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Experiments in Hybridization, with Special Reference to the Effect of Conditions on Dominance

Leonard Doncaster

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V.—*Experiments in Hybridization, with special reference to the Effect of Conditions on Dominance.*

By Leonard Doncaster, *B.A., King's College, Cambridge.*

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1. *Introduction.*

The work described in this paper was begun at the Naples Zoological Station in the winter and spring of 1901, but the greater part of it was done during the year 1902, and, since the methods used in the earlier experiments were not entirely satisfactory, no record of them is included.

The work was suggested by the papers of Vernon (5–9) on Echinoid hybrids, in which he concludes that the differences observed in certain cases between larvæ reared at different seasons, are due to variations in the prepotency of the parents caused by changes in the maturity of the sexual cells. If this is correct, it seemed possible that other conditions acting upon the eggs or spermatozoa might cause alterations in their prepotency, and the experiments were undertaken primarily to test this supposition. That differences in the time during which the genital cells had been kept might affect the extent to which the parental characters were transmitted, was suggested in a paper by Ewart (2) in 1901, and the same thing might be inferred from the tables attached to one of Vernon's papers (9), although no mention is made of it in the text. The conclusion, however, arrived at from the work here described, is that, although the environmental conditions which affect the genital cells influence the form of the larva, yet there is no satisfactory evidence of a change of dominance caused by such conditions.

In addition to the chief object of the work, a few observations were made upon the correlation of characters in the hybrid larvæ, and upon the means used to facilitate cross-fertilisation, and the causes which hinder it, and some account of these subjects is given below.

The experiments described were made with Echinoid larvæ, and the species chiefly used were the same as those employed by Vernon in the part of his work which deals with this subject. Other species were, however, also used for comparison.

VERNON'S papers were published before the Mendelian theory of heredity had attracted the attention of biologists, and he therefore uses the word "prepotency," which has now been replaced to a considerable extent by the more precise term "dominance." "Prepotency" was used of a tendency of one parent to transmit all its characters to its offspring, while "dominance" refers to individual characters taken separately. It will be seen from the work here described, that in any hybrid Echinoid larva, one character may be dominant, while another character, which in the parent was equally well developed, may be recessive. The experiments are therefore an attempt to discover whether dominance can be influenced by the conditions which affect the genital cells, and the word "prepotency" has been omitted as far as possible. When it has been used, it is employed to describe a quality of an individual urchin, or a germ-cell, which tends to transmit a number of its characters to its offspring, while "dominance" is used of individual characters taken separately. Since nothing is known of the next generation of Echinoid hybrids, it cannot be certain that the word "dominance" has here exactly the same meaning as that given to it by MENDEL, but it is clear that "prepotency" cannot properly be used in the case in question, and "dominance" has, therefore, been used in the sense indicated.

I wish to take this opportunity of acknowledging my indebtedness to Mr. BATESON, F.R.S., for reading over this paper, and for much valuable criticism, and also to the authorities of the Naples Zoological Station for their unfailing kindness and assistance.

2. *Material and Methods.*

There are a number of species of sea-urchins found at Naples, belonging to nearly as many genera, and cross-fertilisations have been effected between almost all of them (*cf.* VERNON, 5), but comparatively few of these combinations are of any use for detailed work. In only a few instances can any considerable proportion of plutei be obtained, and some of these have other objections which render them unsuitable. A large proportion of plutei is almost always yielded by the cross *Echinus microtuberculatus* ♀ × *Strongylocentrotus lividus* ♂, and, with more difficulty, the converse cross may give good results, but the larvæ of these two species resemble one another so closely that these combinations are not suited to the present work. This is unfortunate, since these are the only hybrids that were reared beyond the metamorphosis, or, indeed, to the later larval stages. *Sphærechinus granularis* ♀ × *Strongylocentrotus lividus* ♂ yields a good number of plutei, and by far the greater part of the work was done with this cross, which is the one used by VERNON in his work on the effect of maturity on prepotency. The converse cross is exceedingly difficult to obtain, and my efforts were almost entirely unsuccessful, although, curiously enough, it was one of the first Echinoid hybrids known, made by

MARION at Marseilles in 1873, where the conditions are, perhaps, different from those of Naples. *Sphærechinus granularis* ♀ × *Echinus microtuberculatus* ♂ also yields a good number of larvæ; these were the species used by BOVERI and SEELIGER, but the larvæ seem usually less well developed than in the preceding, and I therefore made comparatively few experiments with them. *Arbacia pustulosa* ♀ × *Sphærechinus granularis* ♂ usually gives plutei, and a few experiments were made with these, and also with the same eggs fertilised by *Strongylocentrotus*, but they are not wholly satisfactory. In general, therefore, the work was done with the *Sphærechinus-Strongylocentrotus* hybrid, but occasionally the other crosses mentioned were used for comparison.

Methods.—The methods employed for obtaining and fertilising the eggs were similar to those described by VERNON and others. The urchins to be used were cut open, and the ovaries or testes turned out into small jars of clean water, those from each urchin into a separate jar, and, with a little stirring, the eggs or sperm came out at once into the water. Water from jars containing eggs and sperm of the required species was then mixed, and left for about 15 minutes, in which time the eggs settled to the bottom. As much of the water as possible was then poured off, and replaced by clean, and the eggs again allowed to settle. This was repeated till the water lost its milkiness, and the eggs were then poured into large jars of sea water (usually 3–5 litres), covered with a glass plate, and left. After about 24 hours—but the time varied according to temperature—the young blastulæ were found swimming at the surface, and were poured off from the unfertilised eggs into a jar of 3–5 litres of clean water, covered and left. In order to prevent accidental fertilisation by spermatozoa other than those used in the experiment, the water was generally kept several hours before use, the urchins were well washed in fresh water before being cut open, all instruments were washed in 90 per cent. alcohol and then in fresh water after touching a male, and the jars, &c., were carefully washed with fresh water. It was found, however, that if tank water was used which had not been kept, it was very rare to get eggs fertilised by sperm of the same species, for it had remained so long in the tank that no spermatozoa were left alive; occasionally a pure larva was found in a batch of hybrids, but it was always so easily distinguished at a glance that no mistake could arise from this cause.

A sample of the eggs was examined 12–24 hours after fertilisation, before the blastulæ had begun to swim, in order to estimate roughly the percentage of fertilised eggs and of healthy blastulæ, but exact determinations of this kind were not made. The larvæ were left in the large jars until the 8th day after fertilisation, but were examined at intervals, and note taken of their progress; if a large number died, and there was danger of the water being fouled by their decomposition, the remainder were poured off into clean water. On the 8th day a number of the plutei were removed by means of a syphon into a small beaker, and a few drops of corrosive

sublimate were stirred into the water, which killed them, and caused them to sink. They were then transferred to a small cell, and examined at once with the microscope. Frequently another batch from the same jar was killed and examined for comparison a few days later.

The plutei were examined microscopically in a small cell, covered with a cover-slip. The slide was moved by a mechanical stage, and the characters of each larva recorded in a table as it came into the field, and thus there was no possibility of recording any one twice over. Usually 50, 75, or 100 were so recorded in each batch, sometimes 150, and sometimes fewer than 50 where very few remained alive, but a count was not considered trustworthy unless at least 50 were recorded.

In many cases the larvæ were also measured with an eye-piece micrometer, but this was not considered necessary in every case, for reasons which will be given below.

An account of the characters recorded and the method employed in doing so must be postponed to the next section, in which the larvæ will be described.

The methods adopted for altering the conditions affecting the eggs and spermatozoa must now be explained. The chief differences of conditions to which they were subjected were the following :—

1. Maturity. The experiments lasted over a period from February to July, and some additional ones were made in December, so that considerable differences of maturity in the gonads were involved.
2. Temperature at which the parent urchins were kept. This varied according to season from about 12–23° C.
3. Length of time the parents had been kept in the tanks. This varied from very few hours to nearly five weeks.
4. Age of parents. This was of course not known, but some were small with thin shells and obviously immature ; others strongly developed.

   The above differences of environment affected the parent urchins, but may have had influence on their sexual cells. The following are artificial conditions imposed on the eggs or spermatozoa after being shed.
5. Freshness or staleness. The eggs and spermatozoa were mixed after one or both had been kept varying lengths of time up to 30 hours, or sometimes longer.
6. Temperature. They were kept at various temperatures before fertilisation, and this took place at different temperatures.
7. Concentration of the water. They were kept in sea water to which various proportions of fresh water had been added, or which had been concentrated by evaporation.
8. Shaking. The eggs were in some cases shaken until many began to break up or lose their shape.

Whichever of these last methods was adopted, one part of the eggs was fertilised normally, while the other underwent the treatment required, and the larvæ produced

by the two parts were compared. Usually one female and one male were used in each fertilisation, sometimes the eggs or sperm of more than one were mixed. A difficulty arose about keeping the eggs for a considerable time before fertilisation, for if the same male were used, it would necessitate also keeping the sperm, and if a different male, an error arises owing to the difference of dominance in different individuals. It was found that if one inter-radius was removed, with its testis, and was used to fertilise the fresh eggs, the urchin continued to live and apparently flourish for several days in the tanks, but apparently the sperm was seriously impaired by the body-cavity fluid being replaced by sea water, as necessarily happened, for it only gave exceedingly poor larvæ, both in number and vigour. It was thought best therefore, either to mix the sperm of several males, so as to get an average in each case, or to use one fresh male in each fertilisation, but to test its "prepotency" as far as possible by also using it to fertilise other females. This is of course not a certain method, but if the sum of the experiments were strongly in one direction, the evidence would be nearly as good as if the same urchin could always be used in each experiment of the pair. In the case of the other changed conditions, such as temperature or salinity, the same urchins could be used in each experiment.

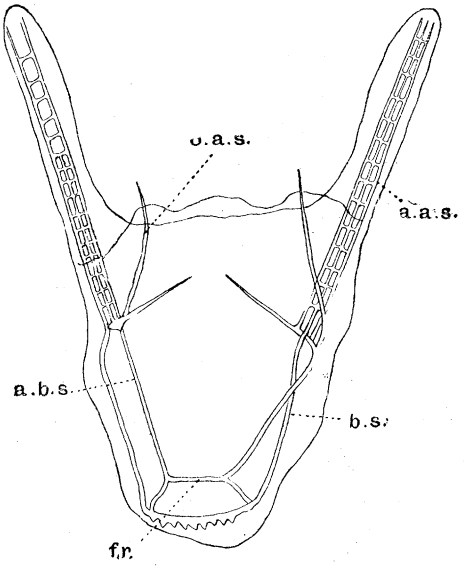
3. *Description of the Larvæ and the Methods of counting them.*

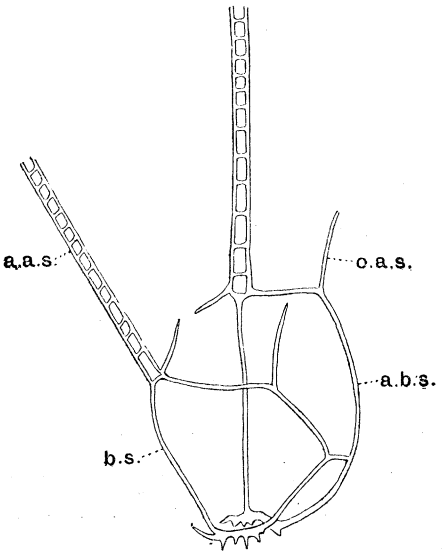
Before explaining the method adopted for estimating the parental characters in the hybrid larvæ, it is necessary to describe the chief characters of the parent species, and of the hybrids. The greater part of the experiments were made upon *Sphærechinus granularis* and *Strongylocentrotus lividus*, and these will be dealt with first, but it is not necessary for me to give such a detailed description as would otherwise be required, for in June, 1902, a paper was published by Steinbrück (4) upon the larvæ of these two species and their hybrids, giving very full descriptions and illustrations of normal and abnormal types.

Figures 1 and 2 illustrate the chief features of normal *Sphærechinus* larvæ, and since full descriptions of these have been published by Vernon (5) and Steinbrück (4) and others, a detailed description is unnecessary. If these figures are compared with those of *Strongylocentrotus lividus* (figs. 3–4) it will be seen that the chief differences are that *Sphærechinus* is more plump in shape, and has arms diverging at a wider angle. In the skeleton the "body-skeleton" is relatively short and without the club at the apex which characterises *Strongylocentrotus*, and it has in addition the bars which I have called "anterior body-skeleton," and a four-sided "frame" at the apex. No trace of the "frame" is ever found in *Strongylocentrotus*, but in rare cases there may be rudiments of the "anterior body-skeleton" attached to the oral arm-bars at the point where they bend up into the arm.

The anal arm-skeleton in the two species differs in that *Sphærechinus* has two or three rods running from the base of the body-skeleton to the end of the arm, and

these are connected by cross-bars which, in healthy larvæ, usually number over 20. In *Strongylocentrotus*, on the other hand, there is typically only one anal arm rod, but





Figs. 1 and 2.—Typical *Sphærechinus* larvæ.*

rarely (fig. 4) there is a rudiment of a second, which arises from the point when the primary rays diverge. The arm-skeleton in well-grown *Sphærechinus* larvæ varies

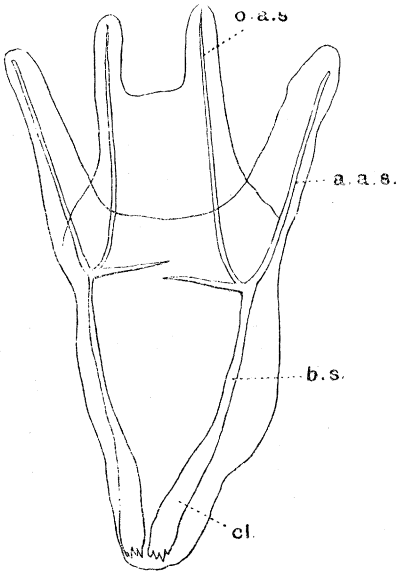


Fig. 3.—Typical *Strongylocentrotus* larva.

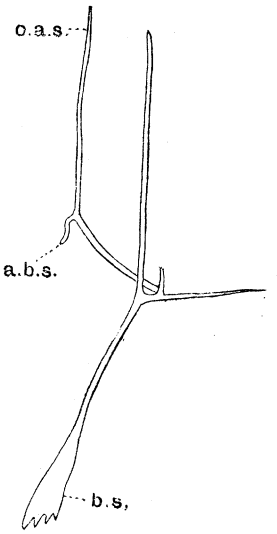
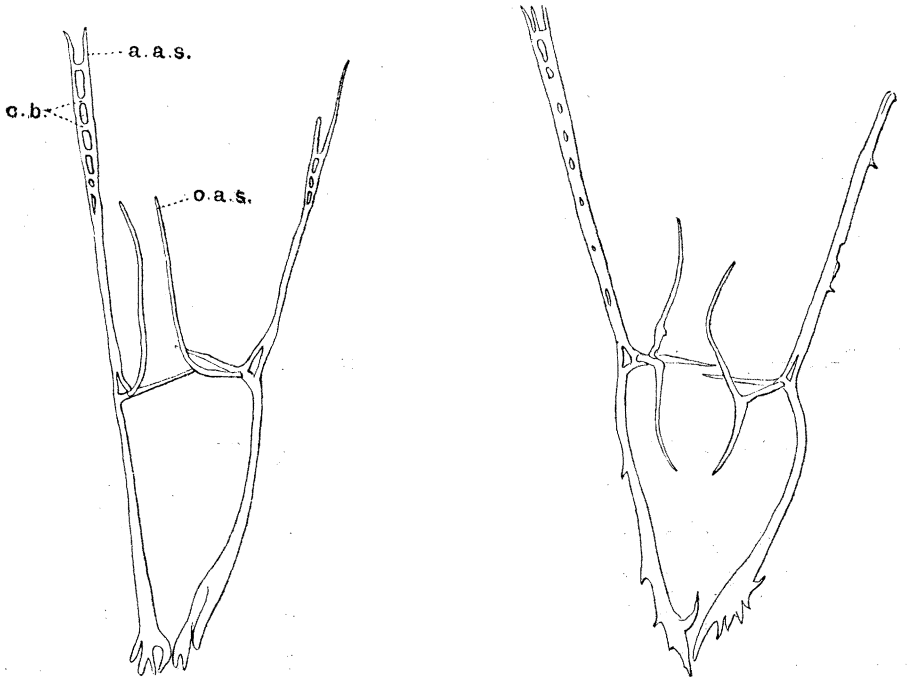


Fig. 4.—Half skeleton of abnormal *Strongylocentrotus* larva.

from less than twice to more than three times the length of the body-skeleton, while in *Strongylocentrotus* the arms are usually about equal in length to the body, but these lengths vary very greatly with age and conditions.

* a.a.s., anal arm-skeleton; a.b.s., anterior body-skeleton; b.s., body-skeleton; cl., club; c.b., cross-bars; r., "frame"; o.a.s., oral arm-skeleton.

Before proceeding to describe the chief types of hybrid larvæ, it may be pointed out that in both *Sphærechinus* and *Strongylocentrotus*, but especially in the latter, abnormalities occur tending towards a condition normal in the other species;* Steinbrück has collected and figured a number of these and lays great emphasis upon their importance, as showing that when similar forms occur in the crossed larvæ, they need not necessarily be due to the influence of the parent which they resemble, but may be due to a tendency in that direction, which is always present, but only called forth under exceptional circumstances. This is undoubtedly an objection to the use of these two species, but it has probably no great weight, for the abnormalities described are so rare that I have never seen a pronounced one among a large number of larvæ examined.

