

ON THE OCCURRENCE OF XX MALES IN *LEBISTES*, WITH SOME REMARKS ON AIDA'S SO-CALLED "NON-DISJUNCTIONAL" MALES IN *APLOCHEILUS*.

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I. INTRODUCTION.

WHILE, as a general rule, the females of *Lebistes reticulatus* are phenotypically plain greyish, and the males coloured in various ways, the former occasionally develop some colour and on the whole show male characters. In certain cases the anal fin is even transformed into the characteristic male gonopodium. This fact was mentioned in one of my earlier papers on *Lebistes* (1927), and Blacher reported similar observations in 1926. Both Blacher and I observed that the sex glands in such circumstances might contain both ovarian and testicular tissue.

As was to be expected, the coloured females assume the colours according to their genetic constitution, as shown in Figs. 15-17 of my 1927 paper.

Further investigation has revealed several cases of such a partial sex alteration, and it is noteworthy that the male secondary sexual features are especially prone to appear in the females of certain *Lebistes* races, sometimes quite a number of sisters without exception being altered in this way.

As I have formerly (1927) shown, there is nothing to hinder such a partially masculine female from functioning as a female and giving birth to normal offspring.

The fact that this alteration of the females in the male direction was to be observed only in certain *Lebistes* races clearly points to differences in genetical constitution in such races, and hence to the possibility of producing, by suitable crossings, individuals in which the gene combination was of such a kind that the sex alteration becomes total.

As already shown (1922 *a, b*, 1923, 1927), *Lebistes* females are homogametic, XX, while the males are heterogametic, containing an X- and a Y-chromosome in the somatic cells. Both X and Y may contain genes for the development of colour patterns; in fact, the Y-chromosome

always carries a disposition to colour, whereas the *X*-chromosome may be empty. Crossing-over takes place both between the *X*'s in the females and between *X* and *Y* in the males, and this last fact proves that the sole real difference between *X* and *Y* is that the *Y*-chromosome contains a dominant male-determining gene, while *X* contains either a recessive female-determining gene or no specific female gene at all. For, if this were not the case, the difference between *X* and *Y* must be gradually blurred by continuous crossing-over, which does not happen, as genetic experiments clearly show.

Hence, in *Lebistes* there must be a single superior, epistatic male gene in the *Y*-chromosome, decisive for the sex determination; a marked difference from *Drosophila melanogaster*, where the presence or absence of the *Y*-chromosome is immaterial. Further, my earlier investigations made it likely that a series of allelomorphic male-determining genes occurred in the *Y*-chromosome of the various *Lebistes* races, the male gene in the different races giving a different colour pattern.

A series of investigations on the topography of the *Y*-chromosome, especially on the location of the male gene in relation to the rest of the genes, will be published on another occasion. Here we shall deal only with the genotypically conditioned sex shift in the females.

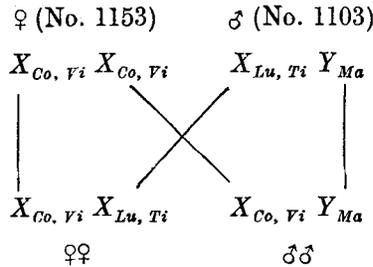
II. PRODUCTION OF XX MALES.

The females which in my experiments have shown a tendency to alter in the male direction are such as are homo- or hetero-zygous for the genes *Coccineus* + *Vitellinus*¹ in the *X*-chromosomes, $X_{Co, Vi} X_{Co, Vi}$ or $X_O X_{Co, Vi}$, also such as possess the genes *Luteus* + *Tigrinus* in *X*, $X_{Lu, Ti} X_{Lu, Ti}$ or $X_O X_{Lu, Ti}$, and sometimes also females with the *Elongatus*-gene, *El*.

