GENE DIFFERENCES BETWEEN THE SEX RATIO AND STANDARD GENE ARRANGEMENTS OF THE X CHROMOSOME AND LINKAGE DISEQUILIBRIUM BETWEEN LOCI IN THE STANDARD GENE ARRANGEMENT OF THE X CHROMOSOME IN DROSOPHILA PSEUDOOBSCURA[1]

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ABSTRACT

The Standard and Sex Ratio gene arrangements of the X chromosome of D. *pseudobscura* differ from each other in allele frequencies at the four X chromosome loci, esterase-5, adult acid phosphatase-6, phosphoglucomutase-1 and octanol dehydrogenase-3. The Standard arrangement which is the common arrangement in all populations is polymorphic at these loci in varying degrees, the geographically less widespread Sex Ratio arrangement has little polymorphism and is genically predominantly $E\text{-}5^{1.04}$ $AP\text{-}6^-$ $Pgm1^{1.0}$ $ODH\text{-}3^{1.0}$. The Sex Ratio arrangement from different populations is alike at all of the four loci, the Standard arrangement shows some gene frequency differences among populations. The Standard and Sex Ratio arrangements differ from each other by three inversions which suggests that the two arrangements are "old". Gene differences between these two chromosome arrangements can be explained due to differential natural selection of alleles in the Standard and Sex Ratio arrangments.——The order and percent recombination among these four loci in the Standard arrangement are: $E\text{-}5$—.294—$AP\text{-}6$—.335—$Pgm\text{-}1$—.024—$ODH\text{-}3$. The Standard X chromosomes from four different wild populations were analyzed for evidence of linkage disequilibrium between pairs of loci at these four loci. No evidence of linkage disequilibrium between pairs of loci was obtained. However, when linkages involving simultaneously three loci, $E\text{-}5$, $AP\text{-}6$ and $Pgm\text{-}1$ are considered, then significant departure from linkage equilibrium is observed.

THE right arm of the X chromosome of D. *pseudoobscura* has two different gene arrangements, Standard (ST) and Sex Ratio (SR). The Sex ratio arrangement differs from the Standard by three inversions. Recombination is suppressed in the right arm of the X chromosome in females heterozygous for the ST and the SR arrangements (STURTEVANT and DOBZHANSKY 1936). Due to reduced recombination in the inversion heterozygotes, natural selection can lead to the formation of different sets of coadapted linked genes in different inversions. In order to detect the extent and magnitude of coadaptation in the X chromosome arrangements, PRAKASH and MERRITT (1972) studied alleles of the Esterase-5 (E-5) and the adult Acid phosphatase-6 (AP-6) loci in the ST and SR arrangements from two different populations. The ST arrangement from both popula-

tions was highly polymorphic for several alleles at the E-5 locus and for two alleles at the AP-6 locus; the SR arrangement had mostly the allele E-$5^{1.04}$ at the E-5 locus and only the allele AP-6^- at the AP-6 locus. In this paper, I present additional evidence of gene differences between the ST and SR arrangements, at the E-5 and AP-6 loci and at two other additional loci, Phosphoglucomutase-1 (Pgm-1) and Octanol dehydrogenase-3 (ODH-3), from several populations of $D.$ $pseudoobscura$. Information on correlations among these four loci in the ST arrangement from several populations is also presented.

MATERIALS AND METHODS

Enzymes: Esterase-5, adult Acid phosphatase-6 and Octanol dehydrogenase-3 were studied by acrylamide gel electrophoresis as described in HUBBY and LEWONTIN (1966) and PRAKASH, LEWONTIN and HUBBY (1969). For the ODH-3 assay, electrophoresis was performed at 360 volts for 45 minutes only. The ODH-3 band moves faster towards the anode than the ODH-1 band. Phosphoglucomutase-1 was studied in starch gel according to the method of AYALA et al. (1972). For allelic identification, several samples from homozygous lines of different alleles were incorporated in the gel.

