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THE ECOLOGY AND GENETICS OF THE "SEX-RATIO" TRAIT IN NATURAL POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

by

David George Baldwin

A Dissertation Submitted to the Faculty of the

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In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
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I hereby recommend that this dissertation prepared under my direction by David George Baldwin

entitled The ecology and genetics of the "sex-ratio" trait in natural populations of Drosophila pseudoobscura.

be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Dissertation Director — Date 4/18/79

As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

	4/18/79 Date
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	4-20-79 Date

Final approval and acceptance of this dissertation is contingent on the candidate's adequate performance and defense thereof at the final oral examination.

11/78

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ABSTRACT

In Drosophila pseudoobscura the "sex-ratio" trait is expressed only in the male, his progeny being almost entirely female. The "sex-ratio" factor is associated with a specific gene sequence (SR) of the X-chromosome. The SR arrangement is apparently in equilibrium in many natural populations. The purpose of the research presented in this paper is to determine the conditions which affect this equilibrium.

In studies of a population in the Santa Catalina Mountains, Arizona, SR frequency was highly correlated with temperature on south-facing slopes but no correlation was found on north slopes. Seasonal fluctuations occurred during every year for which adequate collection data have been obtained. Lowest SR frequencies were found in the autumn and late June, highest frequencies occurred in midwinter, late spring, and midsummer. Demes in different microhabitats were significantly different in SR frequencies in some of the collections.

Laboratory studies included an investigation of the reproductive biology of the species as well as tests of the effects of larval competition and maturation rates upon SR frequencies. The meiotic drive mechanism was found to be a strong factor favoring the increase of SR in populations. This is counteracted, at least partially, by an advantage of the Standard (ST) chromosome in larval viability under low densities and high temperatures. Larvae possessing the SR chromosome were superior under moderately high crowding at 15° C. Developmental times were consistently shorter for ST/ST and ST flies. This would

confer an advantage on the ST chromosome during periods of rapid population expansion.

Results from laboratory and field studies agree with regard to the effect of temperature upon SR frequency. Also, there appears to be agreement on the effect of larval crowding since the densities of natural populations (as judged by numbers of adults attracted to baited traps) are highest during the periods of highest SR frequency. Too little is known of the natural history of this species to determine the effect of colonization opportunities upon SR frequencies in nature. Thus, the role of maturation time differences in natural populations is unknown.

INTRODUCTION

Meiotic drive is the evolutionary force associated with unequal gamete production by the heterozygotes in a population (Sandler and Novitski, 1957). The term is used to describe the effects of any distortion of the gametic ratio of heterozygotes whether the distorting mechanism occurs during meiosis or occurs during the maturation period of the gametes. Genes on a chromosome favored by preferential segregation will be "driven" to increase in frequency even though they may be selectively disadvantageous (for review see Zimmering, Sandler, and Nicoletti, 1970).

If the meiotic drive involves a form of the X-chromosome in the male, the increase in frequency will be relative to the Y-chromosome. This will bring about a distortion of the sex ratio in the population, since more females than males will be produced. The X-chromosome responsible for the distortion will not be meiotically driven against competing, nondistorting forms of the X. The loss of Y-bearing sperm, however, may alter the rate of production or transfer of X-bearing sperm or alter the vigor of such sperm. Thus the frequency of the driven form of the X relative to the other forms may be indirectly influenced by the same mechanism which causes the meiotic drive.

An X-chromosome which distorts the sex ratio of the male's progeny was first described in Drosophila by Morgan, Bridges, and Sturtevant (1925) in D. affinis. Gershenson (1928) discovered the "sex-ratio" trait in D. obscura and Sturtevant and Dobzhansky (1936)

found it in D. pseudoobscura, D. persimilis, D. athabasca, and D. azteca. The trait has subsequently been described in other members of the obscura species group (Novitski, 1947; Jungen, 1967) as well as in other species groups (Stalker, 1961).

The "sex-ratio" trait is expressed as a nearly unisexual progeny of male carriers of the trait, the progeny consisting of 95% or more females. The few male offspring produced are sterile and usually are XO.

The "sex-ratio" factor is consistently associated with a unique gene sequence of the right arm of the X-chromosome (Stalker, 1961). In D. pseudoobscura the Sex Ratio (SR) gene arrangement differs from the Standard (ST) by three inversions in the right arm of the X-chromosome (Dobzhansky and Sturtevant, 1938). The factor causing the segregation distortion is located in one of the two medial inversions (Wallace, 1948). The third inversion is small and nearly terminal. Recombination is greatly reduced in the right arm of the X of heterokaryotypes and recombinants have rarely been found in natural populations. The right arm of the X constitutes approximately 23.5 per cent of the genome (Dobzhansky, Holz, and Spassky, 1942). It is the longest of the five chromosome arms of this species.

