SPECIES CROSSING IN THE GENUS *TENEBRIO.*

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(With One Plate.)

Introduction.

In the course of the extensive investigations carried out by Mr Arendsen Hein of Utrecht on the biology of *Tenebrio* species and the hereditability of variations it was found impossible to cross the various species of *Tenebrio* so that offspring resulted, and it, therefore, became necessary to discover the cause of this. As the sex could already be ascertained in the larvae it was possible to isolate the sexes before puberty. The two sexes were thus separated and after 10 to 12 days in the incubator the species it was desired to cross were brought together, when copulation took place.

♂ *obscurus* × ♀ *molitor*; and reciprocal.
♂ *syriacus* × ♀ *molitor*; and reciprocal.
♂ *obscurus* × ♀ *syriacus*; and reciprocal.
♂ *obscurus* × ♀ *opacus*.
♂ *opacus* × ♀ *molitor*.

The eggs of all these crossings were collected, but out of more than 5000 eggs not a single one developed.

The problems thus confronting Mr Arendsen Hein were threefold and presented themselves as follows:

1. That there was only apparent copulation, the male discharging no spermatozoa into the female of a foreign species.

2. That there was discharge of spermatozoa which did not penetrate the foreign ovum.

3. That fertilisation of the egg took place, but without resulting in development.

Observations.

From my own observations I have ascertained that copulation between the different species of *Tenebrio* has taken place, viz. between

♂ *syriacus* × ♀ *molitor*; ♂ *molitor* × ♀ *obscurus*;
♂ *obscurus* × ♀ *molitor*; ♂ *molitor* × ♀ *syriacus*;

though for lack of time closer investigation was impossible.

I then turned my attention to the first problem quoted above as to whether copulation was only apparent; or, if spermatozoa had been discharged, where they had been deposited. For this purpose a male and a female from the different species under investigation were placed in a separated receptacle and the eggs laid in the buckwheat husks or on the fibres were collected. If these adhered firmly to the husks or fibres, they were fixed for examination and only later separated from them in the alcohol. The following species were thus successfully crossed:

♂ *syriacus* × ♀ *molitor*; ♂ *obscurus* × ♀ *molitor*;
♂ *molitor* × ♀ *syriacus*; ♂ *molitor* × ♀ *obscurus*;
♂ *obscurus* × ♀ *syriacus*.

In all these cases sperm could repeatedly be traced in the spermatheca of the female. This I first ascertained by fixing and examining the sex organs in sections of some of the females. As this method, however, required much time I took to preparing the sex organs and examining them alive under the microscope in Ringer's solution, which method proved exceedingly suitable. By these means I could establish that not only was sperm deposited in the proper place, but that it was alive, which fact could be proved by the rhythmic movements it made, these being clearly perceptible. It is true that in comparative examinations with *Ten. mol.* × *Ten. mol.*, I got the impression that fewer spermatozoa were deposited in the spermatheca in crossing species than in the fertilisation of *Ten. mol.* × *Ten. mol.* Also in crossing different species sperm was not regularly found in the spermatheca, and many females which had lived with males of another species for a considerable time had received no sperm. In a very few instances sperm was found only in the vagina. Thus side by side with females in the species-crosses where fertilisation had exactly the same result as in the case of *Ten. mol.* × *Ten. mol.*, many females being found having little or no sperm in the spermatheca.

The second problem, whether the spermatozoa penetrate the eggs, proved to be more difficult for purely technical reasons. Already in former researches I found that to fix and make microscopical preparations of these eggs, which contain much yolk, required great care. As regards the technical process followed in this investigation I must refer to what has already been stated in connection with the examination of the unfertilised eggs of *Tenebrio* (4, 1924). In this case, too, the difficulty was to obtain sufficiently thin sections in series, which is absolutely necessary to be able to see the two ♂ and ♀ pronuclei in the very earliest

stage. The whole of the egg must be laid out in complete sections under the microscope and in some cases this was successfully done. But the earliest stage is seldom met with, which leads to the conclusion that it must be gone through very quickly. Thus we see in Fig. 2 in one section the egg nucleus engaged in excluding the second polar body, and three sections further a nucleus in a mass of protoplasm in the middle of the yolkgrains (Fig. 2 *a*); since no nuclei are to be met with further in the egg, we may assume that this is the male pronucleus. I have also in a few instances found a structure in the outermost layer of the egg which I take to be the head of the spermatozoon which had penetrated into the egg.

