THE GENETICS OF *ARTEMIA SALINA*.
V. CROSSING OVER BETWEEN THE X AND Y CHROMOSOMES[1]

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IN wild populations of brine shrimps, the eye color is black. A preliminary study of a spontaneous recessive mutation, *w*, which governs white eyes, indicated that the white locus is partially sex-linked; that is, it lies on the homologous segments of the X and Y chromosomes (BOWEN 1963). The female was found to be the heterogametic sex. Females were designated XY, males XX. In matings of $+/w$ females to w/w males, crossing over between the white locus and the sex locus could be detected. The frequency of recombination was low (1/1625 or 0.06%) in the earlier study in which females derived from a mutant laboratory stock were outcrossed to males from two wild populations.

The purpose of the present investigation was to mate white-eyed shrimps to wild-type shrimps from eight bisexual populations to determine if the female is heterogametic in all eight populations and to determine whether the frequency of crossing over is the same on the different genetic backgrounds. One European population appears to be reproductively isolated from the mutant stocks (see Experiment 6). However, when the seven American races are mated to the mutant stocks, fertile hybrids are obtained which can be testcrossed to mutant shrimps (Experiments 1, 4, and 5). It will be shown that the female is heterogametic in the seven American populations and that there is a surprisingly great amount of variation in frequency of recombination between the white locus and the sex locus (values of 0.03% to 20%). Experiments 1, 2, and 3 are designed to demonstrate that a characteristic frequency of crossing over can be transmitted from mother to daughter and that neither the X chromosome nor the autosomes affect the frequency.

The sex chromosomes of Artemia: There are two possible modes of sex determination in Artemia. (1) Sex may be determined by one true sex locus; either by dominance of the gene for femaleness to the allele for maleness at this locus or by a balance between a gene for maleness at the sex locus on the X with genes for femaleness on the autosomes. (2) Sex may be determined by one or more loci on the differential segment of the X and/or the Y chromosome. The apparent "sex locus" would be the junction of the differential segment (carrying the true sex loci) with the homologous segment of the sex chromosome (Figure 1). Both concepts of a sex locus would be in accord with the data obtained from genetic experiments (BOWEN 1963). However, the second model now appears more probable

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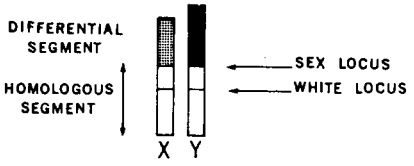

FIGURE 1.—The sex chromosomes of Artemia. In this model, the "sex locus" is the junction between the differential and homologous segments. Crossing over may occur between the white locus and the sex locus.

because STEFANI (1963a) has shown that the sex chromosomes of Artemia are of unequal lengths.

STEFANI (1963a,b,c, 1964) has developed new techniques for observing the oocytes and blastomeres of Artemia. Excellent pictures of the chromosomes of two Sardinian populations have been published: the parthenogenetic population at San Gilla and the bisexual population at San Bartolomeo (STEFANI 1960, 1963a). STEFANI (1963a, p. 628) has outlined his cytological confirmation that the female is the heterogametic sex in Artemia. When he examined late prophase mitotic figures in San Bartolomeo blastulae, there were two types of blastulae: one with 21 pairs of small chromosomes in each blastomere, and another type with 20 pairs of small chromosomes and a heterologous pair consisting of a short and a long chromosome. When he examined the San Gilla parthenogenetic population, the longer heterochromosome was present in all the blastulae. In prophase of the first maturation division in adult males from San Bartolomeo, the longer heterochromosome was missing. He concluded that the longer heterochromosome was the Y. Evidently the differential segment of the Y is longer than that of the X in the San Gilla and San Bartolomeo races.

Symbols used to designate sex chromosomes: To simplify the discussion of the data in the present paper, the second model of the sex chromosomes will be adopted (see Figure 1). Subscripts will be used to designate the allele at the white locus and superscripts will designate the origin of the differential segment. Thus, the genotype $X^{9}_{w}Y^{9}_{+}$ indicates that the X chromosome carries the mutant gene and its differential segment is derived from stock No. 9; the Y chromosome carries the wild allele of white and the differential segment comes from the Quemado wild population. (The symbols used for inbred stocks and wild populations are explained under MATERIALS AND METHODS.) However, one must bear in mind these reservations: (a) The model in Figure 1 shows only one differential segment although in some species, two such segments have been found on a sex chromosome. (b) In Figure 1, the relative lengths of the differential and homologous segments are arbitrary. (c) The origin of the differential segment of the Y also designates the origin of the cytoplasm. Because the female shrimp is heterogametic, the effects of the Y chromosome and of the cytoplasm are confounded. (d) The superscript may represent the racial origin of a true sex locus (see model 1 above). While the cytological studies of Stefani suggested differential segments (see model 2, Figure 1), the Sardinian races which he studied are probably re-

productively isolated from the American races used for studies of recombination between the sex locus and the white-eye locus (see Experiment 6 in this paper).

MATERIALS AND METHODS

Culture techniques: The glassware and standard feeding schedule have been described in an earlier paper (BOWEN 1962). Shrimps were transferred from one vial to the next in glass pipettes. Pipettes were placed in boiling water for more than two minutes before they were used to pick up shrimps of different genotype. Boiling water killed adults and nauplii immediately and cysts in less than one minute. When reared by our standard laboratory techniques, the females usually give birth to free-swimming nauplii. Less than 1 in 100 matings produce a brood of cysts.

Females were isolated for at least two weeks before they were mated to males from another stock. Because the reproductive cycle is completed in less than one week in healthy females, this isolation period insured that the females were not carrying fertilized eggs at the time they were placed in matings. Genetic experiments have shown that females do not store sperm from one reproductive cycle to the next (BOWEN 1962).

The matings described in this paper were single-pair matings in shell vials which contained 5 ml of the culture medium (50 g NaCl per liter of filtered sea water). Once per week, the matings were checked; if nauplii were present, they were transferred to other vials (2 or 3 nauplii per vial). The progeny were classified when they reached sexual maturity (at an age of 2 to 3 weeks). The temperature range was between 21° and 24°C.