The mechanism causing the sex ratio distortion will influence the frequency of SR in a population only if SR males produce their female offspring at a different rate or in different numbers from the ST males. Gershenson (1928) found that D. obscura males with the "sex-ratio" factor produced progenies of size equal to that of progenies of normal males. He suggested that the trait should increase in

populations because a "sex-ratio" male transmits its X-chromosome to all its offspring, whereas a normal male transmits its X to only half its offspring. Unless checked by some counterforce the "sex-ratio" factor should become fixed and the population would face the alternatives of parthenogenesis or extinction (Hamilton, 1967).

Sturtevant and Dobzhansky (1936) reported that the Y-chromosome of D. pseudoobscura "sex-ratio" males is lost during spermatogenesis. They believed the X-chromosome divided twice at the first meiotic division. Thus SR males would produce twice as many X-bearing gametes as the ST males produce.

Novitski, Peacock, and Engel (1965) found no evidence of an extra meiotic division of the X-chromosome. They suggested one of the meiotic poles is nonfunctional. Preferential movement of the X-chromosome to the functional pole during first anaphase would result in inclusion of the X in all the functional gametes.

Policansky and Ellison (1970) reported a loss of half the spermatids in sperm bundles of SR males during spermiogenesis. This indicated that SR males should produce the same number of X-bearing sperm as the ST males produce since the lost sperm are Y-bearing.

Equality in the number of X-bearing sperm produced does not preclude fitness differences between SR and ST males due to the meiotic drive mechanism, however. If sperm compete for nutrition or space, for example, the sperm of SR males may have a faster rate of maturation or may be larger or more vigorous because of reduced competition. If facilitation takes place between X-bearing and Y-bearing sperm, the sperm of ST males would be favored.

Modifier genes might be expected to influence the sex ratio of offspring in either of the cases of gametic selection described above. Sperm competition should favor modifiers which reduce the proportion of male offspring because males produced by the SR male are not fertile. Facilitation favors modifiers with the opposite effect. Modifier genes of the "sex ratio" factor have been found in D. affinis (Novitski, 1947), and D. paramelanica (Stalker, 1961), but Policansky and Dempsey (1978) failed to find them in D. pseudoobscura.

The sex ratio of the progeny of the SR males is also modified by temperature. Darlington and Dobzhansky (1942) found that at 25° C, 6.18% of the progeny were males; at 22° C, 3.74% were males; and at 16.8° C, 1.22% were males. If sperm competition takes place, SR males should have a greater advantage at lower temperatures.

Gametic selection may take place during sperm transfer, storage, and utilization. Policansky (1974) has suggested SR males transfer half as many sperm as are transferred by ST males in each insemination. The smaller amount of sperm might allow the SR inseminated females to become receptive to another mating sooner. This would place the SR male at a disadvantage, especially if sperm displacement occurs during storage (Lefevre and Jonsson, 1962; Beckenbach, 1975). Beckenbach (1978) has found, however, that SR males suffer reduced fertility only during early adulthood or when their sperm supply is depleted by previous matings. The frequency of sperm depletion depends upon the sex ratio of the population, the females' period of nonreceptivity to mating, and the relative mating success of SR males.

SR males produce female offspring at a much faster rate than do ST males, provided SR inseminated females oviposit at the same rate as ST inseminated females (Policansky, personal communication, 1977). During periods of colonization, this would favor the SR chromosome. Thus, sperm utilization may be a gametic selection factor.

Selection acts upon any trait which distorts the sex ratio of progeny (Shaw and Mohler, 1953; Kolman, 1960; Verner, 1965; Spieth, 1974). Selection favoring equality of the sex ratio was first explained by Fisher (1930). In sexually reproducing species the males and females contribute equally to the autosomal portion of the gene pool of the next generation. Thus the total reproductive value of male parents equals that of female parents. Therefore, whenever the sex ratio of the population deviates from equality, parents producing offspring of the rarer sex will have a selective advantage.

Selection of the kind Fisher described may affect the "sex-ratio" trait. Males do not contribute X-chromosomes to their male offspring. Therefore X-linked genes which would increase the proportion of males in the progeny of a male would enjoy no selective advantage. In fact, if no fertility differences are involved, X-linked genes which decrease the proportion of male offspring should be favored (Thomson and Feldman, 1975). As the frequency of the "sex-ratio" trait increases in a population, autosomal genes favoring more males in the progeny should be advantageous in all flies except the SR males. Frequency-dependent mate preference of females might be expected, but Policansky (1974) found no preference for ST males when proportions of SR and females were high in population cages.

Group selection may play a role in determining SR frequencies in natural populations. Wallace (1969) has proposed group selection as a force favoring populations lacking SR. Group selection, however, may act to increase the frequency of SR in a population. In regions where there are frequent colonization opportunities, as in the Sonoran Desert, populations which are capable of distorting their sex ratios in favor of more females might have a selective advantage over populations which do not have this capability. Since the rate of egg production limits the reproductive capacity of a population, a greater number of females would allow the population to expand more rapidly (Giesel, 1972).

Individual selection for a sex ratio distortion may be related to environmental conditions. Trivers and Willard (1973) have proposed that females in poor condition should produce female offspring because young females stressed by the environment will be more likely to be reproductively successful than young males under the same conditions. This is due to the greater variance in reproductive success in males. SR males should be more successful in mating during times of stress.