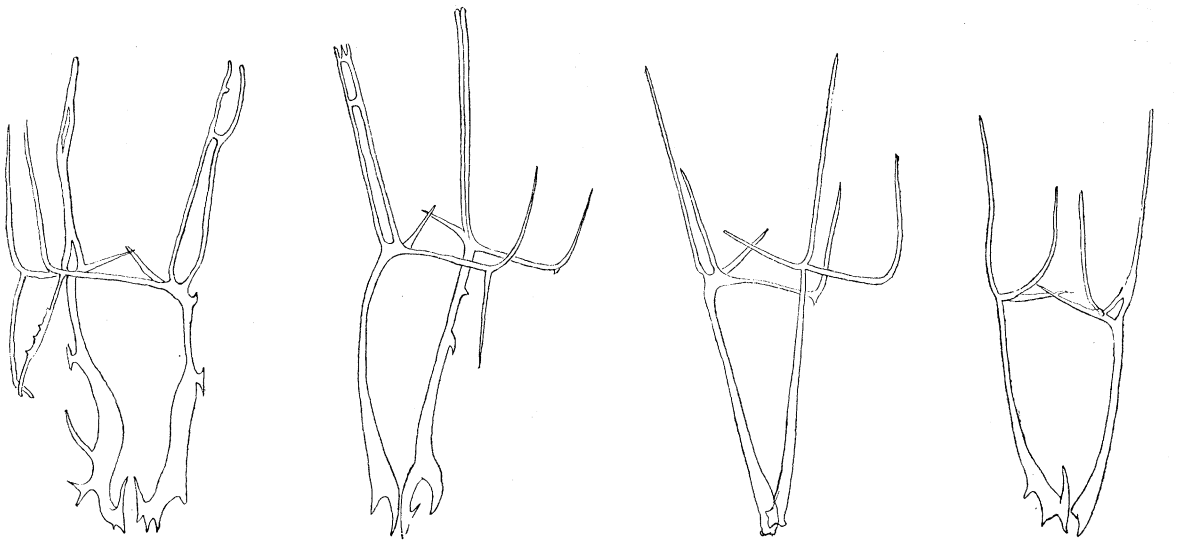


Figs. 5 and 6.—Types of hybrids (*Sphærechinus* ♀ × *Strongylocentrotus* ♂). Only skeleton shown.

Turning now to the hybrids between the two species described, it is to be noticed that they are, on the whole, intermediate between the parent types, and hybrid larvæ exactly resembling either parent never occur. Figs. 5 to 10 represent the chief forms observed, but the minor variations are endless in their combinations, and a large

* It is of some interest that when rudiments of a second arm-bar or anterior body-skeleton occur in *Strongylocentrotus* they rather frequently occur together, *e.g.*, out of 45 abnormal larvæ observed, 22 had a small additional arm-bar, 8 rudiments of anterior body-skeleton, and 15 had the two together. Since many hundreds of larvæ had to be examined to find these cases, it is plainly not chance alone which causes both abnormalities to appear in the same individual in so many instances. It should be noted that the larvæ having these abnormalities frequently showed other irregularities, and were often pathological.

series of figures is given by STEINBRÜCK, almost every one of which might be matched among my larvæ. On the whole, in general shape the *Strongylocentrotus* type is predominant, for the larvæ are narrow with pointed apex, and usually with comparatively short anal arms, while the oral arms are larger in proportion than in *Sphærechinus*. In the skeleton there is the utmost diversity, and very frequently



Figs. 7–10.—Types of skeleton in *Sphærechinus* ♀ × *Strongylocentrotus* ♂ hybrids.

the two sides of the body differ widely from one another. The body skeleton usually resembles that of *Strongylocentrotus* in general form, but the club is very variously developed and there may be no "thorns," or any stage up to large branches pointing up or down; thorns of some kind are, however, generally present. The apical "frame" is never found, as far as my experience goes, but the "anterior body-skeleton" occurs in all stages between complete absence and strong rods clubbed at their apical ends, with or without thorns. The transverse rods of the primary "Dreistrahler" are nearly always long enough to cross. The anal arms may have only one bar, but this is rare, and its appears that in this case the rod always arises from the point of junction of the three primary rays, not from near the base of the vertical body-bar as in *Strongylocentrotus*. Usually there is at least a rudiment of a second bar, which may at once join the chief one, or remain separate from it. This second bar may be of any length up to equality with the other; if shorter than it, it may meet and fuse with it at its end or remain separate, or meet it and separate again higher up. The arm-bar may also bifurcate, so that it is single proximally, double distally, or it may be of double thickness throughout its length, sometimes with perforations indicating that it consists really of two fused bars (fig. 6). There may also be a third bar, usually in the same plane as the other two, which may be shorter than they, or of equal length. There may also in rare cases be rudiments of more

than three bars. Finally there may be cross-pieces as in *Sphærechinus*, usually one, two or three, but not rarely a larger number; these may be associated with any of the above conditions where they are possible.

As mentioned above, a larger or smaller rudiment of the "anterior body-skeleton' may arise from the oral arm-bar at its angle, or there may be traces of more than one and sometimes a backwardly directed rod arises nearer the distal end of the arm-bar; a larger or smaller rod may also arise from near the base of the oral arm-bar, *i.e.*, near its point of origin from the centre of the primary "Dreistrahler," or even from the body-skeleton itself, and point towards the apex, probably both these last conditions are due to the "anterior body-skeleton" appearing in an abnormal position. It may also happen, in some cultures very frequently, that the larva has one or both of the oral arm-bars turned down towards the apex instead of up into the arm; when this is the case the oral arms are usually not developed, and the whole larva is badly grown. The same malformation may occur in pure *Strongylocentrotus* larvæ.

The above account of the chief features of the hybrids will make the method used in recording the characters easily understood. Only skeletal features were recorded, because they are the most definite, and are not affected by the reagents used for preserving or mounting. In the earlier experiments a record was kept of five distinct skeletal characters, and also of the general shape; the characters in question were: (1) The number and length of the anal arm-bars; (2) the presence or absence of cross-bars between them; (3) the length of the "anterior body-skeleton" if present; (4) the size of the club at the apex of the chief body-skeleton; and (5) the size of the thorns or branches at the apex. After some experience, however, the last two, and the general shape, were omitted, owing to the impossibility in the case of the club and shape, of estimating them satisfactorily, and with the branches, because they seemed to have no connection with the other characters, and could not be referred to the influence of either parent with certainty. Each of the characters mentioned was sub-divided, a table drawn up on which the divisions and sub-divisions were marked out, and the characters of each larva as it was, brought into the field of the microscope, were marked down in their appropriate place. The condition of the anal arm-bars was classified into six sub-divisions, according as there were (i) three complete, (ii) two complete, (iii) two on one side and one on the other, (iv) one complete and a second large but incomplete, (v) one and a small piece, and (vi) one only. The condition of the "anterior body-skeleton" was similarly divided into four, viz., (i) large, (ii) small, (iii) very small, and (iv) quite absent. The others have each three divisions, viz., largely developed, poorly developed, and absent.

This classification is somewhat arbitrary, but the time required for the exact measurement of each character made that course quite impracticable, and the method adopted was developed as experience directed. At first only three sub-divisions were used in each class, but it soon became evident that in two of the classes at

least more were needed, and frequently several combinations had to be included in each division in order not to increase their number to an impracticable extent.

The sub-divisions indicated above need, therefore, some further explanation, since in some, conditions are included which should perhaps have been separated from one another. I will take them in the order in which they are described above :—

(1) Arm-bars. (i) "Three" includes three on each side, or three on one and two on the other. (ii) "Two" includes only two on each side, with or without rudiments of a third. (iii) "Two and one" includes cases where on one side there are at least two complete bars, and on the other less than two, but if the second bar on one side is nearly equal in length to the full one, the larva is classified as "two complete." (iv) "One and a-half" means that on one side at least there is one full bar and a second as much as half as long. The second may arise proximally or distally by bifurcation. (v) "One and a piece" (represented on the table as "1*") includes cases where on one side there is one full bar and a rudiment of a second less than half as long; the other arm may be similar, or have only one simple bar. The rudiment may be quite separate, or may join the main bar, or may arise at its end by bifurcation. (vi) "One" means one on each side, with no rudiment of a second, and occurs very rarely in summer, but is commoner in early spring. Where one thick arm-bar was found, as happened rather frequently in the summer, if there were no further indication of its duplicity, it was counted as one (or commonly "1*," for it would nearly always be double just at its origin), but if it had perforations it would be called two, or "one and a-half" if it appeared double through only part of its length, and the bridges separating the perforations would be counted as cross-bars.

(2) The cross-bars were divided into three sub-divisions, "many" if there were more than four, "few" if four or less, and "none" if quite absent; the arm which had the larger number was the one counted, *e.g.*, if there were five on one side and three on the other, the larva was recorded under "many," if one on one side and none on the other, under "few."

(3) Anterior body-skeleton. The classification was into (i) "large" if the rod was more than half the length of the body, (ii) "small" if less than this, but well developed, (iii) "very small" if only a rudiment, and (iv) absent. As with the cross-bars, the side on which the greatest development was found was that recorded. In a considerable number of the later experiments, a record was kept of whether the two sides were symmetrical or not with regard to cross-bars and body-skeleton, and the results observed will be described in the section on the correlation of characters.

The remaining three characters were discarded in the later experiments as being impossible to estimate satisfactorily, and are not included in the tables. My

conclusions are, therefore, founded on a study of the condition of three skeletal characters, viz., the anal arm-bars, the cross-pieces between them, and the "anterior body-skeleton," together with measurements of the length of the anal arms relatively to the body (see next section). Comparatively little use is made of other characters. It will be noticed that in all the characters used, the presence of a certain feature indicates the influence of the *Sphærechinus* parent; its absence, that of the *Strongylocentrotus*. This is an unfortunate circumstance, for, as will be shown below, the absence of these characters may be due to other causes beside the failure to inherit them, or the dominance of the *Strongylocentrotus* type. This defect would be remedied if the converse cross could be obtained, but my efforts were not successful. A few *Echinus* ♀ × *Sphærechinus* ♂ larvæ were reared, but not enough to be of much value, and it was in the hope of escaping from this difficulty that the experiments were made with *Arbacia*.

It remains to describe the larva of *Echinus microtuberculatus* and its hybrids; the only other species used, *Arbacia pustulosa*, will be dealt with below in the account of the experiments made with it. The larva of *Echinus microtuberculatus* is remarkably like that of *Strongylocentrotus lividus*; when young, the two are hardly distinguishable, but later the *Echinus* larva becomes distinguished by a sort of fan of thorns or projections which grow out from the clubbed ends of the body-skeleton, and join one another so as to form a kind of bridge between the two bars of the body-skeleton at the apex. There is no other constant feature to separate the two larvæ, but the average proportions of the body are rather different, and *Echinus* seems less apt to produce additional arm-rods or anterior body-rods.

The hybrids between *Sphærechinus* ♀ and *Echinus* ♂ are not distinguishable with certainty from those of the same female by *Strongylocentrotus* ♂. They seem to have, as a rule, larger thorns and branches on the body-skeleton, and the *Sphærechinus* characters perhaps rather less developed. The larvæ are generally smaller and less healthy than the *Strongylocentrotus* cross, and for this reason were used only rarely.

4. *Measurement of Larvæ.*

Beside the enumeration of characters described in the last Section, in many cases the larvæ were measured for comparison. Vernon, in his earlier papers on *Echinoid* larvæ, based his results almost entirely on this method; the anal arms and length of the animals were measured, and his conclusions derived from the average lengths and their ratios. In the larvæ of *Sphærechinus granularis* of about 8 days old, the arms average about twice the length of the body, and in *Strongylocentrotus lividus* the two lengths are approximately equal; a hybrid, therefore, was said to be of the *Sphærechinus* type, of the *Strongylocentrotus* type, or intermediate, as the ratio of its arm-length to body-length approached that of one or the other, or lay between them.

My experience, however, led me very early to reject this method as untrustworthy,

and VERNON seems to have come to the same conclusion, for in one of his later papers (9) he relies on more purely morphological characters. The reason for this decision is that the arm-length, and hence the ratio between arms and body, varies very widely according to the rate of growth and environment, beside being very variable in larvæ of the same batch. VERNON has given a valuable body of evidence to show how these lengths and ratios differ according to temperature and other conditions under which the larvæ develop, and also according to the condition of the eggs and sperm at the time of fertilisation, and corrections are given in his measurements of hybrids founded on these results. But I think he under-estimated the effect of conditions both in the parent species, and to a much greater extent in the hybrids, for even if the constants for the pure larvæ were correct, they could not be applied with safety to the hybrids, which are much more sensitive. The evidence for these statements will be given below, but I may here point out that a weakly grown larva may be almost or wholly without arms when 8 days old, as was the case with parthenogenetic larvæ of *Strongylocentrotus* which I obtained by LOEB'S methods. The arms may also become actually shorter if the larvæ are kept beyond about 10 days without food, but this almost certainly happens by the dying out of the larger larvæ, not by reduction in individuals.

In consequence, therefore, of these sources of error, measurements were regarded as an untrustworthy indication of parental influence in the hybrids, and in the earlier experiments they were not made at all. Later, however, it was found advisable to have measurements for comparison with the other characters, and since many samples of the earlier larvæ had been kept and mounted, they could be obtained for these also. Following VERNON, I measured only skeletal lengths, since these are not affected by reagents; the arm-length is, therefore, the length of the anal arm-skeleton, from its point of origin from the body skeleton to its extremity, and similarly the body-length is the length from the point of meeting of the three primary rays to the apex. The measurements were made with an eye-piece micrometer, and to be converted into an actual fraction of a millimetre must be multiplied by a constant (about ·013). It was found that the body length was comparatively little affected by external conditions, and that it varied much less than that of the arms; in some cases, therefore, only the arms were measured, in others both arms and bodies. Usually 20, 25 or 30 larvæ were measured, and an average taken; these numbers were found to give a sufficiently accurate result.

5. *General Account of the Experiments.*

The experiments here described were undertaken in order to determine, firstly, the truth of VERNON'S hypothesis that the differences observed in certain hybrids at different seasons were due to changes in the condition of the sexual cells, and secondly, to find out whether by artificially subjecting the cells to various artificial conditions

before fertilisation, differences could be produced in the larvæ. All the experiments from which any result was reached are given in the tables in chronological order, and here only a general account of them will be given, explaining their object and results. In the actual work it frequently happened that one experiment led up to another, but since each lasted over at least 8 days, and sometimes longer, others were carried on in the meanwhile, and hence no logical sequence appears in the tables.

In the tables a summary is given of the conditions under which fertilisation took place, the percentages of each of the characters described above, the number of larvæ counted, the range of temperature during the experiment, and in some cases the average lengths of the body and arm-skeleton. In the last column are added notes on the percentage of eggs fertilised, and on any point of interest which appeared in the experiment. For further explanation of the tables, and the signs and contractions used in them, see the explanation of tables at the end of the paper.

The greater part of the work was done with the hybrid *Sphærechinus granularis* ♀ × *Strongylocentrotus* ♂ *lividus*, so that these experiments may conveniently be taken first. Not all the series of experiments dealt with one set of conditions only, for eggs from the same urchin were often used for different purposes, *e.g.*, to test the effects of staleness, and also of dilution with fresh water, so that in the following account a given series of experiments may be referred to more than once. The experiments made to determine the effects upon the larvæ of different conditions acting upon the eggs will be described in the following order:—(1) freshness and staleness; (2) temperature; (3) concentration of the water; (4) maturity. Finally, experiments made for the purposes not included under any of these heads will be described, the chief of which are those made to discover the effects of conditions acting during the growth of the larvæ, *e.g.*, temperature, overcrowding, &c.

1. *Effects of Staleness of the Eggs and Sperm.*—The chief experiments testing the differences between larvæ reared from fresh and from stale eggs, are included in the series 10–14, 59–63, 71–76, 85–88, 125–127, 131–132 and 184–188.

In some of these, especially the earlier ones, the larvæ from stale eggs differed only slightly from those obtained from fresh, but in others the difference is considerable, and when this is the case, it is always seen that there are fewer larvæ showing the more marked maternal characters among the larvæ obtained from eggs which have been kept. This is seen particularly on comparing the percentage of cross-bars and anterior body-skeleton in Nos. 123 and 125, or in 128 and 132. By itself, then, this would suggest that keeping the eggs for a considerable number of hours tended to reduce their prepotency, but it will be pointed out below that another explanation is possible.

The converse experiment, of keeping the sperm for some hours before fertilisation, was made in the series 147–151, 165–166, 167–168, 187–188, 244–248, 272–277, 354–357, 368–372. The sperm cannot be kept so long as the eggs, since the spermatozoa die much more easily, but if the hypothesis that decreased vitality of the sexual cells

causes a reduction of their prepotency is correct, in the cases where the sperm was kept before it was used the resulting larvæ should show an increase in maternal characters. This is, however, not generally the case; in many of these experiments the larvæ derived from stale sperm have an obvious reduction in maternal characters, although this is not so marked as in some of the experiments with stale eggs, and in no case is there a noticeable increase in the *Sphærechinus* characters in the larvæ reared from stale sperm.

