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THEORETICAL AND APPLIED GENETICS

Sex Determination in *Sarotherodon (Tilapia)*

Part 2: The Sex Ratio as a Tool for the Determination of Genotype – A Model of Autosomal and Gonosomal Influence

I.S. Hammerman and R.R. Avtalion
Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel)

Summary. The simplest possible model of the sex determination process adding autosomal influence to a minimal number of sex chromosomes was developed to explain matings of *Tilapia* (*Sarotherodon*) species. Eighteen different genotypes, each having two autosomes (AA, Aa, or aa) and two sex chromosomes (WX, WY, WW, XY, XX or YY) involved in sex determination, are predicted by the theory. Their sex (10 males and 8 females) were determined using a series of directed graphs, showing the relative strength of the chromosome pairs, developed on the basis of Chen's sex ratio results (Chen 1969). This theoretical model predicts eight different sex ratios (0:1, 1:3, 3:5, 1:1, 9:7, 5:3, 3:1, 1:0 ♀:♂); three of them are not predicted by the WXYZ theory. The greatest part of these sex ratios have been obtained experimentally in extensive series of crosses between related species of *Tilapia* and their hybrids, carried out by several authors. The theory succeeds in explaining all of Chen's results, including those ratios 5:3 and 0:1 seen in certain crosses but not predicted by the WXYZ theory. The importance of the autosomes is seen in comparisons of the genotype pairs (AaWY, aaWY), (AaXY, aaXY) and (AAWW, AaWW) in which the first genotype in each case is male while the second is female as proven by the sex ratio results. The members of the pair differ only in the substitution of one autosome for the other. To test the theory, experiments consisting of hormonal sex reversion and a series of crosses are proposed. Finally, theoretical and practical implications of the theory are discussed.

Key words: *Sarotherodon (Tilapia)* – Sex determination – Theoretical model – Autosomal influence – Sex chromosomes

1 Introduction

Fish belonging to the genus *Tilapia*[1] are currently bred in fish ponds in tropical and subtropical countries and provide an important source of animal protein in developing regions of the world. A free-breeding culture of *Tilapia* in a limited space of water gives rise to an enormous quantity of small fish having no economic importance. Monosex culture of males was found to be the solution. However, the manual separation of males from females is very wasteful and time-consuming (Hickling 1963; Pruginin et al. 1975).

The culture of *Tilapia* greatly progressed when it was found that crosses between certain species of *Tilapia* resulted in all-male broods (Hickling 1960; Fishelson 1966; Pruginin et al. 1975). This unusual sex ratio was explained by Hickling (1960) and Chen (1969) on the basis of Gordon's discovery of a dual mechanism for sex determination in natural populations of platyfish (*Xiphophorus maculatus*). Gordon (1947) found that Mexican platyfish have a homogametic female (XX) – heterogametic male (XY) mechanism while members of the same species from Honduras have a heterogametic female (WY) – homogametic male (YY) system. When the homogametic male and female platyfish were crossed, all males were obtained, and when a reciprocal cross of heterogametic fish was performed, a 1:1 ratio resulted. These results in platyfish are explained by a three-sex chromosome (W, X, Y) theory described by Gordon (1946, 1947) and reviewed by Kallman (1965).

By analogy to this dual system, Hickling (1960) assumed that in *Tilapia* there are species with homogametic males and others with homogametic females that, when

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[1] The genus *Tilapia* (Cichlids) has recently been divided into two genera: (1) *Tilapia*: bottom spawners and (2) *Sarotherodon*: mouth breeders. For convenience in reporting previous work, we will use the older nomenclature which does not distinguish between the two genera

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crossed, give rise to all males. However, in contrast to the ratio (1:1) obtained in the reciprocal cross of *X. maculatus* heterogametic parents, the same cross in *Tilapia* consistently gave a 1 ♀ : 3 ♂ ratio (Chen 1969; Jalabert et al. 1971; Pruginin et al. 1975). This result implies that in the *Tilapia*-system more than the W, X and Y chromosomes have a role in the sex determination process.

A dual system (XX-XY and WZ-ZZ), including the fourth sex chromosome Z, was suggested originally by Bellamy (1936), Kosswig and Öktay (1955) and Anders and Anders (1963) to explain the results obtained in *Xiphophorus* interspecific crosses. By analogy, such a system was applied in *Tilapia* to interpret the results of a very extensive series of crosses between *T. hornorum* and *T. mossambica* (Chen 1969) and between *T. macrochir* and *T. nilotica* (Jalabert et al. 1971). However, these authors were not able to explain an important part of their sex-ratio results using this four-sex-chromosome theory. The involvement of four distinct sex chromosomes in determining the sex in fishes is still controversial, as is the case in *Xiphophorus maculatus*, where no evidence for more than three different sex chromosomes (WXY) could be found, and the Z and Y chromosomes were shown to be synonymous (Gordon 1946, 1947).

