SEX REVERSAL IN APLOCHEILUS LATIPES AND A NEW EXPLANATION OF SEX DIFFERENTIATION

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INTRODUCTION

IN *Aplocheilus latipes* where the male is digametic XY two allelomorphic genes concerning the body color, orange-red (*R*) and white (*r*) are located in the X and Y chromosomes (AIDA 1921). Heterozygous orange-red males X*RY*r in crosses with white females X*r*X*r* produce orange-red females X*R*X*r* and white males X*r*Y*r* in criss-cross manner, though sometimes a few exceptional orange-red males may be found. Some of these abnormal males produce in crosses with normal females offspring with an abnormal sex ratio, the females always predominating in number and the males which are all abnormal like their male parent being very few or not present at all. By carrying on the breeding of these males generation after generation I found that the proportion of males gradually increases (AIDA 1930), and I tried in the former report to explain these abnormal males as the product of non-disjunction in sex chromosomes having XXY constitution.

WINGE (1930) found in *Lebistes reticulatus* sex-reversed males of female genotype XX which in crosses with normal females produced female offspring only. On account of the similarity of breeding results between his observation on sex reversal and ours on abnormal males he suggested that our male fish are also produced by sex reversal, but not by non-disjunction.

To determine which one of these alternative conceptions is more valid further research has been continued. A new fact irreconcilable with the supposition of non-disjunction was found, and at present I am inclined to believe that our males are, as WINGE has assumed, sex-reversed males of the female genotype XX. Moreover I was able to produce at the same time females which are unquestionably due to sex reversal, viz. females of male genotype XY.

The possibility of sex alteration in so well differentiated a gonochorist as Aplocheilus and the breeding results induced me to make a new hypothesis on sex differentiation in our fish.

SEX-REVERSED MALE OF FEMALE GENOTYPE

1. *Effect of individuality of male parent on sex differentiation*

Among a great number of matings of abnormal males in 1927 (table 4, AIDA 1930) the male designated as K10,8 produced male offspring in the highest ratio, viz. 4 ♀ :1 ♂, and male M2 also in pretty high ratio, viz. 10 ♀ :1 ♂. In 1928 some male offspring from these two matings were each mated to five females from the same litter as the male.

TABLE 1

Male offspring of the males K10, 8 and M2 crossed each with 5 females from the same litter as the male. R = Orange-red. r = White.

NO. AND YEAR OF MATING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀♀R	♀♀r	♂♂R	♂♂r	♀♀	♂♂	♀:♂
B.26 '28	R K10, 8, 3	R 5	190	71	27	15	261	42	6
B.27 '28	R K10, 8, 2	R 5	145	68	27	27	213	54	4
B.28 '28	R K10, 8, 4	R 5	149	44	6	1	193	7	28
B.30 '28	R K10, 8, 5	R 5	61	27	3	0	88	3	29
B.31 '28	R K10, 8, 6	r 5	104	83	8	5	187	13	14
B.32 '28	R K10, 8, 7	r 5	92	78	8	4	170	12	14
B.16 '28	R M2, 4	r 5	47	17	3	1	64	4	16
B.17 '28	R M2, 6	r 5	291	284	50	46	575	96	6
B.18 '28	R M2, 2	r 5	37	53	5	5	90	10	9
B.19 '28	R M2, 8	r 5	324	343	62	68	667	130	5
B.20 '28	R M2, 7	r 5	ε0	79	9	7	159	16	10

In the above tabulated matings the ratio of males to females varies from 4 to 29, and no constancy is found notwithstanding the fact that the parents of each mating were taken from the same litter.

All matings were carried on at the same season of the year under similar conditions as possible, so that the difference of external influences is excluded. As in each mating the five females, all taken from the same litter, were crossed with the single male, if some different tendency in producing the male number should exist in the female parent, it might have been mitigated, and would not affect so much the ratio of male number in offspring as we have seen. The actual variation of the ratio, accordingly, may be considered to depend on the nature of the male parent.

2. *Effect of individuality of female parent on sex differentiation*

In the next year (1929) a single male was mated simultaneously to two females in the same aquarium, each from different litters, and the sex numbers were counted in the offspring of each female parent separately.

TABLE 2

Three orange-red males, A, B, and C, from mating 26, 1928, were each mated to single orange-red female from the same litter and also simultaneously to single white female from mating 29, 1928, where only females and no males were produced 170 ♀♀, 0 ♂♂.

R = Orange-red. r = White.

NO. AND YEAR OF MATING	♂	PARENTS ♀	OFFSPRING ♀♀R	♀♀r	♂♂R	♂♂r	TOTAL ♀♀	♂♂	RATIO ♀:♂
B.9 '29	A	*R* from B.26, '28	62	24	33	12	86	45	2
B.12 '29	A	*r* from B.29, '28	38	30	31	29	68	60	1
B.10 '29	B	*R* from B.26, '28	97		21		97	21	5
B.13 '29	B	*r* from B.29, '28	136	1			137	0	No males
B.11 '29	C	*R* from B.26, '28	77	18	33	13	95	46	2
B.14 '29	C	*r* from B.29, '28	69	59	6	1	128	7	18

The breeding results in the table 2 show clearly that the same male may produce in matings to different females male offspring in different ratios. That this variation of the ratio is caused by the difference of the individual tendencies of female parents is to be conjectured.