Origin of the wild populations: All shrimps discussed in this paper are from bisexual populations. Parthenogenesis has never been found in any of these populations although extensive genetic tests have been made for parthenogenesis and pseudogamy (BOWEN 1962). Each locality listed below is preceded by the symbol used for it in the text.

H, Hidalgo. Cysts were collected by DR. A. SILVIA COLLA from Salina Grande near Hidalgo, La Pampa, Argentina. They were obtained through the courtesy of DR. A. D'AGOSTINO.

LM, Little Manitou. Cysts from this lake in Saskatchewan, Canada were collected by MR. ALLAN LEVY and sent to me by DR. A. D'AGOSTINO.

P, Pichilingue. Adult shrimps were collected by the author from salterns on Pichilingue Island in the Gulf of California near the harbor of La Paz, Baja California Sur, Mexico.

Q, Quemado. Cysts from the salt lake near Quemado, New Mexico, U. S. A., were collected by MR. THOMAS D. FOSTER.

SB, San Bartolomeo. Cysts from this salt works in Cagliari, Sardinia were sent by PROFESSOR R. STEFANI, University of Cagliari. This population has been extensively studied in regard to chromosome morphology (STEFANI 1963a) and natural history (STEFANI 1961; STEFANI and FALQUI 1963).

SL, Soap Lake. Adult shrimps were collected by MISS FRITZIE DAVIDSON and MR. ROBERT STEPHENS from this lake in Okanogan County, Oregon, U. S. A. Because there are three "soap lakes" in Oregon, it must be specified that this lake lies 13 miles west of Omak Lake and 8 miles south of the city of Okanogan.

SF, San Francisco. Adult shrimps were collected from two salterns on San Francisco Bay, California, U. S. A. by MRS. MARY JANE BAKER and MRS. JEAN CHAPMAN who are studying the ecology of these solar evaporating ponds.

U, Great Salt Lake, Utah. Cysts were collected by MR. C. C. SANDERS.

Origin of the mutant stocks: Stock #5. The origin of this stock has been described (BOWEN 1963). It is homozygous for two mutations: red eyes (*r*) and crinkle eyes (*c*). The eyes of young shrimp in this stock are red; at sexual maturity the eyes turn brown or black. A few weeks later, the pigment in the normal eye field is black, but a new batch of red pigment (the crinkle patch) is laid down on the eyestalk. In 1963, this stock was divided into two sublines: 5A and 5B, each descended from a single female. When 5A females are mated to white males, the Y_{+}^{5A} chromo-

some in the F_1 females is characterized by a frequency of crossing over of 6/1749, or 0.3%. The $Y_+{}^{5B}$ chromosome is characterized by very low frequency (0/2161).

Stock #9. The origin of this stock from a single #5 stock female has been described earlier (BOWEN 1963). The X chromosome carries the recessive gene *w* for white eyes; the Y carries its wild allele, +. The frequency of crossing over between the sex chromosomes is very low (1/3025 or 0.03%) and therefore the stock breeds true for white-eyed males (X_wX_w) and pigmented females (X_wY_+). The shrimp in one sub-line of this stock have the genotype *r/r; c/c*. Because the gene *w* when homozygous is epistatic to *r* and *c*, the males are white-eyed and the females are red, crinkle-eyed.

Stock #11. One +/+ female shrimp from San Francisco was mated to a white male. The F_1 females (X_wY_+) were crossed to white males and in the progeny of this testcross, the white-eyed males (noncrossovers) and the white-eyed females (crossovers) were selected to make up stock #11. Because the stock is descended from one parental female, the differential portion of the Y chromosome (and the origin of the cytoplasm) is the same throughout this "single-female-line." When #11 females are crossed to wild males, the Y^{11} chromosome in the F_1 females is characterized by high frequency of crossing over (141/965, or 15%).

Stock #13. This white-eyed stock was obtained in the same manner as was stock #11, however the one +/+ parental female came from Great Salt Lake, Utah. The Y^{13} chromosome is characterized by low frequency of crossing over (29/2296, or 1.3%).

Stock #12. This white-eyed stock was obtained in the same manner as was stock #11, however the one parental +/+ female came from Quemado, New Mexico. The parental *w/w* male was derived from repeated backcrossing to Quemado shrimp and therefore one would expect half the autosomes from this stock to come from Quemado. The Y^{12} chromosome is characterized by high frequency of crossing over (893/7518, or 11%).

RESULTS

The term *pigmented* eyes is used below to describe non-white-eyed shrimps. Because some of the white-eyed parents were carrying the gene *r* for red eyes, and/or the gene *c* for crinkle eyes, some of the pigmented testcross progeny were wild-type and some were red-eyed, crinkle-eyed or red-crinkled. The segregation data for the genes *r* and *c* indicated that they were not linked with *w*. The gene *w*, when homozygous, is epistatic to other genes for eye color.

Table 1 summarizes the segregation data compiled in this laboratory up to the present time. Viability of the mutant phenotype is 615/721 in males and 571/687 in females, a value of 0.8 in both sexes. In certain experiments reported below, the viability ranged from 0.5 to 1.0.

