H-Y antigen and the evolution of heterogamety

ABSTRACT: In natural populations of platyfish, Xiphophorus maculatus, there are three sex chromosomes—W, X, and Y. Females may have one of three genotypes: WY, WX, or XX, and males may have either XY or YY. The W chromosome can be considered a modified X that blocks the male-determining function of the Y. The platyfish may represent an evolutionary stage at which female heterogamety arises through a single mutation, in the midst of a male heterogametic system. Our serological analysis revealed presence of H-Y antigen in XY and YY males, but not in XX, WX, or WY females, indicating that H-Y antigen may not be associated invariably with the heterogametic-type gonad—especially in transitional systems.

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SEX-SPECIFIC H-Y (H-W) antigen, the putative inducer of the heterogametic gonad in vertebrates, is normally expressed in males of mammals (XY), and in females of reptiles and birds (WZ)27. The same correlation is found in urodeles (Pleurodeles waltlii, Ambystoma mexicanum, WZ-ZZ, Triturus vulgaris, XX-XY) and in anurans (Xenopus laevis, Bufo bufo, Pyxicephalus adspersus, WZ-ZZ, Rana ridibunda, XX-XY)5. Among teleosts, the antigen is restricted to the heterogametic sex in the eel (Anguilla anguilla, WZ-ZZ)29, medaka (Oryzias latipes, XX-XY)23, and guppy (Poecilia reticulata, XX-XY)26, but in several other species with unknown sex-determining mechanisms, no difference in H-Y antigen phenotype was detected in males and females19.

Male and female heterogamety both are found in teleosts, urodeles, and anurans. Therefore22, both sex-determining modes must have arisen repeatedly throughout vertebrate evolution. As a consequence, restriction of H-Y antigen activity to the heterogametic sex must have occurred independently in the different lineages. Our lack of knowledge about how H-Y activity has become associated with the heterogametic sex is paralleled by a poor understanding of how female heterogamety may have evolved from male heterogamety or vice versa. Thus, it may be asked whether a change in the mode of sex determination is necessarily accompanied simultaneously by a change in H-Y antigen activity.

Bull and Charnov2 have described several single-locus models incorporating a mutation at the sex-determining locus (e.g., X → W) through which populations might acquire three sex chromosomes: W, X, and Y. This polymorphic sex chromosome mechanism would have the potential to evolve into a new mode of sex determination (e.g., from XX ψ-XY δδ to WY ψ-YY δδ) when either genotype of the new recurrent pair has a greater viability than the ancestral genotypes have. A polymorphic system involving W, X, and Y sex chromosomes is found in the teleost Xiphophorus maculatus10,11. Females are WY, WX, or XX and males are XY or YY. To our knowledge, X. maculatus is the only species of this genus with multiple sex chromosomes. Seven other species of Xiphophorus possess the XX ψ-XY δδ mode of sex determination, and in the remaining species the sex determining mechanism is not characterized.

According to an alternative set of models (two-loci models), a mutation arises on an autosome and the new mutation influences sex determination2. Again, if one of the new recurrent genotypes has a higher viability than that of any of the original genotypes, a new system of sex determination could evolve in which the ancestral sex chromosomes would be inherited as autosomes, and the chromosome bearing the new mutation would become the sex chromosomes (e.g., XX AA ψ-XY AA δδ → XX Aa ψ-XX aa δδ). This model differs from the first in that the change from...
male to female heterogamety involves the evolution of a new pair of sex chromosomes from autosomes; in the first model, the change only affects the original pair of sex chromosomes. Autosomal factors that have a strong effect on sex determination are reported for several species of Xiphophorus, but in the eight species with known sex determining mechanisms, the gonosomes are homologous \(^{15}\). The same chromosome pair functions as gonosomes in all eight species.

In this paper we examine the expression of H-Y antigen in 13 species of Xiphophorus (Poeciliidae, Atheriniformes, Teleostei), including the five sex chromosome types of X. maculatus.