Many researchers agree that an autosomal influence on the sex determination process must be taken into account in fish. Winge (1934) and Winge and Ditlevsen (1948) proposed that sex-determining genes of different potency, some pulling in the female direction and others in the male, are distributed over the majority of the autosomes in *Lebistes*. They claim that usually the additive effect of these autosomal factors is insufficient to upset the switch mechanism, but rare fortuitous combinations of autosomal genes could override the action of the sex chromosomes. Similar explanations have been suggested for *Xiphophorus* (Kosswig and Öktay 1955; Kosswig 1964; Öktay 1959; Dzwillo and Zander 1967; Anders and Anders 1963) and *Oryzias* (Yamamoto 1963, 1969). Jalabert et al. (1971) have suggested that the autosomes may play a role in sex determination in *Tilapia*.

Kallman (1968), on the basis of his findings on *X. maculatus*, proposed a different autosomal mechanism in which autosomal transformer genes acting epistatically to the W chromosome may lead to males in WY and WX genotypes which are usually females.

The sex-chromosome system in *Tilapia* is far from being understood. As in most fish, identification of sex chromosomes in *Tilapia* by distinct morphology in a karyotype analysis is not possible. Furthermore, no sex-linked color markers are known in this genus. What is known about the sex-chromosomes in *Tilapia* has been found by sex-reversal and interspecies breeding experiments. Guerrero (1975) proved that the female of *T. aurea* is heterogametic based on the sex-ratio of between 2 to 3.1 ♀♀ : 1 ♂ in crosses of the androgen sex-reversed females with normal females. On the other hand, the female of *T. mossambica* was proved to be homogametic by Clemens and Inslee (1968) since all females were obtained in crosses of methyltestersterone sex-reversed females with normal females. Jalabert et al. (1974) obtained similar results in *T. nilotica*. In crosses between related species of *Tilapia* all-male-generating parents could be identified as homogametic, while those pairs giving a 1 ♀ : 3 ♂ ratio could be identified as being heterogametic. In this respect *T. hornorum*, *T. macrochir* and *T. aurea* could be considered as homogametic in the male and heterogametic in the female, with the opposite being the case for *T. mossambica, T. nilotica* and *T. nigra* (reviewed by Pruginin et al. 1975).

2 Object of Theory and Assumptions

The object of this paper is to explain the results of the careful and comprehensive experiments performed by Chen (1969) in which hybrids of *T. mossambica* and *T. hornorum* were extensively crossed between themselves and were back-crossed with parents for up to four generations. The sex ratios obtained by Chen in all these crosses are summarized in Table 1. We have used the sex-ratios from a small subset of these crosses to determine the sex of all the genotypes. The resulting predictions for the sex-ratio from any possible cross were then compared with the experimental data for the remaining crosses not used in the derivation. As will be seen, the theory is in complete agreement with all the experimental results.

This theory extends the above-mentioned dual model proposed by Gordon (1946) for *X. maculatus*, where two types of genetic mechanisms for sex determination were found. That model is based on three different sex chromosomes, as follows:

Female	Male	
XX	XY	Type 1
WY	YY	Type 2

2.1 *Assumptions of Theory*

1) It assumed, in an extension of Gordon's model, that three gonosome types (W, X, Y) and 2 different autosome types (A, a) are involved in the sex determination in *Tilapia*.

2) Within one species the pair of autosomes are identical (AA or aa).

Table 1. Summary of results of Chen's crosses in *T. mossambica-T. hornorum*-hybrid system

Source of male parent		Source of female parent	No. of offspring in each pair-mating		Sex ratio % ♀ : % ♂	Cross no.
			♀	♂		
T. hornorum	X	*T. mossambica*	N.G.	N.G.	0:100	1a
T. mossambica	X	*T. hornorum*	20	66	23: 77	1b
1a	X	*T. mossambica*	N.G.	N.G.	50: 50	2a
1a	X	*T. hornorum*	N.G.	N.G.	50: 50	2b
1b	X	*T. hornorum*	10	62	14: 86	2c
			53	295	15: 85	
			99	256	28: 72	
1b	X	1b	79	68	54: 46	3a*
1b	X	1b	29	109	21: 79	3c*
			34	129	21: 79	
			56	181	24: 76	
			49	131	27: 73	
			54	140	28: 72	
			56	146	28: 72	
			39	95	29: 71	
1a	X	1b	198	169	54: 46	3d
			127	85	60: 40	
			220	125	64: 36	
			156	77	67: 33	
T. hornorum	X	3c	53	48	52: 48	4a
T. mossambica	X	3c	122	307	28: 72	4b
4a	X	*T. mossambica*	0	68	0:100	5a
			0	74	0:100	
T. mossambica	X	4a	19	64	23: 77	5b
T. hornorum	X	3a	415	487	46: 54	6a*
			34	38	47: 53	
			117	131	47: 53	
T. hornorum	X	3a	2	441	0.5: 99.5	6b*

N.G. = Number of offspring not given by Chen

* Crosses 3a-c and 6a-b are divided into separate groups due to the significant differences between the groups

3) The complement of chromosomes present in the two species would be as follows:

Female	Male	
Either Model I:		
AAXX	AAXY	*T. mossambica*
aaWY	aaYY	*T. hornorum*
Or Model II:		
AAXX	AAXY	*T. mossambica*
aaWX	aaWW	*T. hornorum*

In these models there are six possible gamete types (*AX, AY, AW, aX, aY, aW*). Consequently the number of genotypes resulting from the combination of these gametes is eighteen (Table 2).

4) Each chromosome has a fixed strength, which is not influenced by the presence or absence of any other chromosome.