When the females are taken from the wild stock and bred to any abnormal male, the offspring are always all female with none or very few males. Even the males which in crosses with colored females produce many male offspring yield in simultaneous crosses with wild females female offspring

only. The breeding results of the same male mated to the colored and wild females at the same time in the same aquarium are summarized in the next table.

TABLE 3

Abnormal male crossed with colored and wild females.

BR=Brown, the wild color. *B'R*=Orange-red variegated with black. *B'r*=White variegated with black. *R*=Orange-red. *r*=White.

NO. AND YEAR OF MATING	PARENTS ♂	PARENTS ♀	OFFSPRING ♀♀	OFFSPRING ♂♂	TOTAL ♀♀	TOTAL ♂♂	RATIO ♀:♂
B.25 '32	*B'r* (B.20,'31)	*R*	*B'R* 16 *B'r* 16 *R* 7 *r* 10	*B'R* 16 *B'r* 17 *R* 12 *r* 13	49	58	1
B.34 '32		*BR* (Wild)	*BR* 120	0	120	0	
B.1 '33	*R* (B.28,'32)	*r* (B.28,'32)	*R* 74 *r* 81	*R* 19 *r* 22	155	41	4
B.2 '33		*BR* (Wild)	*BR* 153	*BR* 0	153	0	
B.15 '33	*B'R* (B.26,'32)	*r* (B.26,'32)	*B'R* 29 *B'r* 29 *R* 23 *r* 22	*B'R* 0 *B'r* 3 *R* 6 *r* 3	103	12	9
B.16 '33		*BR* (Wild)	*BR* 310	0	310	0	

Table 3 shows that the very same males which have produced in crosses with colored females males in the ratio of one-fifth, one-tenth or even more than one-half of the total offspring, yielded in mating to wild females, females only and no males at all. The wild females, nevertheless, do not produce constantly females only, thus sometimes when the number of the offspring reared is large enough a few males may be found. For instance, in mating 2, 1931, where an abnormal male was mated to three wild females, only one male was found among 719 offspring, the remaining ones being females.

From these results the inference may be drawn that the females of our colored varieties have some tendency to produce a greater number of male offspring than the wild normal females.

3. *Seasonal influence on sex differentiation*

The ratio of males produced varies according to the different seasons. Our fish spawn every morning throughout the whole warm season, and

the sex ratio in the offspring from the same parents differs according as they are hatched out in early or mid-summer. In the next table the numbers of male and female offspring from the same parents reared in each period are separately listed. The water temperature at the surface in the morning before sunrise was in the first period 23–26°C. and in the second 25–28°C.

TABLE 4

Offspring of the same parents hatched out in earlier and later periods of summer.

BR, *B'R*, *B'r*, *R'r* same as in former table. *Br*=Blue.

NO. AND YEAR OF MATING	PARENTS ♂	PARENTS ♀	HATCHED OUT PERIODS	OFFSPRING ♀♀				OFFSPRING ♂♂				TOTAL ♀♀	TOTAL ♂♂	RATIO ♀:♂
B.6 '29	*R*	*R* (B.31,'28)	From June 26 to July 20	*R*	54	*r*	20	*R*	0	*r*	1	74	1	70
			From July 21 to August 10	*R*	52	*r*	21	*R*	18	*r*	7	73	25	3
B.7 '29	*R*	*R* (B.31,'28)	From June 26 to July 20	*R*	38	*r*	15	*R*	9	*r*	2	53	11	5
			From July 21 to August 16	*R*	16	*r*	6	*R*	17	*r*	7	22	24	1
B.8 '29	*R*	*R* (B.31,'28)	From June 26 to July 23	*R*	53	*r*	24	*R*	4	*r*	1	82	5	16
			From July 24 to August 17	*R*	40	*r*	20	*R*	30	*r*	10	60	40	1.5
B.23 '32	*BR*	*r* (B.2, '31)	From July 4 to July 20	*BR*	11	*Br*	9	*BR*	9	*Br*	8			
				R	10	*r*	9	*R*	1	*r*	6	39	24	2
		From normal stock	From July 25 to August 6	*Br*	11	*Br*	11	*BR*	1	*Br*	4			
				R	17	*r*	12	*R*	1	*r*	3	51	9	6
B.27 '32	*B'R*	*r* (B.16,'31)	From July 10 to July 20	*B'R*	9	*B'r*	9	*B'R*	2	*B'r*	1			
				R	7	*r*	5	*R*	0	*r*	0	30	3	10
			From July 24 to August 6	*B'R*	24	*B'r*	10	*B'R*	8	*B'r*	9			
				R	26	*r*	11	*R*	5	*r*	8	71	30	2