The widespread distribution of the "sex-ratio" factor found among species of Drosophila is evidence that the trait is quite old in this genus. It has probably originated independently in each species since it is associated with different inversions in these species.

The geographical distribution of SR in D. pseudoobscura shows a north-south cline (Dobzhansky and Epling, 1944). The highest frequencies occur at the United States-Mexico border, particularly in the Sonoran Desert, where frequencies in excess of 30% have been recorded. Frequencies decline northward from this region and SR is rarely found

in northern California, Oregon, or Washington. SR apparently decreases in frequency southward from the border but collections have been too few there to allow detection of a cline.

Short-term temporal changes in SR frequency have been found in several natural populations. In the San Jacinto Mountains in California seasonal fluctuations occur (Dobzhansky, 1943; Epling, Mitchell, and Mattoni, 1957). At Aldrich, Texas, monthly as well as yearly changes were found in samples taken during the spring in 1939 through 1940 (Dobzhansky and Epling, 1944). In his study of a number of collecting sites Dobzhansky (1958) found no appreciable changes in SR frequencies over a 17 year period, however.

The equilibrium of SR in natural populations has been explained as a balance of the effects of the meiotic drive mechanism, which are assumed to favor the SR chromosome, with selection factors acting to decrease SR frequency (Wallace, 1948; Beckenbach, 1975). Wallace found that in population cages at 16.5° C, equilibria were reached at very low frequencies of SR. At 25° C, ST always became fixed. In an attempt to explain the selective forces responsible for these results, Wallace examined five major components of fitness; larval competition, longevity of adults, fecundity, sexual activity, and egg hatchability. He concluded that heterozygotes were superior to homozygotes, SR/SR being least fit, and SR males were generally inferior to ST males. SR was more successful at 16.5° C than at 25° C.

Shaw (1959) and Edwards (1961) have determined equilibrium values for SR using Wallace's fitness estimates. Their estimated values of SR are much higher than those established in Wallace's population

cages or those found in natural populations. Barker (1958) has questioned the validity of summing several fitness estimates, the method used to calculate these equilibrium values.

The selective forces which affect the equilibrium of SR in natural populations are unknown. This report describes an effort to determine at least some of the factors involved and the influence they have upon SR frequencies.

The studies described hereinafter have been divided into two groups; those which were performed in the field and those performed in the laboratory. The field studies were designed to evaluate the relationship between environmental factors, such as temperature and microhabitat changes, and SR frequencies in natural populations. The laboratory studies have included attempts to determine the effects of larval competition and developmental time upon the frequency of SR. An effort has been made to determine the strength of the effects of the drive mechanism.

FIELD STUDIES

Materials and Methods

Adult flies were collected from 5 gallon plastic buckets baited with fermenting bananas. When flies were numerous they were collected by sweeping over the traps, but in cooler weather, when flies were scarce, they were aspirated. Immediately after the collection the flies were taken to the laboratory where males were separated from females.

Each wild-caught male was pair-mated to a virgin ST/ST female. The sex ratio of their offspring indicated the karyotype of the male.

In all collections prior to 1975 only one chromosome from each female was recorded. The gene arrangement on this chromosome was determined by examining the salivary gland chromosomes of one F_1 female larva. The maternally derived chromosomal arrangement was distinguished by examining the sex ratio of the total F_1 generation.

Beginning in 1975 the karyotype of each female was determined. This was done through the examination of salivary gland chromosomes of seven female F_1 larvae. The karyotype of the paternal male was inferred from the daily sex ratio of emerging F_1 adults.

In some collections the thorax length was measured and the ovarioles were counted for each female before she was discarded. The thorax was measured from the anterior margin of the mesonotum to the posterior tip of the scutellum. Measurements were made at a magnification of 30x with an ocular micrometer of 60 divisions with one unit equal to 0.0167 mm. All thorax lengths in this paper are expressed in millimeters.

Collections were made at four sites in the Santa Catalina mountains, Pima County, Arizona. The habitats of three of these are described by Patton, Heed, and Lowe (1966). These include Soldier Trail, at 880 m (2900 ft) elevation; Molino Basin, at 1300 m (4300 ft); and Mt. Bigelow, at 2530 m (8300 ft). The fourth site is at Bear Canyon, at 1650 m (5400 ft).

Soldier Trail is in the foothills of the south side of the mountain. Traps were set about 75 m apart on the north- and south-facing slopes of a moderately steep hill. Vegetation, dominated by saguaro and palo verde, differed little on these slopes. Traps were also set in the arroyo below the hill. Here the riparian vegetation, featuring mesquite and a number of species of large shrubs, was dominant.

Molino Basin has a much greater variability in vegetation due to slope differences. The north-facing slope has a well-developed oak and juniper woodland. The south-facing slope collecting site is about 200 m from the north-facing site. It has few trees and is covered with grass, nolina, and sotol. The ravine which runs between the slopes has a woodland consisting primarily of Emory oak and Mexican blue oak.

Traps at Bear Canyon were set on the crest of a low hill. The vegetation is dominated by ponderosa pine, alligator juniper and silverleaf oak.

