EVIDENCE FOR THE EXISTENCE OF TRANSFORMER GENES FOR SEX IN THE TELEOST *XIPHOPHORUS MACULATUS*¹

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THE sex-determining mechanism in Poeciliid fishes of the genus *Xiphophorus* has been studied for more than forty years (BELLAMY 1928; GORDON 1927), yet many problems remain unsolved. In the southern platyfish, *Xiphophorus maculatus*, native to Mexico and adjacent parts of Central America, there are three types of females (XX, WX, WY) and two types of males (XY and YY) (GORDON 1947, 1954; KALLMAN 1965a). The W chromosome carries essentially a stronger female determining potency than the X, because the WY constitution gives rise to females and the XY constitution to males. Two other species, *X. variatus* (BELLAMY 1936; KOSSWIG 1937; RUST 1939) and *X. milleri* (KALLMAN 1965b) possess an XX ♀ —XY ♂ ♂ system. Their sex chromosomes are homologous with those of *X. maculatus* (KALLMAN and ATZ 1967). In *X. hellerii*, the swordtail, no sex-linked characters have been discovered, but because of widely fluctuating sex ratios between and even within stocks (BREIDER 1935; PETERS 1964) this species is thought to possess a polygenic mode of sex determination (KOSSWIG 1964; KOSSWIG and ÖKTAY 1955). Occasionally, in *X. maculatus*, *X. variatus*, and *X. milleri* genetic sex reversals arise: individuals that have either the WY or XX genotypes, but differentiate into functional males (BELLAMY and QUEAL 1951; BREIDER 1942; ÖKTAY 1959) and XY fish that are fertile females (ANDERS and ANDERS 1963; MACINTYRE 1961). The overall incidence of such genetic sex reversals in *X. maculatus* is less than one percent, but their occurrence is not a random event (KALLMAN 1965a). Similar sex reversals have been discovered in the guppy, *Poecilia reticulata* (Poeciliidae) (WINGE 1934) and in the medaka, *Oryzias latipes* (Oryziatidae) (AIDA 1936, YAMAMOTO 1963), species that are normally XX ♀ ♀ and XY ♂ ♂. The occurrence of sex reversals is thus not limited to one species of fish, but may be of general significance.

WINGE proposed that in addition to the sex chromosomes there are also autosomal genes with male- and female-determining potencies (WINGE 1934; WINGE and DITLEVSEN 1948). Usually the additive effect of these autosomal factors is not sufficient to upset the switch mechanism inherent in the XX-XY system of the guppy. Sex reversals are individuals with rare fortuitous combinations of autosomal male or female determining genes whose combined strength overrides the action of the sex chromosomes. Similar explanations have been suggested for

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Xiphophorus (ANDERS and ANDERS 1963; DZWILLO and ZANDER 1967; KOSWIG and ÖKTAY 1955; ÖKTAY 1959) and *Oryzias* (YAMAMOTO 1963).

In 1951 GORDON obtained male sex reversals in *X. maculatus* that apparently required a different explanation. From a cross between a $W_+ Y_{sp}$ (Bh) female (allegedly derived from British Honduras) and an XY male of a strain originating from the Rio Jamapa in Mexico, GORDON (1951a) obtained 11 wild-type females, apparently WX and WY, and 14 spotted (*Sp*) sons, XY and YY. Similarly a $W_+ Y_{sp}$ (Bh) female was mated with a wild-caught male (Cp-18) from the Rio Coatzacoalcos. Instead of the expected 1:1 sex ratio, 23 female and 86 male offspring were produced (ped. 274). Fifty-nine of the males were spotted, having inherited the *Sp* gene located on the Y chromosome of the Bh female, but the remaining 27 were unspotted, as were the females. GORDON assumed that the wild-type males possessed the WY genotype and the females the WX, and concluded that the Y chromosome of *X. maculatus* of the Rio Coatzacoalcos contained stronger sex genes for maleness than the Y chromosome of the Rio Jamapa population. Since then many other populations or commercial stocks of *X. maculatus* have been interbred with one another, but the WY class has always differentiated into females. Several authors, therefore, went beyond GORDON and stated that the Y chromosome of the Rio Coatzacoalcos fish is not only more strongly male-determining than that of the Rio Jamapa but of all other populations as well.

However, since neither the X nor the Y chromosomes of the male parent of ped. 274 was marked by pigment genes, the possibility that both the wild-type males and females of ped. 274 consisted of two genotypes (WY and WX) cannot be ruled out. Moreover, GORDON's conclusion is based upon results obtained with a single male. The Coatzacoalcos basin occupied by platyfish covers several thousand square miles, and there seems to be no justification for extending GORDON's interpretation to the Coatzacoalcos population as a whole. Nevertheless, it has been accepted by most authors and reviewers, most recently by BACCI (1965) and DZWILLO and ZANDER (1967).

The following experiments were designed to reinvestigate this problem.

MATERIALS AND METHODS

The Cp (Coatzacoalcos) males of this study belong to the 17th and 18th laboratory generations of a single strain, all but the first three of which were inbred by brother-sister matings. The strain was derived from fish collected, together with male Cp-18, by GORDON, ARZ and WOOD in 1948 in a small arroyo near Almagres, Veracruz, Mexico. Cp-18, however, is not involved in its ancestry. The Cp stock has no history of genetic sex reversals, i.e. XX males or XY females (KALLMAN 1965a). The X chromosomes are marked either by *Sp* or its wild-type (+) allele and the Y by *Sd*. With a single exception, males of all generations were *Sd* and females *Sp* or + (KALLMAN 1965a, page 157). The origin of the other *X. maculatus* stocks used in the experiments has been described previously (KALLMAN 1965a). Their sex chromosome mechanisms and the relevant marker genes are:

Rio Jamapa stocks:	Jp 163 A	♀ ♀ $X_{Dr} X_{Dr}$	♂ ♂ $X_{Dr} Y_{Sr}$
	Jp 163 B	♀ ♀ $X_{Sp} X_{Sp}$	♂ ♂ $X_{Sp} Y_{Sr}$
Rio Grijalva stock:	Jp 30	♀ ♀ $X_{Sr} X_{Sr}$	♂ ♂ $X_{Sr} Y_{Sr}$
	Gp	♀ ♀ X X	♂ ♂ X Y_{Sd}

The X chromosomes of the Gp strain are either marked by *Sp* or *Sd*.