From the table we can see clearly that the season has a certain influence in determining the ratio of male offspring. In four matings among five the increase of the male number is very large in the second period. Probably high temperature may induce some females to male reversal. WINGE (1934) reports similar seasonal influence on the sex ratio in offspring of sex-reversed males of Lebistes. Thus he found that though in the early summer a relatively large number of males is produced, in other seasons the number of females increases, the females alone being produced in

winter. According to WITSCHI (1929) female tadpoles of *Rana sylvatica* reverse to males at high temperature. Thus the temperature seems to have a similar effect on sex differentiation in lower vertebrates.

4. *Excess of male number*

The mating 9, 1930, yielded male offspring in a high ratio, viz., 140 ♀ ♀ and 81 ♂ ♂. In the next year 10 ♂ ♂ and 19 ♀ ♀ from them were bred in mass (B.20, '31). The result was 105 ♀ ♀ and 82 ♂ ♂; the male ratio increased a little over that of the parents' brood. One male among them, orange-red variegated with black, was paired with a normal white female. This mating produced 143 ♂ ♂ and 45 ♀ ♀; so the male number amounts to more than thrice that of the female, an enormously high male ratio. The details are listed in table 5.

TABLE 5

Mating 26, 1932: B'R male crossed with r female.
B'R, B'r, R, r same as in former tables.

SPAWNING INTERVAL	NUMBER OF FRY ABOUT A MONTH OLD	♀♀				♂♂				TOTAL		RATIO
										♀♀	♂♂	♀ : ♂
From July 9	98	*B'R*	12	*R*	4	*B'R*	16	*R*	16			1
to July 20		*B'r*	4	*r*	3	*B'r*	20	*r*	22	23	74	3
From July 24	72	*B'R*	4	*R*	2	*B'R*	10	*R*	6			1
to August 6		*B'r*	6	*r*	6	*B'r*	6	*r*	12	18	34	2
From August 14	210	*B'R*	0	*R*	2	*B'R*	9	*R*	9			1
to August 29		*B'r*	0	*r*	2	*B'r*	15	*r*	2	4	35	9
								Grand total		45♀♀	143♂♂	

The discrimination of sex in our fish according to external aspects which is not possible in young stages may be made only after maturity, when the male manifests its secondary sexual characters, viz., long dorsal fin and wide anal fin with minute horny processes on fin rays (OKA 1931) and when the female lays eggs. From hatching to maturity there is an interval of about one year, so that the young individuals must pass the cold season, many weak ones dying during this hibernation.

Some suspicion might be raised concerning the sex ratios above in that through some unknown cause the majority of the female offspring might die to cause an unusually high male ratio. In the offspring of the third period the rate of mortality is great, thus only 39 adult fishes were reared from 210 fry. But in those of the first period nearly all of the fry became fully grown, and the disturbance of the sex ratio due to death is not observable. The preponderance of the male offspring in this case, therefore, must be due to the special nature of the parents, and this fact alone suffices

to deny my former supposition of non-disjunction in the origin of abnormal males. If the latter were the products of non-disjunction and had XXY chromosomes, it should be quite impossible to produce more male offspring than female. In our experiment the male number amounts to more than thrice that of the female. It is clear that our former supposition of non-disjunction must be abandoned, and the hypothesis of sex-reversal must be adopted instead of it.

From all these breeding results we can see that the sex ratio of the offspring of abnormal males crossed with any female of colored varieties varies according to the individuality of the two parents and it differs even in different parents taken from the same litter. Through the continual selection of the abnormal male as parent from the litter where the male ratio is high the male offspring may gradually increase until eventually they are in excess. The increase in the proportion of male offspring generation after generation, that I reported in my last paper (1930) is also probably the result of selection, as I have always chosen the male as parent of the next mating from the litter in which a relatively large number of males was found.

SEX-REVERSED FEMALE OF MALE GENOTYPE

A mass mating of normal strain, 1929, where ten white females (X*r*X*r*) were crossed with five orange-red males (X*R*Y*r*) gave the normal offspring composed of 614 orange-red females, 423 white males and a single exceptional white female. The last one was paired with a single normal orange-red male (X*R*Y*r*), the result of which was quite remarkable, as shown in the next table.

TABLE 5

Exceptional white female crossed with single normal orange-red male (XRYr).
Mating 41, 1930

	ORANGE-RED	WHITE	TOTAL
♀♀	55	0	55
♂♂	55	112	167

If the exceptional female were of normal constitution X*r*X*r* the orange-red and white offspring should be female and male respectively. We see however that half of the orange-red offspring are males: thus among 110 orange-red individuals one-half, i.e., 55 are male and another half female; while all white individuals, 112 in all, are male. In total the number of the male offspring is thrice that of female.