A cross between a female $X_{Co, Vi} X_{Co, Vi}$ and a male $X_{Lu, Ti} Y_{Ma}$ produced a very interesting result. "*Ma*" denotes the gene *Maculatus*, which is one of the allelomorphic male genes; this gene has never, even among tens of thousands of individuals, been transmitted to a female. It is firmly attached to the *Y*-chromosome and is transmitted constantly from father to son, generation after generation, and must be considered as identical with the male gene itself.

¹ As misunderstandings have arisen from my formerly designating all the genes with small letters, in consequence of which they have been wrongly regarded as recessive, I shall here designate them with capital letters to signify more clearly that their effect is visible in a single dose. The recessive alternatives (allelomorphs) are all equal to zero.

The result of the cross mentioned might be expected to take the following course:



Hence the colour patterns of all the sons (as pictured in the coloured plates of my 1927 paper) would be expected to show the three genes *Coccineus*, *Vitellinus* and *Maculatus*, while the biotype of the females, as is known, cannot be judged from their appearance.

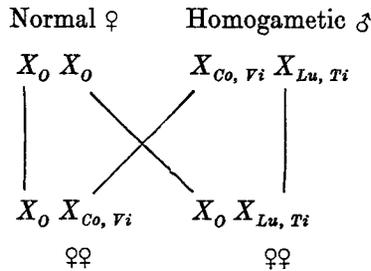
The result was, however, that among 58 sons 55 were of the expected type, while 3, which lacked the *Maculatus* gene, contained *Coccineus*, *Vitellinus*, *Luteus* and *Tigrinus*—viz. the genes that were to be expected in the daughters. As regards sex, these three were completely normal males.

There are two possible explanations. We might suppose crossing-over to have occurred between the *X*- and the *Y*- chromosome of the father, so that *Lu + Ti* had been exchanged with *Ma*; but this eventuality is excluded by the circumstance that the *Maculatus* gene has never once shown crossing-over among tens of thousands of cases. Hence the appearance of 3 cross-over males among 58 is almost inconceivable. The second possibility is that the three males contained *XX* and were chromosomally females. This hypothesis is, of course, verifiable, but it is a not unlikely one since both *Co, Vi* and *Lu, Ti* females, as already mentioned, are apt to develop male characters.

The crucial experiment consists in pairing the supposed *X_{Co, Vi} X_{Lu, Ti}* males with *X_o X_o* females, i.e. with females that contain no colour genes in *X*, and have shown no tendency towards the development of male characters. All the progeny receive two *X*-chromosomes and should be female, though there is, of course, the possibility that some few of the *XX* individuals might be totally or partially masculinized anew.

This pairing was made for the three males (see scheme below), and, as was expected, all the progeny were females. The three males produced 101, 140 and 73 daughters, respectively, a total of 314. They were all characteristic females, though a few showed faint indications of colour,

especially in the caudal fin, where the yellow colour conditioned by the gene *Luteus* was faintly visible.



Considering that my experiments with *Lebistes*, which cover a long series of years, have never before given a progeny of females only, the proportion being generally about 1 : 1 (in several species of Poeliciides there is a surplus of females; cf. Geiser, 1924), this result must be considered a valid proof that the three males mentioned have a XX chromosome set, and thus are homogametic.

Again, half of the daughters of the XX males must contain $X_o X_{Co, vi}$ and the rest $X_o X_{Lu, Ti}$; this cannot be ascertained directly, but the five females taken out at random for a test corresponded to this expectation. By crossing them with $X_o Y_{Ma}$ males, one proved to belong to the former type and four to the latter.

By back-crossing the XX males with their own daughters, so far only females were produced, 107 altogether, several of which, however, showed male characters. No completely male individual has hitherto been observed.

III. THE QUANTITATIVE NATURE OF THE SEX DIFFERENCE.

The possibility of producing XX males in *Lebistes* is interesting in several ways. It shows that not only the Y-chromosome contains a male-determining gene, but that also the X-chromosomes themselves contain genes influencing the sex determination. In certain races, however, this influence may be only faintly female, or it may be as much male as female, or else it may even appear as a male-determining tendency. The theory that the difference between male and female is not of a qualitative but of a quantitative nature, as developed more especially by Goldschmidt and Witchi, receives strong support from these facts.

