8	140	79	34	17·40	264·25	290·0
57	,,	,, ,,	2 *Strongylocentrotus* . . .	,,	25·0	,,	8	117	50	34	18·05	229·22	247·7
58	,,	1 *violet*-white ,,	1 *violet*-white *Sphærechinus*	,,	24·6	,,	8	123	87	8·1	17·93	231·86	241·0
59	,,	,, ,,	1 white ,,	,,	25·2	,,	8	190	97	56	18·75	279·80	339·8
60	9.vii.	5 *Strongylocentrotus* . .	3 *Strongylocentrotus* . . .	,,	23·3	24·1	8	25	92	96	24·49	90·17	94·3
61	11.viii.	8 *Strongylocentrotus* . .	1 *Strongylocentrotus* . . .	,,	23·6	23·5	8	35	100	63	29·97	110·42	115·4
62	,,	,, . .	2 *Dorocidaris*	,,	23·6	,,	8	52	19	2·9	30·07	128·66	130·5
63	20.viii.	8 *Strongylocentrotus* . .	1 *Strongylocentrotus* . . .	,,	23·9	23·2	8	72	100	75	31·09	106·64	118·0
64	,,	,, . .	4 white *Sphærechinus* . .	,,	24·0	,,	8	45	67	51	30·23	164·35	171·7
65	,,	,, . .	5 *violet*-white *Sphærechinus*	,,	24·0	,,	8	68	40	18	27·05	88·07	89·3
66	,,	6 *violet*-white *Sphærechinus*	4 white ,,	25	24·0	,,	8	150	11	1·0	18·37	311·86	313·3
67	31.viii.	3 *Strongylocentrotus* . .	3 *Strongylocentrotus* . . .	50	22·6	22·9	8	75	100	76	30·80	98·84	110·1
68	10.ix.	3 *Strongylocentrotus* . .	2 *Strongylocentrotus* . . .	,,	22·8	23·2	8	178	100	65	32·36	70·18	86·2
69	28.ix.	6 *Strongylocentrotus* . .	3 *Strongylocentrotus* . . .	,,	21·2	20·0	8	45	89	67	32·25	110·58	117·2
70	12.x.	. . . . .	. . . . .	,,	16·5	17·3	8	160	100	97	33·38	88·80	116·3
71	11.xi.	1 *Strongylocentrotus* . .	1 *Strongylocentrotus* . .	,,	14·0	13·9	8	282	100	93	29·53	99·09	151·1
72	,,	,, . .	,, . .	,,	,,	13·0	24	,,	,,	,,	29·89	39·81	..
73	,,	,, . .	2 white *Sphærechinus* . .	10	13·9	13·9	8	97	1·0	0·2	24·83	64·47	64·5
74	,,	3 white *Sphærechinus* . .	2 white ,, . .	50	13·7	,,	8	72	100	88	17·03	186·95	207·6
75	,,	,, ,, . .	1 *Strongylocentrotus* . .	,,	13·9	,,	8	70	21	7·1	21·97	71·48	72·0
76	,,	4 *violet*-white *Sphærechinus*	3 *violet*-white *Sphærechinus*	,,	13·8	,,	8	97	100	100	17·71	181·55	216·8
77	,,	,, ,,	,, ,,	,,	,,	13·0	24	,,	,,	,,	17·13	210·88	..
78	,,	,, ,,	,, ,,	,,	,,	,,	30	,,	,,	,,	17·64	147·11	..

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE (continued).