It is thus proved that the spermatozoa penetrate the ovum and that the egg is fertilised.

There remains the problem why no offspring resulted from the eggs collected from the species-crosses. Not only did Mr Arendsen Hein never see development from any of the large number of eggs he collected, but in my own investigations too the results were negative, notwithstanding the most careful supervision, which makes it absolutely certain that among these eggs there were no *molitor* ♀ fertilised by *molitor* ♂ or *obscura* ♀ by *obscura* ♂, etc.

I now began the cytological examination of these eggs in order to find some explanation for the non-development of the fertilised eggs. By way of introduction to this examination I had beforehand investigated the non-fertilised eggs of *Ten. mol.* (4, 1924) to ascertain the course they followed. It then appeared that in the non-fertilised eggs also a certain development, up to an imperfect blastomere stage, had taken place, showing that not each and every development of the cross-fertilised eggs was to be ascribed to cross-fertilisation.

Lecaillon had already obtained the same result with the non-fertilised egg of hen and peacock (1910).

It now appeared that the cross-fertilised eggs took a much more regular course than the non-fertilised eggs. Here in most cases there was no imperfect blastomere formation, but all the transitions from one stage of development to another were to be found, ranging from cases in which the cells varied to some extent in size up to those in which the whole circumference of the egg is covered with exquisite and sometimes almost exactly similar and regular blastoderm cells, which often scarcely differed in any respect from the eggs of the *Ten. mol.* × *mol.*

It is true that, side by side with these, irregular eggs are also met with, but this can also happen with the eggs of *Ten. mol.* × *mol.*, where

the eggs do not all appear to receive the spermatozoa either; this is just as likely to occur in the species-crosses, though perhaps on a larger scale, seeing that amongst these the number of eggs that degenerate at an early stage is much greater, but I cannot be quite certain of that. We find, however, that eggs which look normal when slightly magnified, show irregularities in the cells when highly magnified, principally in the mitosis in the blastomeres. These irregularities do not occur in all the cells and the stages at which they begin to appear may also vary considerably. Moreover, the extent of the deviations from the normal, *i.e.*, the percentage of the cells which show such deviations in the developing eggs, varies very much.

If we compare the position of the chromosomes in the mitosis of the eggs of crossed species with those of the eggs of *mol.* × *mol.*, we notice that while the placing of the chromosomes in the eggs of the *mol.* × *mol.* is very regular, really forming one plane (seen from the side of the section as one line), in the eggs of species-crosses there is generally nothing of the sort. The chromosomes are more scattered, at varying distances from the poles.

In the course of the mitosis, the chromosomes in the eggs of species-crosses also act very differently and move at varying rates towards the two poles, so that they get further and further apart; so much so that in extreme cases (and these are really not so very unusual) certain chromosomes get so far behind that they are not taken up into the new nucleus at all. This means a loss of chromosomes for the new nucleus about to be formed (Figs. 3 and 4).

If this is repeated several times—and it is self-evident that the force once working abnormally will continue so to work in the following mitosis—then it means a very serious loss for the cell.

We are thus reminded of the researches of Boveri into multipolar mitosis in dispermic sea-urchin eggs (1902–7), where through the deviation of the distribution of the chromosomes, an abnormal development took place and—in the case of too great a shortage of normal chromosomes—the embryo either died off or developed abnormally.

In this recurring loss of chromosomes we may presume to have found the cause of the non-development of the eggs under discussion. The fate of the chromosomes which come to be on the outside of the nucleus, in the way described above, is most probably to be re-absorbed into the protoplasm. This conclusion is formed because all the stages can be seen, from chromosomes plainly lying outside the mitotic figure up to dark spots in the protoplasm outside, the resting nucleus hardly showing

chromatic staining. Very occasionally a few smaller nuclei succeed in forming themselves near the real nucleus (*Karyomeren*, *Teilkerne*).