5) The relative strengths of each of the chromosomes could be designated by a set of numbers, each number being proportional to the amount of the gene product(s) of the chromosome times the relative importance of the product(s) in determining one of the sexes. (The sign of the number we arbitrarily take as positive if its influence is in the male direction and minus if in the female direction.)

6) The additive effect of each individual's four sex-influencing chromosomes determines the primary sex.

7) If the sum is greater than a certain threshold, the individual develops into a male; if less, a female (Avtalion and Hammerman, unpublished).

Table 2. The eighteen possible genotypes from combinations of the two autosomes (A, a) and the gonosomes (W, X, Y)

AAXX*	AaXX	aaXX
AAWX	AaWX	aaWX*
AAXY*	AaXY	aaXY
AAWW	AaWW	aaWW*
AAWY	AaWY	aaWY*
AAYY	AaYY	aaYY*

* Genotype of parent species: *T. hornorum* male (aaYY) and female (aaWY), and *T. mossambica* male (AAXY) and female (AAXX), in Model I, while in Model II the *T. hornorum* male is aaWW and the female aaWX

2.2 Mathematical Notation and Conventions

We will use the same letters to designate both the chromosomes and their strength and will designate the threshold value as t. Thus, the fact that AAXY, for example, is male can be represented by the inequality

$$A + A + X + Y > t,$$

or

$$X + Y > t - (A + A)$$

For simplicity, by subtracting t/4 from each chromosome strength, we redefine the variables such that the threshold has the value 0:

$$X \leftarrow X - t/4, \text{etc.}$$

If the sum is greater than zero, the individual is a male; if less, a female. (This simplification has no effect on the results.) For further simplicity of notation we drop the plus signs and the parentheses and the inequalities then take the form:

$$XY > -AA$$

In these inequalities the strength of the various pairs of the three sex chromosomes (X, Y, W) appear on the left side and the negative of the strength of the pairs of the two autosomes (A, a) appear on the right. The knowledge of the sex of any genotype such as AAXY determines the direction (> or <) of the corresponding inequality. The set of all such inequalities must be consistent for this theory to be of value.

3 Derivation of Set of Inequalities from Sex Ratio Data and Determination of Sex of Different Genotypes

A sufficently complete set of inequalities between the possible autosomal and gonosomal pairs, once determined, would allow the easy identification of the sex of any genotype. This set of inequalities can be obtained from an analysis of the sex-ratio results from a sufficiently large number of crosses between hybrids and from backcrosses with parents. In this section we will use Chen's sex-ratio results from crosses 1a, 1b, 2b, 3d and 3a to determine the set of inequalities appropriate to Model I. In the discussion we show the differences in the results if Model II is considered instead. Firstly, the presumed genotypes of the parental species, based on Model I, imply the following four inequalities:

T. hornorum ♂ : $a + a + Y + Y > 0$, thus $YY > -aa$ (1)

T. hornorum ♀ : $a + a + W + Y < 0$, thus $WY < -aa$ (2)

T. mossambica ♂: $A + A + X + Y > 0$, thus $XY > -AA$ (3)

T. mossambica ♀: $A + A + X + X < 0$, thus $XX < -AA$ (4)

Combining (1) and (2) : $YY > -aa > WY$ (5)
and (3) and (4) : $XY > -AA > XX$ (6)
From (5) we learn that: $Y > W$, (7)
and from (6) that : $Y > X$. (8)

3.1 Cross 1a and Determination of Sex of AaXY, aaWX, aaWW, AaYY, AAYY and AaWX Genotypes

Cross 1a, performed between the homogametic *T. mossambica* ♀ (AAXX) and the homogametic *T. hornorum* ♂ (aaYY) results in 100% male hybrids (AaXY) (Table 1), from which we learn the inequality:

$$XY > Aa \tag{9}$$

The set of inequalities so far determined and their logical consequences are summarized in the directed graphs in Figure 1.

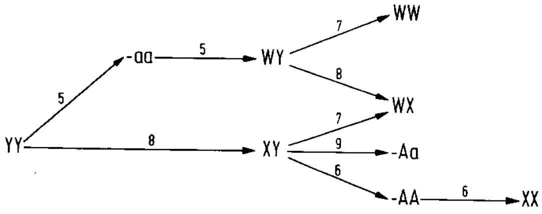


Fig. 1. Directed graph showing relative strength of chromosome pairs in Model I deduced from cross of homogametic parents and parental genotypes. (A directed graph is a convenient way to summarize the mutual relationships among a group of individuals. Each node represents an individual and each arrow the relationship. In the present case the directed graph is a branched chain of inequalities in which each node represents the strength of the chromosome pair designated, and the direction of the arrow describes the direction of decreasing 'maleness'. The strength of the chromosome pair at the tail of each arrow is of higher 'maleness' than the pair at the head of the arrow. Following the chain of arrows permits the deduction of all the relations implied by those relations used to build the graph. This first directed graph permits, for example, the deduction that aaWX is female, since −aa > WY > WX, but does not permit the deduction of the sex of AaXX since the relative strength of −Aa and XX is not implied by the graph.) The numbers above the arrows give the equation from which the relation is derived

By following the chain of arrows we can predict the sex of four other genotypes:

aaWX (♀), aaWW (♀), AaYY (♂) and AAYY (♂).