The collecting site on the north-facing slope at Mt. Bigelow is dominated by Douglas-fir, white fir, and white pine. Traps were also set on the south-facing slope across the crest from the north-face site.

The traps were about 75 m apart. The south-facing slope is dominated by ponderosa pine and features some silver-leaf oak and a few junipers.

Daily temperature extremes were taken from data collected by the National Climatic Center, U.S. Dept. of Commerce, which maintains recording stations at Sabino Canyon and Palisades Ranger Station in the Santa Catalina Mountains. The data from Sabino Canyon were used to indicate temperature extremes at Soldier Trail and Molino Basin. Sabino Canyon is at the foot of the south side of the mountain, about four miles from Soldier Trail and seven miles from Molino Basin. Its elevation is 805 m (2640 ft). The data from Palisades were used to indicate temperatures at Mt. Bigelow. Palisades is about one mile south of the south-facing slope site of Mt. Bigelow. The elevation at Palisades is 2422 m (7945 ft).

Results and Discussion

The data from the field studies are presented in Table 1. The collection dates of the various localities reflects the seasonal distribution of the flies. No D. pseudoobscura have been captured at the higher elevations in the winter nor at the lower elevations in the summer.

The frequencies of SR varied considerably between collections, even within the same locality. The data provide evidence of seasonal fluctuations of these frequencies. SR is most frequent during the late spring and in August. A spring increase is apparent at Molino Basin and SR has reached a high frequency in early June at Mt. Bigelow. A

Table 1. Percentages of SR and SR inseminated females for collections in the Santa Catalina Mountains, Arizona.--The mean daily maximum temperature for two weeks prior to collection and the maximum temperature on the day of collection are included.

Date	Slope	% SR	(N)	% SR insem.	(N)	Max. temp. (^{o}F) $\bar{X}\pm SE$	Temp. col. day (^{o}F)
Soldier Trail							
1. 24 Jan. 77	ravine	16.7	(24)	--	--	66.28±1.43	68
2. 8 Feb. 72		17.8	(73)	8.2	(73)	66.86±1.33	74
	north	20.0	(45)	4.4	(45)		
	south	14.3	(28)	14.3	(28)		
3. 9 Mar. 77	ravine	13.2	(38)	--	--	69.71±1.95	83
4. 12 Apr. 75	ravine	13.2	(76)	13.3	(45)	70.71±1.96	68
5. 1 Nov. 75	ravine	7.5	(40)	6.7	(15)	82.21±1.86	70
6. 4 Nov. 71		4.3	(23)	0.0	(23)	75.71±2.12	87
	north	7.1	(14)	0.0	(14)		
	south	0.0	(9)	0.0	(9)		
7. 14 Dec. 76	ravine	15.6	(186)	2.3	(43)	70.00±1.24	73
Molino Basin							
8. 12 Jan. 73		15.6	(45)	6.7	(45)	59.36±1.52	68
	ravine	13.3	(15)	0.0	(15)		
	north	14.3	(14)	7.1	(14)		
	south	18.8	(16)	12.5	(16)		

Table 1, Continued. Percentages of SR and SR inseminated females for collections in the Santa Catalina Mountains, Arizona.

Date	Slope	% SR	(N)	% SR insem.	(N)	Max. temp. (°F) $\bar{X}\pm SE$	Temp.col. day (°F)
Molino Basin							
9. 21 Jan. 72		7.4	(27)	5.0	(27)	70.36±0.84	69
	north	5.3	(19)	0.0	(19)		
	south	12.5	(8)	12.5	(8)		
10. 1 Mar. 74		5.3	(76)	1.0	(76)	73.00±2.10	86
	north	0.0	(40)	0.0	(40)		
	south	11.1	(36)	2.4	(36)		
11. 16 Mar. 77		7.7	(117)	1.7	(119)	71.93±1.90	73
	north	5.5	(55)	0.0	(31)		
	south	9.7	(62)	2.3	(88)		
12. 7 Apr. 76	north	15.4	(143)	2.0	(148)	79.64±1.74	80
13. 22 May 76	north	21.6	(23)	16.7	(12)	92.64±1.75	91
14. 4 Oct. 76	ravine	11.7	(120)	9.8	(51)	87.57±1.69	83
15. 12 Oct. 72		6.9	(58)	5.2	(58)	90.07±2.10	94
	ravine	33.3	(6)	0.0	(6)		
	north	3.0	(33)	9.1	(33)		
	south	5.3	(19)	0.0	(19)		
16. 10 Nov. 75	ravine	8.6	(93)	2.0	(49)	84.57±1.71	83
17. 8 Dec. 75	ravine	11.7	(68)	0.0	(24)	71.36±2.04	79

Table 1, Continued. Percentages of SR and SR inseminated females for collections in the Santa Catalina Mountains, Arizona.