This singular result may be well explained by supposing that the exceptional female was produced by sex reversal and has X and Y chromosomes. Such a white female X*r*Y*r* mated to a normal orange-red male

X*RYr* will produce offspring composed of orange-red females X*RXr* and males X*RYr*, as well as white males X*rYr* and Y*rYr* in equal ratio, so that the male number will be thrice that of female if the YY males survive.

If this supposition is correct, half of the white male offspring must have two Y chromosomes and in mating with normal female XX produce male offspring only. To test this supposition, three white males were taken at random and each was mated to three normal females respectively. The results are listed in the next table.

TABLE 6

3 white males from B.41, 1930, crossed each with 3 normal orange-red females.
R, r = same as in former tables.

NUMBER AND YEAR OF MATING	MALE PARENT	OFFSPRING ♀♀		OFFSPRING ♂♂		TOTAL	
B.17, 1931	A	*R*	14	*R*	8	♀♀	25
		r	11	*r*	15	♂♂	23
B.18, 1931	B	*R*	1	*R*	5	♀♀	6
		r	5	*r*	4	♂♂	9
B.19, 1931	C	*R*	0	*R*	13	♀♀	0
		r	0	*r*	9	♂♂	22

Though since the number of offspring reared from each mating above listed is small the results cannot perhaps be regarded as quite conclusive, yet they are in favor of the supposition just stated. Two males, A and B, produced offspring with a normal sex ratio, while C male produced only males. Ten offspring from the C male, i.e., five orange-reds and five whites were tested by pairing to normal white and orange-red females respectively. The results were quite normal, because the former yielded offspring consisting of orange-red females and white males in criss-cross manner, and the latter both orange-red and white females and males in equal ratio. From these facts it may be inferred that all male offspring of C male are of the normal XY constitution and consequently the C male must have two Y chromosomes.

The orange-red males of the same litter as C male (B.41 '30) were all normal. Three fishes among them were crossed with three normal white females respectively. The results showed normal criss-cross inheritance: all orange-red offspring were females and all whites were males, as shown in table 7.

The single exceptional white female in mating 11, 1931, is the second fish produced by sex reversal which I obtained, and breeding experiments similar to the above stated were repeated with this female: it was paired with a single orange-red male from normal stock. The result was quite similar to that of B.41, '30 (table 8).

TABLE 7

Offspring of 3 orange-red males from B.41, '30, crossed each with 3 normal white females.

NO. AND YEAR OF MATING	ORANGE-RED		WHITE	
	♀♀	♂♂	♀♀	♂♂
B.9, '31	219	0	0	204
B.10, '31	154	0	0	182
B.11, '31	105	0	1	95

TABLE 8

Exceptional white female from B.11, '31, paired with a normal orange-red male. Mating 22, 1932.

	ORANGE-RED	WHITE	TOTAL
♀♀	217	0	217
♂♂	222	374	596

The mode of color inheritance was quite normal, orange-red and white offspring being nearly in equal ratio, as the male parent was heterozygous for its color. One half of the orange-reds were males and the other half females. The total number of males was thrice that of the females as in B.41, '30.

Under the expectation that one half of the white males would yield male offspring only, ten individuals among them were paired each with a single female. The results are shown in table 9.

TABLE 9

Offspring of 10 white males from B.22, '32, crossed with orange-red females.
R = Orange-red. *r* = White.

NO. AND YEAR OF MATING	FEMALE PARENT	NUMBER OF FRY ABOUT A MONTH OLD		ADULT OFFSPRING				TOTAL	
	R	*R*	*r*	*R*♀♀	*R*♂♂	*r*♀♀	*r*♂♂	♀♀	♂♂
B.5, '33	from same litter as male	274	263	32	30	28	31	60	61
B.6, '33	"	173	168	0	119	0	116	0	235
B.7, '33	"	84	102	27	23	35	18	62	41
B.8, '33	"	126	142	0	116	1	116	1	232
B.9, '33	"	202	199	85	68	68	75	153	143
	R								
B.10, '33	from normal stock	254	239	94	104	93	90	187	194
B.11, '33	"	195	219	0	118	0	131	0	249
B.12, '33	"	256	264	101	83	88	93	189	176
B.13, '33	"	306	342	69	76	61	66	130	142
B.14, '33	"	93	86	1	63	0	59	1	122

In four matings B.6, B.8, B.11 and B.14 among ten, only male offspring were produced, and in two matings B.8 and B.14 one single exceptional

female was found. The remaining six matings yielded offspring of normal sex ratio, i.e., the female and male nearly in equal proportion. The death rate of young fishes during hibernation is not small, as already stated, and is especially heavy when they are attacked by epidemic disease as in B.5, but in those four matings the greater number of fry was reared up to maturity and there is scarcely any doubt that all females in them were killed and the males alone survived. It is plausible to think that the male parents in those matings carry two Y and no X chromosomes at all.