Populations and genetic analysis: The populations studied were from Okanagan in British Columbia, Canada and from several localities in the United States; namely, Methow (Washington), Mt. St. Helena (California), McDonald Ranch in Napa County (California), Riverside (California), Grand Canyon (Arizona), Prescott (Arizona), Radar Station in Tucson (Arizona) and Portal (Arizona). The X chromosomes of wild males and F_1 males from Riverside, wild males from Grand Canyon, Prescott and Portal and F_1 males from McDonald Ranch were analyzed for karyotype and genotype at the four loci. Wild males and a single F_1 male from each wild female were mated individually to $D.$ $pseudoobscura$ stock females which were homozygous for the ST arrangement and E-$5^{1.12}$ AP-6^- ODH-$3^{1.0}$ and Pgm-$1^{1.0}$ alleles. The fertility of progeny males was checked to ascertain that the males being studied were indeed $D.$ $pseudoobscura$ and not $D.$ $persimilis$ males. The X chromosome karyotype of the male was determined by examining the proportions of the two sexes in the progeny and the genotype of the X chromosome at the four loci was determined from electrophoretic analysis of F_1 females. X chromosomes of F_1 males obtained from wild females were analyzed for the E-5 and AP-6 loci only, in the samples from Okanagan, Methow, Mt. St. Helena and Radar Station.

X chromosome genotypes of F_2 males at the four loci

	E-5	AP-6	Pgm-1	ODH-3	Number observed
Parentals	1.0	+	1.5	1.10	39
	1.12	—	1.0	1.0	33
Recombinants	1.0	—	1.0	1.0	15
E-5, AP-6	1.12	+	1.5	1.10	22
Recombinants	1.0	+	1.0	1.0	21
AP-6, Pgm-1	1.12	—	1.5	1.10	24
Recombinants	1.0	+	1.5	1.0	1
Pgm-1, ODH-3	1.12	—	1.0	1.10	2
Recombinants	1.0	—	1.5	1.10	9
E-5, AP-6; AP-6, Pgm-1	1.12	+	1.0	1.0	3
Recombinants	1.0	—	1.0	1.10	0
E-5, AP-6; Pgm-1, ODH-3	1.12	+	1.5	1.0	1
				Total	170

Gene order E-5 AP-6 Pgm-1 ODH-3

r E-5, AP-6 = .294; r AP-6, Pgm-1 = .335; r Pgm-1, ODH-3 = .024

Linkage measurement: A single ST E-$5^{1.0}$ AP-6+ Pgm-$1^{1.5}$ ODH-$3^{1.10}$ male was mated to homozygous ST/ST E-$5^{1.12}$/E-$5^{1.12}$ AP-6-/AP-6- Pgm-$1^{1.0}$/Pgm-$1^{1.0}$ ODH-$3^{1.0}$/ODH-$3^{1.0}$ females from a tester stock. Virgin F_1 females were mated to males from the tester stock. F_2 males were singly mated to virgin females from the tester stock. Enzyme analysis of F_3 females gave the preceding data on X chromosomes of F_2 males.

<div align="center">RESULTS</div>

Associations of alleles with gene arrangements

Esterase-5: Tables 1 and 2 give E-5 allele frequencies in the Standard and Sex Ratio arrangements. The ST arrangement is highly polymorphic; the allele *1.0* is the most common and the allele *1.07* is the second most common allele in all populations. A x^2 test of heterogeneity involving the alleles *1.0, 1.07* and the rest of the alleles pooled in a third class, in ST arrangements from different populations gives a x^2 of 32.36 with 16 degrees of freedom which has a probability of 1%. This heterogeneity in E-5 allele frequencies is mainly due to samples from Okanagan, British Columbia and Methow, Washington. These samples have a higher frequency of the allele *1.0* than the rest of the populations. Only three E-5 alleles were observed in the SR arrangement. The allele *1.04* is 91% and the

<div align="center">TABLE 1</div>

<div align="center">Gene frequencies at the esterase-5 locus in the Standard gene arrangement of the
X chromosome from different populations of D. pseudoobscura</div>