It is here important to point out that the size of the blastomeres and particularly of the nuclei of these cells differs greatly. This is the natural consequence of the very unequal quantities of chromatin with which, in the long run, the nuclei of the blastomere cells are provided, as it may happen that into one nucleus not one single chromosome is passed, and into another nucleus several (cf. Figs. 5 and 5 *a*). This phenomenon also was observed by Boveri in the investigations quoted above, where the nucleus of various regions of the developing embryo had received varying quantities of chromatin and were consequently of different sizes. We cannot point to any difference in the size of the chromosomes, such as Harrison, Doncaster, Federley, Moenkhaus, and others have described in their researches in hybridisation, as here no great difference can be perceived between the chromosomes of the parents.

Another deviation from the normal which is met with in the later stages and in increasing measure as the egg develops, is the change in the shape of the chromosomes. While in the earlier stage the shape of the chromosomes was pointed as in the eggs of *Ten. mol.* × *Ten. mol.*, the later stages of cross-breeding show large bar-shaped, comma- or S-shaped chromosomes (Figs. 6, 7, 8, 9). The existence of these chromosomes is probably due to the conjunction of the original small forms, as the total number has become smaller, and, from the construction of the chromosomes too in certain clear cases, we are justified in concluding that this conjunction has taken place, since reproduced schematically it is as follows: ∧∧∨

The number of chromosomes in the equatorial plane can be ascertained, but there is no object in doing so, since the shape, and consequently the number, as I have just stated, is variable. Side by side with cases in which the chromosomes are reduced in number, owing presumably to conjunction, nuclei are found where there are apparently the normal pointed chromosomes, but too numerous (Fig. 10). Here, probably, certain chromosomes have fallen apart, forming small ones.

One peculiarity, which is often observed in the resting nucleus of the developing eggs in these crosses can be explained as a direct consequence of the nucleus being constructed of two component parts which differ too much from each other. For instance, very often the chromatin can be seen heaped up in two separate masses in the nucleus (Figs. 11 and 12). This can be seen even more plainly when there are two separate karyomeres (Figs. 13 and 14). These forms are repeatedly met with, sometimes

in many adjacent cells. In the indirect division too it can be seen that the chromosomes remain divided in two separate groups and that the division of these groups takes its course each independent of the other (Figs. 15–21 incl.), which is strongly reminiscent of the mitosis in the developing *Cyclops* eggs described by V. Haecker (1895).

Several varieties may be seen of these double nuclei varying from two separate nuclei lying side by side or two chromatin heaps in a single nucleus up to dumb-bell-shaped ones (Fig. 22). At this point it must be emphasised that all these shapes of nuclei are very often met with, whilst they are scarcely ever found in the *mol.* × *mol.* fertilisation—certainly not more frequently than in any other ordinary tissue. In the stages after blastomere formation, pycnosis very often sets in more and more markedly; further development is thus brought to a standstill and the embryo dies, although the development may be fairly advanced before this takes place.

It will suffice now if I point out—with the aid of a few illustrations (Figs. 26, 27 and 29)—the stages of development—probably normal—which are sometimes gone through in such cases.

If, for instance, we look at Fig. 27, the most advanced stage of development I met with in my sections, it can be seen that a considerably advanced stage may be reached. It is a great pity that the section is not complete, but it is distinct enough for us to see how it agrees with the illustrations given by other writers who give figures of this stage. If this diagram is compared with Fig. 3 of N. de Sélys Longchamps (1904) we see that the embryos from which these figures were made cannot differ greatly in age. De Sélys Longchamps states that the age of the embryo in his Fig. 3 was seven days. We even see what this writer calls "l'appendice du premier segment abdominal" represented in the section I have reproduced here.

Although it has been shown in the preceding pages that the development of the embryo may be fairly normal, it must be emphasised that, side by side with the normal, much irregular development occurs, and this already in the blastomere stage. We see, for instance, a strong irregular growth of the cells on the circumference of the egg where they form a large irregular heap (Fig. 30), or again the cells do not occupy the whole of the circumference, but only a small portion here and there (Fig. 28). It is interesting to observe the agreement between the course of development and the deviations in the mitosis spoken of in the preceding pages.