The fact that four males among ten, i.e., one-half of the whole male offspring carry two Y chromosomes well verifies the supposition that the mother fish of these males, the exceptional white female, is the sex-reversed female of male genotype XY.

For the test of the brother orange-red males (B.22 '32) ten fishes among them were mated to normal white females or orange-red females from the same litter as males. Two matings were unfruitful, the other eight all showed normal inheritance so that all orange-red males of B.22 '32 are to be supposed to have the normal constitution of XY (table 10).

TABLE 10

Orange-red males from B.22, '32 mated to orange-red females of the same litter or to white females from normal stock.

R, *r* same as in former table.

NO. AND YEAR OF MATING	FEMALE PARENT	NUMBER OF FRY ABOUT A MONTH OLD		ADULT OFFSPRING			
	r	*R*	*r*	*R*♀♀	*R*♂♂	*r*♀♀	*r*♂♂
B.18, '33	from normal stock	266	217	247	2	0	197
B.20, '33	"	145	146	138	0	0	135
B.21, '33	"	263	271	187	1	0	179
	R						
B.22, '33	from B.22, '32	297	95	145	67	0	63
B.23, '33	"	442	138	194	100	0	84
B.24, '33	"	454	148	239	114	0	116
B.25, '33	"	216	72	30	22	0	17
B.26, '33	"	252	69	178	63	0	62

Quite similar results were obtained by WINGE (1934) on the sex-reversed female of *Lebistes reticulatus*, which produced in a cross with normal male 21 females and 81 males, i.e., in the ratio of 1:3. One-third of the males were reported to be YY males, and in crosses with normal females produced male offspring only, just as in our case.

It is very interesting to see that in fishes which are well sex-differentiated like Lebistes and Aplocheilus one sex may change to the opposite one, and that YY male may be viable and fertile while it is lethal in Drosophila.

DISCUSSION AND A NEW SUPPOSITION ON SEX DIFFERENTIATION

At present the elucidation of sex differentiation is based on the fundamental conception, proposed by Morgan, of two antagonistic and competing female and male determining factors. Generally it is understood that when the female factor *F* predominates the individual becomes female, while when the male factor *M* prevails it becomes male. But about the locations of these factors in the germ cells the opinions of different authors do not agree.

GOLDSCHMIDT (1934) in his explanation of intersexes in Lymantria (female digamety) locates the *M* factor in X chromosome and the *F* factor in the cytoplasm of the ovum, *FMM* being male and *FM* female. KOSSWIG (1931) to explain sex differentiation in the hybrid between *Platypoecilus maculatus* (female digamety) and *Xiphophorus Helleri*, supposes in the former the *M* factor to be located in autosomes and the *F* in W or Y chromosome; the Z or X chromosome being quite indifferent in sex determination has none of *F* or *M* factor. According to BRIDGES (1925) in Drosophila (male digamety) all autosomes have both female and male genes, and in total the male genes are more effective than the female. The X chromosome in contrast to autosomes contains net female genes, and the sex is differentiated according to the ratio of these two sets of genes. WITSCHI (1929) assumes in European frog (male digamety) that the autosomes have net *M* factor and X chromosome net *F* factor as in Drosophila, and moreover Y chromosome carries a variable *f* factor, the allelomorph of *F* and lower in its strength than the single *M*. WINGE (1934) in the interpretation of sex reversal in Lebistes (male digamety) supposes that the net factor of each autosome is different in quality and strength, some being feminine, and others masculine in various strengths. The X chromosome is feminine, while the Y chromosome is masculine and stronger than a single X. The sex reversal is considered to be caused by the accumulation of autosomes of the same sort in an ovum, whose factors being all feminine or masculine surpass in total the antagonistic effect of sex chromosomes so that an individual XX may be changed to male and the XY to female.

Such diversities of the opinions of different authors about the location of *F* and *M* factors induced me to doubt their real existence. From the facts that in Aplocheilus as well as in Lebistes females of male genotype and males of female genotype may be produced through sex reversal, and that the males of female genotype produce in crosses with normal females offspring with sex ratios varying from all females to a preponderance of males, I am induced to make the following hypothesis on sex differentiation.

The genes which correspond to the primary sexual characters of both sexes are distributed in autosomes, and they become activated by certain

definite genes which act as stimulating genes. We may think that the sex differentiation is due to the difference of the quantity of such stimulating genes. When it is greater than a certain limiting value the feminine genes only are activated and the action of masculine genes is suppressed so that the female characters will develop, while on the contrary when it is smaller just the reverse will take place.

The genes concerned in stimulating, which we may call sexual exciters, are located in the sex chromosomes. In the heterogametic male the total sum of sexual exciters in X and Y chromosomes is less than that contained in the two X chromosomes, and in the heterogametic female their total quantity in X and Y or Z and W chromosomes is greater than that in two X or Z chromosomes; in other words, in the former case the X chromosome has greater exciting quantity or potency than the Y and in the latter case we have the reverse. As thus the differentiation of sexes is considered to be caused by the difference of quantity of the same exciting agency there should be a threshold value between the two sex determining quantities and when either of the latter is greater than that value the action of female genes and in the contrary case that of male genes will be stimulated.