### Materials and Methods

Three stocks were tested: two XX 99-XY \( \delta \delta \) stocks (Jamapa, Jp 163 A, Jp 163 B) of X. maculatus, and one WY 99-YY \( \delta \delta \) stock (Coatzacoalcos, Cp). Pedigree 4394 of X. maculatus represents hybrids between the Belize stock (Bp WY 99-YY \( \delta \delta \)) and Jp 163 B. The \( W \) and \( \gamma \) chromosomes were derived from the Bp stock, and the X from Jp. Two classes of females (WY, WX) and two classes of males (XY, YY) were obtained from a mating involving a WY female and the XY male. The presence of a number of codominant sex-linked pigment genes allowed precise identification of the sex chromosome constitution of individual fish\(^{14}\). The sex of the fish was assigned on the basis of presence or absence of a gonopodium, and was confirmed on examination of the gonads at sacrifice.

Seven of the species used in the experiment are known to be XX 99-XY \( \delta \delta \) on the basis of the inheritance of pigment patterns: (X. variatus, X. xiphidium, X. milleri\(^{16,17}\), X. pygmaeus, X. nigrens\(^{13,35}\), X. montezumae\(^{14}\), and X. andersi, (Kallman, unpub.). The sex-determining mechanism of the remaining six species is unknown. In the laboratory the sex ratio of two species, X. cichlurus and X. celenticae rarely has deviated from unity, but in X. heterix, X. signum, and X. alvearezi the ratio is often skewed in favor of either males or females. Evidently the bias in sex ratio has a genetic basis \(^{15}\). All fish tested were sexually mature.

All stocks are of known ancestry and geographical origin. For 11 of the species, geographical origin has been described previously \(^{14}\). The stock of X. milleri was obtained from Lake Catemaco in 1974 and X. andersi was collected in a spring pool near Atoyac (Rio Atoyac-Jamapa drainage) in 1981. Both locations are in Veracruz, Mexico. The two stocks of X. alvearezi were obtained from the Rio Dolores, Alta Verapaz, Guatemala, in 1968, and from the Rio San Ramon, Huehuetenango, Guatemala, in 1976\(^{24}\). Commercially obtained Xiphophorus stocks often include undetected species hybrids that frequently produce atypical sex ratios \(^{15}\). The use of genetically defined stocks avoids the risk of faulty interpretations based on fish of doubtful identity and unknown sex-chromosome mechanisms.

The H-Y antigen phenotype was evaluated by absorption in the sperm cytotoxicity test \(^{7}\). In brief, batches of H-Y antigen were divided into equal portions, and the portions absorbed with cells from experimental fish or from male or female controls. Absorbing cells were suspended in the antiserum for 30 minutes on ice and then discarded. The absorbed portions of antiserum were then tested for residual cytotoxicity against mouse epididymal spermatozoa. Positive absorption, indicating presence of H-Y antigen on the absorbing cells, was manifested as a fall in cytotoxicity, by comparison with the cytotoxicity of unabsorbed portions of antiserum.

For cell suspensions used in absorption, the livers of individual fish were excised, placed in cold Medium 199, and gently dissociated according to standard techniques. Cells from sibs of defined class were pooled, passed through a 53 \( \mu \)m sieve, and washed 2-4 times. Viability of the suspensions was determined by trypan blue dye exclusion.

![FIGURE 1 Expression of H-Y antigen in fishes of the genus Xiphophorus.](image-url)
FIGURE 2 Expression of H-Y antigen in fishes of the genus Xiphophorus. II. Summary of sperm cytotoxicity tests showing male specific expression of H-Y in four species: A—X. nigrensis; B—X. pygmaeus; C—X. variatus; D—X. xiphidium. See legend to Figure 1 for explanation, and compare with Figures 1 and 3. Statistical analyses of the data are presented in Table I.

FIGURE 3 Expression of H-Y antigen in fishes of the genus Xiphophorus. III. Summary of sperm cytotoxicity tests showing male specific expression of H-Y in four species: A—X. alsorei; B—X. clemenciae; C—X. helleri; D—X. signum. See legend to Figure 1 for explanation, and compare with Figures 1 and 2. Statistical analyses are given in Table I.

dilution. A minimum separation of 9 percent dead sperm was accordingly required for identification of H-Y+ and H-Y- cells at 1/2 dilution (i.e., a minimum difference of 9 percent for male-absorbed and female-absorbed cytotoxic scores); a minimum of 8 percent at 1/4 dilution; and a minimum of 14 percent at 1/8 dilution. Those minimums provided the criteria for evaluating the ability of an individual test to discriminate between H-Y+ and H-Y- phenotypes. Statistical significance was determined for all cytotoxicity assays by appraisal of the data in paired t-tests.