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
79	11.xi.	4 *violet*-white *Sphærechinus*	1 *Strongylocentrotus* . .	25	13·8	13·9	8	133	6·0	1·0	21·29	68·96	69·0
80	,,	2 *white*-brown ,,	2 *white*-brown *Sphærechinus*	50	13·7	,,	8	100	99	100	17·91	175·84	210·8
81	,,	1 *brown*-white ,,	2 *brown*-white ,,	,,	13·6	,,	8	42	100	59	17·24	118·70	124·1
82	,,	,, ,,	1 *Strongylocentrotus* . .	18	13·6	,,	8	50	3·0	3·0	18·19	56·51	56·7
83	24.xi.	2 *Strongylocentrotus* . . .	2 *Strongylocentrotus* . .	50	13·9	12·3	8	82	100	85	30·86	92·40	105·3
84	,,	3 *Echinus*	2 *Echinus*	,,	,,	12·6	3	19	63	53	26·26	68·89	..
85	,,	,, ...	,, ...	,,	,,	12·3	8	,,	,,	,,	31·06	130·92	133·4
86	,,	,, ...	2 *Strongylocentrotus* . .	,,	,,	,,	8	23	87	87	31·53	114·51	119·1
87	,,	,, ...	2 *Sphærechinus*	,,	,,	,,	8	37	62	59	29·80	117·35	122·8
88	,,	,, ...	2 *Arbacia*	,,	,,	,,	8	67	45	45	29·81	114·65	121·4
89	,,	,, ...	2 *Echinocardium* . . .	,,	,,	,,	8	77	52	35	30·72	117·71	124·1
90	,,	2 *Sphærechinus*	2 *Sphærechinus*	,,	,,	,,	8	63	100	95	18·17	150·49	168·6
91	,,	5 *Echinocardium*	2 *Echinocardium* . . .	10	,,	,,	8	9	78	7·0	31·16	139·00	139·2
92	,,	,, ...	2 *Strongylocentrotus* . .	20	,,	,,	8	30	67	2·5	29·86	128·37	128·5
93	,,	,, ...	2 *Echinus*	9	,,	,,	8	17	6	3·5	27·33	130·31	130·5
94	,,	,, ...	2 *Sphærechinus*	6	,,	,,	8	10	50	4·0	23·62	142·10	142·2
95	,,	,, ...	2 *Arbacia*	22	,,	,,	8	23	74	3·0	26·80	132·60	132·8
96	3.xii.	5 violet *Strongylocentrotus*	4 violet *Strongylocentrotus*	50	13·4	13·5	8	38	100	92	32·10	96·22	103·0
97	,,	,, ,,	6 *Echinus*	,,	,,	,,	8	107	16	12	25·79	98·82	101·2
98	,,	3 green ,,	4 green *Strongylocentrotus*	,,	,,	,,	8	67	100	100	33·39	99·88	113·3
99	,,	3 black ,,	5 black ,,	,,	,,	,,	8	107	93	34	31·23	91·85	98·5
100	,,	,, ,,	6 *Echinus*	21	,,	,,	8	68	2·2	0·15	25·24	87·77	88·0
101	,,	2 *Sphærechinus*	5 *Sphærechinus*	50	,,	,,	8	88	100	82	18·39	130·80	149·7
102	,,	7 *Echinus*	6 *Echinus*	,,	,,	,,	8	85	92	69	32·35	133·35	149·0
103	,,	,, ...	4 violet *Strongylocentrotus*	,,	,,	,,	8	129	93	93	31·87	128·60	159·3
104	,,	,, ...	4 green ,,	,,	,,	,,	8	127	87	85	32·44	128·72	156·2

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE (continued).