TABLE 1

Segregation of the gene w

Mating female × male	Number of offspring				
	White male	White female	Pigmented male	Pigmented female	Total
$X_wY_w \times X_wX_w$	166	174	0	0	340
$X_wY_w \times X_+X_+$	0	0	1514	1469	2983
$X_+Y_+ \times X_wX_w$	0	0	898	935	1833
$X_wY_w \times X_wX_+$	615	571	721	687	2594
$X_wY_+ \times X_wX_w$	Each female line has its own characteristic frequency of crossing over				
$X_+Y_w \times X_wX_w$					

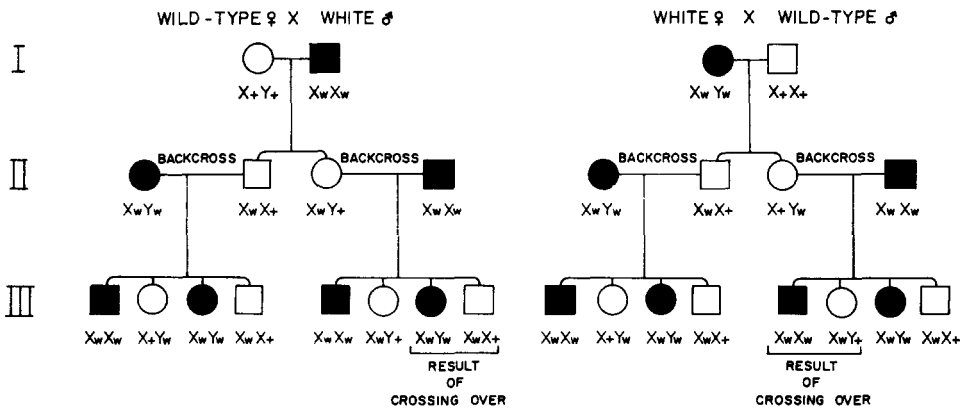

FIGURE 2.—Experimental design for experiments 1, 3, 4, and 5. Solid symbols—shrimps with mutant phenotype; open symbols—shrimps with wild-type phenotype (pigmented eyes). Squares —males; circles—females. The gene determining white eyes is represented by w; its wild allele by $+$.

Design of Experiments 1, 3, 4, and *5:* Reciprocal crosses of wild-type and white-eyed shrimps produced an F_1 generation with wild phenotype (Figure 2). The F_1 males were backcrossed to white females and produced approximately equal numbers of four classes of progeny: white males, white females, pigmented males. and pigmented females. When the F_1 females were testcrossed to white males. they produced two large classes (parental type) and two small classes (recombinants due to crossing over).

From 1 to 10 pairs of parents were used to produce each F_1 generation. After classification, most of the F_1 shrimps were discarded; only the progeny of the one most fertile parental pair were saved for further matings. Thus, generations III, IV, etc., were descended from a single parental pair. The raw data will be presented in the form of pedigrees rather than tables because the latter would require assignment of presumptive genotypes which might prevent the discovery of alternative explanations.

Experiment 1: Matroclinous inheritance of crossover supression: In 1963, reciprocal crosses were made between X_wY_w females from stock #11 and X_+X_+ males from three sources: Quemado, Utah, and stock #5A. The segregation data from these crosses first suggested a mother to daughter inheritance of a characteristic frequency of crossing over.

The data summarized in Figure 3 suggest that reciprocal crosses between the Quemado race and the #11 stock yield similar results. The F_1 females (Generation II) have high frequency of crossing over: 13% and 16% of their progeny are recombinants.

In Figure 4, it is seen that reciprocal crosses of Utah and stock #11 give different results. In the right pedigree, where the female line is descended from one stock #11 female, the frequency is high. In the left pedigree, the female line is descended from one Utah female. The F_1 females exhibit low frequency of crossing over (4/507, or 0.8%). The four exceptional animals in Generation III are

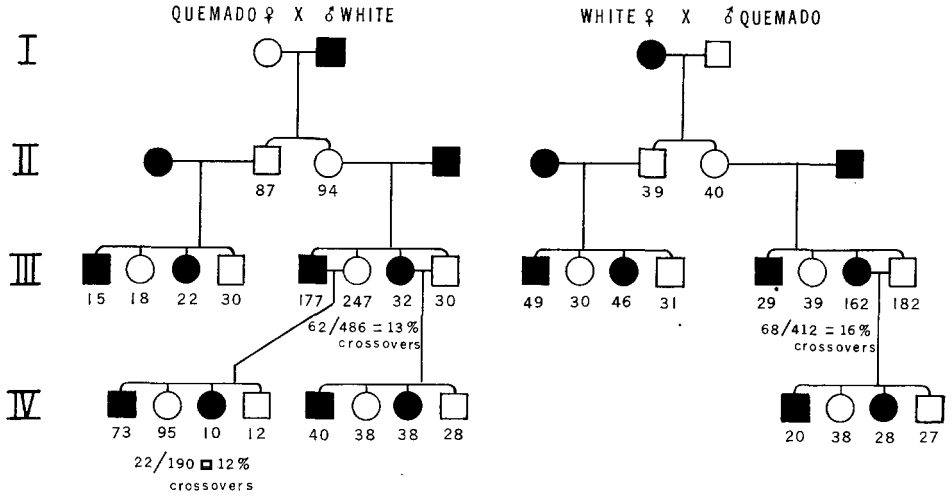

FIGURE 3.—Reciprocal crosses of white-eyed shrimps (stock #11) with wild-type shrimps from Quemado, New Mexico. The symbols are defined in the legend for Figure 2. The number under each symbol indicates the number of progeny in that class. The frequency of crossing over is calculated for the progeny of $+/w$ females.