Results

Fifty-four tests were performed in this study. Three of the tests were uninformative for technical reasons. In 45 of the 54 tests (83 percent), values for male-absorbed controls were located in the H-Y positive region, and values for unabsorbed controls were located in the H-Y negative region. The differences between percent sperm killed with unabsorbed H-Y antiserum and percent killed with B6-male-absorbed antiserum were statistically significant in 37 of those 45 tests (82 percent) (see Materials and Methods). Assignment of H-Y phenotype in Xiphophorus was based on the results of those 37 tests.

Figures 1 to 3 depict a series of tests of H-Y phenotype in 12 species of Xiphophorus, of which seven are known to be male-heterogametic. In each species the males were typed H-Y+, and the females were typed H-Y-. Differences in residual cytotoxicity of male-absorbed and female-absorbed H-Y antiserum were significant at the 95 percent confidence level; differences in cytotoxicity of unabsorbed and female-absorbed H-Y antiserum were insignificant and equal to zero. The differences in cytotoxicity of male-fish absorbed and male-mouse absorbed antiserum were significant in six species: X. couchianus, X. milleri, X. nigrensis, X. signum, X. variatus, and X. xiphidium (Table I). This could represent qualitative or quantitative differences in the cell surface expression of H-Y antigens of fish and mouse.

Figure 4 depicts a series of tests with sera absorbed with cells from X. maculatus. Males (XY and YY) were typed H-Y+ and females (XX, WX, and WY) were typed H-Y-. Differences in cytotoxicity of male-absorbed and female-absorbed serum (Figure 4B) were significant at the 95 percent confidence level; and the same was true when the cytotoxic values of XY-male-absorbed serum were compared with those of the YY-male-absorbed serum. The latter observation could reflect differences in antigen density at the surface of XY and YY cells. Means of tests with cells of two sublines originating in the Rio Jamapa are presented in Figure 4C. A breakdown of those data is given in Table II; and a summary of all of our typing data is shown in Table III.

Discussion

If the W-X-Y system of X. maculatus is considered a representative first step towards
a system of female heterogamety (WY99, YY88), then this species affords an opportunity to evaluate the question of whether heterozygosity and H-Y antigen must always be found together. In the platyfish, XY and YY males are H-Y+, whereas the heterozygous WY and WX females are H-Y− and do not differ serologically from XX females. In fact, H-Y antigen phenotype in Xiphophorus is correlated with sex but not with karyotype.

The occurrence of the XX-YY mechanism in seven species of Xiphophorus and of the W-X-Y system in X. maculatus, and the fact that the sex chromosomes of these species are homologous to each other provide compelling evidence that the ancestral form was XX-XY. The origin of the W chromosome in platyfish can be viewed as a relatively recent evolutionary change. The same argument can be applied to the X* chromosome of the Scandinavian wood lemming (see below). The pattern of H-Y antigen expression in these forms, therefore, may still reflect their recent descent from an ancestral XX-XY system in which H-Y was restricted to the heterogametic (male) sex. The evolution of a W-X-Y or WY-YY system from an XX-XY mechanism may thus precede a change in H-Y antigen expression. It is not clear how the expression of H-Y becomes reversed and ultimately restricted to WY females. Such a change would seem paradoxical because the newly evolved W (X*) chromosome specifically suppresses H-Y activity. There must be additional concomitant changes through which H-Y becomes involved in the development of the heterogametic gonad.

If the above view is correct, expression of H-Y should be restricted to the heterogametic sex in species in which the sex determining mechanism is relatively old, whereas the correlation may be far from perfect in those forms in which male or female heterogamy is of more recent origin. The WY-YY and XX-XY mechanisms are found in different lineages within teleosts, urodeles, and anurans. The different species examined belong to lineages that became separated from each other as early as the Jurassic period in the case of the amphibia; and the anguilliform and atheriniform fishes became separated even earlier. The age of their sex chromosome mechanism cannot be dated and there is no evidence that it is of recent origin. These species, therefore, do not provide any insight as to when or how the heterogametic sex became H-Y+.