(For example, we know from the graph that $-\text{aa} > \text{WY} > \text{WX}$, thus $-\text{aa} > \text{WX}$ or $0 > \text{aaWX}$; thus aaWX is ♀).
It can further be proved that AaWX is female since:

$$2\ \text{AaWX} = 2\ (\text{aaWY}) + (\text{AAXX}) - (\text{aaYY}). \tag{10}$$

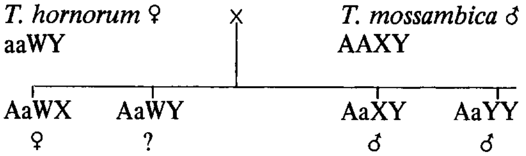
From Eqs. (1), (2) and (4) we know that:

$\text{aaYY} > 0, \text{aaWY} < 0, \text{and AAXX} < 0.$

Thus the right hand side of equation (10) must be less than zero. Consequently AaWX is less than zero and thus female.

3.2 Cross 1b and Determination of Sex of AaWY, AAWY, aaXX and AaXX Genotypes

The reciprocal cross 1b performed by hybridization of the heterogametic parents (*T. hornorum* ♀ x *T. mossambica* ♂), gives a 1 ♀ : 3 ♂ ratio:



Consequently AaWY must be ♂.

Since AaWY is ♂ while aaWY is ♀, we deduce that $\text{A} > \text{a}$ and thus

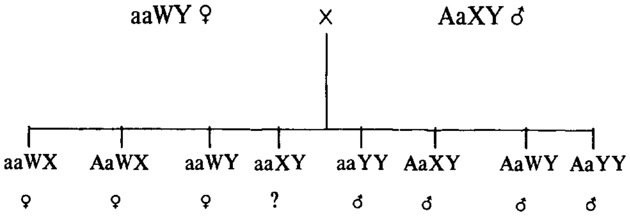
$$-\text{AA} < -\text{Aa} < -\text{aa}. \tag{11}$$

Consequently AAWY is ♂. Furthermore, since AAXX is ♀, AaXX and aaXX must then also be ♀ ♀.

There are now only four genotypes, AAWX, AAWW, AaWW and aaXY, which are still to be determined.

3.3 Cross 2b and Determination of Sex of aaXY Genotype

Chen performed the backcross of the all-male hybrid (AaXY) with the *T. hornorum* ♀ (aaWY). A 1:1 ratio was obtained:



The 1:1 sex ratio implies that aaXY is ♀ and there are now three genotypes (AAWW, AAWX and AaWW) whose sex remain to be determined.

3.4 Cross 3d and Determination of Sex of AAWX Genotype

Chen also crossed the all-male hybrid of cross 1a (AaXY) with the F_1 ♀ of cross 1b (AaWX). The gamete combinations for this cross are as follows:

	AX	aX	AY	aY
AX	AAXX ♀	AaXX ♀	AAXX ♂	AaXY ♂
aX	AaXX ♀	aaXX ♀	AaXY ♂	aaXY ♀
AW	AAWX ?	AaWX ♀	AAWY ♂	AaWY ♂
aW	AaWX ♀	aaWX ♀	AaWY ♂	aaWY ♀

Pooling his results from all four ponds and fish sizes, the sex ratio (677:456) was 60.6% ♀♀ : 39.4% ♂♂. Taking a two-standard deviation confidence range for his results, the expected theoretical values should fall in the range: 57.7% < ♀ ♀ < 63.5%. The predicted ratio depends on the sex of AAWX:

10 ♀ : 6 ♂ (62.5% ♀) if AAWX ♀
9 ♀ : 7 ♂ (56.25% ♀) if AAWX ♂

The experimental results show that AAWX is female. Thus

$$\text{WX} < -\text{AA} \tag{12}$$

3.5 Cross 3a and Sex Determination of the Remaining AAWW and AaWW Genotypes

In cross 3, male and female offspring of the reciprocal cross 1b were paired in individual ponds. In seven of the ponds the sex ratios were consistent with a 1 ♀ to 3 ♂ ratio (3c) and the eighth (Pond B) (3a) gave a ratio of 54% ♀ : 46% ♂.

How can these results be explained? The male parents in cross 3a (offspring of cross 1b) could be AaYY, AaXY or AaWY, and the female must be AaWX.

If the pair in pond B were the pair AaWX ♀ x AaWY ♂, the gamete combinations for this cross (3a) would be as follows:

	AW	aW	AY	aY
AX	AAWX ♀	AaWX ♀	AAXY ♂	AaXY ♂
aX	AaWX ♀	aaWX ♀	AaXY ♂	aaXY ♀
AW	AAWW ?	AaWW ?	AAWY ♂	AaWY ♂
aW	AaWW ?	aaWW ♀	AaWY ♂	aaWY ♀

The resulting sex ratio depends on the sexes of the two genotypes AAWW and AaWW. Since from (11) AA > Aa, the option that AAWW is ♀ and AaWW is ♂ is not possible; thus the three possible combinations are:

	AAWW	AaWW	Ratio (♀ : ♂)	% Females
Option 1	♀	♀	10 : 6	62.5 ♀
Option 2	♂	♀	9 : 7	56.25 ♀
Option 3	♂	♂	7 : 9	43.75 ♀

Only Option 2 is consistent with the 54% experimental result in Pond B. Thus, if the male parent in pond B was AaWY then we would find that AAWW is ♂ and AaWW is ♀. We can indeed prove that AaWY was the male present and not the other males AaYY or AaXY. If AaXY were the male parent, the cross AaWX ♀ X AaXY ♂, would give 62.5% ♀ and if AaYY were the male parent, the cross, AaWX ♀ x AaYY ♂; would give 25% ♀. Neither result is consistent with the 54% ♀ seen in Pond B.