Rio Hondo stocks: Hp-1 ♀ ♀ W₊Y ♂ ♂ Y Y

The Y chromosomes of Hp-1 are either marked *Sd* or +.

Hp-2 ♀ ♀ X₊X₊ ♂ ♂ X₊Y_{Sd}
 Np ♀ ♀ W₊Y ♂ ♂ Y Y

New River stock:

The Y chromosomes of the Np strain are either marked by *N*, *Sd* or *Sr*. The symbols refer to dominant, sex-linked pigment patterns: *N* (*nigra*), *Sd* (spotted-dorsal), *Sp* (spot-sided), *Sr* (stripe-sided) are melanophore patterns; *Dr* (red-dorsal) controls the appearance of erythrophores in the dorsal fin.

Depending upon the stock or particular cross the penetrance of the macromelanophore genes may vary from 0 to 100% (GORDON 1951 a and b; KALLMAN 1965). Fish that are phenotypically wild type but carry a macromelanophore allele are called "non-expression". Such fish can be told from crossovers by appropriate breeding experiments. Most useful are crosses involving strains Jp 163 A and B, because in them or in fish having at least one set of Jp chromosomes the penetrance of the macromelanophore alleles is 100%. This is the reason why the two strains have been used in many of the experiments described below.

Nine crosses were made between WX and WY females belonging to various stocks of *X. maculatus* and XY males of the Cp strain or descendants of it. The origins of the WY and WX females were:

Cross 1: 1557-1 and 1557-2, both W₊Y_{Sr}. F₁ hybrids of a cross between the offspring of a W₊Y₊ female collected in 1963 in the Rio Hondo, British Honduras, and a Jp 163 B male (see also Table 10, KALLMAN 1965a).

Cross 2: 1488-2, W₊X_{Sp}. F₁ hybrid of a cross between a W₊Y₊ female collected in 1963 in the Rio Hondo, British Honduras, and a Gp X_{Sp}Y_{Sd} male (see also Table 10, KALLMAN 1965a).

Cross 3: 1468-3, W₊Y_N. Second laboratory generation descendant of fish collected at Tikal, Rio Hondo drainage, Guatemala, in 1963.

Cross 4: 1524-1, W₊X_{Sp}. F₁ hybrid of the offspring of a W₊Y₊ female collected in Lake Petén, Guatemala, in 1963, that was mated to a Jp 163 B male (see also Table 15, KALLMAN 1965a).

Cross 5: 1469 a-3, W₊Y_{Sr}. Second laboratory generation descendant of fish collected in 1963 in the Rio de la Pasión of the Rio Usumacinta system, Guatemala (see also Table 17, KALLMAN 1965a).

Cross 6: Np W₊Y_{Sd}. Offspring of a female taken from the New River, British Honduras in 1954.

Cross 7: Np W₊Y_{Sr}. A female of the 12th laboratory generation of the Np stock.

Crosses 8 and 9: 1380 b-2, 1380 b-1, W₊Y_{Sr}, W₊X_{Sp}. F₁ hybrids between a W₊Y₊ female collected in the Rio San Pedro de Mártir, Rio Usumacinta system, Guatemala, in 1963, and a Jp 163 b male (see also Table 18, KALLMAN 1965a).

Our method of raising *X. maculatus* and our system of identifying pedigrees and individual fish has been explained previously (GORDON 1950; KALLMAN 1965a). All crosses were performed by the author during 1964 and 1965 except No. 6 which was made by the late DR. MYRON GORDON in 1955.

RESULTS

Crosses 1-5 (Table 1): These crosses involve Cp males and WY and WX females of diverse genetic backgrounds but exclude Np. In the first four experiments all WX (83) and WY (66) offspring developed into females. The single *Sp* male of ped. 1630 was due to non-expression of *Sd* (ped. 1749). In the 5th experiment involving a female derived from fish collected in the Rio de la Pasión, males were obtained in both WY and WX classes (ped. 1633). The difference in the frequency of the two kinds of males is significant (SE = 3.4, P < .01).

Crosses 6-7 (Table 2): Among the offspring of a cross between a wild-caught

TABLE 1

Sex ratios and inheritance of pigment patterns among the offspring of WX and WY females of diverse genetic backgrounds (but not Np) with Cp males

Cross Number	Pedigree of offspring	Pedigree and genotypes of parents		Phenotype of offspring			
		♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1	1643	1557-1, -2 W ₊ Y _{Sr}	Cp/X ₊ Y _{Sd}	Sd: 27 WY +: 26 WX		Sr: 22 XY SdSr: 27 YY	
2	1588	1488-2 W ₊ X _{Sp}	Cp/X ₊ Y _{Sd}	Sd: 3 WY +: 9 WX		Sp: 5 XX SpSd: 4 XY	
3	1617	1468-3 W ₊ Y _N	Cp/X ₊ Y _{Sd}	Sd: 14 WY +: 29 WX		N: 24 XY SdN: 16 YY	
4	1630	1524-1 W ₊ X _{Sp}	Cp/X ₊ Y _{Sd}	Sd: 22 WY +: 19 WX		Sp: 11 XX SpSd: 22 XY	1* XY
	1749	Hp-2 X ₊ X ₊	1630-11/X _{Sp} Y _{Sd}	Sp: 50 XX +: 20* XY		Sd: 32 XY	
5	1633	1469a-3 W ₊ Y _{Sr}	Cp/X ₊ Y _{Sd}	Sd: 3 WY +: 18 WX	8 3	Sr: 15 XY SdSr: 27 YY	

* Sd pattern not expressed.

Np female and a XY male of Cp ancestry, there were both WY (35%) and WX (29%) males (ped. 627). The difference between the frequencies of males in the two classes is not significant (SE = 0.4, P > 0.5).

In pedigree 1571, 48% of the WY and 44% of the WX offspring differentiated into males. Sex reversals thus occurred whether the XY male parent was a pure Cp fish (ped. 1571) or only of Cp ancestry (ped. 627). In order to establish that the Sd and Sp males did not result from non-expression of Sr, one WY and one WX male of ped. 1571 were each crossed to a Jp XX female. Among their offspring more WX males were found (ped. 1701, 24% ♂ ♂; ped. 1704, 33% ♂ ♂). A WX male of ped. 1704 was backcrossed once more to a Jp XX female. In pedigree 1771—two generations removed from the original Np × Cp cross—the frequency of WX males (52%) had not yet diminished. The differences between the percentages of WX males of ped. 1571 and 1704 (SE = 1.1, P = 0.27), ped.