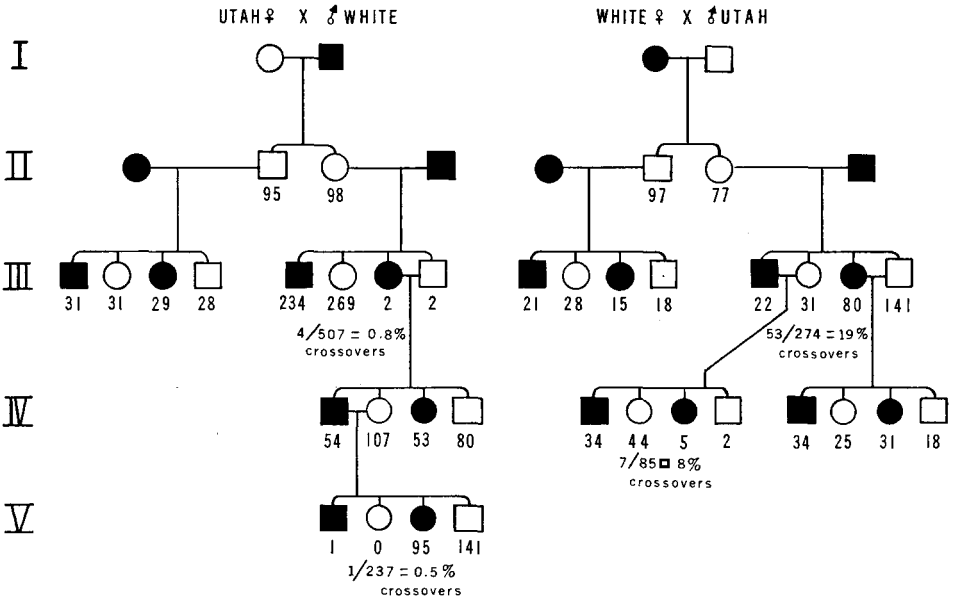

FIGURE 4.—Reciprocal crosses of white-eyed shrimps (stock #11) with wild-type shrimps from Utah. In the line descended from a single female from Utah, the recombination is low (less than 1%); whereas in the line descended from the white female, the percent recombination is high.

evidently not sex reversals but recombinants. The white females must have the X_wY_w genotype; the pigmented males must have the X_+X_w genotype because they produce four classes of progeny. (The low numbers of white shrimps can be attributed to low viability of the white phenotype.) If the four exceptional shrimps in Generation II had been sex-reversals (white-eyed females of X_wX_w genotype and pigmented-eyed males of X_wY_+ genotype), the expected progeny would have been pigmented females and white males.

In Figure 4, note that in Generation II, the $X^{11}_{w}\,Y^{U}_{+}$ females have a frequency of crossing over of 0.8%. In Generation IV, the $X^{11}_{+}\,Y^{U}_{w}$ females have a frequency of 1/237, or 0.5%. This suggests that the position of the alleles at the white locus does not affect the frequency.

In Figure 5, it is evident that reciprocal crosses of stock 5A and stock #11 give different results. In the female line in the left pedigree, the frequency of recombination is low; in the right pedigree, it is high. In the right pedigree, the X_+Y_w females in Generation II produce 38/386, or 10% crossovers. The X_wY_+ females in Generation III produce 18/199, or 9% crossovers. Again, it appears that the position of the alleles at the white locus does not affect crossing over.

Because the two Utah parents in Figure 4 were hatched from cysts collected from Great Salt Lake, they were not related to each other or to stock #5A. Nonetheless, the inheritance patterns are similar in Figures 4 and 5.

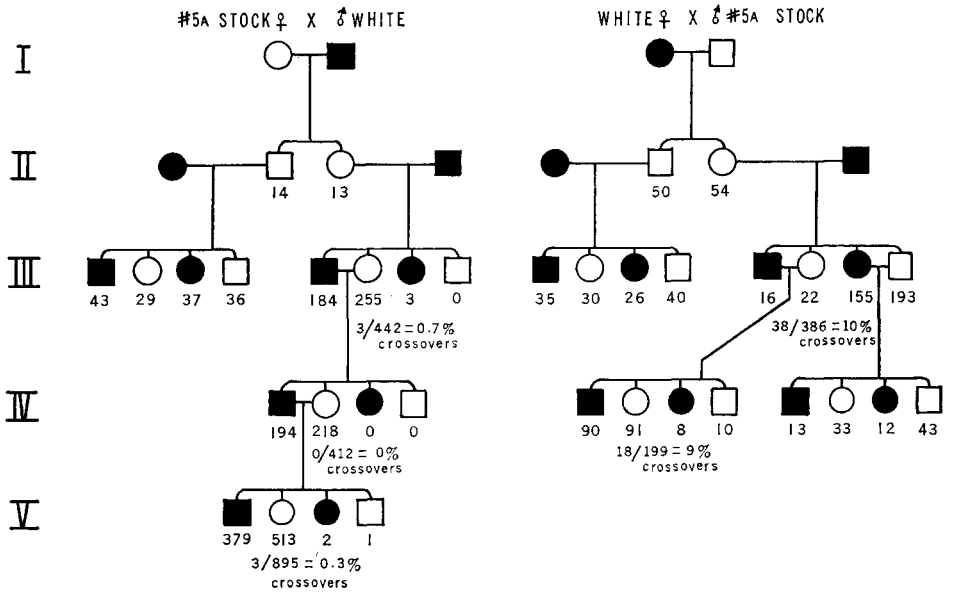

FIGURE 5.—Reciprocal crosses of w/w shrimps (stock #11) with stock #5A which is homozygous for the wild allele of white. In the pedigree on the right, the generation II females (X_+Y_w) have a frequency of crossing over of 38/386, or 10%. The generation III females (X_wY_+) have a frequency of 18/199, or 9%.

The results of Experiment 1 suggest that every line descended from a single female can have a different frequency of crossing over. This has not been proven rigorously because the wild shrimp in the parental crosses (Figures 3 and 4) were unrelated and may have been carrying genes affecting crossing over on the autosomes or the X chromosomes. Experiments 2 and 3 were designed to exclude this possibility.

Experiment 2: Attempt to alter crossover suppression in the #9 stock: Stock #9 has low frequency of crossing over and therefore breeds true for white-eyed males (X_wX_w) and pigmented females (X_wY_+). Only one crossover has been detected in the 3000 progeny of #9 females. This occurred when a #9 female was outcrossed to a X_wX_+ male which was the F_1 offspring of a #9 female and a X_+X_+ male from Quemado (BOWEN 1963). This suggested that dominant autosomal genes from the Quemado race might increase the frequency of crossing over. Furthermore, the results of Experiment 1 suggested high frequency of crossing over in F_1 females from crosses of white and Quemado. In order to test this idea, females of stock #9 were mated to males from stock #12 which is descended from a Quemado female and which is characterized by high frequency of crossing over. Among the 2188 F_1 progeny, no recombinants were observed (Table 2). F_1 females ($X_w^{12}Y_+^9$ genotype) were backcrossed to stock #12 males and among the 634 backcross progeny, no recombinants were found. Examination of Table 2 will indicate that repeated backcrossing to #12 males yields $X_w^{12}Y_+^9$ females which have 7/8 of their autosomes derived from stock #12 which in turn could be expected to have half of its autosomes from the Quemado wild population (see origin of stock #12, MATERIALS AND METHODS). This suggests that Quemado autosomes and the differential portion of the X^{12} chromosome do not account for the high frequency of crossing over which characterizes the F_1 daughters ($X_+Y_w^{12}$) of stock #12 females.