Populations	null	0.85	0.90	0.95	0.97	1.00	1.02	1.04	1.07	1.12	1.16	n^*
Okanagan (British Columbia)	—	—	—	.03	—	.71	—	.016	.21	.016	.016	63
Methow (Washington)	—	—	—	.02	—	.62	—	—	.32	.04	—	50
Mount St. Helena (California)	.026	—	.026	.065	—	.47	.01	—	.25	.13	.026	77
McDonald Ranch, Napa Co. (California)	—	—	.04	.04	—	.35	—	—	.35	.22	—	23
Riverside (California)	.007	.013	—	.10	.007	.50	—	.02	.24	.11	—	297
Grand Canyon (Arizona)	—	.015	—	.09	.015	.43	—	.015	.32	.11	—	65
Prescott (Arizona)	—	.03	—	.08	.01	.47	—	.01	.22	.17	—	76
Radar Station, Tucson (Arizona)	.03	—	—	.09	—	.36	—	.03	.36	.06	.06	33
Portal (Arizona)	—	.076	—	.045	—	.485	—	.03	.27	.076	.015	66

* n is the number of chromosomes studied.

TABLE 2

Gene frequencies at the esterase-5 locus in the Sex Ratio gene arrangement of the
X chromosome from different populations of D. pseudoobscura

Populations	Alleles			n
	0.97	1.0	1.04	
Mount St. Helena	—	—	x	1
McDonald Ranch	—	—	1.00	6
Riverside	.09	.02	.89	56
Grand Canyon	—	.045	.955	22
Prescott	—	—	1.00	11
Radar Station, Tucson	—	—	1.00	3
Portal	.20	.20	.60	5
Pooled data	.06	.03	.91	104

alleles *.97* and *1.0* are 6% and 3% respectively. A total of 168 SR arrangements from nine populations including those studied by PRAKASH and MERRITT (1972), have been examined thus far and the *E-5* allele frequencies in the SR arrangements from different populations are the same with *E-5$^{1.04}$* as the predominant allele.

Adult acid phosphatase-6: Two alleles *AP-6$^+$* and *AP-6$^-$* are present in the ST arrangement and only the − allele is present in the SR arrangement (Table 3). The *AP-6* allele frequencies in ST arrangements from different populations are different ($x^2_{(8)} = 27.96$ p $< .005$). The frequency of the + allele is highest in the northernmost Okanagan and Methow populations and lowest in the Tucson Radar Station, which is at an altitude of 9100 ft. This allele varies in frequency from 14 to 63% in different populations. The 164 SR arrangements studied thus far from several populations including those examined by PRAKASH and MERRITT (1972) are fixed for the *AP-6$^-$* allele.

Phosphoglucomutase-1: Two alleles *Pgm-1$^{1.0}$* and *Pgm-1$^{1.5}$* are present in the ST arrangement. The *Pgm-1$^{1.0}$* allele varies from 56% to 78% in different populations, these differences are not significant ($x^2_{(4)} = 8.12$ p $> .05$). In the ST arrangement, in the pooled data the frequency of allele *Pgm-1$^{1.0}$* is 65% and allele *Pgm-1$^{1.5}$* is 34%; in the SR arrangement the frequencies of the alleles *Pgm-1$^{1.0}$* and *Pgm-1$^{1.5}$* are 95% and 3% respectively (Table 4).

TABLE 3

Gene frequencies at the adult acid phosphatase-6 locus in the Standard and Sex Ratio gene
arrangements of the X *chromosome from different populations of* D. pseudoobscura

Gene arrangements	Alleles	Okanagan	Methow	Mt. St. Helena	McDonald Ranch	Riverside	Grand Canyon	Prescott	Radar St. Tucson	Portal
Standard	+	.635	.53	.44	.43	.37	.44	.39	.14	.37
	—	.365	.47	.56	.56	.63	.56	.61	.86	.63
n		63	47	78	23	270	62	69	29	65
Sex Ratio	+			—	—	—	—	—	—	—
	—			x	1.0	1.0	1.0	1.0	1.0	1.0
n				1	6	55	21	11	3	5

TABLE 4

*Gene frequencies at the phosphoglucomutase-1 locus in the Standard and Sex Ratio gene
arrangements of the X chromosome from different populations of D. pseudoobscura*

Gene arrangements	Alleles	McDonald Ranch	Riverside	Populations Grand Canyon	Prescott	Portal	Pooled data
Standard	1.0	.56	.63	.60	.78	.69	.65
	1.5	.43	.36	.40	.21	.31	.34
	2.0	—	.01	—	.01	—	.008
n		23	299	65	76	64	527
Sex Ratio	0.7	—	.04	—	—	—	.02
	1.0	1.0	.94	1.0	.82	1.00	.95
	1.5	—	.02	—	.18	—	.03
n		6	56	21	11	5	99

Octanol dehydrogenase-3: The ST arrangement has the allele *1.0* as the predominant allele, the allele *1.10* occurs with a frequency of about 7% in this arrangement. The SR arrangement is monomorphic for the allele *1.0* (Table 5).