Thus the possibility drops, which I had not formerly dared to exclude, that the male-determining gene in the Y-chromosome in *Lebistes* was in

the main restrictive of the female sex, and that there were no specifically sex-determining genes in the *X*-chromosome. The experiment proves the presence in the *X*-chromosome of one or more sex-determining genes of a now more female, now more male nature, whereas it is not proved that the male gene of the *Y*-chromosome has an allelomorph in the *X*-chromosome.

What has further been made clear in this experiment is the fact that the autosomes, as I have also formerly presumed (*l.c.* 1927), contain sex-determining genes, for this alone will explain the circumstance that only 3 out of the 58 *XX* animals were males. $X_{Co, Vi} X_{Lu, Ti}$ is not in itself sufficient to bring about male sex. Only about one-sixteenth of the individuals of this formula developed into males, which may be considered a proof of a sex-determining co-operation of autosomal sex genes. To conclude from the evidence that about one-sixteenth of the *XX* animals were males, that just two recessive autosomal sex genes had a share in the sex determination, is, however, hardly permissible.

The case is especially interesting in that the Teleosteans are so far the only example of the fact that within closely related organisms the male sex is heterogametic in certain types (*Lebistes*—Winge, *Aplocheilus*—Aida) and the female sex in others (*Platypoecilus*—Gordon, Fraser). Since it is possible to produce homogametic males in *Lebistes*, the question arises whether it is possible to find or to produce heterogametic females. It is no doubt exclusively a question of the strength of the respective male or female dispositions, whether the male or the female is heterogametic. Schematically expressed, it may be said that if the genes of the *X*-chromosome with a female tendency are stronger than the genes of the *Y*-chromosome with a male tendency, then female heterogamety is possible; whereas, if the masculine tendency of the *Y*-chromosome is stronger than the feminine tendency of the *X*-chromosome, the male heterogamety is possible. As to the homogametic sex, the experiment clearly shows that it is possible by a proper selection to produce both homogametic males and females that are able to function normally. In all circumstances it is obvious that the difference between the two types of heterogamety, generally regarded as essential, may not be significant.

IV. SOME REMARKS ON AIDA'S SO-CALLED "NON-DISJUNCTIONAL" MALES IN *APLOCHEILUS*.

In a paper on his further studies of *Aplocheilus* (1930) Aida gives an account of some interesting results, which he tries to explain on the

supposition of non-disjunction. There are, however, as yet no cytological investigations in support of this hypothesis, and I feel convinced that Aida's results have nothing to do with non-disjunction, but are to be explained in the same way as the aberrant males in *Lebistes* discussed above.

Aida crossed heterozygous red *Aplocheilus* males $X_R Y_r$ with white females $X_r X_r$, and, as was to be expected, found red daughters and white sons. But among some 5000 individuals appeared 5 white daughters and 9 red sons. Only the exceptional sons were fit for a closer analysis, and, in the case of two, proved to be due to crossing-over in the father, through which $X_r Y_R$ sons were produced. The remaining 7 exceptional sons were regarded as non-disjunction males of the formula $X_R X_r Y_r$, it being presumed that both X_R and Y_r had entered with the spermatozoon. These males, crossed with white females of the formula $X_r X_r$, gave almost exclusively females, viz. 996 red and 953 white, and only 19 males, of which 11 were red and 8 white.

In this connection Aida says (*l.c.* p. 5): "The production of the red and white females makes it necessary to consider that the male parent had two X's, an X_R and an X_r . The genetic formula having a double dose of X is characteristic of females, but as these individuals are male, not female, they must necessarily have some male-determining factor which is more powerful than two X's. The phenomenon may be explained fairly well by the supposition that all these exceptional red males are the result of non-disjunction between sex chromosomes and have a Y-chromosome in addition to two X's."