2. *Temperature.*—The chief experiments on differences of temperature affecting the eggs before and during fertilisation are included in the series 10–11, 56–58, 68–69, 71–76, 85–86, 241–242A, 251–254. The eggs were subjected to temperatures considerably above the normal water temperature for a varying number of hours before fertilisation; and the temperature at which fertilisation took place was also varied, and the effects on the larvæ noted. No definite conclusion was, however, arrived at; the results were very contradictory, for in some instances the larvæ from eggs which had been warmed appeared to have a higher percentage of maternal characters, while in the greater number of cases the opposite was observed. In one case also the eggs were killed, although subjected to a temperature which seemed to have no harmful effect upon the eggs of other urchins. Probably the effects observed depended largely on the rapidity with which the change of temperature took place; if it was rapid, the vitality of the eggs was probably reduced, but if gradual, it had no great effect. The temperature of fertilisation had equally contradictory results, so that no definite conclusions can be based upon these experiments.

3. *Concentration or Dilution of the Water.*—In the series 10–12, 23–27, 33–36, 52–54, 59–61, the experiment was made of diluting the water in which the eggs were lying for a few hours before fertilisation. The eggs were placed in sea water mixed with various proportions of distilled or tap water, and before being mixed with sperm were put back into normal sea water. It was found that the water could be diluted up to a very considerable extent without injuring the eggs, but no very constant effects were observed in the larvæ. On the whole, they were perhaps rather less well developed, and had the maternal characters slightly reduced. The most marked effect of the dilution was to render the fertilisation of the eggs by sperm of another species very much more easy (*cf.* percentages fertilised in Nos. 23–27); this fact will be referred to again below.

Dilution of the sperm with fresh water could not be carried on to the same extent without destroying its vitality, but larvæ obtained from sperm which had been in water containing 33 per cent. of tap water for half-an-hour (Nos. 173–175) did not differ greatly from those fertilised by fresh sperm.

A few observations were made on the results of concentrated sea water acting upon the eggs (Nos. 89–90, 135–138, 157–159), but in these again the larvæ were but little affected.

4. *Maturity.*—Apart from the question of the seasonal change observed in the

hybrid larvæ, which will be discussed below, some experiments were made with urchins of which the sexual cells were probably in various stages of maturity. It is not very easy, however, to say to what extent a given urchin is "mature," for its gonads may be very small, and yet contain perfectly functional eggs or sperm. The *Sphærechinus* vary somewhat in the size of the gonads, and in the proportion of immature eggs with large vesicular nuclei, but there does not seem to be a very regular seasonal change. The *Strongylocentrotus*, on the other hand, and also *Echinus*, become almost immature in the summer, so that it is difficult to obtain sperm, and they do not appear to regain their condition until the early spring. In Nos. 263–266 and 278–280 eggs of a ripe female *Sphærechinus* were fertilised respectively by males with well and feebly developed testes, but the difference in the larvæ was only slight, and did not suggest a difference in dominance. It was hoped that by keeping the urchins in tanks without food for a considerable time before use that the ripe cells would be discharged, and others not ripen entirely, and experiments made to compare these with freshly caught specimens are recorded in Nos 93–96, 121–124, 221–223, 258–259. The only general result, however, seems to be that the larvæ of which either parent has been starved are smaller and have fewer of the *Sphærechinus* characters than those from freshly caught parents, and there is again no evidence of a change of dominance.

Attempts were also made to get eggs which were less mature by cutting open the urchins, but not removing the eggs from the ovaries for some hours. This treatment causes a number of eggs to be discharged from the genital pores, and presumably they are the riper ones, and those subsequently taken from the ovaries less ripe, but no definite result was obtained from a comparison of larvæ reared from such eggs with those treated normally. No direct evidence was therefore obtained that dominance of character is influenced by maturity of the eggs or spermatozoa.

A summary has now been given of the more important experiments on exposing the eggs and sperm to treatment of various kinds before fertilisation. No constant result was obtained, but in a number of cases it was found that larvæ from eggs so treated were less well developed, and at the same time had a smaller percentage of the maternal (*Sphærechinus*) characters than those from eggs of the same females, fertilised normally. Most of the remainder of the experiments were therefore made to determine whether this reduction of maternal characters was a consequence of the less vigorous condition of the larvæ, or whether it was due to a diminution of the maternal prepotency or transmitting power, caused by the decreased vitality of the eggs. If the latter hypothesis is correct, no treatment after fertilisation should be able to reduce the maternal characters, for it would act also on the paternal to the same extent. The experiments which will now be described consist therefore of attempts to alter the vitality of the larvæ after fertilisation, in order to compare them with larvæ from eggs treated before fertilisation.

The first attempts in this direction were made by rearing the larvæ in small jars

(one litre) so that they were overcrowded, or by subjecting them in the early stages to high temperatures or considerable dilution of the water for one or two hours. Nos. 69 and 69A, 85 and 85B, 181 and 181A record the results of overcrowding, and Nos. 85 and 85A, 121 and 121A, 159 and 159A, 180 and 180A, 242 and 242C, those of warming or diluting the young larvæ. In the earlier cases it was found that these disadvantageous conditions had the effect of causing the 8th day larvæ to be smaller and less well grown, but there was no reduction in the maternal characters, and this was regarded as evidence that the reduction observed in larvæ from eggs treated before fertilisation was due to a diminution in the dominance of those characters. When the young larvæ (blastulæ or gastrulæ) were warmed up to about 28° or put in water to which an equal volume of fresh water was added, they sank to the bottom and became nearly motionless, but on being restored to normal conditions they recovered, but did not grow to such healthy plutei as those which had undergone no treatment. In subsequent experiments, however, *e.g.*, Nos. 121A, 180A, the diminution in vitality was found to be associated with a distinct decrease in *Sphærechinus* characters, but where the unfavourable treatment had been very severe, or much prolonged, so that there was a very high mortality, *e.g.*, 181A, the *Sphærechinus* features were sometimes actually increased instead of being diminished. It appeared probable, therefore, that diminution of vitality caused a reduction in the development of *Sphærechinus* characters, but that where there was a great selective mortality, only the strongest survived, and these were those in which the *Sphærechinus* characters were most developed. This question will be referred to again below.

The most important experiments in this connection are those in which the larvæ were grown at different temperatures, as seen in Nos. 221 and 221A, 241–242A, 252 and 252A, 268–268C, and the whole series 405–439A. In the experiments made in summer no great difference of temperature could be obtained, owing to limited apparatus, and those made in the winter were carried out to test the conclusions arrived at in the summer. In these experiments it was found that the temperature of growth had an immense effect not only on the size of the larvæ, but also on the extent to which the *Sphærechinus* characters were developed. The larvæ were divided after fertilisation into two lots, one of which was kept at the room temperature (about 19–23° in summer), and the other cooled to 16–17° by running water. As is seen from the tables, while the larvæ at the higher temperature had the normal summer form, with long arms and frequently a great development of cross-bars and anterior body-skeleton, those grown in cooled water were smaller, especially in arm-length, and had the maternal features reduced, sometimes to a great extent, and always appreciably. This reduction is also not a mere retardation of growth, for if kept on beyond the 8 days at the cooler temperature the maternal characters hardly increased at all, but if they were transferred to the warm room, the larvæ rapidly developed into a form approaching that commonly found in summer (*cf.* Nos. 241, 241′, 242A, 242A′, 12th day).

The same thing was found in the winter (*cf.* Nos. 405A, 405B; 409 and 409B, 422 and 422A, &c.); when the larvæ were reared at the summer temperature, by keeping the jars in a cupboard with a glass door, with a temperature maintained at 20–22°; in many cases the plutei were of the typical summer form, although reared in December, while those reared in an unwarmed room at 13–15° were feebly developed, with short arms, and almost of the *Strongylocentrotus* type.

The larvæ were reared in winter at a series of different temperatures, and it was found that below about 13° they did not develop at all beyond the gastrula stage, and remained chiefly at the bottom of the jar; if, however, when they had been in this condition for several days, they were warmed up to 18°, many of them grew into moderately healthy plutei. The larvæ reared at 13–15° were exactly like those reared at the same temperature in March, while those grown at about 18° were better developed and had a higher proportion of cross-bars and anterior body-skeleton, but fewer double-arm bars, and finally the larvæ reared at 20–22° were in many cases like those obtained in summer. The highest development, however, attained in December at 20–22° was not as good as that of the best larvæ reared in summer, and in some cases although grown at this temperature the plutei were weak, with short arms, and a low percentage of *Sphærechinus* features. This may be accounted for by the facts that firstly, neither species was at its true breeding season, and hence the larvæ were less strongly developed, and secondly, the rather rapid rise of temperature to which the eggs were subjected, viz., from about 11° at which the urchins were living to 20° in the jars, was probably enough to reduce their vitality considerably. That this explanation is sufficient to account for the difference between the larvæ reared in December at 18–22°, and those reared in summer, is indicated by the fact that pure-bred larvæ of both the parent species were smaller in the winter than in the summer, although reared at the same temperatures. Temperature has on the pure-bred larvæ an effect similar to that observed on the hybrids, but it is less marked, so that at 10–12° both *Sphærechinus* and *Strongylocentrotus* gave typical plutei, but especially in the case of the former they were very weakly developed, while, as has been mentioned, the hybrid larvæ remained at the gastrula stage at this temperature.

Some other points of interest appear in the records of the experiments, of which perhaps the chief is the differences in the larvæ at different stages of growth. In Nos. 10–13, 33–34, 56, 71–73, 121A, 125, 128, 132, 180–182, 221 and 221A, 225, 241–242A, 252 and 252A, 405B, larvæ of the same batch were examined more than once, viz., on the 8th day, and again on the 10th, 11th or 12th, and in this way the effects of growth and selective mortality can be observed. In the experiments made in the cool weather, *i.e.*, in February, March and December, in most cases on the 10th or 12th day the larvæ had a smaller percentage of double arm-bars, but an increase in cross-bars and anterior body-skeleton. The decrease in double arm-bars must be due largely to selective mortality, for the increase in the length of the arms is slight, but

the increase in the other characters is probably due to a continued growth of the skeleton. In the experiments made in May and June, on the other hand, the decrease in double arm-bars is less marked, and the percentage of cross-bars and anterior body-skeleton is usually either stationary or actually lower, except that in the latter the number of "large" has often increased at the expense of the "small." The cause of this difference between the seasons is probably the increase of temperature. In the earlier experiments, the plutei had not reached the maximum possible growth without food on the 8th day, and so increased in skeletal growth up to the 12th, but as the temperature rose the larvæ grew more quickly, and could not continue to develop beyond the 8th day without food, so that during the next few days the most advanced individuals died off from want of nourishment, and only the more backward remained. It has been pointed out that in the early stages the selective mortality acts in favour of the stronger individuals, which have the *Sphærechinus* features most fully developed (*cf.* 181, 181A, 8th day), but later, unless food is supplied, these die off because they soonest require nourishment, and the weaker survive. These differences of growth, according to the temperature, also account for the fact that in the summer the percentage of larvæ with two or three full arm-bars is much lower than in winter. The reason appears to be that until the arms reach a certain length, the two skeletal bars which are most frequently found at the base grow equally, but later, when the arm gets longer, one of them ceases to grow, and an arm which in the young stage would be classed as "2" would afterwards become "$1\frac{1}{2}$." But in the cool weather the arms remain short, and hence there is a higher percentage with "2" in winter and spring than in summer.

A number of experiments were made to determine whether an individual which proved "prepotent" when crossed with one A of the other species, was also prepotent when crossed with other specimens B and C; records of these will be found in Nos. 121–132, 180–183, 221–227, and the results discussed in a subsequent section.

6. *Experiments with other Hybrids, and with Pure-Bred Larvæ.*

After the hybrids described in the last section, the most important were those made with *Sphærechinus* ♀ × *Echinus microtuberculatus* ♂. These are exceedingly like the larvæ obtained when *Strongylocentrotus* is used for the male parent, but are usually much smaller and more feebly developed, and far more frequently failed to survive till the 8th day.

Experiments Nos. 1–9 (Table III) were made to find out whether the egg of the same female fertilised by different males gave different larvæ and also the effect of keeping the eggs. The eggs fertilised fresh yielded few blastulæ and no larvæ; those after 4 hours rather few of each, and after 23 hours a large proportion. The larvæ from different males differ somewhat, and those from eggs kept 23 hours have a much lower proportion of cross-bars and anterior body-skeleton.

The changes between the 8th and 14th days are like those described above for the *Strongylocentrotus* cross, as is also the case in 200. The remaining experiments are of no great interest, except that the best-grown larvæ and the highest percentage of "maternal" characters both occur early in June, after which the larvæ decrease in size and in July the eggs ceased to segment, apparently in consequence of the complete absence of sperm from the testes of the *Echinus*.

A number of attempts were made to get larvæ from the eggs of *Echinus* and *Strongylocentrotus* fertilised with *Sphærechinus* sperm. Of the first, a few plutei were obtained in March, which were larger than the converse hybrid and resembled pure-bred *Echinus* larvæ except that nearly all had small second arm-bars and anterior body-skeletons. In nearly all other cases no plutei were obtained, though sometimes blastulæ or gastrulæ, the number of which was usually larger if the eggs had been kept several hours or treated with dilute sea water before fertilisation. On June 12, some eggs of *Strongylocentrotus* were fertilised by *Sphærechinus* and yielded a few plutei; these were rather like the converse hybrid in size and shape, but more like *Strongylocentrotus* in the skeleton. They differed from the corresponding hybrid made with *Echinus* in being much smaller.

More than fifty experiments were also made in July with *Arbacia pustulosa*. It was hoped that this species might prove free from the disadvantages associated with *Sphærechinus granularis*, and it has several points in its favour, the chief of which are a very well-marked skeleton, and the fact that when ripe, abundant eggs and sperm are shed by simple pressure of the thumb on the mouth of the urchin. Eggs or sperm can, therefore, be obtained from the same urchin at different times and so the sexual cells which have been kept or otherwise treated can be compared with fresh ones.

Eggs of *Sphærechinus*, *Echinus* and *Strongylocentrotus* all gave a small proportion of blastulæ when treated in July with *Arbacia* sperm, but no plutei were reared. Of the converse crosses, the only satisfactory plutei were obtained from *Arbacia* ♀ × *Sphærechinus* ♂, which closely resembled pure-bred *Arbacia* larvæ, but were smaller and less regular. The eggs of the *Arbacia* were treated in all the ways described in the last section with the *Sphærechinus* ♀ × *Strongylocentrotus* ♂ hybrids, but no marked differences were observed except in size and healthiness. In December, 1900, a few hybrids were made between the same species, and these were also of the *Arbacia* type, but much smaller and feebler than those reared in July, 1902. No further light was, therefore, thrown on the subject under investigation by any of the other crosses attempted.

Some experiments made with pure-bred larvæ of *Sphærechinus*, *Strongylocentrotus* and *Echinus* remain to be described, which are summarised in Table IV.

When it was found that the conditions under which hybrid larvæ after fertilisation were reared had so much effect on their form, some experiments of the same kind were made for comparison with pure-bred larvæ of the parents.

Nos. 189, 189A, and 189B show that keeping the eggs and sperm of *Sphærechinus* for five hours, or treating the eggs with diluted water, reduces the arm-length of the larvæ to a great extent. Nos. 267 and 267A show that if the larvæ are cooled to about 17° (from about 22°) from the 7th to the 10th day, the arm-length is no less than in larvæ reared for 10 days at 20–23°; the experiment made at the same time to test the effect of keeping the same larvæ for 10 days at 16–17° unfortunately failed, owing to an unexplained mortality among the larvæ. But Nos. 319 and 319A show that when reared at the higher temperature the plutei have a rather higher average arm-length, but that they are much less evenly developed. A comparison of No. 318–319 shows that a ripe female gives rather larger plutei, in both body and arm-length, than an immature one.

The experiments made in the winter (425 and 440) show that the larvæ reared at that time have smaller arm-lengths than those obtained in summer, apart from the effect of temperature, as was also found by VERNON. It also appears from them that when grown at very low temperature (11–13°), the larvæ of *Sphærechinus* develop very feebly indeed, and that too high temperature also causes a reduction in size (440*d*).

In the case of *Strongylocentrotus*, the temperature seems to have a greater effect; Nos. 282 and 282A show that larvæ reared for 8 days at 20–23° are much larger, especially in the arms, than those living at 16–17°. The same series, counted on the 11th day, shows that while the plutei reared at 17° have continued to increase both in body and arms, those at 20–23° have now a much lower average, due no doubt to a heavy mortality, for healthy larvæ of this species, when well fed, continue to grow up to about the 20th day in arms and body. No. 282B, which was kept for 8 days at about 16° and then transferred to 22° for 3 days, is remarkable for the enormous increase in length of the arms between the 8th and the 11th day, for on the latter date they average nearly twice as long as 3 days previously, and are longer than the longest arms found in other members of the series. Although the difference between 22° and 16° caused a greater change in *Strongylocentrotus* than in *Sphærechinus*, yet the former is less affected by very low temperatures than the latter, as is seen in No. 406, where the plutei were quite well grown at 12°·5. In 406A, where they were reared at 20°, the arms were very short, probably either in consequence of the bad effect of such an unnatural temperature at that time of year, or because the more advanced larvæ had already died off from want of food at the time when they were measured.

Only one experiment of this kind was made with *Echinus* larvæ, from June 25 to July 3. This indicates that these larvæ develop their arms to a greater extent at 22° than at 16°, while the body-length is appreciably greater at the lower temperature.

7. *Discussion of the Experiments described in Sections* 4 *and* 5.