TABLE 2

Occurrence of WX and WY males among the offspring and descendants of New River (Np) females with Cp males

Pedigree of offspring	Pedigree and genotypes of parents		Phenotype of offspring			
	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
<i>Cross 6</i>						
627	Np W ₊ Y _{Sd} *	557-11 X _{Sp} Y ₊ †	Sp: 17 WX‡	7	SpSd: Sd:	11 XY 19 YY
			+: 13 WY	7		
<i>Cross 7</i>						
1571	Np W ₊ Y _{Sr}	Cp X _{Sp} Y _{Sd}	Sp: 10 WX	8	SpSr: SdSr:	27 XY 33 YY
			Sd: 11 WY	10		
1701	Jp X _{Sp} X _{Sp}	1571-11 W ₊ Y _{Sd}	Sp: 45 WX	14	SpSd:	49 XY
1704	Jp X _{Dr} X _{Dr}	1571-12 W ₊ X _{Sp}	Dr: 37 WX	18	SpDr: 41 XX	
1771	Jp X _{Sp} X _{Sp}	1704-11 W ₊ X _{Dr}	Sp: 31 WX	33	SpDr: 51 XX	2

* Ped. 530, the offspring of a wild-caught female.

† F₁ hybrid of a Cp ♀ X_{Sp}X₊ (4th lab. generation) with Np-11, a wild-caught male.

‡ Most likely sex chromosome constitution.

1571 and 1771 (SE = 0.53, P > 0.5) are not significant; the difference between ped. 1704 and 1771 is significant at the 0.05 level (SE = 2.1, P = 0.04).

It is unexpected that in ped. 1704 which consists of WX and XX offspring, males are restricted to the WX class. A similar result was obtained in ped. 1771. Only two out of 53 XX individuals as compared to 33 out of 64 WX fish were males. These differences are highly significant and indicate that the W chromosome is involved in the production of males.

Cross 8 (Table 3): In pedigree 1503 the YY, XY and WX classes differentiated according to expectation, but two of 14 Sd (WY) fish developed into males. To rule out the possibility that they were the result of crossing over, e.g. between the W and Y chromosomes of the P₁ female, or were due to non-expression of Sr, one of these Sd males was mated with a Np (W₊Y_N) female. If the exceptional male had been YY, all its N young (ped. 1607) should have been males; if it was WY, the N offspring should be females and the N Sd, males. In ped. 1607 the sex chromosome mechanism appears to have broken down. The four phenotypic classes are present in expected frequencies, but the sex ratio is off. The only regular feature of the pedigree is that all YY offspring were males. Since N females occurred and all but two of the 24 wild-type fish were females, it is concluded that the genotype of 1503-11 was W₊Y_{Sd}. Both the N and Sd males of

TABLE 3

Occurrence of WX and WY males among the platyfish having Np and Cp ancestry (Ped. 1607, Cross 8)

Pedigree of offspring	Pedigree and genotypes of parents		Phenotype of offspring			
	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1503	1380b-2 W ₊ Y _{Sr}	Cp X _{Sp} Y _{Sd}	Sp: 22 WX*		Sd: 12 WY	2
			SpSr: 11 XY		SdSr: 9 YY	
1607	Np W ₊ Y _N	1503-11 W ₊ Y _{Sd}	Sd: 15 WY	7	N: 8 WY	9
			+: 22 WW (or WY)	2†	SdN: 18 YY	
1735a	1607-1 W ₊ W ₊	Hp-1 Y ₊ Y _{Sd}	Sd: 16 WY	10	+: 35 WY	15
1735b	1607-2 W ₊ W ₊	Hp-1 Y ₊ Y _{Sd}	Sd: 8 WY	11	+: 8 WY	4
1823	Jp X _{Sp} X _{Sp}	1735a-11 W ₊ Y ₊	Sp: 20 WX	21 XY		
1824	Jp X _{Sp} X _{Sp}	1735a-12 W ₊ Y ₊	Sp: 35 WX	49 WX,XY		
1825	Jp X _{Sp} X _{Sp}	1735b-13 W ₊ Y ₊	Sp: 18 WX	61 WX,XY		
1830	Jp X _{Sp} X _{Sp}	1735a-14 W ₊ Y ₊	Sp: 24 WX	77 WX,XY		
1773	Jp X _{Sp} X _{Sp}	1607 -11 W ₊ Y _N	Sp: 11 WX	1	SpN: 11 XY	
1861	Jp X _{Sp} X _{Sp}	1607 -13 W ₊ Y _{Sd}	Sp: 24 WX	16	SpSd: 36 XY	
1690	Jp X _{Sr} X _{Sr}	1607 -12 Y _N Y _{Sd}	SrN: 26 XY		SrSd: 23 XY	

* Most likely sex chromosome constitution.

† Male 1607-11 was phenotypically wild type when sacrificed at age of 1 year, see ped. 1773.

pedigree 1607 must be considered sex reversals, WY. When two wild-type females of ped. 1607 were crossed to Hp-1 males (YY), 37% of their WY offspring (ped. 1735) were again males. Four pedigrees (1823, 1824, 1825, 1830) demonstrate that the males of ped. 1735 cannot have been YY; in crosses with XX females such males would sire only males (see table 23, KALLMAN 1965a). On the contrary, the presence of female offspring in the four crosses is evidence for the WY genotype of the ped. 1735 males. The deviation from the expected 1:1 sex ratio is significant in ped. 1825 (77% ♂ ♂) and ped. 1830 (76% ♂ ♂), suggesting the existence of WX males, but their exact frequency cannot be determined, since WX and XY classes look alike.

We cannot establish that either of the phenotypically wild-type males of ped. 1607 was WW. One was not tested. The other, 1607-11, gave rise to N progeny (ped. 1773) and was a WY male in which the N gene was not expressed. When two WY males of ped. 1607 were mated with XX females, their XY offspring de-

veloped into males, but the WX class consisted of both sexes (ped. 1773 and 1861). These crosses, in which WX and XY individuals could be told apart, support the conclusion that in ped. 1825 and 1830 WX males also occurred. There were no sex reversals in ped. 1690, all XY and YY fish were males.