If we compare the circumstances in *Lebistes* described above with those observed by Aida in *Aplocheilus*, it is evident that we are dealing with the same phenomenon; but Aida had not at his disposal a sufficient number of genes in the chromosomes concerned to determine which chromosomes entered into the exceptional males. To judge from *Lebistes*, Aida's exceptional males are $X_R X_r$ males, *i.e.* individuals that in spite of their XX constitution have developed in the male direction in consequence of a casual accumulation, in the X-chromosomes and in the autosomes, of genes with a faintly female and stronger male effect respectively. Normal sex determination, depending on XX or XY constitution, is here disturbed, other genes having taken the lead in the sex determination.

Aida's view of non-disjunction also presents other difficulties. Since the progeny of the exceptional males are almost all females, he has to suppose that it is always the two X-chromosomes which conjugate

previous to the reduction division, *Y* remaining isolated, and that "the superfluous solitary *Y*-chromosome lagging in the middle of the spindle might be excluded from the formation of the gametic nuclei" (*l.c.* p. 13). "In *Aplocheilus*, indeed, the frequency of the occurrence of heterosynapsis in non-disjunctional males may be well conceived roughly to be zero" (*l.c.* p. 14). Aida is evidently disturbed by the presence of a *Y*-chromosome.

The very few sons which these exceptional males produce are again "non-disjunctional," *i.e.* they, too, produce almost all daughters; although we find an increasing number of sons as the generations progress. "What caused this increase—whether the presence of some gene or some local external condition—is not known at present" (*l.c.* p. 13). Though inexplicable on non-disjunction, this increase is easily understood on the supposition that the exceptional males have the *XX* formula; for males of this formula must normally produce only daughters, but may give rise to a few sons when accidental circumstances, or inbreeding in certain individuals, cause an accumulation of the genes with a male tendency (*i.e.* in the autosomes). Inbreeding will theoretically explain why the number of males increases generation after generation, but it cannot be seen from Aida's tables whether inbreeding has taken place, or no.

The strangest phenomenon in Aida's material is the red male *N* which was produced as the only son by the crossing of a supposed non-disjunctional male with a white female; this male was the only brother of 125 red females and 91 white females. The progeny of the male *N* consisted of 133 females and 156 males, which suggests that it behaves as a normal male. Its sons, too, behaved as normal *XY* males. Aida considers this male a proof that the father must actually have contained a *Y*-chromosome ($X_p X_r Y_r$). Even if mistakes in the experiments are left out of account here, it seems more likely to me that we have here a case in which a great accumulation of male-determining genes has taken place (through crossing-over) in one of the *X*-chromosomes of an *XX* male, so that the equivalent of a new *Y*-chromosome may be said to have arisen, which for the future takes the lead in the sex determination.

Altogether, it may then be said that, as the experiments on *Lebistes* clearly show that *XX* males may arise, and that these, like Aida's exceptional males, produce practically only daughters, there is every reason to regard the *Aplocheilus* males in question as being of the same nature. Cytological investigation might decide the question.

SUMMARY.

In certain *Lebistes* races the females (XX) are apt to show male characters and develop colour, though normally only males (XY) show colour patterns. By appropriate crossings of such races it proved possible to produce a few XX males. Such males mated with normal females gave only females; through inbreeding it is possible to produce individuals again which are more or less altered in a male direction.

These experiments show that, although genes in the X-Y-chromosome pair normally are alone decisive as regards sex, it is possible through selection to produce types in which other chromosomes take over the sex determination. They show also that both X-chromosomes and autosomes contain genes which have a share in the sex determination.

The remarkable fact that the closely related *Platypoecilus* has homogametic males and heterogametic females is explained on the ground that the sex determination is of a quantitative nature, so that the heterogamety may change from being a male to being a female characteristic.

Aida's so-called non-disjunctional males in *Aplocheilus* (1930), which in like manner produce nearly all females, must be regarded, not as having arisen through non-disjunction, in which case they should contain XXY, but as being XX males.

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