In the two last sections the more important experiments have been described, but very little attempt has been made to indicate the conclusions which may be drawn from their results. It has been explained above that this investigation was undertaken in order to amplify if possible the conclusions arrived at by VERNON respecting the effect of conditions in influencing prepotency. But before deciding that any alteration in the larvæ, following a change of conditions of the eggs or sperm is due to a reduction or increase in prepotency, it is necessary to know certainly what characters in the hybrid larva are safely to be ascribed entirely to the influence of one or the other parent. The larvæ of the parent species and the commoner types of hybrids, have been described in Section 3, and from the differences between the parents it may be inferred that in the hybrids the complete double or treble condition of the arm-bars, the presence of cross-bars, the presence of anterior body-skeleton, and the existence of arms longer than the body, may be taken as *Sphærechinus* characters; while the absence of these and the presence of a marked club at the apex of the body-skeleton, are features inherited from the *Strongylocentrotus* parent. But from the account given above, it will be seen that not all of these characters are of equal value. VERNON in his earlier paper relied entirely on the ratio of arm-length to body-length, and in his later one chiefly on the presence or absence of cross-bars. In the present investigation not much importance has been attached to measurement, and the clubbed body-skeleton has hitherto been passed over without mention, for reasons given below, so that the features chiefly dealt with are the arm-bars, the cross-bars, and the anterior body-skeleton. But it has been mentioned above that these characters are frequently in opposition to one other, for while cross-bars, anterior body-skeleton, and usually the large arm-length are found together, yet frequently an increase of these is associated with a decrease in the percentage of double or treble arms-bars. It must not be inferred from this statement that there is pronounced correlation in the individual larvæ between the characters mentioned, for it will be seen in the section devoted to the subject, that the correlation between any two characters is very low; but nevertheless it has been very frequently found that where differences of conditions have led to differences in the larvæ, those with longer average arm-length have higher percentages of cross-bars and anterior body-skeleton, and fewer double arm-bars. Now it has been shown that although all these characters are subject to great changes during development, owing to growth and selective mortality, yet this is more conspicuously the case with the arm-bars than with the others, and hence where there was contradiction between them and the other characters, they have been neglected.

If then the different classes of experiments are examined in this light, it appears that where the eggs have been kept for a long time before fertilisation (*i.e.*, 15 hours

or more) the larvæ have always fewer *Sphærechinus* characters than those reared from fresh eggs, and at the same time they are always smaller (*cf.* Exps. 62, 74, 75, 87, 125, 126, 235, 7, 9). Eggs, however, which had been kept for a shorter time, or which had been diluted, warmed, or otherwise treated, gave results differing in different instances. In some cases there was a reduction of maternal characters, almost always accompanied by a decrease in size, at others no reduction, and usually no diminution of size. These facts could be explained by two hypotheses; the first, that any treatment of the eggs which reduced their vitality also reduced the dominance of the characters transmitted; or secondly, that a decrease of vitality prevents the larvæ from developing certain characters which require strength and energy for their production. In order to test the truth of the second hypothesis, larvæ were reared in unfavourable circumstances which reduced their size and strength, and at first (69A, 85A) no reduction was observed in the maternal features, and it was supposed that this was evidence in favour of the first suggestion. But later (see Nos. 121A and 121B, 180A, 221A, 241, 242A, 252A, &c.) it was found that unfavourable conditions, especially low temperature, acting during growth did reduce the *Sphærechinus* characters to a marked extent, and that where the contrary seemed to be the case, the cause was almost certainly to be found in selective mortality. This showed that the facts observed might certainly be due, at least partially, to the second hypothesis, and this view is supported by other considerations. For if there is any reduction of dominance caused by the treatment of the eggs, not only should there be a decrease of the *Sphærechinus* characters but also an increase in those of *Strongylocentrotus*, viz.: in the clubs or the body-skeleton and in body-length. Neither of these, however, were found, the clubs varied little and irregularly, but, on the whole, seem to be larger in stronger and better developed larvæ, and smaller in the weaker ones, so that, as a rule, the percentage of clubs increases with that of cross-bars and anterior body-skeleton. Nor is there any marked increase of body-length when the *Sphærechinus* characters are reduced, or at least this is not greater than that observed in larvæ grown at a lower temperature, which tend to have in some cases rather larger bodies associated with shorter arms (*cf.* 221 and 221A).

It was not certain, however, that the whole of the effect was due to slowness or feebleness of growth, and there were some indications that there might also be a reduction in the dominance of the *Sphærechinus* characters, acting at the same time in the same direction.

To test the possibility of this being the case, the experiments were made in which the spermatozoa were subjected to a variety of conditions, and also those in which different crosses were used. The results of the experiments in which sperm was used under different circumstances are not quite consistent, and some of them are rather anomalous, but the conclusion on the whole must be that although stale or immature sperm gives larvæ which are weaker than those from fresh sperm, yet there is no satisfactory evidence that the dominance of the paternal characters is decreased as

the spermatozoa lose in vitality. As has been explained above, the experiments with other crosses threw no further light upon the question, and, therefore, a similar conclusion must be drawn with regard to sexual cells as a whole, namely, *that the evidence from Echinoid hybrids gives no grounds for the belief that a decrease in the vitality of sexual cells is associated with a diminution in the dominance of the characters transmitted*, for although such a diminution sometimes appears to exist, yet it may be sufficiently accounted for by differences of growth and selection.

Turning now to the results of the experiments in rearing the hybrid larvæ at different temperatures, it is seen that they lead to conclusions directly at variance with those drawn by Vernon. From a study chiefly of the reaction to temperature of the larvæ of the parent species, he concluded that temperature was in itself insufficient to account for the seasonal difference observed in the hybrids, and, therefore, supposed that they were caused by changes of "prepotency" accompanying different conditions of maturity. But it has been shown in these experiments not only that great differences are produced in the relative proportions and structure of the skeleton of the hybrids by slight changes of temperature, but also that by rearing them in water raised to about 20° in December, the typical summer form may be got. That sometimes the larvæ remain of the winter type, even when reared in warmer water, is almost certainly due to the fact that neither parent normally breeds in the winter, and, therefore, the larvæ are relatively weakly. This was found to be the case with pure-bred larvæ of both parents, so that it is only likely to be still more marked in the hybrids, since they are more sensitive than either of the parent forms. At first sight it seems unlikely that a difference in the healthiness and rapidity of growth should cause the plutei to resemble one parent or the other, but this is explained by the fact that all the more important features of the *Sphærechinus* pluteus are of a positive nature, while the larva of *Strongylocentrotus* is chiefly distinguished by their absence. And it was shown that the only positive feature of *Strongylocentrotus*, the club at the apex of the body-skeleton, was not reduced in summer, but in some cases at least actually increased. For example, in No. 244 (June 12–20) there were large clubs 8 per cent., small 88 per cent., no club 4 per cent., compared with large 0, small 84 per cent., none 16 per cent. in No. 33 (March 3–11). These are typical examples of well-grown larvæ taken at random, and others taken at the same seasons would give similar proportions.

On the whole, therefore, it must be concluded that the differences observed in the larvæ in winter and summer are due chiefly to the temperature, and that there is no satisfactory evidence of a difference of dominance accompanying changes in maturity.

There is one other point, already mentioned in the account of the experiments, which requires some further explanation. An examination of the series of experiments 121–124, 125–130, 180–183, 221–227, 272–277, shows that where the eggs of the female are fertilised separately by two males, and there are pronounced differences

in the resulting larvæ, then if the eggs of another female are fertilised by the same male, there will be similar differences in the larvæ produced. I thought at first that the ratios between the percentages in the two cases might be approximately equal, but apparently this is not so; the results, however, are sufficiently clear to make it safe to state the rule that if one male shows greater dominance than another in the characters transmitted when each is crossed with one female, then, when both are crossed with another female, the characters of the first will again be more dominant than those of the second. This is well illustrated in the series 121–124. In 121, 122 a female (*brn. wt.*) is crossed with two males (*a*) and (*b*), and while in 121 the larvæ derived from (*a*) have 30 per cent. cross-bars, those from (*b*) have 19 per cent.; in Nos. 123, 124 another female (*vi. wt.*) is crossed with the same males, and the larvæ from (*a*) and (*b*) have respectively 39 and 22 per cent. of cross-bars. Although the ratio 30 : 19 is not equal to the ratio 39 : 22, and in other cases there is a still wider discrepancy, yet it is evident that the dominance of a given character differs in the offspring of different males, and that when it is greater in those of a male A than in those of a male B, when both are crossed with a female Y, it will also be greater in those of A than in those of B, when both are crossed with a female Z. Precisely the same may be said of the female, as is seen in the same series, and perhaps still better in Nos. 221–227; in Nos. 121–124 as the (*vi. wt.*) female transmits the cross-bars more fully than the (*brn. wt.*), when crossed with male (*a*), viz., 39 : 30 per cent., so it does when crossed with (*b*), viz., 22 : 19 per cent.

8. *On the Conditions Favourable to Cross-fertilisation.*

An examination of the notes attached to the tables of experiments shows that the proportion of eggs fertilised varies to an immense extent, and that these variations seem to be influenced in many cases by the external conditions. A considerable number of papers have been written on the causes of such variations, but their conclusions differ greatly from one another, and this is not surprising, since the facts seem frequently to be contradictory among themselves. Vernon, for example, in his earlier papers (5), concludes that eggs which have been kept are more easily fertilised by foreign sperm than fresh eggs, but in his last paper (9) he shows that this is not always the case. Similarly, the effects of temperature and other external conditions seem to vary in different cases, so that conditions which in one instance seem to favour cross-fertilisation, at another, appear to be adverse to it. I will here discuss the various conditions to which the sexual cells were subjected from the point of view of their effect on the percentage of eggs fertilised and of larvæ produced.

As has been said, the effect of keeping the eggs varies very largely in different cases. In the experiments made in 1901, in nearly all cases the number of blastulæ was markedly greater from eggs which had been kept for about 8 hours than from quite fresh eggs, but, on the other hand, No. 13 (1902), only about 2 per cent. of the

eggs segmented which had been kept for 7 hours, while 20 per cent. of the fresh eggs developed, and other similar instances have several times occurred. But there can be no doubt that very frequently eggs which have lain for some time in sea water are more easily fertilised than those quite freshly shed; for example, in Nos. 1–9 (hybrids with *Echinus* ♂) the fresh eggs yielded very few blastulæ and no larvæ; those kept 4 hours gave rather few blastulæ, and of those kept 23 hours 70 per cent. developed. Usually *Sphærechinus* eggs degenerate considerably if kept as much as 20 hours, but this depends upon the temperature; when the water is cool, they may yield good larvæ after a still longer time, but as the temperature rises the eggs survive for a shorter period. There seems usually to be a gradual rise in the percentage of segmenting eggs till they have been kept for a certain time, which depends upon the temperature and probably other causes, and when it is passed not only do fewer eggs develop, but they are also less regular. The eggs of other species can be kept for a longer time, for example, on March 7, eggs of *Strongylocentrotus* which had been kept for 44 hours at about 14° gave a large number of blastulæ when fertilised by *Echinus* sperm, although none of the same eggs had segmented when mixed fresh with sperm of the same species. So, too, on March 14, *Echinus* eggs kept 25 hours gave some blastulæ with *Sphærechinus* sperm, though when they were fresh it had no effect. When the eggs have been kept so long that they can no longer develop, the addition of sperm causes them to break up and decompose much more rapidly than if they are merely left in sea water, so that the spermatozoa must enter the eggs after they have lost the power of developing.

Loeb (3) has recently published an interesting paper on keeping the eggs of *Arbacia sp.* alive by dilute potassium cyanide, and I made some experiments with this. I found on June 2 and again on June 9, that that *Sphærechinus* eggs kept 48 hours in a solution made by adding 20 or 15 c.c. of $\frac{n}{100}$ KCN to 100 c.c. of sea water, yielded abundant blastulæ when fertilised by *Sphærechinus* sperm, and that plutei were very well developed (Exp. 191). The temperature was then about 20°, and eggs in normal sea water decomposed in 30 hours or less. The remarkable thing about these experiments is that the solution of potassium cyanide used was about ten times as strong as Loeb's; this was in consequence of an accidental measurement in the first instance, but the experiment was repeated with a fresh solution in order to be certain that its strength was as stated.

Attempts at obtaining hybrid larvæ from eggs kept in the cyanide solution were not successful, although very few blastulæ were obtained on one occasion when the eggs were 48 hours old, and several times many of the eggs segmented irregularly, and remained undecomposed, when no sperm was added, for over 70 hours. Spermatozoa in the same solution were dead after 22 hours.

The effect of keeping the spermatozoa varies as widely as in the case of the eggs, but they seem always to die off sooner. In rare cases hybrid larvæ were obtained

from spermatozoa kept 15 hours or more, but usually they were dead after a less time; probably the amount of dilution of the sperm, the amount of available oxygen and possibly other conditions are important factors in determining how long they will keep their power. In any case, it appears that when fresh they are always in better condition for fertilising the eggs of another species than when at all stale, as is shown by experiments 147–151, 162–168, 272–276 and others.

The effects of warming the eggs before fertilisation are even more various than those of keeping them, as is seen from a comparison of Nos. 10–11, 56–58, 68–69, 71–73, 85–86. In general it appears that eggs which have been kept at about 27° for a comparatively short time are more easily fertilised than those which have not been warmed, but there are many exceptions to this rule, and it seems that a temperature which is fatal to one set of eggs may render another peculiarly fertile. The temperature of fertilisation, and the time taken in raising the eggs from one temperature to another, probably also affect the percentage of segmenting eggs, but, on the whole, when the 1901 experiments are also taken into account, it seems that those which have been raised to a temperature from 27–30° usually yield a larger proportion of blastulæ than those which have been kept for the same time in cool water (12–15°).

The condition which seems to have the greatest and most uniform effect in facilitating cross-fertilisation is dilution with fresh water. This is illustrated in Nos. 12, 23–26, 33–36, 53, 59–61, in which it is seen that the percentage of *Sphærechinus* eggs which yield blastulæ with *Strongylocentrotus* sperm rises with the amount of dilution of the water in which the eggs have been kept for a short time, until that dilution reaches 50 per cent.; a greater dilution than this is, however, soon fatal. The optimum time during which the eggs should be kept in the diluted water appears to be about 1 or 1½ hours; when kept too long (*e.g.*, No. 35) the proportion of blastulæ falls again. Distilled water acts more vigorously than tap water, as is shown by Nos. 59–61. Although the number of blastulæ is increased by placing the eggs for a short time in diluted sea water, it may happen that many of the larvæ formed die off before the 8th day, but, on the other hand, in other cases the larvæ from diluted eggs are as strong and healthy as those treated normally.

Not only *Sphærechinus* eggs are rendered more easily fertilisable by diluted water, for it was found that those of *Strongylocentrotus* kept for 2 hours in 30 or 50 per cent. tap water gave with *Sphærechinus* sperm a considerable number of morulæ, while of eggs fertilised after lying the same time in sea water hardly any segmented (on July 4 and June 25). So also, on July 3 and 9, *Arbacia* eggs when kept 1 or 2 hours in 30 per cent. tap water yielded a large proportion of blastulæ when mixed with *Sphærechinus* or *Strongylocentrontus* sperm, while of the eggs kept in normal sea water only very few developed. In the case of *Arbacia* 33 per cent. of tap water for 1½ hours appeared to be the optimum (at a temperature of about 23°), but the eggs can stand a much greater dilution for a short time; for on July 2 some eggs of *Arbacia* which

had been for several minutes in pure tap water, gave with sperm of their own species a good proportion of blastulæ which grew up into healthy plutei. In several instances eggs which had been treated with fresh water did not develop, but in those cases the fresh eggs also failed to segment; and it may be taken as a very general rule that the addition of fresh water to the sea water in which the eggs are lying until the mixture contains from 30 to 50 per cent., increases the proportion of eggs which undergo normal segmentation.

Concentration of the sea water in which the eggs are placed seems to have little effect on the number which develop, unless the concentration is considerable. When left in water concentrated to half its volume for one hour (158) fewer blastulæ were produced than from normal eggs, and eggs kept two hours in this solution gave hardly any blastulæ, but nearly all became irregularly broken up.

If the eggs are shaken before the sperm is added, in some cases a much larger proportion develop than from unshaken eggs, *e.g.*, Nos., 292, 342, and some of the 1901 experiments; on the other hand, in other instances shaking seems to have the opposite effect, as was the case on April 20, 1901, when unshaken eggs of the *Strongylocentrotus* and *Echinus* each yielded a moderate proportion of blastulæ with *Sphærechinus* sperm, while shaken eggs remained unsegmented. It is remarkable, however, that several times shaking has been found to give not only a larger proportion of segmenting eggs, but also a much larger number of healthy plutei.

There is no satisfactory evidence that a maturity of the eggs influences their readiness for cross-fertilisation, but there are some indications that a very mature female yields a lower percentage of hybrid larvæ than one which is somewhat immature; if, however, the eggs are very immature, with large vesicular nuclei, they do not develop at all.

It therefore appears that cross-fertilisation is rendered more easy by placing the eggs for from one to two hours in diluted sea water, and that the same result may sometimes at least be achieved by warming the eggs to about 28°, by keeping them for several hours, or by shaking them. Now all these methods of treatment are such that if they were prolonged, or made more intense, they would kill the eggs altogether, and it seems, therefore, that cross-fertilisation is assisted by conditions which tend to reduce the vitality of the eggs, since the same conditions, if continued, destroy the eggs entirely.

There can be no doubt that species whose larvæ are closely similar, are more easily cross-fertilised than are widely different species, for example, *Echinus* eggs are almost as readily fertilised by the spermatozoa of *Strongylocentrotus* as by their own; the converse cross is, however, much harder to effect, and indeed *Strongylocentrotus* eggs seem difficult to fertilise with foreign sperm of any species.