Linkage disequilibrium between loci in the ST arrangement

The X chromosomes of wild males and of a single F_1 male obtained from each wild female were analyzed for alleles at the *E-5*, *Pgm-1*, *AP-6* and *ODH-3* loci. These wild males and females were collected from Riverside California in April 1973. Since the *ODH-3* locus is weakly polymorphic, the data for this locus are not given in Tables 6, 7, and 8. There is no evidence of this locus showing any linkage disequilibrium with the other three loci.

The X chromosomes of wild males and F_1 males show no significant differences either in allele frequencies or in frequencies of different linkages involving the loci *E-5*, *Pgm-1* and *AP-6*; the linkage data of X chromosomes of wild males and F_1 males were therefore pooled. The observed and expected frequencies of different linkages involving pairs of loci *E-5*, *Pgm-1*; *E-5*, *AP-6*; and *Pgm-1*, *AP-6* are given in Tables 6, 7, 8; linkages involving only the four most frequent *E-5* alleles were considered. There is no evidence of linkage disequilibrium between the

TABLE 5

*Gene frequencies at the octanol dehydrogenase-3 locus in the Standard and Sex Ratio gene
arrangements of the X chromosome from different populations of D. pseudoobscura*

Gene arrangements	Alleles	McDonald Ranch	Riverside	Populations Grand Canyon	Prescott	Portal
Standard	.90	—	.003	—	.01	.015
	1.00	.83	.923	.94	.91	.925
	1.10	.17	.073	.06	.08	.06
n		23	286	80	76	67
Sex Ratio	1.0	1.0	1.0	1.0	1.0	1.0
n		6	56	21	11	5

TABLE 6

Observed and expected (in parenthesis) frequencies of different linkages between esterase-5 and phosphoglucomutase-1 loci

Phosphoglucomutase-1 alleles	Esterase-5 alleles				Total
	0.95	1.0	1.07	1.12	
1.0	18	96	41	20	175
	(18.6)	(90.4)	(44.9)	(21)	
1.5	11	45	29	13	98
	(10.4)	(50.6)	(25.1)	(12)	

$\chi^2_{(3)} = 2.11$
N.S.

TABLE 7

Observed and expected (in parenthesis) frequencies of different linkages between esterase-5 and adult acid phosphatase-6 loci

Adult acid phosphatase-6 alleles	Esterase-5 alleles				Total
	0.95	1.0	1.07	1.12	
+	7	48	25	12	92
	(9.2)	(47.7)	(24)	(11)	
—	18	81	40	18	157
	(15.8)	(81.3)	(41)	(19)	

$\chi^2_{(3)} = 1.03$
N.S.

TABLE 8

Observed and expected (in parenthesis) frequencies of different linkages between phosphoglucomutase-1 and adult acid phosphatase-6 loci

Adult acid phosphatase-6 alleles	Phosphoglucomutase-1 alleles		Total
	1.0	1.5	
+	65	32	97
	(61.9)	(35.1)	
—	99	61	160
	(102.1)	(57.9)	

$\chi^2_{(1)} = 0.689$
N.S.

E-5, Pgm-1 (Table 6), *E-5, AP-6* (Table 7) and *Pgm-1, Ap-6* (Table 8) pairs of loci. The observed and expected frequencies of linkages involving the *E-5, AP-6* and *Pgm-1* loci simultaneously are given in Table 9. The *E-5* alleles were grouped in two classes, the $E-5^{1.0}$ allele which is the most frequent allele versus the rest of the alleles pooled together. The observed frequencies of different linkages are not significantly different from expectation either in the wild males or in the F_1 males; the direction of deviations of the observed from the expected frequency however is the same in the wild males and the F_1 males. The wild male and the F_1 male linkage frequencies are not different from each other; these data therefore have been pooled (bottom of Table 9). The pooled linkages show significant deviation from expectation $(\chi^2_{(3)} = 9.05 \text{ p} = .03)$.