Experiment 3: Test for effects of autosomes and X chromosomes on crossover suppression: Three inbred stocks were used: stocks #12 and #13 which have the w/w genotype and stock #5B which is homozygous for the wild allele of w. Results of reciprocal crosses between stocks #5B and #13 are summarized in Figure 6. Results of crosses between stocks #5B and #12 are summarized in Figure 7.

TABLE 2

Results of repeated backcrossing of X_WY_+ females from stock #9 (characterized by low frequency of crossing over) to X_WX_W males from stock #12 (high frequency of crossing over)

Parents		Progeny				
Fraction of mother's autosomes derived from stock #12	Mating: Female X_wY_+ × Male X_wX_w	White male	White female	Pigmented male	Pigmented female	Crossovers/ total progeny
none	stock #9 × stock #12	1021	0	0	1167	0/2188
1/2	F_1 (#9×#12) × stock #12	271	0	0	363	0/634
3/4	backcross female × stock #12	102	0	0	112	0/214
7/8	2nd backcross female × stock #12	289	0	0	314	0/603

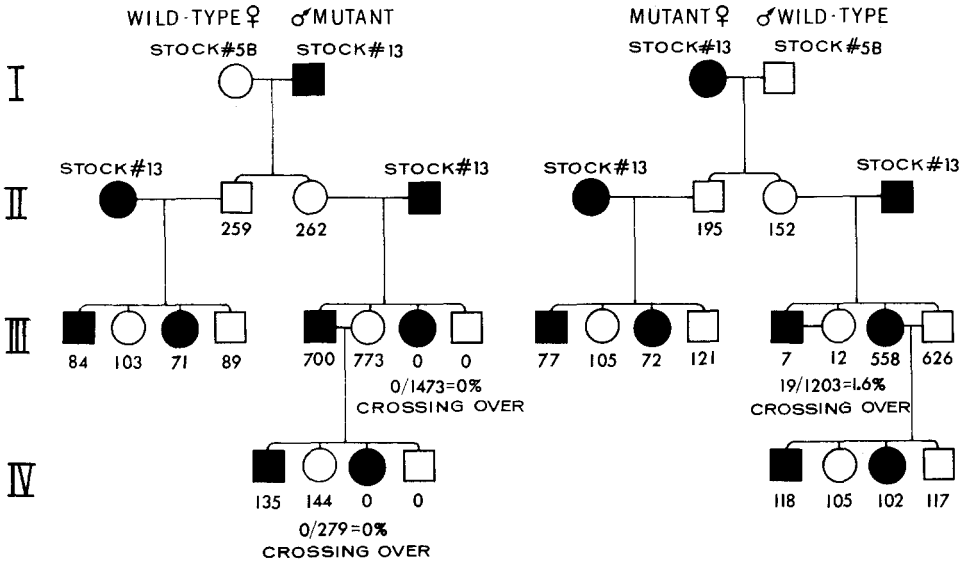

FIGURE 6.—Reciprocal crosses between +/+ stock #5B and *w/w* stock #13. The pedigree notations are the same as in Figure 3.

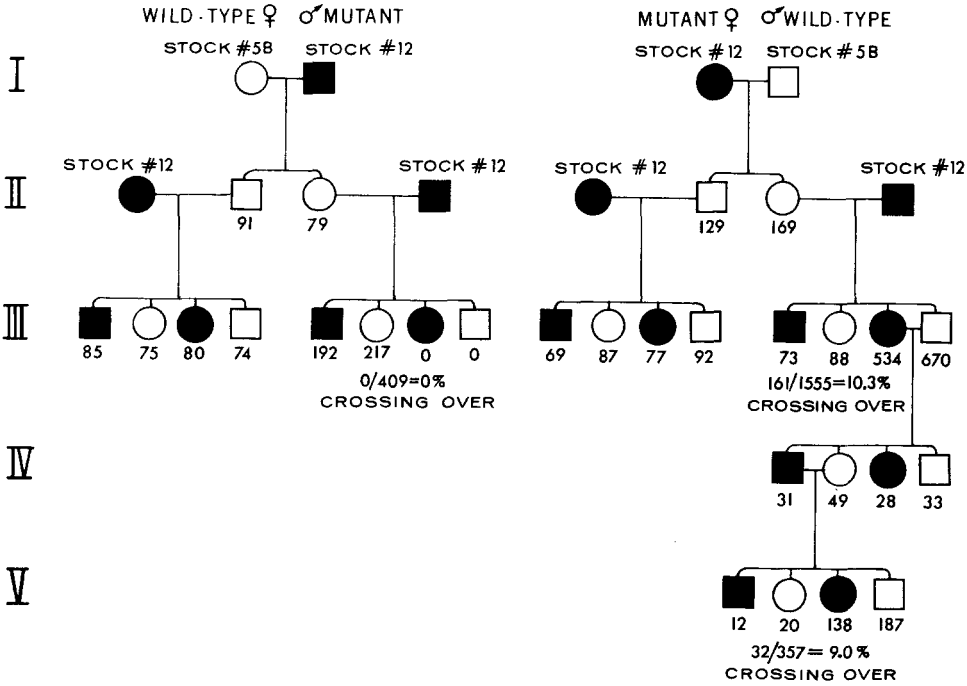

FIGURE 7.—Reciprocal crosses between +/+ stock #5B and *w/w* stock #12. The Y^{12} chromosome had previously been associated with high frequency of crossing over.