Some experiments were made to determine whether the gelatinous envelope has any influence in preventing cross-fertilisation. That this may be the case is indicated by the fact that eggs which have been shaken, so as at least partially to remove the

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envelope, were sometimes more easily fertilised. But this was not always the case, and it can easily be shown that the gelatinous envelope is not the sole cause, for if eggs of *Strongylocentrotus* are mixed with large quantities of *Sphærechinus* or *Arbacia* sperm, often enough spermatozoa traverse the envelope to cause the egg inside it to rotate, and yet the egg is not fertilised.

This was also shown by another method. Early in January, 1903, eggs of various Echinoids were treated with sea water saturated with carbon dioxide, as described by Yves Delage (1) in his experiments on Artificial Parthenogenesis of *Asterias*. It was found that this solution has the power of removing the gelatinous coat in a few minutes. If the eggs of *Strongylocentrotus* are mixed with sperm of their own or another species immediately after the treatment, they are unable to keep out the spermatozoa and polyspermy results. But if they are left for some hours, and then mixed with sperm, foreign spermatozoa have no effect, while those of their own species fertilise them normally, but more slowly than with untreated eggs. It appears, therefore, that the gelatinous coat has comparatively little effect in keeping out foreign spermatozoa, since when it is removed the eggs can still not be fertilised by them, but that it assists the spermatozoa of the same species in reaching the egg, for when it is absent the eggs are only fertilised slowly by them.

No parthenogenetic larvæ were obtained by the use of CO_2 solution, as Delage also found with Echinoid eggs, but in many cases it caused the eggs to segment or break up irregularly after being put back into normal sea water, and this effect was the same whether foreign spermatozoa were present or not.

9. *Note on the Correlation of Characters in the Larvæ.*

In recording the characters of the hybrids, those of each larva were kept separately in order that it might be possible to discover to what extent there was correlation between them. It was expected that where a larva inherited one character strongly from the female parent, other characters of *Sphærechinus* would also tend to be present, but this is only the case to a limited extent. No exact measurements are available of the features chiefly studied, since they were classified into divisions which are not certainly of the same value, and therefore it is not possible to work out the extent of the correlation with real accuracy, but it is possible to see to some extent how large it would be. Even had accurate measurements been made, it is probable that the results obtained would have been untrustworthy, for it has been shown how greatly the larvæ are affected by conditions, and even if these had been kept as far as possible the same for all larvæ used for this purpose, yet their effect could not be eliminated. For even if the external conditions are identical, yet the eggs differ among themselves in vitality, and the more vigorous would give rise to larvæ which developed characters different from those found in the weaker. For these reasons an exact determination of the coefficient of correlation would be useless, if not misleading, but it may nevertheless be of interest to notice some of the facts.

Table I. is a correlation table including larvæ reared under very various conditions, for determining the correlation between the number of anal arm-bars and the length of the anterior body-skeleton. The line of regression when worked out is found to be roughly horizontal, so it may be concluded that in this case there is no correlation.

Together with the sums of the rows and columns in this table, are given the numbers of larvæ in each row or column which have cross-bars, and the ratio which they bear to the whole. It is seen that the ratios fall fairly steadily when traced from the "large" anterior body-skeleton downwards, and hence there is some correlation between these characters. There is very little trace of it, however, between the cross-bars and the number of arm-bars, except that the larvæ with "1*" and "1" arm-bar naturally have much fewer cross-bars than the others. There are indications, however, that when a batch of larvæ is grown under constant conditions, there would be some correlation between these characters, but where varied batches of larvæ are combined in one table, it does not appear, for it has been pointed out that favourable conditions, such as tend to increase the percentage of cross-bars, cause a decrease in that of double arm-bars, because where the arms are long, the second bar rarely reaches the end of the arm.

It seems, therefore, that the number of arm-bars is not correlated with the size of the anterior body-skeleton, and only to a very small extent with the presence of cross-bars, but that between the cross-bars and the anterior body-skeleton there is quite perceptible correlation. The data available are, however, not sufficient to work this out with numerical accuracy.

There is another question of correlation in these hybrid larvæ, which is of considerable interest, namely, that of the correlation between the two sides of the same larva. It is seen from the tables how large a proportion come under the class "2 and 1," that is, with two full arm-bars on one side, and less than two on the other. The records of this character were, however, not kept in sufficient detail for statistical examination, but during the last month of the work at Naples, a record was kept with regard to the cross-bars and anterior body-skeleton, in order to find out what proportion of the larvæ were symmetrical in respect of these features. Altogether, 1471 larvæ were so recorded, from experiment 242 (12th day) onwards. From these the following figures are obtained. The cross-bars will be dealt with first. It was found that out of the 1471 larvæ 275 have cross-bars, and of these 57 have them symmetrically. Therefore 18·7 per cent. of the larvæ have cross-bars, and 3·9 per cent. have them symmetrically.

If, then, the larvæ are regarded as each composed of two independent halves, out of every 1000 larvæ, there will be $187+39\,(=226)$ arms with cross-bars.

Therefore in every 1000 half-larvæ there will be 113 arms with cross-bars, or ·113 of the half-larvæ have cross-bars.

If now the half larvæ are imagined as being fitted together in pairs quite by chance,

that is with no correlation between the two halves, $\cdot113^2$ ($\cdot0128$) of the resulting complete larvæ would have cross-bars on both sides.

If, on the other hand, there were complete correlation between the two sides, all the larvæ with cross-bars would have them on both sides, and therefore $\cdot113$ of the whole number of larvæ would have them so.

But, actually, $\cdot039$ of the whole number of larvæ have them symmetrically, and therefore a measure of the correlation is found by the difference between $\cdot039$ and $\cdot0128$ divided by the difference between $\cdot113$ and $\cdot0128$ $\frac{\cdot039-\cdot0128}{\cdot113-\cdot0128}=\cdot2614$, which is therefore a measure of the extent of the correlation, no correlation being represented by 0, and complete correlation by 1.

If the anterior body-skeleton is treated in the same way, it is found that out of 1471 larvæ 982 have a.b.s. and 310 have it symmetrically developed, that is, $\cdot668$ of the whole number have a.b.s., and $\cdot211$ have it symmetrical. Therefore, out of 2000 half-larvæ, there are $\cdot668+\cdot211$ ($=\cdot879$) with a.b.s. or $\cdot439$ of them have it. The measure of the correlation is, therefore, in just the same way $\frac{\cdot211-\cdot439^2}{\cdot439-\cdot439^2}=\cdot0743$.

In this case, therefore, the correlation appears to be very low, but possibly the cause of this is that where the a.b.s. on one side was "large" and on the other "very small," the larva was recorded as asymmetrical, though the feature was present on both sides, but very differently developed. Had the larvæ been recorded in greater detail for this purpose, the correlation would doubtless have appeared greater.

It appears, therefore, from these observations that there is comparatively little correlation between the two sides of the body in respect of the three characters which have been chiefly studied in this work. This fact has been noticed before, especially by Steinbrück, who says that in the larvæ which he examined a large proportion were differently developed on the two sides, but he gives no statistics from which the actual frequency of such asymmetry can be found. It is of considerable interest to discover that in these hybrid larvæ such a very large proportion should develop, and therefore presumably inherit the maternal features differently in the two halves of the body, for these differences can hardly be the result of differences of conditions affecting the two sides of the larvæ. It can only be supposed, therefore, that the halves of the skeleton are largely independent, and may differ in the way in which their characters are inherited from the parents.

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Summary.

The following are the chief conclusions which may be drawn from the experiments described :—

(1) There is a considerable difference in the relative and actual size of the parts, and in the skeletal characters, between hybrid larvæ of the cross *Sphærechinus* ♀ × *Strongylocentrotus* ♂, grown in the winter or early spring, and those grown in summer. This difference is not found in every larva, or even in every batch of larvæ, but if the average for the different seasons is considered, it is very distinct. The experiments have shown that temperature has a very great influence in determining the development of the characters considered, and that by rearing larvæ in December in water warmed to 20–22° C, they approach and frequently attain the typical summer form. It is probable, therefore, that temperature is the chief, if not the only cause of the seasonal change.

(2) A similar difference, generally less conspicuous, is found between larvæ obtained from fresh eggs and from eggs which have undergone treatment which reduces their vitality, but this probably arises from the decreased vigour of the larvæ in the second case, and there is no ground for supposing that there is an alteration of dominance accompanying the diminished vitality.

(3) There is a distinct difference in the dominance of characters in the off-spring of different individuals in Echinoids, and it appears that this is a definite quality of the individual urchin, so that if A shows greater dominance than B, when both are crossed with a specimen Y of the other sex, then A will also show greater dominance in the character considered than B, when they are crossed with an individual Z.

(4) There is only a low correlation in the hybrids between the different characters inherited from the same parent, but some pairs of characters show a greater correlation than others. There is also little correlation between the presence or absence of a character on the right and left sides of the same pluteus, although both the parent species are symmetrical in respect of the characters considered. An Echinoid pluteus is, of course, not entirely symmetrical, but the asymmetry only affects the structures derived from the cœlom, and except in rare and abnormal cases the two halves of the skeleton in a pure-bred pluteus are exactly alike.

(5) In experiments made to discover the causes which hinder or prevent cross-fertilisation, no positive conclusions were reached. It was shown that various forms of treatment, especially that of diluting the water in which the eggs were lying, render cross-fertilisation more easy, and that the gelatinous envelope of the egg is not the chief agent in excluding foreign spermatozoa. Species whose larvæ resemble one another are more easily cross-fertilised, and yield stronger plutei than those whose larvæ differ widely.

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Explanation of Tables.

Table I.—Correlation tables. Showing the correlation between the number of arm-bars and the length of the "anterior body-skeleton." In the first two tables the number of larvæ with cross-bars is also shown in each square, and in all three tables the totals of cross-bars, and their ratio to the totals of larvæ, are given in the "totals" columns.

A. Larvæ in Experiments 10–96 (February and March).
B. Larvæ in Experiments 200–372 (June and July).
C. A. and B. combined.

Table II.—Summary of Experiments with *Sphærechinus* ♀ × *Strongylocentrotus* ♂ hybrids.

Table III.—Summary of Experiments with *Sphærechinus* ♀ × *Echinus* ♂ hybrids.

Table IV.—Summary of Experiments with pure *Sphærechinus*, *Strongylocentrotus*, and *Echinus* larvæ.

Explanation of Signs and Contractions used in Tables II., III., and IV.

In the "Conditions" column, "♀ same" or "♂ same" means that the female or male used was the same as that used in the previous experiment. "Fresh" means that the eggs or sperm used were freshly shed. The colours of the *Sphærechinus* urchins are indicated by contractions; "vi" = violet, "wt" = white, "brn" = brown. The predominating colour is in italics, *e.g.*, *vi*-wt means that the spines were chiefly violet, with white tips. When different males were used in different experiments of the same series, they are frequently lettered (*a*), (*b*), (*c*), &c.

A horizontal bracket indicates that the two sub-divisions which it connects were counted together.

In the temperature column, the temperatures given are the whole range of the tank water during the experiment. Where the actual temperatures of the water in which the larvæ were living are given, they are marked with an asterisk.*

The "arm-length" and "body-length" given are measured in micrometer units, and are the average of a number of larvæ. The actual maxima and minima observed are sometimes given in the "Notes" column.

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TABLE I.—Correlation Tables. *Sphærechinus* ♀ × *Strongylocentrotus* ♂ Hybrids Showing Correlation between Number of Arm-bars and Length of Anterior Body-skeleton. (The figures marked "×" in brackets show the Number of Larvæ in each Division which also had Cross-bars.)

Anterior body-skeleton.	Arm-bars. 3.	2.	2 and 1.	$1\frac{1}{2}$.	1*.	1.	Totals of rows.	Totals cross-bars.	Ratios of cross-bars to totals.
A. Table of Larvæ from Experiments 10–96 (February and March).									
l.	3 (1×)	24 (10×)	24 (8×)	36 (3×)	33 (1×)	1	121	23×	·190
s.	12	114 (9×)	111 (15×)	110 (6×)	117	26	490	30×	·061
v.s.	6	102 (9×)	78 (3×)	81 (4×)	100	25	392	16×	·041
o.	19	247 (18×)	247 (11×)	193 (7×)	365 (2×)	160	1231	38×	·031
Totals of columns	40	487	460	420	615	212			
Totals (cross-bars)	1×	46×	37×	20×	3×	0×	2234	107×	·048
Ratio of cross-bars to totals	·025	·095	·080	·048	·005	·000			
B. Table of Larvæ from Experiments 200–372 (June and July).									
l.	4	40 (24×)	116 (53×)	150 (84×)	238 (7×)	0	548	168×	·307
s.	7 (3×)	57 (23×)	140 (62×)	134 (56×)	187 (6×)	2	527	150×	·285
v.s.	4 (1×)	68 (35×)	119 (45×)	99 (33×)	131 (4×)	1	422	118×	·280
o.	6 (1×)	95 (37×)	226 (66×)	176 (53×)	244 (3×)	5	752	160×	·213
Totals of columns	21	260	601	559	800	8	2249		
Totals (cross-bars)	5×	119×	226×	226×	20×	0×		596×	
Ratio of cross-bars to totals	·238	·456	·376	·405	·025	·000			·266
C. Table made by combining A and B. (Only the Totals of Cross-bars, and their Ratios to the Totals of Larvæ, are given; not the Numbers in each Division.)									
l.	7	64	140	186	271	1	669	191×	·2855
s.	19	171	251	244	304	28	1017	180×	·177
v.s.	10	170	197	180	231	26	814	134×	·1646
o.	25	342	473	369	609	165	1983	198×	·0998
Totals of columns	61	747	1061	979	1415	220	4483		
Totals (cross-bars)	6×	165×	263×	246×	23×	0×		703×	
Ratio of cross-bars to totals	·0984	·2209	·2479	·2513	·0163	0			·1568

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TABLE II.—Summary of Experiments with *Sphærechinus* ♀ × *Strongylocentrotus* ♂ Hybrids.

No. of experiment	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton per cent.			Number of larvæ counted.	Range of temperature during experiment.	Average body length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.				
10	Feb. 21—Mar. 1. 8 days.	♀ *vi.* wt. ♂ } both fresh.	53		43		3		0	0	100	3	20		77	30	13°·4 to 14°·0	..	..	About 20 p. c. of eggs segmented. Fair number of larvæ.
11	,,	♀ same. Eggs 1½ hrs. at 25—32°. ♂ same as 10.	33		57		10		0	0	100	0	20		80	30	,,	..	..	Over 50 p. c. segmented. Fair number of larvæ.
12	,,	♀ same. Eggs 1½ hrs. in 50 p. c. tap-water. ♂ same as 10.	30		47		23		0	0	100	0	27		73	30	,,	..	..	About 50 p. c. segmented. Fair number of larvæ.
13	,,	♀ same. Eggs kept 7 hrs. ♂ same. Sperm fresh.	43		52		5		0	5	95	0	49		51	75	,,	..	..	About 2 p. c. segmented. Many well-grown larvæ.
14	Feb. 22.....	♀ same. Eggs kept 23 hrs. ♂ same kept alive. Sperm fresh.	..	..	..	..	..	..	..	..	..	..	..		..	..	,,	..	..	About 50 p. c. segmented. Died off rapidly.
10	Feb. 21—Mar. 3. 10 days.	Same as 10 above.	0	22	8	44	18	8	0	0	100	4	36		60	23	,,	..	..	Rather few survived till 10th day.
11	,,	Same as 11 above.	4	22	14	34	14	12	0	0	100	0	34		66	50	,,	..	..	Fair number on 10th day.
13	,,	Same as 13 above.	0	12	12	54	10	12	0	8	92	6	52		42	50	,,	16·0	15·0	Many good larvæ on 10th day.
11	Feb. 21—Mar. 7. 14 days.	Same as 11 above.	0	14	16	41	14	15	0	2	98	2	36		62	44	13°·4 to 14°·5	..	..	
13	,,	Same as 13 above.	0	10	16	40	16	18	0	18	82	12	46		42	50	,,	..	..	
23	Feb. 26.....	♀ *brn*-wt. Eggs kept 1½ hrs. ♂ fresh.	..	..	..	..	..	..	..	..	..	..	..		..	..	..	..	..	About 18 p. c. segmented. Died off rapidly.
24	,,	♀ same. Eggs 1½ hrs. with 20 p. c. tap-water. ♂ same as 23, fresh.	..	..	..	..	..	..	..	..	..	..	..		..	..	..	..	..	About 15 p. c. good blastulæ. Died off rapidly.