Owing to the entirely fortuitous emission of various chromosomes

the course of development varies greatly. Taking into account the altered shape of the chromosomes in the hybrids various chromosome-combinations may result when a "*Sammel-chromosom*" is emitted. Development may thus come to a standstill at a very early or a very late period, there may be abnormal irregular growths with embryo-formation, etc., and it would, therefore, not be surprising if it were found possible from a very large number of crossings between various species of *Tenebrio*, to get larvae or even imagines. Comparing the development of the cross-fertilised egg with that of the non-fertilised egg of *Tenebrio* as described in a former article of mine (1924), it is found that, although in this case too large a proportion of the eggs degenerate early, yet the greater proportion reach a fairly complete and fairly regular blastomere formation. A small proportion get as far as embryo formation before degenerating. No distinct difference can be traced between different crossings.

DISCUSSION.

On a survey of similar researches recorded in biological literature it is seen that disturbances of the mitosis of hybrids set in at very different periods of development.

Arranged according to the time at which such disturbances set in, a systematically graduated series may be drawn up which runs parallel with the closer or more distant kinship of the species crossed. At one end of this series appear species differing so little from each other that under normal circumstances they have a normal and fertile offspring. Cannon (1903) states that in the crossing of *Gossypium Barbadense* × *herbaceum*, as a rule normal mitoses are met with, but that in material fixed late in the year, irregular mitoses appeared just as in the case of completely sterile hybrids. Then come the crossings which always for the greater part result in sterile hybrids, or those which have exclusively sterile offspring. This calls to mind the experiments of Guyor (1902–3) with pigeons, Geoffrey Smith (1914) with pigeons and pheasants, Poll (1911) with ducks, Woodsalek's researches on the mule, H. Federley's crossings of Lepidoptera (1914–15), and the researches of Harrison and Doncaster (1914). All the above-mentioned show deviations in the chromosome pairing, which appears either in the syndesis, or in the meiosis, or in both. To this group belong, in the province of botany, the crossings of *Hieracium* and *Drosera* described by Rosenberg, the experiments with roses carried out by Täckholm, Blackburn and Harrison and many others. For a survey of the action of chromosomes in hybrids attention

is drawn to Wilson, *The Cell*, pp. 841–853 (1925), and Tischler, *Allgemeine Pflanzen Karyologie*, pp. 430–452.

On the other hand, for example, it must be pointed out that at the opposite end of the series there is the case related by Godlevski, of the fertilisation of a sea-urchin egg by the sperm of an annelid or mollusc. Here there is no question of crossing, as in the heterogeneous fertilisation, the sperm only plays the part of activator of the egg cell, whilst its chromosomes play no part in the further development. When the difference between egg and sperm-cell is not quite so great, then what occurs is similar to what Baltzer (1909) describes in the crossing of *Paracentrotus* × *Sphaerechinus*, where nearly all the male chromosomes were unable to adapt themselves to the female egg-plasm and were ejected. The peculiarity of the case is that in reciprocal crosses a similar occurrence did not take place. Moenkhaus's examination (1904) into the development of crosses between *Fundulus* and *Menidia* is very interesting on account of the various shapes of the chromosomes. In these cases development ceases already during the segmentation or gastrulation, and abnormal developments are numerous.

Comparing the present researches with this bird's-eye view of hybrid cytology, it becomes evident that in our case development never reaches the stage at which the larvae leave the egg, and that development may cease or degeneration set in at any of the stages at which such is possible, which is not surprising considering the very irregular loss of chromosomes in the mitosis.

A similar loss of chromosomes in the mitosis is described by Baltzer. But there the character of the chromosome-loss is very different, since the 16 or 17 chromosomes which are unable to adapt themselves to the new plasma are all emitted at the same time at the first division. In *Chromosomestudien an Mischlingen* (1914–15), H. Federley describes in the first meiotic division of *Dicr. ermina* × *vinula*, a phenomenon similar to the one I have described above, namely the union of several chromosomes, which explains the altered shape of many of them and the frequent decrease in numbers. Federley takes it to be a chromatolytic phenomenon (Fig. 8 in the paper just cited). Doncaster and Gray (1913), too, describe this cohesion of chromosomes in crossing *Echinus acutus* × *esculentus*.

The phenomenon I have observed, that the figures in the mitosis of hybrids are not so regular as those of the parents, that the equatorial plate, as seen from the side, covers a greater surface (thus giving an effect of irregularity), is confirmed by most investigators (see fig. 10 in Federley's article just quoted, compared with his figs. 2, 3, 5, 6), which


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shows in one hybrid the same deviation first occurring in the meiosis, which in another already occurs in the embryo-development. A cross which took a course similar to the one here described by me is *Ech. miliaris* ♀ × *esculentus* ♂, and *miliaris* ♀ × *acutus* ♂, which Doncaster and Gray have described. Here, too, side by side with very regular mitoses, there were mitoses where chromosomes were ejected.