Date	Slope	% SR	(N)	% SR insem.	(N)	Max. temp. (°F) $\bar{X}\pm SE$	Temp. col. day (°F)
Molino Basin							
18. 27 Dec. 72		12.0	(25)	0.0	(25)	68.21±1.61	66
	north	10.0	(20)	0.0	(20)		
	south	20.0	(5)	0.0	(5)		
Bear Canyon							
19. 30 Apr. 75		21.4	(14)	--	--		
20. 28 May 76		15.6	(32)	8.3	(12)		
21. 21 June 76		11.8	(17)	0.0	(16)		
22. 13 Aug. 76		28.6	(7)	0.0	(7)		
Mt. Bigelow							
23. 8 June 75	north	11.1	(36)	8.0	(45)	73.57±1.78	71
24. 8 June 76		19.1	(194)	6.9	(101)	75.36±0.95	77
	north	15.2	(66)	0.0	(36)		
	south	21.1	(128)	10.8	(65)		
25. 28 June 75		8.5	(224)	4.8	(124)	76.07±1.57	81
	north	6.1	(180)	4.1	(97)		
	south	18.2	(44)	7.4	(27)		

Table 1, Continued. Percentages of SR and SR inseminated females for collections in the Santa Catalina Mountains, Arizona.

	Date	Slope	% SR	(N)	% SR insem.	(N)	Max. temp. (°F) $\bar{X}\pm SE$	Temp. col. day (°F)
Mt. Bigelow								
26.	28 June 76		12.6	(143)	3.8	(130)	81.36±1.01	84
		north	14.1	(64)	3.2	(63)		
		south	11.4	(79)	4.5	(67)		
27.	5 Aug. 75		18.6	(113)	4.2	(71)	77.21±1.21	85
		north	21.4	(28)	5.9	(17)		
		south	17.6	(85)	3.7	(54)		
28.	5 Aug. 76		17.6	(119)	7.3	(82)	71.64±1.35	80
		north	15.2	(99)	4.7	(64)		
		south	22.2	(20)	16.7	(18)		
29.	20 Sep. 75	south	13.7	(22)	0.0	(12)	71.57±0.94	75

1. (N) following %SR represents the number of chromosomes examined. When it follows any other value, (N) represents the number of flies examined.

2. In this and all the following tables the measure of dispersion around the mean is the standard error of the mean.

decline in frequency occurs during June in the Mt. Bigelow and Bear Canyon collections.

At the lower elevations SR is consistently infrequent in autumn collections. A midwinter peak occurs at Soldier Trail and, perhaps, at Molino Basin. SR frequencies during the early spring (March) are low in the two collections from Molino Basin but moderately high in one collection from Soldier Trail.

Epling et al. (1957) found seasonal fluctuations in SR frequencies in their studies of three sites in the San Jacinto Mountains in southern California. At Pinon Flat and Vandeventer Flat, sites under the climatic influence of the Sonoran Desert, they found SR at low frequency in the early spring, increasing through June, and decreasing rapidly in July. Although the peaks do not quite coincide, this pattern is similar to that of the data in Table 1, if the Molino Basin, Bear Canyon, and Mt. Bigelow collections are considered together. No August rise in frequency was found at Pinon Flat but a considerable increase occurred in early August at Vandeventer Flat. At both localities the frequency of SR was high in October. This conflicts with the data from Molino Basin.

Keen Camp, at 1383 m (4500 ft) on the Pacific side of the mountain, was much less variable in respect to SR frequency. There was an increase in frequency from March until May but little fluctuation from May through September. Mt. Bigelow collections were less variable in SR frequency than were the collections at lower elevations in the Santa Catalinas. Thus Mt. Bigelow resembles the Keen Camp collections in respect to variability of SR frequency. Both of these localities have

smaller day-to-day temperature variances and smaller differences in daily temperature extremes than the other collecting localities in their respective mountain ranges.

Within each locality the positions of the baited traps relative to the slope of the terrain seemed to have an important bearing on the SR frequency. SR was most frequent on the north-facing slope at Soldier Trail but at Molino Basin, SR was most frequent on the south-facing slope in all seven collections in which the slopes can be compared. No consistent pattern of SR frequency is to be found in comparing the slopes at Mt. Bigelow. SR was most frequent on the south-facing slope in three of the five collections.

The mean maximum temperature for the 14 days prior to the time of collection is given for each collection in Table 1. The frequency of SR at Mt. Bigelow seemed to be influenced by mean temperature to a much greater extent on the south-facing slope than on the north-facing slope. SR frequency was negatively correlated with mean temperature at the south site, if the late September collection was excluded ($r = -0.9510$, $P < 0.01$). The excluded collection may be influenced by factors other than temperature since autumn collections at the other localities showed a very low frequency of SR, and flies were difficult to capture this time of the year. The correlation of SR frequency with temperature at the north-facing site was nonsignificant ($r = +0.1119$, $P > 0.05$).

At Molino Basin the correlations were the same as those at Mt. Bigelow. The negative correlation for the south-facing slope was

significant ($r = -0.8530$, $P < 0.02$). For the north-facing slope the correlation was nonsignificant ($r = +0.2014$, $P > 0.05$).

The sample sizes at Soldier Trail were inadequate to allow a comparison of the slopes. The combined data of all collections there indicate a strong negative correlation between SR frequency and mean temperature ($r = -0.8462$, $P < 0.01$).