Cross 9 (Table 4): Only two males with unexpected phenotypes were obtained in pedigree 1500. The *Sp* male resulted from non-expression of *Sd* (see ped. 1712); its genotype was XY. The exceptional *Sd* male was crossed to a W_+Y_N female of the *Np* stock. The sex ratio of their progeny (ped. 1612) was abnormal: many WW and WY fish developed into males, but the occurrence of wild-type and *N* females indicates that male 1500-11 was W_+Y_{sd} . The frequency of the four pigment classes also differed significantly from expectation ($N = 3$, $x = 12.23$, $P < .01$) which can be attributed primarily to a deficiency of *N* individuals. The *N* pattern was often weakly developed and did not appear until long after the fish had reached sexual maturity suggesting that several wild-type and *Sd* males might have also possessed the *N* gene. This possibility was tested and the presence of *N* demonstrated in two cases (ped. 1756, 1757). Therefore, the frequency of W_+W_+ and W_+Y_{sd} males is somewhat lower and that of W_+Y_N and $Y_{sd}Y_N$ males slightly higher than appears from Table 4, but the main conclusion that a high proportion of WW and WY individuals are sex-reversed males still holds. The absence of *N* and *Sd* offspring in ped. 1759 and the sex ratio of 75 ♀ ♀ : 1 ♂ can be taken as further evidence for the WW genotype of the wild-type females of ped. 1612. Since WW males had never before been reported in *X. maculatus*, additional crosses were performed to obtain unequivocal evidence for their existence. Three phenotypically wild-type males were crossed to $Jp X_{sp}X_{sp}$ females, because it is known that *N* and *Sd* are strongly enhanced in their expression when introduced into the *Jp* 163 stocks and thus never go undetected (KALLMAN, unpublished). Thus if the males were actually W_+Y_N , approximately one half of their offspring would be *N* and these *N* individuals would be males (XY). It was unlikely that the males were YY, the products of simultaneous crossovers in both P_1 parents, for the frequency of crossing over between the W and Y chromosomes involving the sex differential segment is only 0.2% (KALLMAN 1965a). Furthermore, if they were YY males, all-male broods are expected with XX females (see table 23, KALLMAN 1965a). There can be little doubt that two of the wild-type males (1612-13, -15) possessed the WW genotype, since they sired only *Sp* offspring of which 25% were males. However, a third male, 1612-11, was W_+Y_N . A fourth male, 1612-12, was phenotypically *Sd*, but breeding tests showed it to be YY. The WX class of four pedigrees (1787, 1756, 1849, 1772) consisted of both sexes, a result not unexpected since in similar crosses (ped. 1701, 1704, Table 2; and ped. 1773, 1861, Table 3) WX males also occurred.

Five WX males of ped. 1772 were then mated to *Jp* XX females to determine whether more "normal" sex determination would reappear as the number of Jamapa chromosomes increased. This was not the case: the frequency of WX males in the five crosses (ped. 1834, 1836, 1837, 1839, 1840) ranged from 25 to 53%. The results again implicate the W chromosome interacting with autosomal factors in the production of sex reversals, since the percentage of WX males was

TABLE 4

*Occurrence of WX and WY males among platyfish having Np and Cp ancestry
(Ped. 1612, Cross 9)*

Pedigree of offspring	Pedigree and genotypes of parents		Phenotype of offspring			
	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1500	1380b-1 W ₊ X _{Sp}	Cp X ₊ Y _{Sd}	Sp: 40 XX*	1† XY	Sd: 62 WY	1
			+: 61 WX		SpSd:	35 XY
1644	1500-1 W ₊ Y _{Sd}	Jp X _{Sp} Y _{Sr}	Sp: 23 WX		Sr: 18 WY	1
			SpSd:	22 XY	SdSr:	16 YY
1753	Hp-2 X ₊ X ₊	1644-11 W ₊ Y _{Sr}	+: 46 WX		Sr:	46 XY
1712	Jp X _{Sr} X _{Sr}	1500-12†X _{Sp} Y _{Sd}	SpSr: 28 XX		SdSr:	31 XY
1612	Np W ₊ Y _N	1500-11 W ₊ Y _{Sd}	Sd: 21 WY	32	N: 12 WY	23
			+: 29 WW‡ (or WY)	10	SdN:	23 YY
1759	1612-1 W ₊ W ₊	Hp-1 Y ₊ Y ₊	+: 75 WY	1		
1757	Jp X _{Sp} X _{Sp}	1612-12§ Y _{Sd} Y _N	SpSd:	42 XY	SpN:	41 XY
1787	Jp X _{Sp} X _{Sp}	1612-14 W ₊ Y _N	Sp: 26 WX	3	SpN:	43 XY
1756	Jp X _{Sp} X _{Sp}	1612-11§ W ₊ Y _N	Sp: 27 WX	4	SpN:	35 XY
1849	Jp X _{Sp} X _{Sp}	1612-15 W ₊ W ₊	Sp: 78 WX	24		
1772	Jp X _{Sp} X _{Sp}	1612-13 W ₊ W ₊	Sp: 40 WX	16		
1834	Jp X _{Dr} X _{Dr}	1772-11 W ₊ X _{Sp}	DrSp: 39 XX	3	Dr: 17 WX	17
1836	Jp X _{Dr} X _{Dr}	1772-12 W ₊ X _{Sp}	DrSp: 69 XX	1	Dr: 30 WX	29
1837	Jp X _{Dr} X _{Dr}	1772-13 W ₊ X _{Sp}	DrSp: 62 XX	3	Dr: 29 WX	33
1839	Jp X _{Dr} X _{Dr}	1772-14 W ₊ X _{Sp}	DrSp: 55 XX	10	Dr: 23 WX	8
1840	Jp X _{Dr} X _{Dr}	1772-15 W ₊ X _{Sp}	DrSp: 55 XX		Dr: 44 WX	15

* Most likely sex chromosome constitution.

† This fish (1500-12) was Sp in phenotype, Sd not expressed (sacrificed when 10 months old); see ped. 1712.

‡ See pedigree 1849, 1772.

§ See pedigree 1757, 1756, ♂ 1612-12 phenotype Sd, N not expressed (sacrificed when 11 months old); ♂ 1612-11 phenotype +, N not expressed (sacrificed when 11 months old).

significantly higher than that of XX males (41% WX ♂♂; 5.7% XX ♂♂).