TABLE 9

Observed and expected (in parenthesis) frequencies of different linkages between E-5, Pgm-1 and AP-6 loci in X chromosomes of wild males, F₁ males obtained from wild females and in the pooled samples from Riverside (California)

	$E\text{-}5^{1.0}$ $Pgml^{1.0}AP\text{-}6+$	$E\text{-}5^{1.0}$ $Pgm\text{-}1^{1.0}AP\text{-}6-$	$E\text{-}5^{1.0}$ $Pgm\text{-}1^{1.5}AP\text{-}6+$	$E\text{-}5^{1.0}$ $Pgm\text{-}1^{1.5}AP\text{-}6-$	$E\text{-}5^{others}$ $Pgm\text{-}1^{1.0}AP\text{-}6+$	$E\text{-}5^{others}$ $Pgm\text{-}1^{1.0}AP\text{-}6-$	$E\text{-}5^{others}$ $Pgm\text{-}1^{1.5}AP\text{-}6+$	$E\text{-}5^{others}$ $Pgm\text{-}1^{1.5}AP\text{-}6-$	Total	
Wild males	20 (21.5)	30 (28.5)	9 (8.6)	11 (11.4)	21 (16.75)	18 (22.25)	8 (11.2)	18 (14.8)	135	$\chi^2_{(3)} = 3.7$ N.S.
F₁ males	9 (10.5)	27 (25.5)	8 (4.65)	8 (11.35)	13 (10.75)	24 (26.25)	4 (8.1)	24 (19.9)	117	$\chi^2_{(3)} = 7.3$ N.S.
Pooled	29 (31.4)	57 (54.6)	17 (13.1)	19 (22.9)	34 (27.75)	42 (48.25)	12 (19.7)	42 (34.3)	252	$\chi^2_{(3)} = 9.05$ p=.03

The Standard and Sex Ratio arrangements differ from each other at the four loci we have studied; the kind and magnitude of differentiation between the arrangements is different depending upon the locus. At the E-5 locus, only three alleles—*1.04*, *.97* and *1.0*—are present in the SR arrangement, whereas eleven alleles are present in the ST arrangement. In the ST arrangement the alleles *1.0*, *1.07*, *1.12* and *.95* are the four most common alleles and the allele *1.04* which is about 90% in the SR arrangement is rare. At the *Pgm-1* locus the alleles *1.0* and *1.5* are present in both arrangements; the allele *1.5* is about 34% in the ST arrangement but only ~ 3% in the SR arrangement. In contrast to the E-5 and *Pgm-1* loci, the SR arrangement is fixed for the AP-6^- and ODH-$3^{1.0}$ alleles. The ST arrangement is quite polymorphic at the four loci, the SR arrangement is genically predominantly E-$5^{1.04}$, Pgm-$1^{1.0}$, AP-6^-, ODH-$3^{1.0}$. The SR arrangements from all the populations are quite similar in allele frequencies at the four loci. The ST arrangements, however, seem to have the highest frequencies of E-$5^{1.0}$ and AP-$6^{\pm}$ alleles in the northernmost populations from Okanagan and Methow. The ST arrangements from several populations in California and Arizona have similar allele frequencies at the four loci. Most of the populations of *D. pseudoobscura* examined for gene variation by electrophoretic analysis of proteins are from California, Nevada, Arizona, Colorado and Texas. Analysis of these populations shows similar allele frequencies for loci which are not associated with inversions (PRAKASH, LEWONTIN and HUBBY 1969; S. PRAKASH, R. C. LEWONTIN and D. W. CRUMPACKER, unpublished). Extensive analysis of northern populations from British Columbia, Washington and Oregon and southern populations from Mexico may reveal the presence of allele frequency gradients in the ST arrangement related to latitude etc.

In view of the associations of alleles with the ST and SR arrangements, DOBZHANSKY and AYALA's (1973) observation of seasonal allele frequency changes at the *Pgm-1* locus in *D. pseudoobscura*, may be a consequence of seasonal changes in SR frequency in these populations. Since no independent analysis of *Pgm-1* locus in the ST and SR arrangements has been done, it is not clear whether the allele frequencies within the ST and SR arrangements vary seasonally.