These correlations indicate that temperature is an important factor influencing SR frequency on the south slopes of Mt. Bigelow and Molino Basin but is relatively unimportant on the north slopes. These differences may be explained by natural selection or by behavioral differences between the genotypes.

To account for these correlations, selection on the south slope must favor the SR genotypes when the temperature is low but ST genotypes must be at an advantage in warmer temperatures. Selection on the north slope must be independent of temperature. Selection would have to be quite intense to bring about the magnitude of SR frequency changes found in these data. The proximity of the north and south sites is well within the mean daily dispersal distances of about 170 m as established for D. pseudoobscura by Powell et al. (1976) and Crumpacker and Williams (1973). Therefore flies must be able to move from one slope site to the other quite readily. This would tend to decrease the effects of selection differences between the slope sites.

Taylor and Powell (1977) have concluded that in their collection areas, habitat selection provided the most likely explanation for the genetic variance observed in Drosophila persimilis. This is an attractive explanation for the correlation differences described above.

The north and south sites are near one another but are in very different habitats at Mt. Bigelow and Molino Basin. These localities are apparently more finely subdivided by the flies than by the investigator. Identical traps placed a few meters apart have attracted much different numbers of flies.

Karyotypic differences in habitat preference could result in migration out of the south-facing habitats by SR flies during periods of high temperature and their return during cooler periods. This behavior would affect SR frequencies at the north-facing sites only if the migrants constituted a fairly large percentage of total flies at these sites.

Comparisons of maximum temperatures on the days of collection with SR frequencies produce a significant correlation only for the south site at Molino Basin ($r = -0.8193$, $P < 0.05$). The same comparison for the south site at Mt. Bigelow had the correlation $r = -0.7065$, which is nonsignificant. Therefore short term phenomena such as adult feeding activity differences between genotypes provide an unsatisfactory explanation for the mean temperature-SR frequency correlation at Mt. Bigelow.

Two points of caution must be given in regard to the SR frequencies presented in Table 1. First, little reliability should be placed on any sample of much less than one hundred chromosomes. All comparisons of slope sites for a single collection date involve at least one small sample.

Second, the present method of determining the genotype of a wild-caught female's mate would be inaccurate if a great amount of

sperm mixing frequently takes place and multiple inseminations are common. Table 2 presents the circumstances under which errors may be expected. Errors arise because the inseminating male is assumed to be ST if the daily sex ratio of a female's progeny is near equality. With sperm mixing, however, a female inseminated by both kinds of males could produce male and female offspring each day. Thus the genotype of this female may be misscored.

Mistakes of this sort may involve heterozygous or SR/SR females but such errors would be so infrequent they may be disregarded. Some of these errors would tend to slightly increase SR frequencies, some would slightly decrease them.

Mistakes involving ST/ST females would occur more often. An estimate of the distortion of SR frequencies due to these errors may be derived given certain assumptions about the population. If the frequency of double inseminations is about 50% (Anderson, 1974; Cobbs, 1977), the frequency of double inseminations involving both genotypes of males would be twice the product of the frequencies of the SR and the ST males, multiplied by 0.5.

Assume the frequency of SR in a population in Hardy-Weinberg equilibrium is 0.20, which is near the maximum frequency found in these studies. The frequency of mixed double inseminated females would be 0.16. The ST/ST genotype would occur in 64% of the females. Therefore the frequency of ST/ST females inseminated by both genotypes of males would be about 10%. Since half of the chromosomes of these females would be misscored, the distortion favoring the frequency of SR would be about 5%.

Table 2. Comparison of actual and recorded karyotypes of multiply-inseminated wild-caught females, under the assumption that sperm mixing frequently occurs.--Larval karyotypes indicate all the different karyotypes which would be found in seven larvae examined per wild-caught female.

Larval karyotypes	Female karyotypes	
	Actual	Recorded
*SR/ST, *ST/ST	ST/ST	SR/ST
*SR/SR, *SR/ST, *ST/SR, *ST/ST	SR/ST	multiple insemination or SR/SR or SR/ST
*SR/SR, *ST/SR	SR/SR	multiple insemination

* Chromosome of father shown first

The results of the female receptivity test reported in this paper show that sperm displacement is far more common than mixing in females inseminated in the laboratory. There may be a considerable amount of sperm mixing in females mated in the field, however (W. W. Anderson, personal communication, 1978).

Table 1 shows the percentage of SR inseminated females for each of the collections. Of 39 collections, 35 have a lower percentage of SR inseminated females than SR chromosomes. Policansky (1974) discovered that the frequency of SR males in each of his collections was twice that of SR inseminated females. In the present study the discrepancy may be explained by a great amount of sperm mixing in frequent multiple inseminations. In fact, the differences between the percentages of SR and the SR inseminated females may be used to estimate the frequency of multiple inseminations in these populations.