Sex reversals in the Np stock: Since all crosses involving females of Np and males of Cp ancestry gave rise to a high incidence of sex reversed males, it became important to establish whether WY males also occur in the Np stock and in hybrid offspring of Np females with males of diverse genetic backgrounds but not involving Cp. The Np strain (WY ♀♀, YY ♂♂) is maintained by three types of matings: $Sr \times SdN$, $Sd \times NSr$ and $N \times SdSr$. Since the W chromosome is unmarked, males should inherit two patterns and females one. In reviewing the history of this stock KALLMAN (1965a) pointed out that the ratio of 240 ♀♀ : 276 ♂♂ was in good agreement with the expected 1:1 sex ratio. But some males and females exhibited no pattern and some males only one. The unmarked fish are undoubtedly due to the non-expression of a macromelanophore gene, but the possibility that some males with only one pattern are due to crossing over in the female parent or are WY males could not be excluded. Additional crosses have since been performed with similar results (Table 5). Two males with exceptional phenotypes were tested and their genotypes identified as WY (ped. 1702, 1738, Table 5). According to these data, the frequency of WY males in the Np stock may be as high as 13%, but is probably lower since some of the males with a single pattern may be due to other causes. Nevertheless, an incidence of 13% is significantly lower than the frequency of WY males among the offspring of Np females and Cp males which ranged from 35–63% (crosses 6–9).

During the last 10 years five crosses have been performed that clearly show that WY males do not usually arise when Np females are outcrossed to stocks other than Cp (Table 6). In ped. 1087, the wild-type female and the *Sd* male are undoubtedly due to non-expression of *Sr* which was poorly developed in the other eight males. In ped. 1194, all the WX fish were female, but the WY and YY offspring possessed the same pigment pattern and cannot be told apart. There is, however, nothing in the sex ratio that would indicate that any significant number

TABLE 5

Sex ratio and inheritance of sex-linked patterns in the Np strain of Xiphophorus maculatus (13th-17th Lab. Generation) and test for sex reversal

Type of cross					Phenotype of offspring					
Female	Male	<i>Sd</i>	<i>N</i> ♀♀	<i>Sr</i>	+	<i>Sd</i>	<i>N</i>	<i>Sr</i> ♂♂	<i>NSr</i>	<i>SdSr</i> <i>NSd</i>
WY	YY									
<i>Sr</i> × <i>SdN</i>		19	14	..	1*	3†	7†	1*	17	19 ..
<i>N</i> × <i>SdSr</i>		4	..	4	..	1	..	3	5	.. 9
<i>Sd</i> × <i>SrN</i>		1*	27	21	..	1*	..	..	..	29 22
Total:		91	♀♀			Total:	117	♂♂		
<i>Test for Sex Reversal:</i>										
ped. 1702	Jp $X_{Sp}X_{Sp}$ ♀ × Np <i>N</i> ♂†					offspring: 4 <i>Sp</i> ♀♀ 2 <i>SpN</i> ♂♂				
ped. 1738	Jp $X_{Sp}X_{Sp}$ ♀ × Np <i>Sd</i> ♂†					39 <i>Sp</i> ♀♀ 43 <i>SpSd</i> ♂♂				

* Offspring with exceptional phenotypes are italicized; those with an asterisk are due to non-expression of macromelanophore pattern and/or crossing over.

† One *Sd* and one *N* fish were tested; they are WY males; see ped. 1702, 1738.

of the WY fish are males. The four wild-type females of ped. 1200 are probably due to non-expression of *N*, but the two *N* males could have been sex reversals but this was not tested. One of the exceptional males of ped. 1461 b possessed the WY genotype (ped. 1636, 1668, Table 6), but no crosses were performed with the other. The two males with the exceptional phenotypes in ped. 1482 b were both YY (see ped. 1689 and 1737, Table 10, KALLMAN 1965a). Thus, of 175 WX and WY hybrids (from Table 6 and ped. 1702 and 1738 of Table 5) at best 4 (2.3%) are sex reversed males.

DISCUSSION

The experiments were designed to test critically the hypothesis that the Y chromosome of the *Xiphophorus maculatus* population of the Rio Coatzacoalcos is stronger in male determining potency than that of other platyfish populations. According to this theory, fish with the WY constitution develop into females unless the Y has been derived from the Rio Coatzacoalcos. The crosses described in this paper demonstrate decisively that this hypothesis is not correct and that the origin of the WY males requires another explanation. This is clearly shown by 1) the occurrence of both WX and WY males among the offspring of Cp (XY) males and WY females (ped. 1633, Table 1; ped. 627 and 1571, Table 2), 2) the differentiation into females of all or almost all WY fish of six pedigrees that had inherited their Y chromosome from the Cp strain (ped. 1643, 1588, 1617, 1630, 1503, 1500, Table 7), 3) the occurrence in pedigrees 1607 and 1612 (Tables 3 and 4) of two types of WY males some of which had obtained their W from the Np and Y from the Cp stock and others which had received their W from the Rio San Pedro population and Y from Np, 4) the presence of WW males in at least one cross (ped. 1612, Table 4), and 5) the appearance of many WX males among the descendants of sex reversals that were of both Np and Cp ancestry.

TABLE 6

Incidence of WX and WY males in X. maculatus hybrids. Crosses between Np females and males of stocks other than Cp

Pedigree of offspring	♀ ♀	Parents	♂ ♂	♀ ♀	Phenotypes of offspring	♂ ♂
1087	Np W ₊ Y _{Sd}	Jp X _{Sr} Y _{Sr}	Sr: 14 +: 1		SdSr: .. Sd: ..	8 1
1194	Np W ₊ Y _{Sr}	C-30 X _{Sd} Y _{Sr}	Sd: 17 Sr: 14	.. 10	SdSr: ..	15
1200	Np W _N Y _{Sr}	Hp-1 Y ₊ Y ₊	N: 44 +: 4	2 ..	Sr: ..	44
1461b	Np W ₊ Y _{Sd}	1358b-11 Y ₊ Y _N	N: 14 +: 12	.. 2	Sd: .. NSd: ..	26 12
1668	Jp X _{Sp} X _{Sp}	1461b-11 W ₊ Y ₊	Sp: 33	20		
1636	Hp-1 W ₊ Y _{Sd}	1461b-11 W ₊ Y ₊	Sd: 11	3	+: 14	..
1482b	Np W ₊ Y _N	1358a-12 Y ₊ Y _{Sd}	Sd: 13 +: 9	1* 1*	N: .. SdN: ..	8 3

* In these two fish *N* is not expressed; genotype was YY, see Table 10, KALLMAN 1965a.