Though some external or internal conditions might influence these exciting potencies of sex chromosomes, in normal case their disturbing action is not so great as to cause the total sum of the potencies of sex chromosomes to pass over the threshold value and produce abnormal sex differentiation.

According to my opinion the sex reversal in our fish may be explained by an unusual disturbance of the potency of the X chromosome. In our breeds of colored varieties, I think, the X chromosome loses the constancy of its potency by some unknown cause, and so fluctuates always in varying range that in some fishes which happened to have lower fluctuants of the X chromosome the total sum of their potencies falls under the threshold value, so that the masculine genes only are activated notwithstanding their female genotype, and the sex is reversed.

The range of fluctuation is supposed not to be equal in all X chromosomes and it varies even in those of offspring produced from the same parents. The variation of the number of sex-reversed males due to the individuality of both parents in the offspring of the XX male crossed with the female of colored varieties will be the result of the varying ranges of fluctuation in the X chromosomes of the latter. The wider the range of their fluctuation in both parents, the greater number of males will be produced.

From the fact that in crosses with wild females any sex-reversed male yields generally female offspring only, rarely together with a few males, we may infer that the range of fluctuation is not very wide in negative direction, hence most of the lower fluctuants, excepting the lowest ones, in combination with a normal X chromosome of the wild species are unable to

depress the total sum of potencies so much as to let it pass over the threshold value and effect sex reversal.

The fluctuation of the X chromosome in our breed, in my view, would have taken place at first in one of two X chromosomes, and on account of many years' close inbreeding all colored varieties in our breeds came to get two fluctuating X chromosomes, whereupon some females which happened to carry two lower X chromosomes were first detected as the sex-reversed ones. By constant breeding of the sex-reversed males selected out from the litters where their number is high, the fluctuation of X chromosome may be shifted further in the negative direction, so that the number of the male offspring will be increased. The matings in table 5 are the results of such processes, where the excess of males may be found.

Probably the fluctuation of the X chromosome might be influenced easily by outer conditions, and the difference of the rate of male production from the same parents in different seasons is to be explained as the effect of different climate.

The fluctuation takes place in both directions either positive or negative, starting from normal value. The effect of the positive fluctuation cannot be recognized in the offspring of sex-reversed males crossed with normal females as then both parents carry X chromosomes only, and the increase in the potency of that chromosome results in nothing but to produce the females which are not different at all from the normal ones.

The sex-reversed female of male genotype XY is to be explained as the effect of the increase in the total potency of X and Y chromosomes. When the total sum of potencies increases and passes over the threshold value to the female side, a female will be produced in spite of the male genotype. Whether this increase of potency is caused by the fluctuation occurring in the X chromosome only or in both X and Y is not yet fully decided. But even through the consideration of the fluctuation in the X chromosome only the fact is easily understood. The single X chromosome of extreme fluctuation on the positive side, which will very rarely occur, accompanied by a Y chromosome, may have sufficient total potency to excite the female genes only. The rarity of sex-reversed females favors this supposition.

The experimental results of the sex reversal phenomenon in our fish are thus fairly explainable by the supposition that sex differentiation is due to differences in the total quantities of exciting factors in the sex chromosomes, and the fluctuation of the potency of the X chromosome.

Now to test how far this supposition of sex differentiation is conformable to the other facts about sex differentiation, I will try in the following to interpret some well known complex facts based on this supposition.

The most perplexing facts are those of Goldschmidt's intersexes and sex reversal in the hybrids between the different local races of *Lymantria dispar*. The results of the crosses designated as "Basic" by him and the

crosses between the very weak and strong races (GOLDSCHMIDT 1934) can easily be interpreted on the basis of our hypothesis with an auxiliary one that the maternal cytoplasm influences slightly the potencies of sex chromosomes: the cytoplasm of the strong race strengthens and that of the weak weakens the potencies of sex chromosomes.

To simplify the explanation we may assume schematically some numbers for the potencies of sex chromosomes and the threshold value in different races:

Japanese or strong race..... X20 Y40=60, X20 X20=40, the threshold value=52.
European or weak race ..... X25 Y37=62, X25 X25=50, the threshold value=55.
Very weak race..... X26 Y30=56, X26 X26=52, the threshold value=53.

In the hybrids between these races of different potencies of X and Y chromosomes there will appear some individuals in which the total potency of sex chromosomes is enough large to excite one group of genes, either female or male, in the autosomes introduced from one parent, so that the corresponding sexual characters will develop. For the sexual genes in another group of autosomes introduced from another parent it is at the threshold value or very near to it, so that some characters of both sexes may develop, and in such a case a female or male intersex will be produced. Sex reversal will be produced when the total potency of sex chromosomes passes over the threshold values in both parents to that side, male or female, which is opposite to the sexual constitution of the hybrid.