TABLE 3

Crossing over in F_1 females derived from reciprocal crosses of stock #5B(+/+) with white-eyed stocks #12 and #13

Genotype of F_1 females		Classification of their backcross progeny (1964) recombinations/total progeny		Frequency of crossing over associated with the differential segment of the Y in previous generations (1963) recombinations/total progeny	
Sex chromosomes*	Autosomes†				
$X_w{}^{12}Y_+{}^{5B}$	1A from stock #12 1A from stock #5B	0/409	(0.0%)	1/2096	(0.05%)
$X_+{}^{5B}Y_w{}^{12}$	1A from stock #12 1A from stock #5B	161/1555	(10.3%)	25/171	(14.6%)
$X_w{}^{13}Y_+{}^{5B}$	1A from stock #13 1A from stock #5B	0/1473	(0.0%)	1/2096	(0.05%)
$X_+{}^{5B}Y_w{}^{13}$	1A from stock #13 1A from stock #5B	19/1203	(1.6%)	10/1093	(0.9%)

* Subscripts indicate the allele present at the white locus on the sex chromosome. Superscripts indicate the origin of the differential segment of each chromosome. (The origin of the differential segment of the Y is the same as that of the cytoplasm.)

† 1A designates one haploid set of autosomes.

The data are correlated with the genotype of the F_1 females in Table 3. The Y^{12} chromosome was associated with a frequency of crossing over of 25/171, or 14.6% in 1963; by 161/1555, or 10.3% in 1964; and by 700/5606, or 12.4% in 1965. The last value was obtained from Experiment 4 (Table 4). In Table 3, there is good agreement between the value in the left column and that in the right column. The differential segment of the Y is more important than the autosomes or the X chromosome in determining the frequency of crossing over.

Experiment 4: Similarity of the X chromosomes of wild populations: White females from stock #12 were mated to +/+ males from seven localities (listed in Table 4). The F_1 females (genotype $X_+Y_w^{12}$) were testcrossed to #12 *w/w* males. (The experimental design is the right pedigree in Figure 2.) The percent recombinations (pigmented females and white males) were calculated for each F_1 female. In all instances, the F_1 daughters of a single wild male had similar frequencies of crossing over. This suggests that each male parent was carrying two similar X_+ chromosomes. The data from all the daughters of a single mated pair are pooled in Table 4.

Only two X chromosomes from Pichilingue were tested: X^{P1} and X^{P2}. X^{P2} was obtained from a highly inbred stock of wild shrimps and was tested in three independent experiments in order to estimate experimental error. Values of 10, 11, and 13% were obtained (Table 4).

In Table 4, the frequency of crossing over varies between 8% and 21% with

TABLE 4

Crossing over in F_1 females derived from matings of w/w *females (from stock #12) with +/+ males from seven localities*

Parental matings					
Number of matings	Origin of male parent: Source†	Origin of male parent: Locality	Genotype* of F_1 females	Progeny of F_1 females × *w/w* males: recombinations/total progeny	
1	cyst	Little Manitou	$X_+^{LM1}\ Y_w^{12}$	23/244	(9%)
1	cyst	Little Manitou	$X_+^{LM2}\ Y_w^{12}$	5/48	(10%)
1	cyst	Great Salt Lake	$X_+^{U1}\ Y_w^{12}$	53/274	(19%)
1	cyst	Great Salt Lake	$X_+^{U2}\ Y_w^{12}$	29/228	(13%)
1	cyst	Great Salt Lake	$X_+^{U3}\ Y_w^{12}$	17/125	(14%)
1	cyst	Great Salt Lake	$X_+^{U4}\ Y_w^{12}$	21/141	(15%)
2	inbred stock	San Francisco	$X_+^{SF1}\ Y_w^{12}$	67/625	(11%)
1	cyst	San Francisco	$X_+^{SF2}\ Y_w^{12}$	113/1253	(9%)
1	cyst	San Francisco	$X_+^{SF3}\ Y_w^{12}$	11/146	(8%)
1	inbred stock	Soap Lake	$X_+^{SL1}\ Y_w^{12}$	29/141	(20%)
1	inbred stock	Soap Lake	$X_+^{SL1}\ Y_w^{12}$	7/43	(16%)
2	cysts	Quemado	$X_+^{Q}\ Y_w^{12}$	68/412	(16%)
1	cyst	Pichilingue	$X_+^{P1}\ Y_w^{12}$	57/267	(21%)
1	inbred stock	Pichilingue	$X_+^{P2}\ Y_w^{12}$	23/179	(13%)
1	inbred stock	Pichilingue	$X_+^{P2}\ Y_w^{12}$	39/362	(11%)
1	inbred stock	Pichilingue	$X_+^{P2}\ Y_w^{12}$	51/514	(10%)
1	cyst	Hidalgo	$X_+^{H}\ Y_w^{12}$	87/604	(14%)
		TOTAL	$X_+\ Y_w^{12}$	700/5606	(12.4%)

* Subscripts indicate the allele at the white locus. Superscripts indicate the origin of the differential segment of the sex chromosome.

† The source of the wild parent (either an inbred laboratory stock or a cyst collected from the natural habitat) is given to enable the reader to determine the number of independent genotypes sampled.

a mean of 700/5606, or 12.4%. This value is close to the figure of 161/1555, or 10%, which characterized the Y^{12} chromosome earlier (see Table 3). Evidently, the X chromosomes of these wild races are homologous with the pairing segment of the Y^{12} chromosome in the region between the two genetic markers.

Experiment 5: Evidence for female heterogamety and great variation in the Y chromosomes of wild populations: White males from stock #9 were mated to +/+ females from seven different sources (listed in Table 5). The F_1 females (genotype $X_w{}^9Y_+$) were backcrossed to #9 *w/w* males (see the left pedigree in Figure 2). In all cases, there were more parental type progeny than recombinants. This indicates that the female is the heterogametic sex in all of these wild populations. This is in contrast to the interesting situation in the platyfish *Xiphophorus (Platypoecilus) maculatus* where the male is heterogametic in some races, the female in others (GORDON 1947, 1954).