Thus we have proven that AAWW is ♂ and AaWW ♀. From these sexes we obtain the inequalities:

$$-\mathrm{Aa} > \mathrm{WW} > -\mathrm{AA}, \tag{13}$$

and from a comparison of the sex of AAWW ♂ and AAWX ♀ we learn that:

$$\mathrm{W} > \mathrm{X} \tag{14}$$

The final directed graph of the relative strengths of the gene combinations is shown in Figure 2. The sex of all the genotypes have now been determined and are summarized in Table 3. There are eight male genotypes and ten female genotypes between which eighty distinct crosses are theoretically possible.

$$\mathrm{YY} \xrightarrow{5} -\mathrm{aa} \xrightarrow{5} \mathrm{WY} \xrightarrow{14} \mathrm{XY} \xrightarrow{9} -\mathrm{Aa} \xrightarrow{13} \mathrm{WW} \xrightarrow{13} -\mathrm{AA} \xrightarrow{12} \mathrm{WX} \xrightarrow{14} \mathrm{XX}$$

Fig. 2. Final directed graph showing relative strength of chromosome pairs in Model I. Arrows indicate the direction of decreasing values. The numbers above the arrows give the equation from which the relation is derived. This graph permits the deduction of the sexes of all the genotypes as follows: All genotypes for which the value of their sex chromosome pair (YY, WY, XY, WW, WX, or XX) is greater than the negative of the value of their autosomal pair (–AA, –Aa or –aa) are males (e.g., AAYY and AaWY), while those for which the value is less are females (e.g., aaWX and AaXX)

Table 3. Sexes of the genotypes in *T. mossambica-T. hornorum* hybrid system, according to Model I (————) and Model II (– – – – – –)

Males					
AAYY	AAWY	AAXY	AAWW	AAWX	AAXX
AaYY	AaWY	AaXY	AaWW	AaWX	AaXX
aaYY	aaWY	aaXY	aaWW	aaWX	aaXX
					Females

4 Test of Predicted Genotype Sexes in Nine Additional Crosses

The sexes of the eighteen possible genotypes were determined above on the basis of the assumed genotype of the four parents and on the results of five crosses: the two F_1 crosses 1a and 1b, the backcross 2b and two of the F_2 crosses 3a and 3d. If the present theory is correct, the results of Chen's nine remaining crosses and backcrosses (2a, 2c, 3c, 4a, 4b, 5a, 5b, 6a and 6b), should be in agreement with the sex ratios predicted on the basis of the genotype sex assignments given in Table 3. The test of these predictions is presented below.

4.1 Cross 3c

In the crosses of pairs of male and female offspring of the reciprocal cross 1b we noted that the female has the genotype AaWX while the males must be either AaYY, AaXY or AaWY. Cross 3a, seen in Pond B and giving 54% females, was explained above by the male being AaWY. The results in the remaining seven ponds are explained by the male in each case being of the genotype AaYY. The AaWX x AaYY gives a 1 ♀ : 3 ♂ ratio (2 females: aaWY, aaXY; 6 males: 2 AaWY, 2 AaXY, AAWY, AAXY) in agreement with the experimental data. (The third possible cross, AaWX x AaXY, is predicted to give a 5 ♀ : 3 ♂ ratio and was evidently not performed.)

4.2 Crosses 6a and 6c

Chen took four female offspring from Pond B and crossed them with the homogametic *T. hornorum* male (aaYY) in four separate ponds. A sex ratio close to 1:1 (47% or 46% ♀) (cross 6a) was found in 3 ponds and an almost all male brood (0.5% ♀) in the fourth pond (cross 6b). By our analysis above, the cross in Pond B was AaWX x AaWY and the resulting female offspring in the pond had the seven different genotypes as shown in Table 4. The first cross in Table 4 explains the essentially all male brood seen in cross 6b and the next four crosses in the table predict the 1:1 ratio seen in the other ponds (crosses 6a). If more than the four crosses were made, it is to be expected that the all-female ratios of the last two crosses in the table would also have been seen.

Table 4. Predicted sex ratios from the crosses of the seven possible female genotypes from pond B with the male aaYY

Females from pond B	Genotype of offspring from cross with aaYY		Predicted sex ratio (♀:♂)	
	Females	Males		
AAWX	–	AaWY AaXY	0:1	In agreement with experimental results of cross 6b
AaWX	aaWY aaXY	AaWY AaXY	1:1	In agreement with experimental results of cross 6a
AaWW	aaWY	AaWY	1:1	
aaWY	aaWY	aaYY	1:1	
aaXY	aaXY	aaYY	1:1	
aaWX	aaWY aaXY	–	1:0	Not seen
aaWW	aaWY	–	1:0	

4.3 Cross 2c

The *T. hornorum* ♀ (aaWY) was crossed with three of the male hybrids from reciprocal cross 1b. The male could be either AaYY, AaXY or AaWY. The case AaYY predicts a 1 ♀ : 3 ♂ ratio (females: aaWY; males: AaWY, aaYY, AaYY), which is close to the experimentally observed 21% ♀ : 79% ♂ ratio (average of three ponds). The latter two possibilities predict a 1:1 ratio (which crosses were evidently not performed).