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
105	3.xii.	7 *Echinus*	5 black *Strongylocentrotus*	50	13·4	13·5	8	145	88	81	30·98	127·04	156·9
106	,,	,,	5 *Sphærechinus*	,,	,,	,,	8	57	84	84	30·22	116·04	127·2
107	13.xii.	3 *Strongylocentrotus*	2 *Strongylocentrotus*	,,	12·3	12·6	8	104	94	80	30·72	101·47	118·3
108	,,	,,	,,	,,	,,	11·0	31	,,	,,	,,	30·81	55·87	..
109	,,	,,	,,	,,	,,	20·5	8	70	93	93	35·55	110·79	125·7
110	,,	1 *Strongylocentrotus*	,,	,,	12·1	12·6	8	257	34	29	25·31	87·47	100·5
111	,,	3 *Strongylocentrotus*	2 *Strongylocentrotus*	,,	12·1	,,	8	112	21	12	34·10	77·28	79·3
112	,,	3 white *Sphærechinus*	2 white *Sphærechinus*	,,	12·3	,,	8	110	100	85	16·11	104·97	124·2
113	,,	,, ,,	,, ,,	,,	,,	20·5	8	140	100	100	17·56	288·41	369·7
114	,,	2 *violet*-white *Sphærechinus*	6 *violet*-white *Sphærechinus*	,,	,,	12·6	8	120	100	78	18·62	163·38	194·0
115	,,	2 *brown*-white ,,	5 *brown*-white ,,	,,	,,	,,	8	56	100	100	17·52	165·21	184·2
116	,,	7 mixed *Sphærechinus*	2 *Strongylocentrotus*	28	,,	,,	8	70	1·0	0·43	20·08	32·85	32·9
117	,,	,, ,,	,,	23	,,	,,	8	70	7·1	1·1	22·42	38·56	38·6
118	,,	,, ,,	2 *Echinus*	4	,,	,,	8	90	0·2	0·05	21·45	37·45	37·5
119	,,	4 *Echinus*	,,	50	,,	,,	8	77	95	88	30·58	154·97	176·0
120	,,	,,	,,	21	,,	11·0	31	,,	,,	,,	30·52	38·52	..
121	,,	,,	2 *Strongylocentrotus*	50	,,	12·6	8	95	89	89	36·92	101·69	118·9
122	,,	,,	,,	,,	,,	11·0	31	,,	,,	,,	35·06	70·58	..
123	,,	,,	2 *Strongylocentrotus*	25	,,	12·6	8	104	6·7	0·67	34·98	97·49	97·6
124	22.xii.	4 *Strongylocentrotus*	2 *Strongylocentrotus*	50	10·4	10·0	8	56	96	41	27·94	63·20	66·1
125	,,	,,	,,	,,	,,	24·0	8	56	96	30	34·10	132·41	137·8
126	,,	,,	6 *Echinus*	,,	,,	10·0	8	33	36	9·1	25·59	69·24	69·6
127	,,	4 *Sphærechinus*	1 *Sphærechinus*	,,	,,	,,	8	80	100	71	15·63	84·75	94·6
128	,,	3 *Echinus*	6 *Echinus*	,,	,,	,,	8	43	86	46	28·58	96·08	100·0
129	,,	,,	2 *Strongylocentrotus*	,,	,,	,,	8	90	80	39	31·46	91·46	97·9
130	,,	4 *Echinocardium*	2 *Echinocardium*	17	,,	10·3	5	3	10	6·7	20·12	111·84	111·9

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE (continued).

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
131	24.xii.	1 *E. microtuberculatus* . .	1 *Echinus acutus*	50	9·2	9·7	8	218	99	13	22·24	80·24	84·8
132	31·xii.	2 *Strongylocentrotus* . . .	3 *Strongylocentrotus* . . .	,,	10·1	11·4	8	168	99	40	29·41	65·75	74·7
133	,,	,, . . .	,, . . .	,,	,,	15·9	8	,,	,,	8·9	33·39	104·02	107·1
134	,,	,, . . .	,, . . .	,,	,,	20·4	8	,,	,,	57	35·47	98·41	117·1
135	,,	,, . . .	,, . . .	,,	,,	23·7	8	,,	,,	38	36·04	111·24	111·4
136	,,	,, . . .	1 *Strongylocentrotus* . . .	,,	10·4	11·4	8	317	87	85	32·24	74·63	115·0
137	,,	,, . . .	1 *Strongylocentrotus* . . .	,,	10·7	,,	8	223	95	81	26·09	50·56	52·3
138	,,	1 *Strongylocentrotus* . . .	3 *Strongylocentrotus* . . .	,,	,,	,,	8	222	93	33	30·43	41·80	47·8
139	,,	2 *Strongylocentrotus* . . .	,, . . .	,,	,,	,,	8	107	91	36	30·01	62·29	67·0
140	,,	,, . . .	2 *Echinus*	13	10·1	,,	8	130	0·8	0·46	23·53	95·78	95·9
141	,,	,, . . .	,, ...	36	,,	,,	8	85	1·8	0·8	25·94	64·21	64·3
142	,,	,, . . .	1 *Echinus*	50	11·4	,,	8	243	55	1·0	25·91	67·57	67·9
143	,,	3 *Sphærechinus*	2 *Sphærechinus*	50	10·1	11·4	8	112	100	36	16·87	102·24	110·6
144	,,	,, ...	,, ...	,,	,,	15·9	8	,,	,,	100	18·46	258·94	317·2
145	,,	,, ...	,, ...	,,	,,	20·4	8	,,	,,	88	17·65	302·55	361·8
146	,,	,, ...	,, ...	,,	,,	23·7	8	,,	,,	99	16·97	352·34	427·6
147	,,	,, ...	3 *Strongylocentrotus* . . .	32	,,	11·4	8	30	5	2·5	21·36	37·22	37·4
148	,,	,, ...	1 *Strongylocentrotus* . . .	26	11·4	,,	8	45	33	3·0	21·81	31·26	31·3
149	,,	3 *Echinus*	2 *Echinus*	50	10·1	,,	8	36	96	96	28·38	138·14	147·8
150	,,	,, ...	,, ...	,,	,,	15·9	8	,,	,,	98	32·18	158·67	171·9
151	,,	,, ...	,, ...	,,	,,	20·4	8	,,	,,	100	35·34	141·61	157·5
152	,,	,, ...	,, ...	,,	,,	23·7	8	,,	,,	75	35·16	159·48	168·1
153	,,	,, ...	3 *Strongylocentrotus* . . .	,,	,,	11·4	8	27	92	70	33·38	99·25	102·9
154	,,	,, ...	,, ...	,,	10·7	,,	8	98	89	66	32·79	85·46	97·2
155	,,	,, ...	2 *Echinus acutus*	,,	10·1	,,	8	32	91	22	24·44	119·38	121·1