The mean thorax lengths, given in millimeters, of wild-caught females are presented in Table 3. These are negatively correlated with mean temperatures ($r = -0.6123$, $P < 0.02$). Most of the variance in size of adults is due to the temperature and the nutritional conditions during the third instar. Females collected during the winter months (Oct. through Apr.) had a mean thorax length of 1.043 ± 0.004, $N = 492$; those collected during the summer months (May through Sept.) had a mean of 1.036 ± 0.005, $N = 440$, all sites included. This difference is not significant ($t = 0.354$, $P > 0.05$). There is no consistent size difference between flies collected on the north-facing slope and those collected on the south slope.

Table 3. Mean thorax length and mean ovariole number of wild-caught female D. pseudoobscura.

Date	Slope	Thorax, mm $\bar{X} \pm SE$	(N)	Ovarioles $\bar{X} \pm SE$	(N)
Soldier Trail					
24 Jan. 77	ravine	1.047±0.015	(40)	--	
9 Mar. 77	ravine	1.088±0.022	(20)	--	
1 Nov. 75	ravine	0.996±0.026	(14)	33.93±1.62	(14)
14 Dec. 76	ravine	1.011±0.011	(67)	--	
Molino Basin					
16 Mar. 77		1.100±0.009	(100)	--	
	north	1.066±0.017	(34)	--	
	south	1.115±0.001	(66)	--	
7 Apr. 76	north	1.055±0.008	(159)	34.90±1.55	(21)
22 May 76	north	0.989±0.038	(11)	30.70±2.73	(10)
4 Oct. 76	ravine	1.051±0.012	(51)	--	
10 Nov. 75	ravine	1.049±0.014	(41)	39.41±1.00	(42)
8 Dec. 75	ravine	--		37.09±1.81	(22)
Bear Canyon					
28 May 76		1.011±0.041	(11)	33.36±2.27	(11)
21 Jun. 76		1.003±0.029	(16)	--	
13 Aug. 76		0.983±0.023	(16)	--	
Mt. Bigelow					
8 Jun. 76		1.040±0.008	(101)	34.16±0.68	(101)
	north	1.052±0.015	(36)	33.92±1.15	(36)
	south	1.033±0.010	(65)	34.29±0.85	(65)
28 Jun. 76		1.024±0.009	(120)	--	
	north	1.028±0.001	(66)	--	
	south	1.019±0.015	(54)	--	
2 Jul. 77	north	1.093±0.015	(32)	--	

Table 3, Continued.

Date	Slope	Thorax, mm $\bar{X} \pm$ SE	(N)	Ovarioles $\bar{X} \pm$ SE	(N)
Mt. Bigelow					
5 Aug. 75		1.027±0.014	(44)	--	
	north	1.021±0.018	(17)	--	
	south	1.032±0.016	(27)	--	
5 Aug. 76		1.055±0.009	(76)	--	
	north	1.055±0.010	(58)	--	
	south	1.047±0.019	(18)	--	

These data indicate there are considerable differences in size of flies from different localities during the same year. In fact significant differences in size may occur between trap sites within a locality, as seen in the data from the 16 Mar. 77 collection at Molino Basin ($t = 2.877$, $P < 0.01$).

Sokoloff (1966) caught D. pseudoobscura adults in a number of localities during two successive years. Several body characters were measured on these flies (although thorax length was not included). For a given year flies from the various localities varied little in size but striking differences were found between sizes in successive years. Sokoloff concluded that the phenotype of D. pseudoobscura is flexible in regard to climate but inflexible in regard to habitat differences. The data of Table 3 indicate the phenotype of this species may vary with habitat as well as with climatic changes.

Table 3 presents the ovariole numbers of flies for some of the collections. Ovariole number correlates significantly with thorax length ($r = +0.7344$, $P < 0.001$) which reflects an appreciable variation in larval environmental conditions (Robertson, 1957). The correlation of ovariole number and mean temperature is nonsignificant ($r = -0.4587$, $P > 0.05$).

The mean thorax lengths and mean ovariole numbers of flies of the various SR and ST genotypes are given in Table 4. A comparison of the frequency of SR at each site with the ratio of thorax lengths of SR/ST and ST/ST females at the same site yields a significant correlation ($r = +0.9061$, $P < 0.02$). Sokoloff (1966) has suggested that size is probably largely independent of third chromosomal inversions. This

Table 4. Mean thorax length and mean ovariole number of flies of various karyotypes.

Date	Slope	Karyotype	Thorax, mm $\bar{X} \pm SE$	(N)	Ovarioles $\bar{X} \pm SE$	(N)
Soldier Trail						
14 Dec. 76	ravine	SR/ST	1.008±0.029	(12)	--	
		ST/ST	1.012±0.013	(43)	--	
		SR	0.961±0.023	(15)	--	
		ST	0.950±0.009	(58)	--	
Molino Basin						
4 Oct. 76	ravine	SR/ST	1.030±0.026	(9)	--	
		ST/ST	1.055±0.023	(18)	--	
10 Nov. 75	ravine	SR/ST	1.040±0.025	(9)	37.50±2.49	(8)
		ST/ST	1.056±0.015	(33)	40.09±1.15	(32)
8 Dec. 75	ravine	SR/ST	--		33.83±2.60	(6)
		ST/ST	--		38.31±2.26	(16)
Mt. Bigelow						
8 Jun. 76	north	SR/ST	1.045±0.039	(9)	33.78±2.83	(9)
		ST/ST	1.046±0.017	(22)	33.23±1.45	(22)
	south	SR/ST	1.047±0.014	(22)	37.09±1.17	(22)
		ST/ST	1.020±0.015	(34)	32.59±1.22	(34)

apparently is not true of the X-chromosome gene arrangements. Heterozygotes are larger than ST homozygotes when the frequency of SR is high, but they are smaller than ST/ST flies when the SR frequency is low.