4.4 Crosses 4a and 4b

The F_2 female from the sibling cross 3c was crossed with the *T. hornorum* ♂ (aaYY) (4a) and with the *T. mossambica* ♂ (AAXY) (4b). 52% females were obtained in the first cross (4a) and 28% females in the second cross (4b). In the present theory the F_2 female from the cross 3c could be either the aaWY (identical to the *T. hornorum* ♀) or aaXY. By crossing these two females with *T. hornorum* ♂ (4a) or *T. mossambica* ♂ (4b) 1:1 or 1:3, ♀:♂, ratios are predicted respectively, as follows:

4a: (aaWY or aaXY) × (aaYY) → aaWY ♀ + aaYY ♂ or aaXY ♀ + aaYY ♂, respectively.

4b: (aaWY or aaXY) × (AAXY) → AaWX ♀ + AaXY, AaYY, AaWY ♂♂ or AaXX ♀ + 2AaXY, AaYY ♂♂, respectively. These predictions are in agreement with the experimental results.

4.5 Cross 5a

The F_3 male offspring of cross 4a is of genotype aaYY (similar to the *T. hornorum* ♂). By crossing these males with the *T. mossambica* female (AAXX), all males (AaXY) are predicted in conformity with the experimental results.

4.6 Cross 5b

The F_3 female offspring of cross 4a are predicted to have the same genotype as their mothers, aaWY or aaXY. When they are crossed with the *T. mossambica* male (AAXY) 23% ♀♀ are obtained. This is in agreement with our prediction of a 1 ♀ : 3 ♂ ratio for either of the two female genotypes (see 4.4).

4.7 Cross 2a

Finally, in the backcross 2a of the F_1 male hybrid (AaXY) with the *T. mossambica* ♀ (AAXX) a 1:1 ratio was obtained in agreement with the theory (females: AAXX, AaXX; males: AAYY, AaYY).

5 Discussion

5.1 Comparison of Theories and Experimental Results

As was demonstrated above, the present (autosomal) theory explained all of Chen's experimental results; moreover, the results of crosses 3a, 3d and 6b are better explained by this theory (Table 5). In cross 3d the WXYZ theory predicts 75% females, while the present theory predicts 62.5% females, close to the experimental result of 61% females.

In crosses 6a and 6b the WXYZ theory predicts broods of either equal male-female populations or of all-female populations. However, the experimental results in one pond gave an essentially all-male brood (99.5% ♂), while in the other three ponds the ratio was close to 1:1. These results are in agreement with the present theory which predicts an all-male brood in addition to the ratios predicted by the WXYZ theory.

Chen explains the deviations from the predictions of the WXYZ theory on the basis of an increased mortality of female fingerlings. The present theory does not require such an assumption.

The predictions of the two theories disagree also in the ratio predicted for cross 3a, for which the WXYZ predicts a 1:1 ratio (50% females) while the present theory predicts 56.25% ♀, which is in slightly better agreement with the experimental ratio of 54% female.

In all other crosses performed by Chen, the prediction of the two theories are identical.

Table 5. Comparison of sex-ratios as predicted by the WXYZ and autosomal theories (Model I) with the experimental sex-ratio (female: male) results

Cross	Experimental Results		Predicted sex ratio from theories	
	% female ± S.D.*	Approximate small number ratio	WXYZ	Autosomal
1a	0	(0:1)	0:1	0:1
1b	25 ± 4	(1:3)	1:3	1:3
2a	50	(1:1)	1:1	1:1
2b	50	(1:1)	1:1	1:1
2c	21 ± 3	(1:3)	1:3	1:3
3a	54 ± 4	(1:1 or 9:7)**	1:1	9:7****
3b	n.o.	–	3:1	5:3****
3c	25.4 ± 1.2	(1:3)	1:3	1:3
3d	60.6 ± 1.4	(5:3)**	3:1	5:3****
4a	52 ± 5	(1:1)	1:1	1:1
4b	28 ± 2	(1:3)	1:3	1:3
5a	0	(0:1)	0:1	0:1
5b	23 ± 5	(1:3)	1:3	1:3
6a***	46.3 ± 1.4	(1:1)	1:1	1:1
6b***	0.5 ± 0.3	(0:1)**	n.p.	0:1
6c	n.o.	–	1:0	1:0

n.p. = Experimental results are not predicted by the theory

n.o. = Sex ratio attributed to these crosses were not observed in experimental results

* The standard deviation is that for the binomial distribution: $S.D. = \sqrt{\frac{f\,(1\text{-}f)}{N}}$, where f is the fraction of females and N is the total number of offspring

** Experimental results which are better explained by autosomal theory

*** The crosses we designated 6-a,b,c are the crosses of pond B female offspring with *T. hornorum* males, which Chen failed to designate with a number

**** The ratios 9:7 and 5:3 in percents are 56.25:43.75, and 62.5:37.5, respectively. These ratios are not predicted by the WXYZ theory

5.2 Further Predictions of Theory

Knowing the sex of all the eighteen genotypes (Table 3) we can predict the sex-ratio from any possible cross (Avtalion and Hammerman 1978).