TABLE 7

Incidence of WY and WX males in X. maculatus hybrids involving the Cp stock

Pedigree	Data from table	WY class		WX class	
		♀ ♀	♂ ♂	♀ ♀	♂ ♂
Crosses between males or females of the Cp strain (or of Cp ancestry) with fish of stocks other than Np					
1643	1	27	..	26	..
1588	1	3	..	9	..
1617	1	14	..	29	..
1630	1	22	..	19	..
1633	1	3	8	18	3
1503	3	12	2	22	..
1500	4	62	1	61	..
1644	4	18	1	23	..
1753	4	..	..	46	..
Crosses between Cp males (or of Cp ancestry) and Np females					
627	2	13	7	17	7
1571	2	11	10	10	8
1607	3	23	17	..	..
1612	4	33	55	..	..

With one exception (ped. 1633, Tables 1 and 7), a high incidence of sex reversals (WX and WY males) was encountered neither among broods sired by Cp males with females of strains other than Np, nor among the descendants of Np females mated with males of stocks other than Cp (Table 6; see also ped. 1702, 1738, Table 5). There is overwhelming evidence that normal sex ratios are the rule when members of different geographical populations of *X. maculatus* are crossed and that the WX, WY and XX combinations give rise to females and the XY and YY genotypes to males (for a summary of a large number of interpopulation crosses see tables 22 and 23, KALLMAN 1965a).

With the exception of pedigree 1633 (Table 1 and 7) broods with a high percentage of WX and WY males were produced only when Np females were mated to males of the Cp stock (ped. 1571, Table 2 & 7) or males of Cp ancestry (ped. 627, Table 2; 1607, Table 3; 1612, Table 4 and 7). This suggests that most of the WY and WX males result from the interaction of autosomal factors, derived from these strains. By themselves the factors have little effect; among 397 WY and WX offspring of eight pedigrees involving Cp but not Np ancestry, only four were males (ped. 1643, 1588, 1617, 1630, 1503, 1500, 1644, 1753, Table 7). No sex reversals have been found in the Cp stock, which lacks the W chromosome (see Table 4, KALLMAN 1965a). Similarly the incidence of WY males in the Np stock is at the most 13% (Table 5) and probably much lower, since sex reversals cannot be distinguished from crossovers and phenotypic non-expression in this strain. Sex reversals are even rarer among the young of Np females mated with males of any stock other than Cp (Table 6). It has not been ascertained whether the slight difference in the incidence of WY males between pure Np fish and Np hybrids (Table 6) is significant, since the precise number of Np WY males

is not known. In the inbred Np strain the factor or factors responsible for sex reversals might be present in homozygous condition; hybrids would be heterozygous. The results of cross 5 (Table 1) which apparently do not fit this scheme, may be explained by assuming that the factors that are present in Np may also be found in other populations.

The numerous WY males of pedigrees 1607 and 1612 (Tables 3 and 4) cannot be attributed to the male parents having themselves been sex reversals, since WX and WY males in similarly high frequencies were produced by XY males (ped. 627, 1571, Table 2). Moreover, it must be emphasized that when sex reversals (WY ♂♂) of Cp, but not Np, ancestry or of Np, but not Cp, ancestry were crossed to Jp or Hp-1 females, only normal offspring were produced (ped. 1753, Table 4; ped. 1702, 1738, Table 5; ped. 1636, 1668, Table 6).

These experiments do not permit the formulation of an exact model that would explain the occurrence of sex reversals in Np × Cp crosses and subsequent generations. The crosses suggest, however, that the Cp stock contributes few factors, perhaps only one. For example, Cp-18, tested by GORDON in 1948 was a wild-caught male not involved in the ancestry of our strain. When it was mated to a Bh female as high a proportion of sex reversals was obtained as in crosses conducted 16 years later with Cp males of the 17th-18th inbred generations (ped. 1571, 1607, 1612). A mating involving a Cp female of the 4th generation yielded similar results (ped. 627, Table 2). It seems unlikely that a combination of several factors could be responsible for the sex reversals: certainly the genotype of the wild-caught male must have been different from that of the males of the highly inbred Cp stock. The frequency of the factor or factors in natural populations is not known, but it might have been present in more than one fish of the Almagres collection and have become incorporated into our Cp stock. Similar considerations apply to the Np stock. Ped. 627 involved the 1st laboratory generation of Np females, while ped. 1571, 1607, and 1612 were produced by members of the 12th.

If the sex reversals are attributed to the interaction of two factors, one derived from Np and the other from Cp, one parent of ped. 1571 (Table 2) must have been heterozygous for its factor, since only 46% of the WY and WX offspring were males. The male parents of ped. 1607 and 1612 certainly were heterozygous for the Cp factors, since they possessed only one set of Cp chromosomes. In ped. 1607, the actual result of 45% WY males does not differ significantly from the expectation of 50% males ($\chi^2 = 0.6$, $0.5 > P > 0.3$), but in pedigree 1612, there is a slight excess of males ($\chi^2 = 5.4$, $P = 0.02$). For pedigree 612 (31.8% sex reversals) it can be assumed that both parents were heterozygous for them and in this case only 25% of the W fish are expected to be males ($\chi^2 = 1.07$, $P = 0.3$).