The frequency of crossing over varied between 0.03% and 20% (Table 5). The most extreme suppression of crossing over is associated with the Y chromosomes from stock #9, Little Manitou Lake, and Great Salt Lake.

An inbred laboratory stock was obtained from one mating of wild shrimps from a saltern on San Francisco Bay. All females in this stock had the same Y

TABLE 5

Crossing over in F_1 females derived from matings of w/w *males (from stock #9) with +/+ females from seven different sources*

Parental matings					
Number of matings	Origin of female parent: Source†	Origin of female parent: Population	Genotype* of F_1 females	Progeny of F_1 females × *w/w* males recombinations/total progeny	
1	inbred stock	stock #9	$X_w{}^9Y_+{}^9$	1/3025	(0.03%)
1	cyst	Little Manitou	$X_w{}^9Y_+{}^{LM}$	2/554	(0.4%)
1	cyst	Great Salt Lake	$X_w{}^9Y_+$	1/426	(0.2%)
1	cyst	Great Salt Lake	$X_w{}^9Y_+{}^{U2}$	5/744	(0.7%)
1	cyst	Great Salt Lake	$X_w{}^9Y_+{}^{U3}$	3/124	(2.4%)
1	cyst	San Francisco	$X_w{}^9Y_+{}^{SF1}$	7/433	(1.6%)
2	inbred stock	San Francisco	$X_w{}^9Y_+{}^{SF2}$	11/338	(3.0%)
1	inbred stock	San Francisco	$X_w{}^9Y_+{}^{SF2}$	7/155	(4.5%)
1	cyst	San Francisco	$X_w{}^9Y_+{}^{SF3}$	8/180	(4.4%)
1	cyst	San Francisco	$X_w{}^9Y_+{}^{SF4}$	243/1488	(16%)
1	inbred stock	Soap Lake	$X_w{}^9Y_+{}^{SL1}$	16/102	(16%)
1	inbred stock	Soap Lake	$X_w{}^9Y_+{}^{SL1}$	8/40	(20%)
1	cyst	Quemado	$X_w{}^9Y_+{}^{Q1}$	23/151	(15%)
1	cyst	Quemado	$X_w{}^9Y_+{}^{Q2}$	25/171	(15%)
2	inbred stock	Quemado	$X_w{}^9Y_+{}^{Q3}$	84/676	(12%)
1	inbred stock	Pichilingue	$X_w{}^9Y_+{}^{P1}$	26/312	(8%)
1	inbred stock	Pichilingue	$X_w{}^9Y_+{}^{P1}$	37/335	(11%)
1	inbred stock	Pichilingue	$X_w{}^9Y_+{}^{P1}$	4/26	(15%)

* Subscripts indicate the allele at the white locus. Superscripts indicate the origin of the differential segment of the sex chromosome.
† Either an inbred laboratory stock or a cyst taken from the natural habitat.

chromosome. This chromosome, $Y_+{}^{SF2}$, was tested in two independent experiments in order to estimate the experimental error. Values of 3.0 and 4.5% were obtained (Table 5). Three other females taken from the same saltern gave values of 1.6%, 4.4% and 16%. This suggests that the San Francisco race may carry several Y chromosomes, each characterized by a different frequency of crossing over.

Another estimate of variation in testing of one Y chromosome can be obtained by noting the values obtained for one Y from Pichilingue which was tested in three independent experiments (Table 5).

The data in Table 5 suggest that the Y chromosomes in wild races of Artemia show great variation in frequency of crossing over. The values which characterize each chromosome cannot be estimated with certainty from Table 5 because the influence of autosomal dominant genes cannot be ruled out in this experiment.

In Experiments 4 and 5, reciprocal crosses of white-eyed shrimps with those from seven wild populations yielded F_1 progeny of normal viability and fertility with no significant deviation from the expected value of 50% males. The test-cross segregation ratios were those expected from matings between diploid races within a single species. This indicates that the seven populations (those listed in Table 4) are geographical races of a single species. This confirms an earlier study

TABLE 6

Fertility of six types of matings during a three week period

Parental cross male × female	fertile pairs/total matings
+/+ San Bartolomeo × *w/w* stock #12	0/86
w/w stock #9 × +/+ San Bartolomeo	0/59
+/+ San Bartolomeo × +/+ San Bartolomeo	22/26
w/w stock #12 × *w/w* stock #12	14/31
w/w stock #9 × +/+ Pichilingue	26/54
+/+ Pichilingue × *w/w* stock #12	8/23

in which the autosomal mutation, *r*, which governs red eyes was used as a marker when mutant stocks were crossed to wild populations (BOWEN 1964).

Experiment 6: Attempts to mate white-eyed shrimps with wild shrimps from San Bartolomeo: Eighty-six single-pair matings were made up of wild-type San Bartolomeo males and white-eyed females from stock #12, and 59 single pair matings were made up of white-eyed males (stock #9) and San Bartolomeo females. In both types of matings, the males clasped the females but viable progeny were not produced. This suggests that the San Bartolomeo population is reproductively isolated from all the other populations used in this study (those listed in Table 4). The mechanism of isolation is not known because the eggs in the uterus of the females were not examined to see if fertilization and segmentation had occurred.

These data cannot be evaluated unless they can be compared with fertility data from other kinds of matings. In Table 6, it is seen that one can expect at least 30% of laboratory matings to be fertile. The matings listed in Table 6 were made at the same time and according to the same experimental design. Matings were checked weekly and if the males had died, they were replaced by males from the same stock. If the females died, the matings were discontinued. At the end of 1 week, 25% of the females were dead. At the end of 3 weeks, about 50% of the females were dead and the experiment was terminated.

About half of the San Bartolomeo shrimps used in the hybridization attempts (the first two lines in Table 6) were hatched from cysts collected from the Sardinian ponds in order to ensure that a large number of independent genotypes were sampled. The others came from a single inbred stock characterized by high fertility (line 3 in Table 6).