In the next table the statements of GOLDSCHMIDT on the different crosses are cited in the first column and the respective interpretation for each cross is described in the second. The cytoplasmic influence is denoted by the number 2; when the female parent is from the strong race 2 is added, and when it is from the weak or very weak race 2 is subtracted from the total sum of potencies in each zygote.

TABLE 11

Interpretations of Goldschmidt's basic crosses and crosses between strong and very weak races of Lymantria dispar.

J=JAPANESE STRONG RACE E=EUROPEAN WEAK RACE	j=AUTOSOME SET FROM JAPANESE RACE e=AUTOSOME SET FROM EUROPEAN RACE ⚥=INTERSEX. Rev=SEX REVERSAL
1. J♀×E♂=F_1 normal	X20 Y40×X25 X25=X20 X25, X25 Y40 45+2=47 65+2=67 ♂ ♀
2. E♀×J♂=F_1♂ normal, ♀ intersex	X25 Y37×X20 X20=X25 X20, X20 Y37 45−2=43 57−2=55 ♂ j♀, e⚥
3. (J×E)²=♀ normal, ♂ up to ½ of their number intersexual	X25 Y40×X20 X25= X20 Y40, X25 Y40, X20 X25, X25 X25 60+2=62 65+2=67 45+2=47 50+2=52 ♀ ♀ ♂ e♂, i⚥

J=JAPANESE STRONG RACE E=EUROPEAN WEAK RACE	j=AUTOSOME SET FROM JAPANESE RACE e=AUTOSOME SET FROM EUROPEAN RACE ⚥=INTERSEX. Rev=SEX REVERSAL
3a. (J×E)×(E×J)=exactly like No. 3	X25 Y40×X25 X20= X20 Y40, X25 Y40 X20 X25 X25 X25 60+2=62 65+2=67 45+2=47 50+2=52 ♀ ♀ ♂ e♂, j⚥
4. (E×J)²=♂ normal, ♀ ½ normal and ½ intersexuals	X20 Y37×X20 X25= X20 X20, X20, X25, X25 Y37, X20 Y37 40−2=38 45−2=43 62−2=60 57−2=55 ♂ ♂ ♀ j♀, e⚥
4a. (E×J)×(J×E)=exactly like No. 4	X20 Y37×X25 X20=same as No. 4
5. J×(J×E), J×(E×J): always normal	X20 Y40×X20 X25= X20 X20, X20 X25, X20 Y40, X25 Y40 40+2=42 45+2=47 60+2=62 65+2=67 ♂ ♂ ♀ ♀
6. E×(J×E), E×(E×J): exactly like F_2 No. 4, namely males normal, females ½ normal, ½ intersexual	X25 Y37×X20 X25= X20 X25, X25 X25, X25 Y37, X20 Y37 45−2=43 50−2=48 62−2=60 57−2=55 ♂ ♂ ♀ j♀, e⚥
7a. (J×E)×J=all normal	X25 Y40×X20 X20=X25 X20, X20 Y40 45+2=47 60+2=62 ♂ ♀
7b. (J×E)×E=♀ normal, ♂ all intersexual	X25 Y40×X25 X25=X25 Y40, X25 X25 65+2=67 50+2=52 ♀ e♂, j⚥
8a. (E×J)×J=all ♂ normal, ♀ intersexual	X20 Y37×X20 X20=X20 X20, X20 Y37 40−2=38 57−2=55 ♂ j♀, e⚥
8b. (E×J)×E=all normal	X20 Y37×X25 X25=X20 X25, X25 Y37 45−2=43 62−2=60 ♂ ♀
a. Very weak ♀×Strong ♂=only ♂	X26 Y30×X20 X20=X20 X26, X20 Y30 46−2=44 50−2=48 ♂ Rev♂
b. Strong ♀×Very weak ♂=normal	X20 Y40×X26 X26=X20 X26, X26 Y40 46+2=48 66+2=68 ♂ ♀
c. Very weak ♀×F_1 ♂ from b=3♂:1♀	X26 Y30, X20 X26= X20 X26, X20 Y30, X26 X26, X26 Y30 46−2=44 50−2=48 52−2=50 56−2=54 ♂ Rev ♂ ♂ ♀
d. (Strong ♀×Very weak ♂)²=2♀, 1♂, 1♀ by sex-rev. (hardly viable)	X26 Y40×X20 X26= X26 X20, X20 Y40, X26 X26, X26 Y40 46+2=48 60+2=62 52+2=54 66+2=68 ♂ ♀ Rev ♀ ♀
e. (Strong ♀×Very weak ♂)♀×Very weak ♂=only ♀	X26 Y40×X26 X26=X26 X26, X26 Y40 52+2=54 66+2=68 Rev ♀ ♀

The appearance of abnormal sex in *D. melanogaster* (BRIDGES 1922, 1925) may also be elucidated on the basis of our present supposition. The triploid intersex carries triploid autosomes and two X chromosomes (3A, 2X). The total of the potencies of two X chromosomes may be here considered to be equal or very near to the threshold value for three sets of autosomes, and consequently both sexual characters are revealed. The super-female (2A, 3X) and male (3A, X) are considered to be caused by extremely excessive and scanty quantities of the exciting factor respectively, of which the sterility is the result.