However, the question of whether the sex determining system evolves before the pattern of H-Y antigen activity may be examined in the poecilid fishes, in which the heterogametic mode of sex determination may be of relatively recent origin. Thus, the XX-XY system is known not only from seven species of Xiphophorus, but also from three species of Poecilia (P. reticulata, P. latipinna, and P. velifera). The W-X-Y system occurs in X. maculatus, but based upon preliminary evidence it also is present in P. sphenops. Female heterogamety has been demonstrated in four species of Gambusia. The known distribution of the W-X-Y sex-determining mechanism and female heterogamety argues for their independent origin. If the ancestral condition in poecilid fishes was XX-XY, the relative common occurrence of female heterogamety in Gambusia would suggest that the WY-YY mechanism in this genus is older than the W-X-Y system in Xiphophorus or Poecilia. The pattern of H-Y antigen activity in Gambusia would therefore be of great interest. As to the mechanism whereby synthesis of H-Y is switched from one sex to another, there are instances of induction of H-Y in the homogametic sex by application of sex steroid hormones in at least four female heterogametic species: Gallus domesticus, the chicken; Coturnix japonica, the quail; Xenopus laevis, the South African clawed frog; and Pleurodeles waltl, the ribbed newt. There are indications of induction of H-Y in the "homogametic" sex, by temperature, in two reptilian species: Emys orbicularis, the European pond turtle, and Alligator mississippiensis, the American alligator (Nakamura, Lance, and Wachtel, unpub.). So the structural genes for H-Y are present in both sexes in the nonmammalian vertebrates, at least, but are normally activated only in the heterogametic sex.

The view that female heterogamety could arise in a male-heterogametic species, with H-Y antigen synthesis following suit embodies some difficulties. First, the function of H-Y was deduced on the basis of invariant expression of the molecule in the heterogametic sex, or more correctly, association with the heterogametic-type gonad; if H-Y is the inducer of the XY testis it is not clear how synthesis of H-Y and induction of the XY testis can be separated. The new hypothesis requires the presence of an alternative YY (ZZ) testis inducer ready to switch on when H-Y function is transferred to the WY (WZ) female. Second, the necessity of limiting H-Y to the heterogametic sex is brought into question by the observation, cited above, that H-Y is found in the gonads of females of either sex chromosome constitution in temperature sex-reversed
Expression of H-Y antigen in Xiphophorus species

Species with known sex-determining mechanism

- Xiphophorus maculatus
  - 8 XX
  - 7 XY

- Xiphophorus couchianus
  - 7 XX
  - 6 XY

- Xiphophorus milleri
  - 7 XX
  - 10 XY

- Xiphophorus montezumae
  - El Salto
  - 5 XX

- Hybrids
  - Xiphophorus xiphidium
  - Ped. 4311

Species with unknown sex-determining mechanism

- Xiphophorus clemenciae
  - San Ramon
  - 10

Table I. Statistical analyses of tests for expression of H-Y in Xiphophorus fishes

<table>
<thead>
<tr>
<th>Species</th>
<th>Compare* values for</th>
<th>Reciprocal of antiserum dilution</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. alvarezi</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.001 &lt; P &lt; 0.005</td>
</tr>
<tr>
<td>X. andersi</td>
<td>♂♀</td>
<td>2</td>
<td>0.005 &lt; P &lt; 0.010</td>
</tr>
<tr>
<td>X. clemenc?</td>
<td>♂♀</td>
<td>4</td>
<td>0.001 &lt; P &lt; 0.005</td>
</tr>
<tr>
<td>X. couj?anu?s</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.025 &lt; P &lt; 0.050</td>
</tr>
<tr>
<td>X. helleri</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.001 &lt; P &lt; 0.005</td>
</tr>
<tr>
<td>X. milleri</td>
<td>♂♀</td>
<td>4</td>
<td>0.001 &lt; P &lt; 0.005</td>
</tr>
<tr>
<td>X. montezumae</td>
<td>♂♀</td>
<td>2</td>
<td>0.010 &lt; P &lt; 0.025</td>
</tr>
<tr>
<td>X. nigrensis</td>
<td>♂♀</td>
<td>4</td>
<td>0.010 &lt; P &lt; 0.025</td>
</tr>
<tr>
<td>X. pygmaeus</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.005 &lt; P &lt; 0.010</td>
</tr>
<tr>
<td>X. signum</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.025 &lt; P &lt; 0.050</td>
</tr>
<tr>
<td>X. variatus</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.010 &lt; P &lt; 0.025</td>
</tr>
<tr>
<td>X. xiphidium</td>
<td>♂♀</td>
<td>2 and 4</td>
<td>0.001 &lt; P &lt; 0.005</td>
</tr>
</tbody>
</table>