TABLE (continued).

Number of experiment.	Date of fertilisation.	Female Echinoids used.	Male Echinoids used.	Number of larvæ measured.	Temperature of impregnation.	Mean temperature during development.	Days of development.	Number of ova per 10 cub. centims.	Per cent. number of blastulæ.	Per cent. number of larvæ.	Mean body-length.	Mean arm-length.	Corrected arm-length.
					°	°							
156	31.xii.	2 *Echinocardium mediterraneum*	1 *Echinocardium mediterraneum*	50	10·1	11·2	5	154	54	0·4	17·87	118·83	120·0
157	,,	2 *Echinocardium mediterraneum*	3 *Strongylocentrotus* . . .	2	,,	,,	5	78	17	0·06	13·60	42·90	42·9
158	,,	2 *Echinocardium mediterraneum*	2 *Echinus*	2	,,	,,	5	280	46	0·03	10·50	62·90	62·9
159	7.i.	2 *Strongylocentrotus* . . .	2 *Strongylocentrotus* . . .	50	10·9	12·2	8	195	84	65	30·95	88·04	110·0
160	,,	,, . . .	1 *Strongylocentrotus* . . .	,,	11·5	12·2	8	195	81	63	30·38	85·98	107·1
161	,,	1 *Strongylocentrotus* . . .	2 *Strongylocentrotus* . . .	,,	11·7	,,	8	103	97	95	28·00	111·23	133·1
162	,,	,, . . .	1 *Strongylocentrotus* . . .	,,	11·8	,,	8	122	88	90	29·91	88·14	108·9
163	,,	2 *Strongylocentrotus* . . .	1 *Echinus*	8	10·9	,,	8	165	2·1	0·9	27·59	61·59	61·8
164	,,	,, . . .	,, ...	25	,,	,,	8	142	2·1	0·5	29·15	65·25	65·4
165	,,	,, . . .	1 *Echinus*	45	11·7	,,	8	170	5·9	3·0	28·85	67·52	67·8
166	,,	4 *Sphærechinus* . . .	1 *Sphærechinus*	50	10·9	,,	8	300	97	92	18·29	142·35	221·3
167	,,	,, . . .	,, ...	,,	,,	20·2	8	,,	,,	100	17·34	190·37	304·8
168	,,	,, . . .	2 *Strongylocentrotus* . . .	19	,,	12·2	8	356	2·0	0·06	22·03	47·29	47·3
169	,,	6 *Echinus*	1 *Echinus*	50	,,	,,	8	157	94	75	29·24	134·99	166·7
170	,,	,, ...	,, ...	,,	,,	20·2	8	,,	,,	93	32·19	108·85	140·9
171	,,	4 *Sphærechinus* . . .	,, ...	4	,,	12·2	8	1500	0·7	0·03	23·17	27·05	27·1
172	,,	5 *Echinocardium* . . .	1 *Echinocardium* . . .	6	,,	,,	8	13	8	1·5	25·67	147·50	147·5
173	,,	,, . . .	1 *Echinus*	10	,,	,,	8	10	6	1·4	27·25	146·36	146·4