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body length (micrometer units).	Average arm length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
25	Feb. 26	♀ same. Eggs 1½ hrs. with 33 p. c. tap-water. ♂ same.	..	..	..	..	..	..	..	..	..	..	..		..	..	..	..	..	About 35 p. c. good blastulæ. Died off in 6 days.
26	Feb. 26—Mar. 6. 8 days.	♀ same. Eggs 1½ hrs. in 43 p. c. tap-water. ♂ same.	0	15	20	15	10	40	0	0	100	5	25		70	20	13·3 to 14·5	..	..	About 40 p. c. blastulæ. Many larvæ on 8th day, not well developed.
27	Feb. 26	♀ same. Eggs 1⅓ hrs. in 50 per cent. tap-water. ♂ same.	..	..	..	..	..	..	..	..	..	..	..		..	..	..	..	..	About 60 p. c. blastulæ. Died off gradually.
26	Feb. 26—Mar. 10. 12 days.	See No. 26 above.	0	4	8	26	30	32	0	6	94	10	58		32	50	„	..	..	Larvæ well developed on 12th day, but rather a large proportion irregular.
33	Mar. 3—11 ..... 8 days.	Eggs kept 3 hrs. Several ♂ mixed.	13	51	16	14	4	2	0	8	92	0	43	34	23	100	13 to 14·5	..	..	About 75 p. c. good blastulæ. Many good larvæ on 8th day.
34	„	♀ same. Eggs 1 hr. with 50 p. c. tap-water. ♂ same as 33.	5	52	13	28	1	0	0	1	99	1	25	39	35	75	„	..	..	Nearly all segmented. Mostly good blastulæ. Many larvæ on 8th day, but fewer and less well developed than 33.
35	„	♀ same. Eggs 2 hrs. in 50 p. c. tap-water. ♂ same.	4	58	13	17	8	0	0	6	94	4	44	27	25	100	„	17·8	10·8	About 40 p. c. segmented. Mostly good blastulæ. Larvæ intermediate between 33 and 34.
33	Mar. 3—13 ..... 10 days.	See 33 above.	3	41	16	23	16	1	0	12	88	4	56	31	9	100	12·8 to 14·5	..	..	Apparently not changed since 8th day.
33′	„	Same as last, but only *small* ones counted.	0	30	4	48	13	4	0	4	96	0	48	26	26	23	„	..	..	
34	„	See 34 above.	2	36	20	20	22	0	0	9	91	9	47	27	18	45	„	..	..	Little change since 8th day.

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36	Mar. 3	♀ same as 33. Eggs 1 hr. with 66 p. c. tap-water. ♂ same, fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	No segmentation.
52	Mar. 10.....	♀ *vi*-wt. Eggs kept 2½ hrs. ♂ fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Few blastulæ. Culture accidentally destroyed.
53	Mar. 10—18 ..... 8 days.	♀ same. Eggs 2¼ hrs. in 50 p. c. tap-water. ♂ same, fresh.	0	18	45	32	0	5	0	0	100	0	0	9	91	22	12·6 to 13	..	..	About 60 p. c. good blastulæ. Well developed larvæ on 8th day.
54	Mar. 10	Same as 53, but fertilised in 33 p. c. tap-water.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	No segmentation.
56	Mar. 11—19..... 8 days.	♀ *vi*.-brn. Eggs 1 hr. at 26—29°. ♂ fresh. Fertilised at 18·5°.	1	12	23	7	2	30	0	1	99	1	9	4	85	75	12·6 to 13·0	..	..	All segmented. Mostly good blastulæ. On 8th day mostly good, but some very poor.
57	,,	♀ same. Eggs 1¾ hr. at 26—29°. ♂ same. Fertilised at 18·5°.	0	21	34	12	9	24	0	0	100	0	24	12	64	33	,,	..	..	Rather few segmented.
58	,,	♀ same. Eggs 3 hrs. at 26—29°. ♂ same. Fertilised at 16°.	0	33	21	5	9	32	0	1	99	0	15	12	73	66	,,	..	..	Mostly segmented, but some irregularly. Larvæ less well developed than 56 and 57.
56	Mar. 11—21..... 10 days.	See 56 above.	0	6	22	12	22	38	0	2	98	2	16	20	62	50	,,	..	..	
59	Mar. 12—20..... 8 days.	♀ *vi*.-wt. Eggs kept 3 hrs. ♂ fresh.	0	3	7	12	45	33	0	0	100	0	9	25	66	150	,,	..	..	About 50 p. c. blastulæ. Larvæ on 8th day very good.
60	Mar. 12	♀ same. Eggs 1¼ hrs. in 50 p. c. distilled water. ♂ same, fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	,,	..	..	Very few blastulæ, died off rapidly.
61	Mar. 12—20..... 8 days.	♀ same. Eggs 3 hrs. in 43 p. c. distilled water. ♂ same, fresh.	0	6	17	17	41	19	0	0	100	1	8	12	79	150	,,	..	..	Nearly all blastulæ. 8th day larvæ abundant, but less well developed than 59.
62	Mar. 13—21..... 8 days.	♀ same. Eggs kept 23 hrs. ♂ same, kept alive. Sperm fresh.	0	0	17	17	34	34	0	0	100	0	0	17	83	6	,,	..	..	Fair number segmented, but irregularly. Only 6 larvæ survived till 8th day.
63	Mar. 13.....	♀ same. Eggs kept 30 hrs. ♂ same, kept alive. Fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	,,	..	..	Irregularly segmented. Died off at once.

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
68	Mar. 14—22..... 8 days.	♀ eggs kept 2 hrs. (at 14°). ♂ fresh. Fertilised at 14°.	4	46	25	17	6	2	0	1	99	0	6	12	82	100	12·6° to 13·4	..	..	About 70 p. c. good blastulæ. Many larvæ on 8th day; large proportion with oral arm skeleton small or absent.
69	„	♀ ♂ both 2 hrs. at 23°. same. Fertilised at 22·5°, and cooled slowly afterwards.	3	44	24	17	6	6	0	3	97	0	9	17	74	100	„	..	..	Nearly all good blastulæ. Many larvæ on 8th day; larger and better than 68.
69A	„	69 reared in 1 litre jar.	9	33	31	13	7	7	1	0	99	0	23	20	58	75	„	..	..	Smaller and less well developed than 69 reared normally.
71	Mar. 17—25..... 8 days.	♀ *vi.*-wt. Eggs 1½ hrs. at 13°. ♂ fresh. Fertilised at 13°.	0	2	12	23	56	7	0	10	90	0	16	14	70	150	12·8 to 13·9	..	..	About 20 p. c. blastulæ. Many larvæ on 8th day, mostly good.
72	„	♀ same. Eggs 1½ hrs. at 25—29°. ♂ same. Fertilised at 22°.	0	3	7	29	49	12	0	4	96	0	26	15	59	190	„	..	..	About 20 p. c. blastulæ. Many larvæ on 8th day, but less well developed than 71.
73	„	♀ same. Eggs 1½ hrs. at 22°. ♂ same. Fertilised at 22°.	0	8	13	21	52	5	0	1	99	0	24	13	63	75	„	..	..	About 75 p. c. blastulæ. Many larvæ on 8th day, about like 72.
74	Mar. 18—26..... 8 days.	♀ same. Eggs kept 21 hrs. ♂ different, fresh. Fertilised at 13°.	0	0	5	13	82	0	0	2	98	0	12	12	76	60	„	..	..	Many segmented, but rather irregularly.
75	„	♀ same. Eggs kept 19 hrs., then 2 hrs. with 43 p. c. tap-water. ♂ same as 74, fresh.	0	0	9	9	73	9	0	0	100	0	9	18	73	11	„	..	..	Many segmented. Few on 8th day.
76	„	♀ same. Eggs kept 19 hrs., then 2 hrs. at 22—27°. ♂ same as 74. Fertilised at 20°.	0	0	6	22	72	0	0	3	97	0	9	9	82	32	„	..	..	Moderate number segmented, but irregularly. Few survived till 8th day.

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71	Mar. 17—29..... 12 days.	See 71 above.	0	0	6	14	68	12	0	2	98	4	18	38	40	50	12·8 to 14·3	..	..	Many survived till 12th day, but proportion of badly developed larger than on 8th.
72	„	See 72 above.	0	0	2	12	76	10	0	2	98	0	24	44	32	50	„	..	..	Same remarks as last.
73	„	See 73 above.	0	2	4	14	70	10	0	2	98	2	26	42	30	50	„	..	..	Same remarks as 71, 12 days.
85	Mar. 25—Apr. 3 .. 9 days.	♀ eggs kept 2 hrs. ♂ fresh.	4	30	36	15	14	1	0	7	93	0	18	13	69	100	13·5 14·5	..	..	Over 70 p. c., good blastulæ. Large number of good 9th day plutei.
85A	„	85 warmed to 29° for 2 hrs., from 2nd to 4th hr. after fertilisation.	4	46	29	13	3	5	0	6	94	0	22	12	66	100	„	..	..	About 70 p. c. blastulæ, some irregular. Many plutei.
85B	„	85 reared in 1 litre jar.	4	46	18	20	12	0	0	6	94	0	20	12	68	50	„	..	..	Well developed plutei.
86	„	♀ same. Eggs at 28—30°. For few minutes reached 33°. ♂ same, fresh.	4	39	31	4	13	9	0	13	87	4	18	4	74	23	„	..	..	About 50 p. c. irregularly segmented and broken up. Few blastulæ. Few larvæ on 9th day, but well developed.
87	Mar. 26—Apr. 5 .. 10 days.	♀ same. Eggs kept 23 hrs. ♂ different, fresh.	6	27	36	27	4	0	0	6	94	0	6	30	64	30	14 to 15	..	..	About 50 p. c. good blastulæ. Moderate number of larvæ on 10th day.
88	Mar. 26.....	♀ same. Eggs kept 29 hrs. ♂ same as 87, fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Fully 70 p. c. segmented, mostly well. Good number of blastulæ, but died off later.
89	Mar. 27—Apr. 7 .. 11 days.	♀. Eggs kept 3 hrs. ♂ fresh.	4	29	27	25	15	0	0	19	81	60	23	12	5	75	„	..	..	Good number of good larvæ.
90	„	♀ same. Eggs 3 hrs. in sea water concentrated to five-sixths of its volume. ♂ same, fresh.	0	20	21	36	21	1	0	21	79	53	31	8	8	75	„	..	..	Plutei unhealthy, mostly at bottom of jar.
93	Apr. 1—9..... 8 days.	♀ *vi*-wt, fresh. Urchin newly caught. ♂ fresh (*a*).	0	11	26	17	38	8	0	9	91	12	44	22	22	100	14·4 to 15·1	20·6	16·9	Moderate number of good blastulæ. Good number of good larvæ.
94	„	♀ same, fresh. ♂ different (*b*), fresh.	0	8	17	20	48	7	0	8	92	13	42	8	37	60	„	..	..	Moderate number of blastulæ. Good number of larvæ, but less well developed than 93, 95, or 96.
95	„	♀ same, fresh. ♂ different (*c*), fresh.	0	13	25	34	26	2	0	7	93	2	36	30	32	100	„	19·1	15·2	Moderate number of blastulæ. Good number of good larvæ.
96	„	♀ different. vi-wt. Urchin at least 5 days in tank. ♂ same as 95 (*c*), fresh.	0	17	41	22	17	3	0	1	99	3	25	11	61	150	„	18·6	17·3	Many blastulæ. Many well-developed plutei.

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Condition.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
121	May 14—21 ..... 7 days.	♀ *brn*-wt. 5 weeks in tank. Eggs fresh. ♂ (*a*) 5 weeks in tank. Fresh.	1	17	25	22	26	9	0	30	70	9	39	29	23	100	15°3 to 16°4	..	..	All segmented. Very many larvæ, very well developed.
121	May 14—22 ..... 8 days.	See 121 above.	1	0	25	27	32	5	1	28	71	19	37	15	29	100	„	19·8	20·1	
122	„	♀ same, fresh. ♂ s (*b*), 3 mixed. 5 weeks in tank.	1	25	35	21	10	8	0	19	81	1	21	27	51	100	„	..	..	Less than 10 p. c. blastulæ. Many larvæ, but less well developed than 121.
123	„	♀ vi-wt, newly caught, fresh. ♂ (*a*). 5 weeks in tank.	0	6	20	25	43	6	1	38	61	18	35	21	26	100	„	20·2	17·5	About 5 p. c. blastulæ. Many good larvæ.
124	„	♀ same as 123, fresh. ♂ s (*b*) same as 122, fresh.	0	12	40	8	38	2	2	20	78	2	18	18	62	50	„	17·9	18·0	About 70 p. c. blastulæ. Good number of good larvæ.
121A	„	121 after 48 hrs. mixed with equal volume (50 p. c.) tap-water for 1½ hrs.	0	9	22	23	37	9	0	17	83	6	42	25	27	100	„	..	..	Poorly developed, many very bad.
121B	„	121 after 48 hrs. heated for 2 hrs. to 34°.	0	48	36	4	12	0	0	0	100	8	12	12	68	25	„	..	..	Same remarks as 121A, but more badly developed.
125	May 15—23 ..... 8 days.	♀ same as 123. Eggs kept 23 hrs. ♂ (*c*) different, newly caught.	1	18	28	14	37	2	0	9	91	5	31	31	33	100	15 to 16°4	18·2	13·5	About 50 p. c. blastulæ. Many evenly developed larvæ.
126	„	♀ same. Eggs kept 23 hrs. ♂ (*d*) different. Newly caught.	2	26	39	19	11	0	0	27	73	0	13	31	56	100	„	..	..	Nearly all blastulæ. Many good larvæ, larger and better than 125.
127	May 15	♀ same as 121. Eggs kept 24 hrs. ♂ (*c*) same as 125.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2 or 3 p. c. blastulæ. Died off.

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128	May 15—23 ..... 8 days.	♀ *vi*-wt. Newly caught, fresh. ♂ (*c*) same as 125, fresh.	0	12	30	25	24	9	0	8	92	7	35	41	17	100	,,	15·1	17·9	About 20 p. c. good blastulæ. Many good larvæ.
129	,,	♀ same, fresh. ♂ (*d*) same as 126.	0	35	27	24	14	0	1	27	72	2	23	19	56	100	,,	..	21·8	40 p. c. good blastulæ. Many good larvæ.
130	,,	♀ same, fresh. ♂ (*e*) different.	0	12	30	18	38	2	0	0	100	2	30	16	52	50	,,	..	18·1	Most segmented and gave good blastulæ. Moderate larvæ on 8th day.
121A	May 14—26..... 12 days.	See 121A above.	0	6	20	16	50	8	0	4	96	20	34	24	22	50	,,	..	..	Not much changed since 8th day.
125	May 15—26..... 11 days.	See 125 above.	3	21	24	18	33	0	0	6	94	3	45	21	30	50	,,	..	..	Little changed since 8th day.
128	,,	See 128 above.	0	10	28	24	28	10	0	12	88	30	18	36	16	50	,,	..	..	Little changed since 8th day.
131 (*cf.* 128 above)	May 16—24..... 8 days.	♀ same as 128. Eggs kept 24 hrs. ♂s (2 mixed) (*a*) fresh.	0	26	30	12	30	2	0	2	98	0	16	22	62	50	15·4 to 16·4	..	15·8	About 80 p. c. blastulæ. Many good larvæ.
132	,,	♀ same. Eggs kept 26 hrs. ♂ (*b*), different, fresh.	0	22	27	18	33	0	0	2	98	0	6	17	77	100	,,	15·5	16·5	About 20 p. c. blustulæ. Many good larvæ.
132	May 16—26..... 10 days.	See 132 above.	0	8	20	24	48	0	0	8	92	6	22	22	50	50	,,	..	..	Rather better developed than on 8th day.
135	May 16—24..... 8 days.	♀ wt. Newly caught. Eggs kept 3 hrs. ♂ (*b*), same as 132.	0	14	28	24	28	6	0	6	94	6	16	18	60	50	,,	..	20·0	About 20 p. c. blastulæ. Many good larvæ.
136	,,	♀ same. Eggs 1 hr. in sea water, concentrated to three-quarters of its volume. Then 2 hrs. in normal. ♂ (*b*), same, fresh.	0	14	38	18	28	2	0	2	98	0	12	10	78	50	,,	..	..	About 35 p. c. blastulæ. Plutei very good.
137	,,	♀ same. Eggs 2 hrs. in concentrated sea water. ♂ same (*b*), fresh.	0	16	32	20	22	10	0	4	96	8	10	16	66	50	,,	..	..	About 40 p. c. blastulæ. Plutei less good than 135 and 136.
138	,,	♀ same. Eggs 2¾ hrs. in concentrated sea water (three quarters of its volume). ♂ same, fresh.	0	22	38	14	24	2	0	8	92	2	12	8	78	50	,,	..	..	About 10 p. c. blastulæ. Larvæ better than 137.
147	May 21—28..... 7 days.	♀ *vi*-wt., fresh. ♂s (2 mixed), fresh.	0	31	37	14	15	3	0	10	90	5	18	20	57	100	15 to 16·4	18·8	17·2	All segmented, but many irregular. Larvæ mostly good.

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars. per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
148	May 21—28 ..... 7 days.	♀ *vi*-wt. Different from 147, fresh. ♂s (2). Different from 147, fresh.	2	8	14	24	48	4	0	14	86	2	14	40	44	50	15° to 16·4	..	..	About 40 p. c. good blastulæ. Moderate larvæ.
151	,,	♂ ♀ same as 147, eggs and sperm both kept 5 hrs.	0	35	35	11	14	5	0	3	97	0	14	24	62	100	,,	19·2	13·6	About 20 p. c. good blastulæ. Good number of plutei.
147	May 21—30 ..... 9 days.	See 147 above.	1	31	25	22	16	5	0	23	77	4	26	32	28	80	15 to 17	..	..	
150	,,	♀ ♂ same as 147. Eggs and sperm kept 4 hrs.	0	12	24	22	26	16	0	10	90	2	16	28	54	50	,,	..	..	About 25 p. c. good blastulæ. Larvæ well developed.
157	May 23—31 ..... 8 days.	♀ *vi*-wt. Eggs 1 hr. in sea water, concentrated to half its volume. ♂ fresh.	1	12	17	32	37	1	0	10	90	15	41	20	24	100	15 to 17·5	..	..	About 40 p. c. blastulæ. Many eggs irregularly broken up. Larvæ moderate.
159	,,	♀ same. Eggs kept 2 hrs. in normal sea water. ♂ same, fresh.	0	5	11	33	48	3	0	12	88	14	50	17	19	100	,,	..	..	About 80 p. c. blastulæ. Very many larvæ, most well developed.
159A	,,	159 in 50 p. c. tap-water for 1½ hrs. when 24 hrs. old.	0	20	24	32	20	0	0	24	76	0	40	24	36	25	,,	..	..	Very poorly developed.
162	May 26—June 3 .. 8 days.	♀ *vi*-wt., fresh. ♂ 3 mixed, fresh.	8	28	38	10	16	0	0	22	78	32	20	18	30	50	15·7 to 18·8	..	..	About 25 p. c. blastulæ. Moderate larvæ.
163	,,	♀ *vi*-wt., fresh. Different from 162. ♂ 10 days in tank. Very ripe, fresh.	1	32	36	20	11	0	0	16	84	52	11	17	20	75	,,	..	..	About 10 p. c. blastulæ. Many 8th day larvæ, poorly developed.