As has been discussed before (PRAKASH and MERRITT 1972), the observed allelic differences between the ST and SR arrangements must have been maintained by natural selection. The accumulation of three inversion differences between the SR and ST arrangements suggests that the two arrangements are quite old. If different alleles were not favored by natural selection in different inversions then at equilibrium due to recombination and mutation one would expect similar allele frequencies in the two arrangements. The ST and SR arrangements are adapted to different environmental conditions as is evident from geographical clines and seasonal changes in their frequencies (DOBZHANSKY and EPLING 1944; DOBZHANSKY 1943). The ST arrangement is predominant and the SR arrangement is either absent from many populations or is present in lower frequencies than the ST arrangement. The more widespread ST arrangement has a

greater degree of polymorphism than the SR arrangement. These two observations—i.e., differences in the genic content and in the degree of polymorphism between the ST and SR arrangement—can be explained on the basis of natural selection of different alleles in different arrangements.

Our data provide no evidence of linkage disequilibrium when linkages involving pairs of loci in the ST arrangement are considered (Tables 6, 7, 8, 10). MUKAI, METTLER and CHIGUSA (1971) did not find any evidence of linkage disequilibrium between pairs of four different loci in the wild type second chromosome in *D. melanogaster*. CHARLESWORTH and CHARLESWORTH (1973) studied linkages involving pairs of five different loci on the third chromosome in *D. melanogaster*. The chromosomes obtained in two successive years from a wild population gave two significant linkage disequilibria with a $P < 0.05$ out of a total of 20 comparisons. In the chromosomes extracted from a population cage which had been maintained for several years in the laboratory, they found two highly significant linkage disequilibria ($p < 0.01$) out of a total of 18 comparisons. In our study we observe significant disequilibria only when linkages involving the three loci are considered (Table 9). However, the three loci *E-5, AP-6*

TABLE 10

Observed and expected (in parenthesis) frequencies of different linkages in Standard
X chromosomes of wild males from three different populations

Populations	$E\text{-}5^{1.0}Pgm\text{-}1^{1.0}$	$E\text{-}5^{1.0}Pgm\text{-}1^{1.5}$	$E\text{-}5^{rest}Pgm\text{-}1^{1.0}$ Linkages	$E\text{-}5^{rest}Pgm\text{-}1^{1.5}$	χ^2 with 1 d.f.
Grand Canyon	13 (15.35)	12 (9.65)	22 (19.65)	10 (12.35)	1.66
Prescott	29 (27.53)	6 (7.47)	30 (31.47)	10 (8.53)	.687
Portal	23 (20.48)	7 (9.52)	20 (22.52)	13 (10.48)	1.865
	$E\text{-}5^{1.0}AP\text{-}6^+$	$E\text{-}5^{1.0}AP\text{-}6^-$	$E\text{-}5^{rest}AP\text{-}6^+$	$E\text{-}5^{rest}AP\text{-}6^-$	
Grand Canyon	11 (10.53)	14 (14.47)	13 (13.47)	19 (18.53)	.064
Prescott	12 (11.74)	18 (18.26)	15 (15.26)	24 (23.74)	.017
Portal	10 (10.82)	21 (20.18)	12 (11.18)	20 (20.82)	.187
	$Pgm\text{-}1^{1.0}AP\text{-}6^+$	$Pgm\text{-}1^{1.0}AP\text{-}6^-$	$Pgm\text{-}1^{1.5}AP\text{-}6^+$	$Pgm\text{-}1^{1.5}AP\text{-}6^-$	
Grand Canyon	16 (16.2)	20 (19.8)	11 (10.8)	13 (13.2)	.011
Prescott	21 (20.74)	32 (32.36)	6 (6.26)	10 (9.74)	.023
Portal	11 (13.67)	30 (27.33)	9 (6.33)	10 (12.67)	2.475

and *Pgm-1* are very loosely linked with each other. The existence of correlations even among loci which are so far apart must mean that different linkages are acted upon differentially by natural selection. If environment plays an important role in the maintenance of several of the allozyme polymorphism then one would expect to find a change in the frequency of chromosomes with different allele combinations in different seasons. The situation would be analogous but not as pronounced as observed in seasonal changes in the frequency of different inversions.

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