In bees and many other Hymenoptera the diploid egg develops into a female and the haploid into a male. These facts are intelligible when we assume a cytoplasmic influence upon the potencies of sex chromosomes. We suppose that the cytoplasm in Hymenoptera resists the exciting action of sex chromosomes and weakens it somewhat. In a diploid egg the sum of the potencies of two sex chromosomes, 2x, decreased by the quantity c, the cytoplasmic resistance, i.e. 2x-c, might still be greater than the threshold value for two sets of autosomes, and stimulate the female genes to their activity, while in the haploid form x-c is less than the threshold value for one set of autosomes, and the male genes only will be excited.

Thus the hypothesis of quantitative differences in the degree of sensitivity of the male and female sexual genes in autosomes and corresponding differences in the potencies of sex chromosomes in different sexes explains well many complex facts of sex differentiation, and I think that it is the general mode of sex differentiation.

Through this hypothesis the phylogenetic relationship among the hermaphrodite, rudimentary hermaphrodite (KOSSWIG 1931, WITSCHI 1929) and the gonochorist may be well cleared up. In the hermaphrodite the sensitivity of male and female genes to stimulating action is equal, and all of them are consequently excited to the same degree. In the rudimentary hermaphrodite, however, the sensitivity of sexual genes and the corresponding total potency of sex chromosomes is different in male and female sexes but this difference is small and the total potency of sex chromosomes in each sex draws very near to the threshold value. In such animals some slight influence of outer or inner conditions will induce the disturbance of sex differentiation, sex reversal or intersexuality being easily produced. In the gonochorist this difference is so great that normally any condition is unable to cause such sexual disturbances.

The three sexual types i.e., Protenor ♀XX♂XO, Drosophila ♀XX-♂XY and Abraxas type ♀XY♂XX show according to our hypothesis only the differences in respect to the accumulation of stimulating genes in two sex chromosomes. In the Protenor type all genes are contained in the X chromosome only and in the latter two types they are distributed

between two chromosomes X and Y. In the Drosophila type the X chromosome carries a greater number of genes than Y, and in the Abraxas type on the contrary the Y chromosome is greater in its potency than the X. Crossing over of the stimulating genes, if possible, might produce one type from another. The difficulty that opposite types of sex determination appear in very nearly related species, for example, the Drosophila type in Lebistes and the Abraxas type in Platypoecilus may thus be easily explained.

SUMMARY

Further breeding investigations on the abnormal males of *Aplocheilus latipes*, which were assumed in my former paper (1927) to be the products of non-disjunction of the sex chromosomes are described. These males produce in crosses with normal females offspring in which the number of females always exceeds that of males in varying ratios.

The sex ratios in these offspring vary according to the individualities of male and female parents. The different males from the same litter yield offspring of different sex ratios, and also the different females mated to the same male produce offspring of varying sex ratio.

Temperature influences the sex ratio which may differ in the offspring of the same parents produced early in the year or during mid-summer. Generally under a hotter climate the number of male offspring is greater than under a colder one.

By continued breeding of males selected out from the litters with high ratios of males the number of males increases until eventually some males are obtained whose male offspring far exceed the females in number. This fact denies the supposition of non-disjunction as accounting for the origin of abnormal males, so that it must be discarded and the alternative of sex reversal adopted.

The sex-reversed female of male genotype XY was found. It produced in crosses with normal males female and male offspring in the ratio of 1:3. One-third of its male offspring were males of the constitution YY, and these produced only male offspring.

For the interpretation of these results a new hypothesis of sex differentiation is proposed. The female and male primary sexual characters have all their own respective corresponding genes; these are distributed in the autosomes, and are set into activity by a certain amount of stimulating genes. The degree of sensitivity of female and male genes to the stimulating genes is various, and the female genes require a greater amount of stimulation than the male genes to become active. Sex differentiation is caused by differences in quantity of the stimulating genes. When the difference is great the female genes alone are activated and the action of male genes suppressed, while if it is small just the reverse takes place. Between these

two quantities is a threshold value, above or below which the female or male genes are stimulated to produce female or male respectively. The genes of stimulation in the gonochorist are located in the sex chromosomes, and the total stimulating capacity or potency is greater in female sex chromosomes than in male ones.

Sex reversal in our fish and the differences in the sex ratios among the offspring of sex-reversed males are explained by the fluctuation of the stimulating power or potency of the X chromosome.

Other abnormal facts of sex differentiation are explained on the basis of our present hypothesis.

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