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165 and 166	May 26	♀ same as 162. Eggs 5 and 8 hrs. in ovary. ♂ same as 162. Sperm kept 5 and 8 hrs. respectively.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	„	..	..	Moderate number of eggs, segmented, and then died off.
167	May 26—June 3 .. 8 days.	♀ same as 163. Eggs kept 8 hrs. ♂ different, fresh.	0	17	43	15	24	1	0	11	89	51	32	11	7	75	„	19·7	..	Mostly good blastulæ. 8th day larvæ not well developed.
168	May 27—June 4 .. 8 days.	♀ *vi*-wt., fresh. ♂ same as 167. Sperm kept 15 hrs.	0	2	23	10	65	0	0	10	90	80	7	3	10	60	16 to 19	20·7	..	Very few blastulæ. Most of larvæ very badly developed.
169 to 172	May 27	♀ same as 163. Eggs kept 24 hrs. both in water and in ovary. Fertilised with fresh and stale sperm.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Some gave a few bastulæ, but all died off rapidly.
173	May 28—June 5 .. 8 days.	♀ *vi*-wt., fresh. ♂ newly caught, fresh.	2	14	33	25	26	0	15	41	44	60	16	10	14	100	16·4 to 19	..	..	Many blastulæ. Many well developed larvæ on 8th day.
174	„	♀ same. ♂ same. Sperm ½ hr. in 20 p. c. tap-water. Fertilised in 15 p. c.	3	28	33	14	22	0	11	56	33	54	11	17	20	36	„	..	..	Less than 1 p. c. blastulæ. Few 8th day larvæ.
175	„	♀ same. ♂ same. Sperm ½ hr. in 35 p. c. tap-water. Fertilised in 25 p. c.	0	35	21	28	14	0	7	56	35	49	28	7	14	14	„	..	..	Very few segmented. Very few larvæ.
174 and 175	„	174 and 175 added together for comparison.	2	30	30	18	20	0	10	56	34	52	16	14	18	50	„	..	..	
180	May 29—June 6. 8 days.	♀ green (very rare colour), fresh. ♂ (*a*), fresh.	0	1	4	20	73	2	0	19	81	24	24	24	28	100	16·6 to 19·2	18·0	20·3	Many good blastulæ. Many well-developed larvæ.
181	„	♀ same. ♂ different (*b*).	0	4	5	31	60	0	0	25	75	25	24	24	27	75	„	17·5	16·6	Larvæ rather less well developed than 180.
182	„	♀ *brn*-wt. Fresh. ♂ (*a*), same as 180.	0	0	4	10	86	0	0	10	90	22	18	28	32	50	„	18·2	20·2	Same remarks as 180.
183	„	♀ same as last. ♂ (*b*), same as 181.	0	2	16	32	50	0	0	32	68	34	14	16	36	50	„	17·9	12·9	Many good blastulæ. Rather few 8th day larvæ, poorly developed.
180A	„	180 for 1 hr. in 50 p.c. tap-water, when 48 hrs. old.	0	0	6	12	82	0	0	16	84	24	14	28	34	50	„	18·7	16·0	Well-developed 8th day larvæ.

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
181A	May 29—June 6. 8 days.	181 kept for several days in overcrowded jar, with dirty water, so that many died off.	2	8	36	32	22	0	0	40	60	18	30	12	40	50	16°6 to 19·2	17·6	13·1	Large proportion died off during development.
180	May 29—June 9. 11 days.	See 180 above.	0	1	15	9	75	0	0	12	88	25	19	16	40	75	16·6 to 19·8	..	..	
180A	,,	See 180A above.	0	0	8	14	78	0	0	10	90	40	8	16	36	50	,,	..	..	
182	,,	See 182 above.	0	0	12	6	82	0	0	8	92	34	10	16	40	50	,,	..	..	
184 to 186	May 30.....	♀ same as 180, 181. Eggs kept 23 hrs. ♂s different. Fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Some of the eggs segmented, but died off.
187	May 30—June 7. 8 days.	♀ *brn*-wt., same as 181. Eggs 23 hrs. in ovary. ♂ same as 180 (*a*), 23 hrs. in testis.	0	2	8	30	60	0	0	24	76	18	18	8	56	50	17 to 19·2	..	..	Few blastulæ and segmented eggs. Few larvæ on 8th day, mostly poor.
188	,,	♀ same. Eggs 23 hrs. in ovary. ♂ different. Fresh, newly opened.	0	11	19	32	38	0	0	25	75	11	25	24	40	75	,,	..	..	Good number of blastulæ. Good number of larvæ, much better developed than 187.
221	June 9—17..... 8 days.	♀ *brn*-wt. ♂ (*a*) } both fresh.	0	11	36	28	25	0	8	52	40	19	28	31	22	75	19·2 to 19·9	19·4	21·3	Many good larvæ (greatest arm-length 28, smallest 13).
221A	,,	221 reared at about 16°·5	0	20	40	18	22	0	0	32	68	2	18	38	42	50	16·5*	19·7	13·2	(Greatest arm-length 20, smallest 8.)
222	,,	♀ same as 221. Eggs shaken for 2 minutes. ♂ same as 221.	0	18	24	34	22	2	8	54	38	16	24	34	26	50	19·2 to 19·9	..	..	Good number of blastulæ. Larvæ rather less well developed than 221.

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223	„	♀ same. ♂ (*b*), different. 25 days in tank.	0	18	36	34	12	0	14	66	20	44	34	14	8	50	„	19·1	24·2	Good number of blastulæ. Larvæ very good.
224	„	♀ same. ♂ (*c*), different.	0	8	36	40	16	0	2	76	22	22	52	16	10	50	„	19·2	16·6	Moderate number of blastulæ. Larvæ much less good than 223.
225	„	♀ *vi*-wt. (different). ♂ (*a*), same as 221.	0	0	5	23	35	37	0	35	65	10	17	22	51	75	„	20·5	20·4	Good number of blastulæ. Very well-developed larvæ.
226	„	♀ same as last. ♂ (*b*), same as 223.	0	12	36	36	18	0	6	54	42	24	30	12	36	17	„	..	..	Moderate number of blastulæ. Larvæ good, but very few.
227	„	♀ same. ♂ (*c*) same as 224.	0	16	20	26	38	0	2	40	58	4	44	26	26	50	„	..	..	Moderate number of blastulæ. Larvæ well developed.
221	June 9—21..... 12 days.	See 221 above.	0	26	17	29	29	0	23	34	43	40	17	32	11	35	18·6 to 19·9	..	22·0	Many had died since 8th day (greatest arm-length 22, least 9).
221A	„	221 kept 12 days at 16—17°	0	12	20	32	36	0	0	30	70	20	42	16	22	50	16* to 17	..	17·0	Greatest arm-length 25, least 8.
225	„	See 225 above.	0	10	25	30	35	0	0	38	62	30	28	30	12	40	„	..	..	
235	June 10—18..... 8 days.	♀ same as 221. Eggs kept 24 hrs. at 16°. ♂ new, fresh.	0	15	25	40	20	0	0	25	75	30	25	15	30	20	19·2 to 19·9	..	15·4	Few blastulæ. Few larvæ, moderately developed.
241	June 11—19..... 8 days.	♀ wt. fresh. Fertilised at 17°·3 and reared at 17—18°. ♂s, 3 mixed, newly caught, fresh.	0	10	28	14	40	8	0	18	82	2	22	22	54	50	17* to 18	..	18·2	About 10 p. c. good blastulæ. Good number of well-developed larvæ.
241A	„	241 reared at 21—23° from 1 hr. after fertilisation. Towards end of 8 days temp. fell to 20—21°.	2	8	34	34	20	2	0	30	70	12	20	18	50	50	21* to 23	..	20·4	Good number of blastulæ. More advanced than 241.
242	„	♀ ♂ same, fertilised at 23°. raised for 3 mins. to 26°. Reared at 22—23°, later 20—21°.	0	14	38	16	32	0	0	14	86	14	14	14	58	50	„	..	17·9	Many good gastrulæ. Larvæ well but unevenly developed.
242A	„	242 reared at 17—18° from 1 hr. after fertilisation.	0	18	42	12	26	2	0	10	90	0	16	26	58	50	17* to 18	..	14·3	Moderate number of blastulæ. Larvæ poorly developed.
242A′	„	Larvæ from 242A with arm-length above 18, counted separately.	0	15	50	20	15	0	0	10	90	5	10	15	70	20	„	..	..	

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
242B	June 11—19..... 8 days.	242 for 2 hrs. in 50 p. c. tap-water when 30 hrs. old.	0	12	24	21	42	0	0	24	76	21	9	24	45	33	21° to 23	..	..	Both 242B and 242C were kept in 1 litre jars.
242C	,,	242B for 1 hr. in 50 p. c. tap-water for 2nd time time when 48 hrs. old.	0	4	32	12	52	0	0	20	80	16	8	16	60	25	,,	..	..	
242B and 242C	,,	242B and 242C added together.	0	10	27	17	47	0	0	23	77	18	8	20	54	60	,,	..	..	
241	June 11—23..... 12 days.	See 241 above. 12 days at 17—18°.	2	8	44	14	32	0	0	10	90	8	12	20	60	50	17* to 18	..	16·8	
241′	,,	241 at 17—18° till 8th day; from 8th to 12th day at 21°.	0	12	14	16	58	0	0	22	78	14	24	6	36	50	17* to 21	..	15·2	
241A	,,	See 241A above. 12 days at 21—23°.	0	4	26	28	42	0	0	14	86	20	16	14	50	50	20* to 23	..	18·8	
242A	,,	See 242A above. 12 days at 17—18°.	4	8	44	20	24	0	0	8	92	0	20	12	68	25	17* to 18	..	12·7	
242A″	,,	242A, 1st 8 days at 17—18°, 8th to 12th day at 21°.	0	8	28	16	48	0	0	32	68	36	16	4	44	25	17* to 21	..	15·3	
244	June 12—20..... 8 days.	♀ brn-wt., fresh. ♂ s, 3 mixed, fresh.	6	36	32	10	14	0	0	26	74	30	24	20	26	50	18·6 to 19·7	18·7	14·5	Good number of blastulæ. Larvæ moderate.
245	,,	♀ } same. ♂ } eggs and sperm kept 4½ hrs.	0	12	48	6	30	3	0	12	88	15	36	15	33	33	,,	..	..	Moderate number of blastulæ. Larvæ less well developed than 244.

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246	„	♀ } same. ♂ } eggs and sperm kept 7 hrs.	0	16	40	14	30	0	0	12	88	22	44	12	22	50	„	19·2	18·9	Moderate number of blastulæ. Larvæ better than 244, much better than 245.
247	„	♀ same. Eggs kept 7 hrs. ♂ different, very ripe, fresh.	0	10	34	26	18	12	0	10	90	20	46	22	12	50	„	..	..	Moderate number of blastulæ. Larvæ good.
248	„	♀ same. Eggs kept 7 hrs. ♂s, 3 (including that used in 247), fresh.	4	20	24	32	20	0	0	10	90	40	18	26	16	50	„	20	22·9	Moderate number of blastulæ. Larvæ very good.
251	June 16—24..... 8 days.	♀ brn-wt. Eggs kept 4 hrs. at 16°·5. ♂s, 2 mixed, fresh. Fertilised at 18°.	0	1	10	32	57	0	0	6	94	58	28	10	4	100	18·6 to 20·3	..	22·4	Many blastulæ. On 8th day very many good larvæ. (Greatest arm-length 29, least 11, but very few below 16.)
252	„	♀ same. Eggs kept 4 hrs. at 16°·5. ♂ same. Fertilised at 25°.	0	1	10	24	65	0	0	13	87	55	27	9	9	100	„	..	25·1	Many blastulæ. Larvæ like 251. (Greatest arm-length 32, least 18.)
252A	„	252 reared at 16° after 2nd day.	0	7	24	33	35	1	1	8	91	8	47	27	19	75	16*	..	19·9	(Greatest arm-length 27, least 9.)
253	„	♀ same as 251. Eggs 4 hrs. at 26—27°. ♂ same, fresh. Fertilised at 22°.	0	1	3	27	68	0	0	9	91	64	25	5	5	75	18·6 to 20·3	..	23·0	Many blastulæ. Larvæ like 251. (Greatest arm-length 37, least 13.)
254	„	♀ same. Eggs kept 4 hrs. at 26—27°. ♂ same, fresh. Fertilised at 25°.	0	2	13	20	65	0	0	12	88	50	33	7	10	40	„	..	..	Many blastulæ. Larvæ like last.
252A	June 16—28..... 12 days.	See 252A above (all 12 days at 16°).	0	3	21	21	54	0	0	10	90	21	33	36	9	33	16* to 17	19·7	17·3	
252A	„	252 till 8th day at 16°; from 8th to 12th day at 22—23°.	0	0	15	20	65	0	0	20	80	60	13	7	20	40	16* to 23	..	24·8	
252	„	252 12 days at 20—23°.	0	0	10	25	65	0	0	30	70	90	5	5	0	20	20* to 23	..	22·6	
258	June 18—26..... 8 days.	♀ brn-wt. Eggs kept 5 hrs. at 16°. ♂s (3) newly caught, full of food, fresh. Fertilised at 21°.	0	0	26	18	56	0	0	16	84	42	20	18	20	50	18·6 to 20·5	..	..	Larvæ not very well developed.

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TABLE II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			4.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.					
259	June 18—26..... 8 days.	♀ same. Eggs 5 hrs. at 16°. ♂s (3) 34 days in tank. Gut empty. Fertilised at 21°.	0	17	18	25	40	0	0	2	98	38	15	17	30	25	18°·6 to 20·5	..	..	Larvæ very badly developed. Almost all those with "2" arm-bars had very short arms.
263	June 19—27..... 8 days.	♀ wt. Very ripe. ♂s (2) rather immature.	0	4	32	8	56	0	0	8	92	8	24	16	52	25	18·6 to 20·8	..	10·8	Good number of blastulæ. Larvæ few and badly developed.
266	,,	♀ same. ♂s (2) different, very ripe.	0	10	22	30	38	0	0	10	92	2	18	16	64	50	,,	..	14·1	Good number of blastulæ. Larvæ poorly developed, but better than 263.
268	June 20—27..... 7 days.	♀ wt. fresh. ♂ fresh. } Fertilised at 19° and reared at 20—22°.	0	8	26	36	30	0	0	28	72	14	18	14	54	50	20* to 22	..	21·4	Many blastulæ. On 7th day many larvæ, moderately developed.
268A	,,	268 reared at 16—17° from 5 hrs. after fertilisation.	0	7	45	38	7	0	0	15	85	0	0	15	85	13	16* to 17	..	15·6	Very few survived till 7th day. Only 13 counted.
268B	,,	268A removed and reared at 20—22° after being 18 hrs. at 16°.	0	10	30	20	30	0	0	20	80	0	10	10	80	10	16* then 20–22	..	15·5	Very few survived. Only 10 counted.
268C	,,	268A removed and reared at 20—22° after being 70 hrs. at 16°.	0	0	12	25	63	0	0	12	88	2	12	18	68	40	,,	..	26·2	Rather few survived, but these were well developed.
272	June 23—July 1.. 8 days.	♀ vi-wt. rather immature with thin shell. ♂s (3) fresh. (*a*).	0	0	14	48	38	0	2	54	44	58	8	4	30	50	21* to 23	..	19·7	Many blastulæ. Many larvæ, unevenly developed (greatest arm-length 30, least 11).
274	,,	♀ ♂ } same. Eggs and sperm kept 6 hrs.	0	7	27	20	47	0	3	30	67	30	33	20	17	30	,,	..	15·4	Less than 1 p. c. blastulæ. Very few 8th day larvæ, very unevenly developed (greatest arm-length 30, least 3).