* ♂ Male Xiphophorus fish; ♀ Female Xiphophorus fish; B6 C57BL/6 male mouse

Table II. Results of sperm cytotoxicity tests after absorption of H-Y antiserum with cells of Xiphophorus maculatus from the Jamapa River

<table>
<thead>
<tr>
<th>Subline</th>
<th>Sex</th>
<th>1/H-Y antiserum dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jp 163</td>
<td>♂</td>
<td>45.0 ± 0.0*</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>44.5 ± 0.7</td>
</tr>
<tr>
<td>Jp 163</td>
<td>♂</td>
<td>47.0 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>42.0 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>59.5 ± 0.7</td>
</tr>
</tbody>
</table>

* Percent dead sperm ± standard deviation at dilution; a summary of these data is presented in Figure 4C which includes values for positive and negative controls and for background; each value is a mean of values from two tests for each subline.

Table III. H-Y antigen expression in Xiphophorus

<table>
<thead>
<tr>
<th>Species</th>
<th>Stock or pedigree</th>
<th>No., sex, and genotypes of fish tested</th>
<th>H-Y expressed</th>
<th>No. generations stock was in lab.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. maculatus</td>
<td>Jp 163 A</td>
<td>8 XX 7 XY 6 XY</td>
<td>male</td>
<td>60</td>
</tr>
<tr>
<td>X. maculatus</td>
<td>Jp 163 B</td>
<td>7 XX 6 XY</td>
<td>male</td>
<td>57</td>
</tr>
<tr>
<td>X. maculatus</td>
<td>Cp</td>
<td>30 WY 33 YY</td>
<td>male</td>
<td>13</td>
</tr>
<tr>
<td>X. maculatus</td>
<td>ped. 4394</td>
<td>10 WY, 10 WX 9 XY, 6 YY</td>
<td>male</td>
<td></td>
</tr>
<tr>
<td>X. andersi</td>
<td>19 XX 22 XY</td>
<td>male</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>X. milleri</td>
<td>8 XX 10 XY</td>
<td>male</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>X. montezumae</td>
<td>El Salto</td>
<td>5 XX 9 XY</td>
<td>male</td>
<td>18, 19</td>
</tr>
<tr>
<td>X. nigrensis</td>
<td>Rio Coy</td>
<td>12 XX 19 XY</td>
<td>male</td>
<td>9, 10</td>
</tr>
<tr>
<td>X. pygmaeus</td>
<td>10 XX 7 XY</td>
<td>male</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>X. variatus</td>
<td>Ped. 4311</td>
<td>10 XX 8 XY</td>
<td>male</td>
<td>16</td>
</tr>
<tr>
<td>X. xiphidium</td>
<td>San Carlos</td>
<td>7 XX 7 XY</td>
<td>male</td>
<td>20</td>
</tr>
</tbody>
</table>

Species with known sex-determining mechanism

- X. alvarezi
- X. andersi
- X. couj?anu?s
- X. xiphidium

Species with unknown sex-determining mechanism

- X. clemenciae
- X. maculatus
- X. alvarezi
- X. couj?anu?s
- X. helleri
- X. xiphidium

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the potential to generate alternate modes of sex determination.

References

7. GOLDBERG, A. BOYSE, D. H., E. H., E. H. GROPP, FREDGA, G. SCHMID. The genetics of poly- }