The morphology of San Bartolomeo shrimps is slightly different from that of American shrimps. The frontal knob on the basal segment of the antenna is more elongated in San Bartolomeo males than it is in American males. The two small spikes on the ventral surface of the ovisac of the female and on the penes of the male in American races are missing or greatly reduced in San Bartolomeo shrimps.

DISCUSSION

The frequency of crossing over between two loci on the X and Y chromosomes

can vary greatly from one inbred stock to another. This fact has been demonstrated in four genera: Lebistes, Oryzias, Culex, and Artemia.

Crossing over between the X and Y chromosomes was first clearly demonstrated in the guppy, *Lebistes reticulatus* (WINGE 1923). WINGE (1934, p. 38) reported three examples of variation in recombination between two markers on the sex chromosomes.

In the medaka, *Oryzias (Aplocheilus) latipes*, the locus governing white *vs.* normal red body pigmentation is on the homologous portion of the sex chromosomes. The male is XY; the female XX (AIDA 1921). YAMAMOTO has continued the work of AIDA on this egg-laying cyprinodont fish. In stock rr-d, the frequency of crossing over between the sex locus and the *r* locus is about 0.2%. Therefore, the stock breeds true for orange-red males (X^rY^R) and white females (X^rX^r). This affords ideal material for the testing of chemical compounds for their efficacy in bringing about sex reversal. YAMAMOTO (1963, 1964b) has used estrogens to transform genotypic males into phenotypic females. When these are mated to normal males, some YY progeny result. Whereas Y^rY^r males are fully viable, Y^RY^R males have low viability. A recessive lethal gene may be closely linked to a suppressor of crossing over or may be incorporated within an inversion in the Y^R chromosome. YAMAMOTO (1964a, p. 54) has concluded that the Y^R chromosome contains an "inert section" which "contains few or no major genes for viability." Recombination between the *r* locus and the differential segment is different in X^rY^R males and in X^RY^r males.

In the mosquito, *Culex molestus*, the mutant gene *w* which determines white eye color is partially sex linked (GILCHRIST and HALDANE 1947). The male is the heterogametic sex. Crossing over between the white locus and the sex locus can be detected when *w*/+ males are mated to *w*/*w* females. The frequency of crossing over varied greatly from one male to the next, the values ranging from 0 to 16%. The authors suggested that their results could be explained by inversions which could be lost by occasional crossing over.

Extensive studies of crossing over between the X and the short arm of the Y in the male *Drosophila melanogaster* (reviewed by BROSSEAU 1960), have shown that recombination is rare. Clusters of exceptional progeny may be recovered from one culture (HUGHES, HILDRETH, and BECKER 1964). This has been interpreted as evidence that recombination between the X and Y takes place in spermatogonial mitoses.

In *Artemia salina*, there are at least three Y chromosomes which have different frequencies of crossing over: Y^9, 0.03%; Y^{13}, 1.3%; and Y^{12}, 12%. The characteristic frequencies are independent of the position of the allele at the white locus (Experiment 1) and of the X and the autosomes (see Experiments 2 and 3 in this paper). The mutant X_w chromosome was first found in stock #5 where crossing over is suppressed. When this X_w is combined with unrelated Y_+ chromosomes from Pichilingue or Quemado, the frequency of recombination is high (see Experiment 5). This suggests that the spontaneous mutation of X_+ to X_w was not accompanied by chromosomal aberration.

Several mechanisms can be proposed to account for the matroclinously trans-

mitted suppression of crossing over. First, the cytoplasm may account for the suppression. THODAY and BOAM (1956) have found evidence for a cytoplasmic suppression of crossing over in *Drosophila melanogaster*. Second, genes at one or more loci on the Y chromosome might suppress crossing over. If these are located on the homologous segment, they would necessarily be dominant and closely linked to the sex locus. Third, chromosomal rearrangements might account for the suppression. For example, an inversion located entirely in the homologous section of the Y chromosome would impair synapsis and reduce frequency of chiasmata. An inversion which included portions of the differential and homologous regions would make the nonhomologous region longer and would reduce the recombination between the sex locus and the white locus.

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SUMMARY

Wild-type shrimps from Argentina, Mexico, Canada, and four localities in the United States were mated to mutant white-eyed stocks. The F_1 progeny of reciprocal crosses were viable and fertile and equal numbers of males and females were seen. The testcross segregation ratios were those expected from matings between diploid races within a single species. However, repeated attempts to mate white-eyed shrimps with wild shrimps from San Bartolomeo (Sardinia) failed. It is possible that this population is reproductively isolated from the American shrimps.

Data from more than 10,000 testcross progeny are in accord with the hypothesis that the mutant gene w, which determines white eyes, is recessive and partially sex-linked. The female is the heterogametic sex in the seven American races studied. Females will be represented as XY; males as XX. In matings of $w/+$ females to w/w males, crossing over can be detected between the sex locus and the white locus. Subscripts are used to designate the allele at the white locus and superscripts designate the racial origin of the differential segment of the X and Y chromosomes.

The X_+ chromosomes from the seven wild races show high frequency of crossing over when paired with a standard Y_w. The Y_+ chromosomes, when paired with a standard X_w, show great variation: from 0.03% to 20% crossing over. Each line descended from a single female has its own characteristic frequency of crossing over which is transmitted matroclinously. The position of the alleles at the white locus does not affect recombination; that is, within a stock descended from a single female, X_+Y_w females do not differ from X_wY_+ females in regard to frequency of crossing over. By reciprocal crosses and repeated backcrossing, it was shown that neither the autosomes nor the X chromosomes affected frequency of crossing over in five inbred stocks which were extensively studied.

The matroclinously inherited suppression of crossing over may be due to in-

versions, deletions, or suppressor genes in the Y chromosomes; this mechanism has been implied by the use of chromosome symbols in the text. However, because the female brine shrimp is heterogametic, one cannot rule out cytoplasmic inheritance of the suppression of crossing over.

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