In addition to the sex ratios (♀:♂) which could theoretically be obtained on the basis of the WXYZ theory (0:1, 1:3, 1:1, 3:1, 1:0) the present theory predicts three other ratios (5:3, 9:7, 3:5) seen in certain crosses. For example, when the female AaWX, obtained in the cross *T. mossambica* ♂ x *T. hornorum* ♀, is crossed with its brothers AaXY, AaWY the ratios 5:3 and 9:7, respectively, are obtained, and when crossed with one of the male offspring, AAWY, of these crosses, the ratio 3:5 could be obtained. The ratio 9:7 could occur only in the cross AaWX ♀ x AaWY ♂. This cross is seen in Chen's cross 3a.

Of the eighty possible crosses, fourteen result in all male broods. Ten of these are crosses between the 'supermale' AAYY and any of the females. The other four involve the crosses between the females AAWX (F_2 hybrid) with AAXX (*T. mossambica*) and the males AaYY (F_1 hybrid) with aaYY (*T. hornorum*).

All female broods could result from certain crosses of the *T. hornorum* ♂ aaYY and the F_2 hybrid ♂ AAWW with, for example, the F_2 hybrid aaWX.

It is interesting to note that three 'subspecies' of *Tilapia* may be obtained in which the offspring are identical to their parents: AAWW ♂ x AAWX ♀, aaYY ♂ x aaXY ♀, and AAWW ♂ x AaWW ♀. In the third example both the male and female would have identical sex chromosomes WW. (Such a system is analogous to that seen by Winge and Ditlevsen (1948) in strains of *Lebistes* with XX males and XX females.) In this 'subspecies' the decisive role in sex determination is obviously played by the autosomes.

These three examples show that the generation of subspecies of *Tilapia* could occur in nature through hybridization in addition to through mutation.

In Table 3 we see that for chromosome pairs WY, XY and WW, the sex depends on the particular pair of autosomes present, whereas for the YY, WX and XX pairs the sex does not vary despite the autosomal influence.

The relative influence of the different chromosomes on the sex determination could be seen partially by considering one possible group of values for their strengths, e.g. $A = 5$, $a = 0$, $W = -4$, $X = -7$ and $Y = 3$. This set is consistent with the inequalities seen in Figure 2. The full set of groups of consistent values is derived elsewhere (Avtalion and Hammerman, unpublished) along with a quantitative theory which reconsiders the gene balance hypothesis developed by Goldschmidt (1955).

5.3 *Possible Tests of Theory*

The theory presented here makes definite predictions beyond those given for the sex ratios in the crosses performed by Chen. These predictions in many cases differ from those of the WXYZ theory and thus the theory may be tested as described below.

5.3.1 Test Crosses for Ratios not Predicted in WXYZ Theory

In order to test the theory we propose the following series of pairmating crosses:

I(F_1): *T. hornorum* ♂ (aaYY) x *T. mossambica* ♀ (AAXX) gives a 0:1 sex ratio (♀:♂) having the genotype AaXY.

II(F_1): *T. mossambica* ♂ (AAXY) x *T. hornorum* ♂ (aaWY) gives a 1:3 sex ratio having the genotypes: ♀ (AaWX) and ♂♂ (AaXY, AaWY, AaYY). These sex ratios in F_1 are also predicted by the WXYZ theory.

III(F_2): Cross the males from cross II with the female offspring of the same cross in sufficient ponds in order to be confident of obtaining as parents all the three phenotypically indistinguishable males, which, however, could be identified on the basis of the distinctive sex ratios predicted by the theory:

a) AaWY x AaWX → 9:7
b) AaXY x AaWX → 5:3
c) AaYY x AaWX → 1:3

The corresponding ratios for these crosses in the WXYZ theory are as follows:

a) WY x WX → 1:1
b) XZ x WX → 3:1
c) YZ x WX → 1:3

These crosses correspond to those of Chen (3a, 3b and 3c).

The following crosses (IV and V) not performed by Chen would be of great importance to test the present theory since they predict sex ratios distinctly different from those of the WXYZ theory.

IV(F_3): The male offspring of cross IIIa (AaXY, AAXY, AaWY, AAWY and AAWW) would be crossed with the female AaWX from cross II. The resulting ratios are predicted to be:

a) AaXY x AaWX → 5:3 .
b) AAXY x AaWX → 1:1
c) AaWY x AaWX → 9:7
d) AAWY x AaWX → 3:5
e) AAWW x AaWX → 3:1

Only 1:1 ratios are predicted for the corresponding crosses in the WXYZ theory:

XY x WX → 1:1
WY x WX → 1:1

V(F_3): The female offspring of cross IIIa (AaWW, aaWW, AAWX, AaWX, aaWX, aaWY and aaXY) would be crossed with the male AaXY from cross I. The resulting ratios are predicted to be:

a) AaXY x AaWW → 5:3
b) AaXY x aaWW → 3:1
c) AaXY x AAWX → 1:1
d) AaXY x AaWX → 5:3
e) AaXY x aaWX → 3:1
f) AaXY x aaWY → 1:1
g) AaXY x aaXY → 1:1

The corresponding ratios in the WXYZ theory are:

XZ x WW → 1:0
XZ x WX → 3:1

VI(F_3): In the WXYZ theory the genotype WW is female. However, in the present theory AAWW is a male. This male could be identified among the males involved in cross IV, above, as that giving a sex ratio close to 3:1. Crossing that same fish with the *T. mossambica* female (AAXX) should give all female offspring (AAWX), also a significant test of the autosomal theory.