CONCLUSIONS.

In species-crosses in the genus *Tenebrio* fertilisation of the egg does take place.

Owing to the deviations from the normal in mitosis the embryo does not reach the larval stage.

Embryonic development differs greatly in abnormality and in the stages it reaches.

This greatly differing development, taken in conjunction with the greatly varying emission of chromosomes, may be taken as supporting the theory of chromosome-heredity.

EXPLANATION OF PLATE XXVIII.

All the numbers of oculars and objectives refer to the Leitz microscope.

Fig. 1. Section of spermatheca of *T. obscurus* fertilised by *T. molitor*.

The cut tube of this organ occurred six times in the part of the section here sketched, and the four central of these contained sperm; all the other cells and nuclei have not been drawn. Fixing Bouin. Colouring haematox. orange G.

Fig. 2. Egg of *T. syriacus* fertilised by *T. mol.* Emission of polar body. Formalin. Haematox. Eosin. Oc. 3; obj. 8.

Fig. 2 *a*. ♂ pronucleus: same egg.

Fig. 3. Blastomeres from crossing *T. obsc.* ♂ × *T. syriacus* ♀. Emission and retention of chromosomes. Sublim. formal. Haematox. Bord. red.

Fig. 4. Ditto.

Fig. 5. Irregular size of cells in cross *T. obsc.* ♂ × *T. mol.* ♀.

Fig. 5 *a*. Regular cells in *T. mol.* × *mol.*

Fig. 6. Cell from cross *T. syr.* ♂ × *T. mol.* ♀. S-shaped chromosome.

Fig. 7. Equatorial plate from cross *T. syr.* ♂ × *T. mol.* ♀. Oc. 4; ol. imm. $\frac{1}{12}$.

Fig. 8. Greatly altered shape of chromosome in cross *T. obsc.* ♂ × *T. syr.* ♀.

Fig. 9. Abnormal chromosomes (in pairs!). Cross *T. syr.* ♂ × *mol.* ♀. Oc. 4; imm. $\frac{1}{12}$.

Fig. 10. Equatorial plate. Cross *T. obsc.* ♂ × *syr.* ♀. Number of chromosomes too many. Oc. 5; imm. $\frac{1}{12}$.

Fig. 11. Chromatin in two groups. Cross *T. syr.* ♂ × *mol.* ♀. Oc. 5; imm. $\frac{1}{12}$.

Fig. 12. Ditto. Sublim, formalin. Oc. 3; obj. 8.

Fig. 13. Double nucleus. Cross *T. syr.* ♂ × *mol.* ♀. Oc. 3; imm. $\frac{1}{12}$.

Fig. 14. Ditto.

Fig. 15. Chromosomes in two groups. *T. syr.* ♂ × *mol.* ♀. Oc. 3; imm. $\frac{1}{12}$.

Figs. 16–19. Ditto.
Figs. 20–21. Ditto. Two nuclei in section lying near each other.
Fig. 22. Dumb-bell-shaped nuclei *T. mol.* ♂ × *syr.* ♀.
Fig. 23. Abnormal chromosome shapes. *T. syr.* × *mol.* Oc. 4; imm. $\frac{1}{12}$.
Fig. 24. Supernumerary nuclei. *T. mol.* ♂ × *syr.* ♀. Oc. 3; obj. 8.
Fig. 25. Equatorial plate *T. obsc.* ♂ × *obsc.* ♀.
Fig. 26. Developed embryo from cross *T. mol.* ♂ × *obsc.* ♀. Rest of egg omitted. Oc. 2; obj. 4.
Fig. 27. Most advanced stage met with. Section incomplete. *T. obsc.* ♂ × *syr.* ♀.
Fig. 28. Irregular blastomere formation. *T. syr.* ♂ × *mol.* ♀.
Fig. 29. Section embryo. *T. syr.* ♂ × *T. mol.* ♀. Oc. 2; obj. 3.
Fig. 30. Growth in a single place on circumference of egg. *T. obsc.* ♂ × *syr.* ♀.

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