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275		,,	♀ same. Eggs kept 6 hrs. ♂s (3 mixed) (*b*) fresh.	0	10	25	25	40	0	0	30	70	15	25	20	40	2	,,	..	17·4	Few blastulæ. Very few survived, moderately developed (greatest arm-length 27, least 10).
276		,,	♀ *brn*-wt. fresh. ♂s same as 272A. Sperm kept 7 hrs.	0	16	28	20	36	0	4	26	70	18	18	30	34	50	,,	..	22·9	Few blastulæ. Moderate number of well-developed larvæ.
277		,,	♀ same as 276. Fresh. ♂ (*b*) same as 275. Fresh.	0	8	22	34	36	0	2	26	72	24	20	24	32	50	,,	..	19·6	Many blastulæ. Good number of 8th day larvæ.
277′		,,	Dead skeletons of 277, from bottom of jar.	0	15	15	45	25	0	0	25	75	5	40	25	30	20	,,	..	19·6	
278	June 24—July 2 .. 8 days.		♀ fresh. ♂s (several mixed) mature.	2	20	22	28	25	0	2	20	78	54	22	20	4	50	22* to 23·5	..	19·4	Many blastulæ. 8th day larvæ well developed.
280		,,	♀ same. ♂s (2 mixed) immature.	4	24	30	32	10	0	0	16	84	54	18	18	10	50	,,	..	17·0	Many blastulæ. 8th day larvæ less well developed than 278.
291	June 25.....		♀ ♂ fresh.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Rather few blastulæ. All the larvæ died off.
292	June 25—July 3 .. 8 days.		♀ same. Eggs shaken till they began to lose their shape and break up. ♂ same. Fresh.	0	7	30	27	36	0	0	36	64	57	20	10	13	30	22* to 23·5	..	18·9	Many blastulæ. Many 8th day larvæ, unevenly developed. No dwarfs (greatest arm-length 35, least 8).
354	July 7—14 ..... 7 days.		♀ *vi*-wt. Fresh. ♂s (3 mixed) sperm kept 7 hrs.	0	5	45	40	10	0	0	30	70	5	0	10	85	20	22·5* to 25	..	12·4	Fair number of blastulæ. 7th day larvæ very poor.
355		,,	♀ same. Fresh. ♂ (3 mixed) different. Fresh.	8	42	17	17	17	0	25	25	50	8	25	8	58	12	,,	..	18·0	Fair number of blastulæ. Very few survived to 7th day.
356		,,	♀ (2 mixed). Eggs kept 4 hrs. ♂ same as 354. Sperm kept 7 hrs.	0	16	16	8	60	0	0	16	84	4	4	20	72	25	,,	..	16·3	Fair number of blastulæ. Few larvæ, but these good.
357		,,	♀ same as 356. Eggs kept 4 hrs. ♂ same as 355. Fresh.	2	18	28	30	22	0	0	22	78	16	12	16	56	50	,,	..	14·5	Few blastulæ. Few larvæ, but these good.
368	July 8—15 ..... 7 days.		♀ Eggs 7 hrs. at 24°. ♂s (3 mixed). Fresh.	0	14	28	35	21	0	0	35	63	0	14	21	63	14	23* to 25·7	..	15·0	Fair number of blastulæ. Very few larvæ, poorly developed (greatest arm-length 21, least 11).
370		,,	♀ (2 mixed). Fresh. ♂ sperm 7 hrs. at 24°.	10	20	30	20	20	0	0	30	70	10	20	5	65	20	,,	..	11·1	Fair number of blastulæ. Very few larvæ, poorly developed (greatest arm-length 15, least 8).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table II.—*continued.*

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars. per cent.			Anterior-body skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average body-length (micrometer units).	Average arm-length (micrometer units).	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few	None.	Large.	Small.	Very small.	None.					
372	July 8—15 ..... 7 days.	♀ ♂ } Both fresh.	0	21	26	16	37	0	0	32	68	10	32	16	42	19	23* to 25·7	..	15·9	Few blastulæ. Very few larvæ, but these rather well developed (greatest arm-length 27, least 9).
405A	Dec. 8—15 ..... 7 days.	*Sph.* ♀ *brn*-wt., not very mature. *Strong.* ♂ fairly ripe. Reared at 18—20°.	2	43	29	14	9	2	0	14	86	2	32	23	43	40	—	..	..	About 40 p. c. blastulæ. Plutei unevenly developed, some very good.
405	Dec. 8—16 ..... 8 days.	Same. Reared at 12—13°.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	—	..	..	No larvæ good enough to count.
405A	„	Same. 8 days at 18—20°.	2	24	38	30	6	0	0	16	84	12	30	28	30	50	—	17·2	15·9	See 405A above (max. arm-length 28, min. 10).
405B	„	Same. 8 days at 13—15°.	7	30	37	13	13	0	0	3	97	0	3	30	67	30	—	17·3	11·7	Poorly developed. Many too bad to count. (Max. arm-length 16, min. 7.) 405B on Dec. 23, 15 days, was still in same condition.
407A	Dec. 9—17 ..... 8 days.	*Sph.* ♀ *vi*-wt. *Strong.* ♂ not very ripe. Reared at 19—20°.	0	5	8	30	57	0	5	24	71	57	24	5	14	40	—	18·4	18·4	Very few eggs segmented. Very few plutei, but those well developed. (Greatest arm-length 30, least 11.)
409	Dec. 10—18 ..... 8 days.	*Sph.* ♀ *brn*-wt. *Strong.* ♂ (2 mixed, not very ripe). 8 days at 13—15°.	3	51	24	6	9	6	0	3	97	0	3	6	90	33	—	17·5	9·4	About 10 p. c. segmented. Plutei poor.
409B	„	Same 1st 2 days at 13—15° afterwards at 19—20°.	0	12	12	36	36	4	0	32	68	20	12	24	44	25	—	19·6	18·1	Very few survived, but these good.
412	Dec. 11—19 ..... 8 days.	*Sph.* ♀ *vi*-wt. *Strong.* ♂. 8 days at 17—18.°	0	36	32	12	20	0	4	4	92	4	16	20	60	25	—	18·5	12·9	About 5 p. c. of eggs gave blastulæ. Plutei few and poor.

420	Dec. 13—20 …… 7 days.	*Sph.* ♀ 1 hr. in 50 p. c. tap-water. *Strong.* ♂. Reared at 17—18°.	2	35	40	17	5	0	0	5	95	0	15	8	67	40	—	17·1	10·6	About 30 p. c. blastulæ. Moderate number of plutei; poorly developed. (Max. arm-length 19, min. 5.)
420A	,,	Same reared at 20°.	0	32	34	12	12	0	2	26	72	0	16	36	48	50	—	17·8	13·9	Not very well developed. (Max. arm-length 19, min. 6.)
421A	Dec. 15—30 …… 15 days.	*Sph.* ♀. *Strong.* ♂. 7 days at 13—15°; then 8 days at 22°.	0	6	19	6	50	19	0	0	100	19	19	19	43	16	—	17·6	13·5	Very few survived till 15th day, and some of these irregular. 421, 7 days, at 13—15°, was closely like 422 reared at same temperature.
422	Dec. 15—22 …… 7 days.	*Sph.* ♀ *vi*-wt. *Strong.* ♂ several mixed, not very ripe. Reared at 13—15°.	15	57	20	0	3	5	0	0	100	0	5	3	92	40	—	16·2	11·8	About 25 p. c. blastulæ. Many good plutei, but arms short.
422A	,,	Same reared at 20°.	2	38	30	14	10	6	2	50	48	16	16	20	48	50	—	16·5	18·2	Many very good plutei.
424	,,	*Sph.* ♀ rather immature. *Strong.* ♂ also not very mature. Reared at 20—22°.	0	0	4	8	88	0	0	4	96	8	16	32	44	25	—	17·0	19·4	Moderate number of blastulæ. Few plutei survived, but these very well developed.
426	Dec. 18—27 …… 9 days.	*Sph.* ♀ 2 mixed, not very ripe. *Strong.* ♂ 3 mixed, not very ripe. Reared at 13—15°.	0	40	36	0	24	0	0	0	100	0	16	20	64	25	—	16·2	10·7	Many blastulæ. Fair number of plutei, poorly developed.
426A	,,	Same, reared at 15—18°.	0	28	34	12	26	0	0	16	84	2	22	24	52	50	—	17·8	16·6	Many plutei. Fairly well and evenly developed.
426B	,,	Same, reared at 20—22°.	0	18	30	26	24	2	0	20	80	10	26	30	34	50	—	17·3	17·2	
431	Dec. 21—29 …… 8 days.	*Sph.* ♀. *Strong.* ♂. Reared at 15—18°.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	—	..	..	Fair number of larvæ, but very bad.
431A	,,	Same, reared at 20—22°.	0	6	24	18	52	0	2	14	84	38	20	20	22	50	—	19·5	15·6	Good number of larvæ, but not well developed.
438	Dec. 29—Jan. 5 .. 7 days.	*Sph.* ♀ 3 mixed, ripe. *Strong.* ♂ 2 mixed, fairly ripe. Reared at 18°.	0	25	25	20	30	0	0	15	85	0	0	10	90	20	—	19·3	12·9	Few blastulæ. Rather few plutei.
438A	,,	Same. Reared at 21—23°.	0	15	35	30	20	0	0	10	90	0	15	5	80	20	—	19·2	11·0	Very few plutei.
439	Dec. 29—Jan. 6… 8 days.	*Sph.* ♀ unripe, *vi*-wt. *Strong.* ♂ same as 438. Reared at 14—16°.	5	25	25	20	20	0	0	5	95	0	15	10	75	20	—	18·6	8·6	Few plutei, very poor. (Max. arm-length 13, min. 0.)
439A	,,	Same. Reared at 21—23°.	4	22	22	30	20	2	0	34	66	8	10	22	60	50	—	19·4	14·2	Not many plutei, not well developed. (Max. arm-length 30, min. 9.)

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TABLE III.—Hybrids between *Sphærechinus* ♀ and *Echinus* ♂.

No. of experiment.	Date and duration.	Conditions.	Arm-bars, per cent.						Cross-bars, per cent.			Anterior body-skeleton, per cent.				Number of larvæ counted.	Range of temperature during experiment.	Average arm-length.	Notes.
			3.	2.	2 and 1.	1½.	1*.	1.	Many.	Few.	None.	Large.	Small.	Very small.	None.				
1—3	Feb. 20, 1902.....	♀ fresh. 3 different ♂s, fresh.	..		..		..		..	..	..	..	..		..	..	° —	..	Very few segmented. Died off.
4	Feb. 20—28..... 8 days.	♀ same. Eggs kept 4 hrs. ♂ (*a*), fresh.	54		31		15		0	8	92	0	46		54	13	13·4 to 14	..	About 2 p. c. segmented. Few plutei, very poor.
5	„	♀ same. Eggs kept 4 hrs. ♂ (*b*), fresh.	25		55		20		0	5	95	0	61		39	20	„	..	About 1 p. c. segmented. Few larvæ, very poor.
7	Feb. 21—Mar. 1. 8 days.	♀ same. Eggs kept 23 hrs. ♂ (*c*), fresh.	58		25		7		0	0	100	0	7		93	40	„	..	Over 70 p. c. segmented. Many plutei on 8th day, but bad.
9	„	♀ same. Eggs kept 23 hrs. ♂ (*d*), urchin dying, but sperm still alive.	21		42		35		0	0	100	0	14		84	14	„	..	About 70 p. c. segmented. Larvæ poor.
5	Feb. 20—Mar. 3. 11 days.	See 5 above.	0	15	0	55	30		0	0	100	0	45		55	20	13·4 to 14·5	..	Still bad.
7	Feb. 21—Mar. 3. 10 days.	See 7 above.	0	28	12	28	32		0	0	100	0	32		68	25	„	..	Still very poor larvæ.
9	„	See 9 above.	0	16	4	44	36		0	0	100	4	28		68	25	„	..	
7	Feb. 21—Mar. 7. 14 days.	See 7 above.	0	8	6	42	16	28	0	0	100	0	32		68	50	13 to 14·5	..	Still many plutei, but majority too bad to count.
29	Feb. 27—Mar. 10. 11 days.	♀ eggs kept 8 hrs. ♂ fresh.	0	8	12	30	30	32	0	6	94	10	58		32	50	„	..	About 40 p. c. segmented. Good number of larvæ, but very irregular.
200	June 3—11 ..... 8 days.	♀ *vi*-wt. Freshly caught. Rather immature. ♂s 3 mixed. Fresh.	0	4	20	24	52	0	2	32	66	16	14	28	42	50	18·8 to 19·9	..	Good blastulæ.

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200	June 3—15 ..... 12 days.	See 200 above.	0	6	12	44	38	0	0	42	58	20	8	34	38	50	,,	..	
206	June 4—12 ..... 8 days.	♀ *vi.*-wt. Immature. Eggs kept 9 hrs. at 22°. ♂ 2 mixed. Fresh.	0	0	6	8	86	0	0	2	98	6	8	26	60	50	19 to 19·9	..	Many good blastulæ. Plutei poor.
207	,,	♀ same. Urchin kept alive. Eggs 9 hrs. in ovary. ♂s same. Fresh.	0	0	20	7	73	0	0	7	93	7	7	40	46	15	,,	..	Very few blastulæ. Very few plutei, but these better developed than 206. Large clubs on body-skeleton.
215	June 5—13 ..... 8 days.	♀. Eggs 7½ hrs. in ovary. ♂s 3 mixed. Fresh.	2	10	35	23	30	0	3	49	49	2	10	37	51	60	,,	..	Few blastulæ. Plutei moderate on 8th day.
218	June 7—16 ..... 9 days.	♀ *brn*-wt. Fresh. ♂s, 3 mixed. Fresh.	0	10	17	20	53	0	5	30	65	7	20	18	55	40	,,	..	Good blastulæ. Plutei mostly good.
219	,,	♀ same. Fresh. ♂s same. Sperm kept 4 hrs.	0	8	27	33	32	0	5	33	62	7	17	28	48	60	,,	..	Few blastulæ. Mostly good.
342	July 4—10 ..... 6 days.	♀ Eggs shaken till they began to lose their shape. ♂s several mixed, very immature.	0	10	55	15	20	0	0	15	85	5	5	15	75	20	21·9 to 23·6	8·0	Good number of blastulæ. 8th day plutei very poor (same eggs unshaken gave few blastulæ, and no plutei).

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TABLE IV.—Experiments upon the Size of *Sphærechinus*, *Strongylocentrotus* and *Echinus* Larvæ reared under different conditions.

No. of experiment.	Date and duration.	Conditions.	Range of temp.	Body-length. Average.	Body-length. Max. and min.	Arm-length. Average.	Arm-length. Max. and min.
		A.—*SPHÆRECHINUS.*					
189	June 2—10..... 8 days.	*Sphærechinus.* ♀ ♂ both fresh.	18°·6 to 19·8	..	..	25·7	31 13
189A	,,	♀ and ♂ same. Eggs and sperm each kept 5 hrs.	,,	..	..	22·2	26 18
189B	,,	189A for ¾ hr. in 50 p. c. tap water when 50 hrs. old.	,,	..	..	28.4	32 25
189	June 2—14.....	See 189 above.	,,	..	..	25·8	31 19
189A	,,	See 189A above.	,,	..	..	20·8	25 15
191	June 2—10..... 8 days.	Eggs 44 hrs. in 100 c.c. sea water and 15 c.c. $\frac{n}{10}$ KCN. ♂ fresh.	,,	..	..	27·7	33 21
267	June 20—30..... 10 days.	♀ ♂ both fresh. Larvæ 10 days at 20—23°.	20* to 23	14·9	16 14	29·1	37 21
267A	,,	267 7 days at 20—23°, then 3 days at 16—17°	23* to 16	14·8	15 14	29·3	38 21
318	June 30—July 8.. 8 days.	♀ rather young. ♂ adult.	About 23*	13·5	15 11	32·5	38 15
319	,,	♀ very ripe. ♂ same.	,,	14·6	16 13	34·0	43 22

No. of experiment.	Date and duration.	Conditions.	Range of temp.	Body-length. Average.	Body-length. Max. and min.	Arm-length. Average.	Arm-length. Max. and min.
		B.—*STRONGYLOCENTROTUS.*					
282	June 24—July 2.. 8 days.	*Strongylocentrotus.* ♀ ♂. Reared at 16—17°.	16°* to 17	22·9	27 17	11·6	19 5
282A	,,	282 reared at 21—23°.	21* to 23	24·0	29 19	18·7	27 10
282	June 24 to July 5. 11 days.	Reared at 16—17° for 11 days.	16* to 17	24·9	28 17	15·9	22 12
282A	,,	282 reared at 21—23° for 11 days.	21* to 23	21·8	25 18	13·2	17 6
282B	,,	282 8 days at 16—17°, and then 3 days at 21—23°.	16* to 23	22·3	28 15	21·5	35 13
406	Dec. 8—15..... 7 days.	Reared at about 12°·5	12·5*	20·3	25 16	15·5	22 8
406A	,,	Same. Reared at about 20°.	20*	24·2	28 19	11·1	21 5
406B	Dec. 8—18..... 10 days.	406 7 days at 12°·5, then 3 days at 20°.	12·5* to 20	21·9	..	18·3	..

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319A	„	319 kept at 17° from 3 hrs. after fertilisation.	About 17*	14·7	16 13	33·6	37 27									
								C.—*ECHINUS.*								
425	Dec. 18—27	♀ ♂ fresh. Reared at 12—13°·5.	12* to 13·5	13·0	..	Ab't 9·0	14 0	About June 25—July 3	*Echinus.* ♀ ♂. Reared at 22°.	22*	23·3	28 19	21·0	25 17		
425A	„	Same. Reared at 18°.	18*	12·9	14 11	14·2	25 8	„	Same. Reared at 16—17°.	About 16*	24·2	26 22	17·0	24 10		
440A	Dec. 29 to Jan. 5.. 7 days.	♀ ♂ fresh. Reared at 13—14°	13* to 14	14·2	..	14·3	..									
440B	„	Same. Reared at 14—16°	..	14·1	..	20·9	..									
440C	„	Same. Reared at 18°	..	14·1	..	25·4	..									
440D	„	Same reared at 22—23°	..	14·2	..	18·0	..									