5.3.2 Sex Reversal

a) It is predicted by the present theory that if the male AaXY (obtained from the cross of the homogametic parents) is sex-reversed by estrogen treatment in the fingerling stage (as previously performed by Yamamoto (1975) in goldfish and Nakamura and Takashashi (1973) in *T. mossambica*), it will give a 1 ♀:3 ♂ ratio when crossed with the *T. hornorum* male (aaYY):

aaYY ♂ x AaXY ♀ → aaXY ♀, AaXY ♂, AaYY ♂, aaYY ♂.

This differs from the 0♀:1♂ ratio predicted by the WXYZ theory:

ZZ ♂ x XY ♀ → XZ ♂, YZ ♂

b) Furthermore, crossing of this sex-reversed AaXY with the *T. mossambica* ♂ (AAXY) will give as one of the male offspring the 'supermale' AAYY. This 'supermale' could be identified among the male offspring as that giving all males when crossed with *T. hornorum* ♀. Generation of such a 'supermale' is not predicted by the WXYZ theory, using the same crosses.

5.3.3 Evaluation of the Theory for Another Chromosome System (Model II)

Another chromosomal formula could be assigned to *T. hornorum*, and the system with *T. mossambica* (Model II) would be as formulated in Section 2.1 above.

Following the same analytic pathway described for Model I, the directed graph describing the relationships among the chromosome pair strengths was found (Fig. 3). In Model II there are only four female genotypes (Table 3).

This system was also found to be consistent with Chen's experimental results. It gives identical predictions to that of Model I for all crosses except crosses 2c and 3a. In cross 2c the sex-ratios (♀:♂) 1:1, 1:7 and 3:5 are predicted instead of 1:1 and 1:3, as predicted in Model I. In cross 2c Chen performed three pair matings and the experimental results from the three ponds (14:86%, 15:85% and 28:72%) could be interpreted as consistent with a 1:3 ratio if their mean (21:79%) is considered or with the 1:7 (12.5:87.5%) 3:5 (37.5:62.5%) ratios if they are separately considered. It is of great interest to determine which of the two models is closer to reality. For this purpose, it is necessary to perform cross 2c with more pairs and to carry out the cross IV described above. The sex ratios (♀:♂) predicted in that cross (not performed by Chen) are 5:3, 1:1, 9:7, 3:5 and 3:1 for Model I and 1:1 for the second model.

In cross 3a model II (like the WXYZ theory) predicts a 1:1 ratio instead of the 9:7 predicted by Model I.

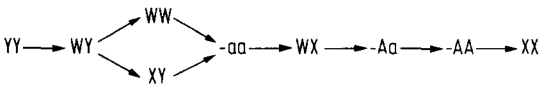


Fig. 3 Final directed graph showing relative strength of chromosome pairs in Model II. In this model one cannot determine the position of WW relative to XY. This, however, does not prevent the determination of the sex of the various genotypes, as explained in the legend of Figure 2

5.3.4 Conclusions

We have proposed here the simplest possible model adding autosomal influence to a minimal number of sex chromosomes. This theory has succeeded better than the known four-sex chromosome theory to explain Chen's results in crosses of *T. mossambica* and *T. hornorum*. Similarly, this theory, when applied to another system of interspecific crosses between *T. nilotica* (homogametic female) and *T. macrochir* (homogametic male) and their hybrids, performed by Jalabert et al. (1971), could explain as well most of the experimental results. However, in this case it was found that the relative strength of the W chromosome differs significantly from that in the *hornorum-mossambica* system. Detailed analysis of the *nilotica-macrochir* system will be the object of a separate report.

The present simple theory, which considers only a minimal number of sex-influencing chromosomes and does not take into account regulatory genes or crossing-over, is successful in explaining the existing data. Further experiments such as those proposed above may show the need for considering additional autosomal pairs or transformer genes acting epistatically on autosomes and sex chromosomes (Kallman 1968). It would be important to see to what extent similar methods of analysis could be applied to other species, and it would be interesting to know whether those sex ratios (1:0 and 3:1), not seen in the existing experimental results, could be obtained by performing the appropriate crosses (6c and IV), and whether any of the genotype combinations not yet encountered may consist of lethal gene combinations.

The understanding of sex-determination in *Tilapia* is not only of theoretical importance but it also permits the designing of new series of crosses which would more reliably provide the all-male broods needed in the practical farming of these fish.

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Dr. I.S. Hammerman
Department of Life Sciences
Bar-Ilan University
Ramat-Gen (Israel)