If the two-factor hypothesis is correct, then 25% of the WX offspring should be males when WW, WX, or WY males of the above pedigrees are mated to Jp XX females. Of eight such crosses, all but one conformed to expectation (Table 8). In pedigree 1861, there was an excess of males. Five WX males of ped. 1772 and one of ped. 1704 were bred with Jp XX females. Again 25% males with a W chromosome are to be expected. But this was not the result: in four of these crosses one half of the WX individuals were males (Table 8). Thus the number

TABLE 8

*Occurrence of WX males among the first and second generation descendants of
Np × Cp sex reversals*

Pedigree	Data from table	WX class		χ^2 and P for an expectation of 25% WX σ^σ if two factors cause sex reversal	
		$\phi \phi$	σ^σ	χ^2	P
One generation removed from Np $\times$ Cp cross					
1701	2	45	14	0.05	$0.9 > P > 0.8$
1704	2	37	18	1.7	$0.2 > P > 0.1$
1773	3	11	1	1.0	$P = 0.3$
1861	3	24	16	4.0	$P = 0.04$
1787	4	29	3	2.5	$0.3 > P > 0.1$
1756	4	27	4	1.8	$0.2 > P > 0.1$
1849	4	78	24	0.12	$0.8 > P > 0.7$
1772	4	40	16	0.4	$P = 0.5$
Two generations removed from Np $\times$ Cp cross					
1771*	2	31	33	22	$P < 0.01$
1834†	4	17	17	10.5	$P < 0.01$
1836†	4	30	29	18	$P < 0.01$
1837†	4	29	33	23	$P < 0.01$
1839†	4	23	8	0.1	$P = 0.9$
1840†	4	44	15	0.5	$0.8 > P > 0.7$

* Derived from ped. 1704.

† Derived from ped. 1772.

of factors and their transmission are not known and the solution of the problem must await further experiments.

One could also assume that the Np and Cp strains each contribute more than one factor, but that only one factor from each is needed to cause sex reversals. However, this interpretation also fails to explain why in pedigrees two generations removed from the original Np × Cp crosses, the incidence of sex reversals is frequently as high as 50% (Table 8).

An added difficulty is presented by the fact that some fish can carry both Np and Cp factors yet be functional females. This is clearly demonstrated by two matings involving WW females and Hp-1 males (ped. 1735 a & b, Table 3); their WY offspring consisted of 37.3% males. When four of them were crossed to Jp XX females, WX and XY young were produced which could not be told apart phenotypically. Nevertheless, from their sex ratios it is apparent that in ped. 1824 and 1830 about 25% and in ped. 1835 about 50% of the WX fish were males (Table 3). Thus, potent factors for sex reversals were present in the WW females, yet remained unexpressed. It is also interesting that the two WW females (ped. 1735) gave rise to a higher incidence of males with a W chromosome than the two WW males (ped. 1772, 1849, Table 4) ($SE = 2.21, 0.03 > P > 0.02$).

KOSSWIG and ÖKTAY (1955) and KOSSWIG (1964) have presented a theory of polygenic sex determination that has a direct bearing on these experiments. "The genetic system in cases of so-called polygenic sex determination is based upon a series of pairs of alleles (*sic*); each allele works together with its partner in the

homologous chromosome and with all others localized in other chromosomes. This means that sex determination is assured by a system of polygenes with an additive polymeric action of each of its members" (page 5, Kosswig 1964). They believe there are autosomal male (*M*) and female (*F*) determining genes in *X. maculatus* and other fishes in addition to sex chromosomes. In rare instances the sum of autosomal *F* genes together with one X chromosome may surpass a certain threshold so that XY individuals differentiate into females instead of males; conversely the combined effect of autosomal *M* genes and the Y chromosome may be sufficiently strong to cause WY fish to become males. In the Jp strains of *X. maculatus* XY females arise sporadically (ANDERS and ANDERS 1963; KALLMAN 1965a; MACINTYRE 1961). One could interpret this as evidence that the stock harbors a significant number of autosomal *F* genes. It was, therefore, paradoxical to find a few XX males among the offspring of Jp XX females and XY males of Lake Petén (KALLMAN 1965a). As has already been pointed out by KALLMAN (1965a) this finding is difficult to reconcile with Kosswig's ideas of polygenic sex determination. DZWILLO and ZANDER (1967) state that in addition to autosomal *F* genes, there are also a small number of autosomal *M* genes in *X. maculatus* populations with homogametic females (e.g. Jamapa stocks), but that these *M* genes have no effect within their own population. According to these investigators the situation is reversed in populations in which males are homogametic (WY ♀ ♀ YY ♂ ♂). Here the *F* genes not located on the W chromosome are without importance. If the *M* genes of the Jamapa stocks were not allelic to those of the Lake Petén population, the Jamapa × Petén hybrids might possess more *M* genes than either parent. According to their interpretation the XY Petén males must have carried an unusually large number of *M* genes. DZWILLO and ZANDER explain this by postulating that the Lake Petén platyfish are of hybrid origin; that they have been derived from two populations, one homogametic in females and the other homogametic in males. Because of random segregation of sex genes some Lake Petén platyfish could possess a large number of *M* genes.

Underlying these theories is the tacit assumption that there exist separate *X. maculatus* populations, some with XX-XY and others with WY-YY sex-determining mechanisms. However, fish with the W and X chromosomes have been collected together in Lake Petén in 1954 and 1963, in the Rio de la Pasi6n 160 (km) to the south in 1963, in the Rio Grijalva near Villahermosa 360 km to the west in 1952, in the Rio San Pedro de M6rtir 75 km to the northwest in 1963, in the Rio Hondo system 200 km to the northeast in 1954 and 1963 (KALLMAN 1965a), and in 1966 near the city of Belize 200 km to the east, at the eastern limit of the range of *X. maculatus* (KALLMAN unpublished). The only drainage system from which the X has not yet been demonstrated is the New River from which only 3 fish were tested. There is no evidence that the Lake Petén population is of hybrid origin.

The autosomal Np and Cp factors are not sex genes *per se*, i.e. they do not carry a male (or female) valence as do the postulated sex genes of the theory of polygenic sex determination (DZWILLO and ZANDER 1967; Kosswig 1964; 6KTAY

TABLE 9

Incidence of males in seven pedigrees consisting of WX and XX classes

Pedigree	Data from table	WX			XX		
		♀ ♀	♂ ♂	% ♂ ♂	♀ ♀	♂ ♂	% ♂ ♂
1704	2	37	18	33	41	..	0
1771	2	31	33	52	51	2	4
1834	4	17	17	50	39	3	7
1836	4	30	29	49	61	1	2
1837	4	29	33	53	62	3	5
1839	4	23	8	26	55	10	15
1840	4	44	15	25	55	..	0
Total: 211		153	42		364	19	5

1959). There is also no evidence that the action of the Np and Cp factors is additive to that of the sex chromosomes. On the contrary our crosses point towards an epistatic interaction of at least two autosomal factors with the sex chromosomes, in this case preferentially with the W. It is significant and unexpected that in all pedigrees which consisted of two classes, WX and XX, males that arose were preferentially WX (364 WX fish—42% ♂ ♂, 383 XX fish—5% ♂ ♂, Table 9). There was also no correlation between the frequency of WX and XX males (Table 9) as would be expected, if the sex reversals were due to the additive effect of *M* genes. These results are unusual because the W chromosome is thought to be more strongly female determining than the X: WY individuals are normally females and XY individuals males. One cannot assume that the W chromosome is inactivated and that the male phenotype is due to the presence of one Y chromosome (in the case of WY fish), since no significant difference in the incidence of WX and WY males was demonstrated in pedigrees in which both classes occur (ped. 627 and ped. 1571, Table 2). Perhaps the combination of Cp and Np factors specifically promotes a male-determining tendency of the W chromosome that is normally not detectable. In WW individuals the factors may interact with each W chromosome independently thereby accounting for the lower incidence of males in this class as compared with WY fish (ped. 1612, Table 4). WW fish may not develop into males unless the interaction is with both W chromosomes. This may explain why some WW fish were functional females, although the factors were present (ped. 1735, Table 3).

The idea that autosomal factors epistatic to the sex chromosomes are responsible for the occurrence of genetic sex reversals, is also consistent with the results of ÖKTAY's (1959) experiments. But the existence of such epistatic factors is difficult to demonstrate in ÖKTAY's case, since both males and females possessed the XX genotype. However, one important difference exists between our and ÖKTAY's experiments. In ÖKTAY's stock (of hybrid origin) XX males arose predominantly after inbreeding and few, if any, such males were obtained when XX males or females of the exceptional line were outcrossed to fish derived from the Jamapa stock. In our experiments a high percentage of genetic sex reversals occurred in

fish that were of both Np and Cp ancestry and also when such fish were outcrossed to other stocks. Thus homozygosity for at least some of the autosomal factors could have been a prerequisite for the occurrence of ÖKTAY's XX males while heterozygosity was the rule in our experiments. It is not known how ÖKTAY's factors would interact with a W chromosome nor how inbreeding would affect the incidence of genetic sex reversals in Np $\times$ Cp crosses.

The existence of autosomal factors that specifically change the sex-determining tendency of the W chromosome from female to male has important evolutionary implications. In the swordtail, *X. hellerii*, sex-linked traits have not been discovered. Because sex ratios fluctuate widely within different stocks and pedigrees of swordtails, sex determination is thought to be controlled by a large number of sex genes scattered over many chromosomes. As pointed out by KOSSWIG (1964), the contradictory results (sex ratios) obtained found an easy interpretation through KOSSWIG's (1935) assumption that there are no sexually different gonosomes in *X. hellerii*, but that merely polygenes and their manifold recombinations decide the sex of each fish. KALLMAN (1965a) has already briefly indicated that the sex-determining mechanism of *X. hellerii* may have been derived from such a system as exists today in *X. maculatus*. In the crosses described herein, sex ratios have been obtained for *X. maculatus* that do not differ from those of *X. hellerii* (compare the sex ratios of crosses listed in Tables 2, 3 and 4 with the sex ratios of *X. hellerii* obtained by PETERS (1964)). Had our experiments been performed with stocks of unknown geographic origin and unknown sex-determining mechanism, and had sex-linked marker genes been absent, it would not have been possible to demonstrate that *X. maculatus* has W, X, and Y chromosomes and autosomal factors that specifically interact with the W to produce males. Instead I might have come to the same conclusion as KOSSWIG (1935) did for *X. hellerii*, that sex in *X. maculatus* is determined by many sex genes with *M* or *F* values scattered over many chromosomes. Sex determination in *X. hellerii* may not be basically different from that in *X. maculatus*. Possibly *transformer genes* may be much more common in the swordtail and obscure its sex-chromosome mechanism. No critical experiment has been performed that could rule out the possibility that sex chromosomes exist in the swordtail.

Few instances are known in which specific autosomal genes have been implicated as the cause of genetic sex reversals. The best known are *tra* of *Drosophila melanogaster* which in homozygous condition causes XX individuals to develop into sterile males (Sturtevant 1945), *Hr*, a dominant allele of *tra* which is responsible for hermaphroditism of XX flies (GOWEN and FUNG 1957), and *ix* of *D. virilis* which in homozygous condition causes XX flies to differentiate into hermaphrodites (Lebedeff 1934). None of these, however, is strictly comparable to the situation in platyfish; since *Drosophila* has only two sex chromosomes, X and Y, one cannot determine whether *tra* and *ix* act by themselves or through the sex chromosome.

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SUMMARY

The teleost *Xiphophorus maculatus* possesses three sex chromosomes. In natural populations the sex chromosome constitution of females is either WY, WX, or XX and that of males YY or XY. In laboratory stocks genetic sex reversals occur, i.e. females with the genotype XY and males with the genotypes WY, WX or XX, but the overall incidence of such exceptions is less than 1%. However, when, XY males of the Cp stock or of Cp ancestry are crossed to WY females of the Np stock, approximately 50% of the WX and WY offspring are fertile males. Some functional WW males were produced by crossing a WY Np female with a WY male. Breeding experiments have shown that some WW females of Np and Cp ancestry carry potent factors for male sex reversals. This situation is highly specific, since almost only normal offspring are produced when fish of the Cp stock or of Cp ancestry are crossed to stocks other than Np and, conversely, when fish of the Np strain or of Np ancestry are crossed to stocks other than Cp. No sex reversals occur in the Cp stock which lacks the W chromosome and the incidence of WY males in the Np strain is less than 13%. When exceptional males of both Np and Cp ancestry are outcrossed to other stocks, the frequency of male sex reversal does not diminish and WY, WX and XX male offspring are obtained that have inherited their sex chromosomes from stocks other than Np and Cp. The high incidence of male sex reversals appears to be due to a combination of autosomal factors derived from the Np and Cp strains. These factors act preferentially through the W chromosome, since seven crosses of XX females with WX males resulted in 364 WX offspring of which 153 (42%) were males and 383 XX offspring of which only 19 (5%) were males. No evidence has been found that the Y chromosome of *X. maculatus* of the Rio Coatzacoalcas contains stronger sex genes for maleness than the Y chromosomes of other populations.

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