There are several possible explanations for this correlation. The SR frequency and the sizes of SR/ST flies may be reflecting the relative qualities of developmental conditions for the various genotypes. Differences in larval conditions may result from competitive differences or from habitat selection.

Another possible explanation is that large flies may be more likely to be caught in the baited trap. Therefore, the larger the SR/ST females relative to ST/ST females, the more likely SR/ST females would be captured. In this case, SR frequencies in these data would reflect relative fly size and not the true frequencies of the inversion.

When SR frequency is compared with the ratio of ovariole number of SR/ST and ST/ST flies for each of four sites (Table 4), the correlation is significant ($r = +0.9087$, $P < 0.05$). This is not surprising since a strong correlation exists between size and ovariole number.

LABORATORY STUDIES

The laboratory studies consisted of three experiments: (a) a female receptivity test to investigate pertinent questions regarding the reproductive biology of D. pseudoobscura. This was done in order to determine the strength of the meiotic drive effect in influencing SR frequencies; (b) larval competition tests to determine how larval densities and developmental times might influence SR frequencies; and (c) a female choice test to detect female mating preferences as well as maturation rate differences between adult males of different karyotypes.

Origin and Maintenance of Fly Stocks

The SR chromosome used in all of the tests was derived from a single female captured at Mt. Bigelow, Arizona. The SR stock was homokaryotypic for all the autosomes. The Arrowhead (AR) arrangement was fixed on the third chromosome. The SR stock was maintained by crossing SR/SR females with ST males to obtain SR males each generation.

The wildtype ST chromosome was derived from the same Mt. Bigelow collection from which the SR chromosome was obtained.

Two ST stocks with X-linked markers were used. The stock with vermilion was started with a single mutant female captured in the desert near Tucson. All vermilion flies used were homokaryotypic for AR. The stock containing short was provided by Dr. Wyatt W. Anderson. These flies were homokaryotypic for the Standard arrangement on the third chromosome.

All stocks were maintained on banana, agar, molasses, and yeast medium in eight dram shell vials at 23° C.

Female Mating Receptivity Test

Materials and Methods

Virgin four-day-old females, having either an ST/ST or an SR/ST karyotype, were each pair-mated with an ST or an SR male. The ST chromosomes carried the marker gene vermilion. The males were four- or five-day-old virgins. Each pair was placed into an eight dram vial containing medium of banana, yeast, malt, and agar. For the first 63 pairs set up, the duration of copulation was recorded. After 24 hours the male of each pair was discarded and the female was transferred to a fresh vial to pair with a virgin male having a different karyotype from that of the female's first mate. This pair was transferred to a fresh vial every 24 hours for 12 days. The vials in which eggs had been laid were kept at 23° C until all F_1 adults were scored for sex and eye color.

An SR male used as a mate for an ST/ST female is expected to produce only wild-type female offspring because the vermilion allele is recessive. The ST male mated with an ST/ST female produces vermilion male and female offspring. Therefore it is easy to determine which male's sperm has fertilized the eggs.

Some ambiguity arises when the SR/ST female is used in these crosses. The SR male will produce only wild-type females but the ST male also produces wild-type females as well as vermilion females and wild-type and vermilion males. Without further testing the father of

wild-type female offspring is unknown. Therefore, determination of the fertilizing male has been based upon the presence of vermilion F_1 flies or the sex ratio of F_1 progeny.

Results

The frequency distributions of periods of female nonreceptivity are described in the histograms of Figures 1 and 2. The value representing the period of nonreceptivity is the number of days from the first insemination until eggs are laid which produce progeny of the second insemination. These data include nonreceptivity periods for females which failed to remate. The periods in these cases were taken to be the number of days until the series of transfers were terminated. The mean nonreceptive period for 50 females mated first to ST males was 6.20 ± 0.32 days. For the 33 females mated first to SR males the mean was 5.42 ± 0.33 days. These means do not differ significantly ($t = 1.696$, $P > 0.05$).

The histogram of Figure 2 indicates a bimodal distribution of nonreceptive periods induced by ST males. This bimodality was largely caused by females which did not remate. The first inseminating male was ST in 9 of the 11 cases in which females failed to produce offspring from a second mating. Thus 18% of the ST males were able to prevent the female from remating within 9 days of the insemination, whereas only 6% of the SR males induced such a sustained nonreceptive period.

The mean nonreceptive periods of the females which remated are given in Table 5. None of these means differ significantly. The

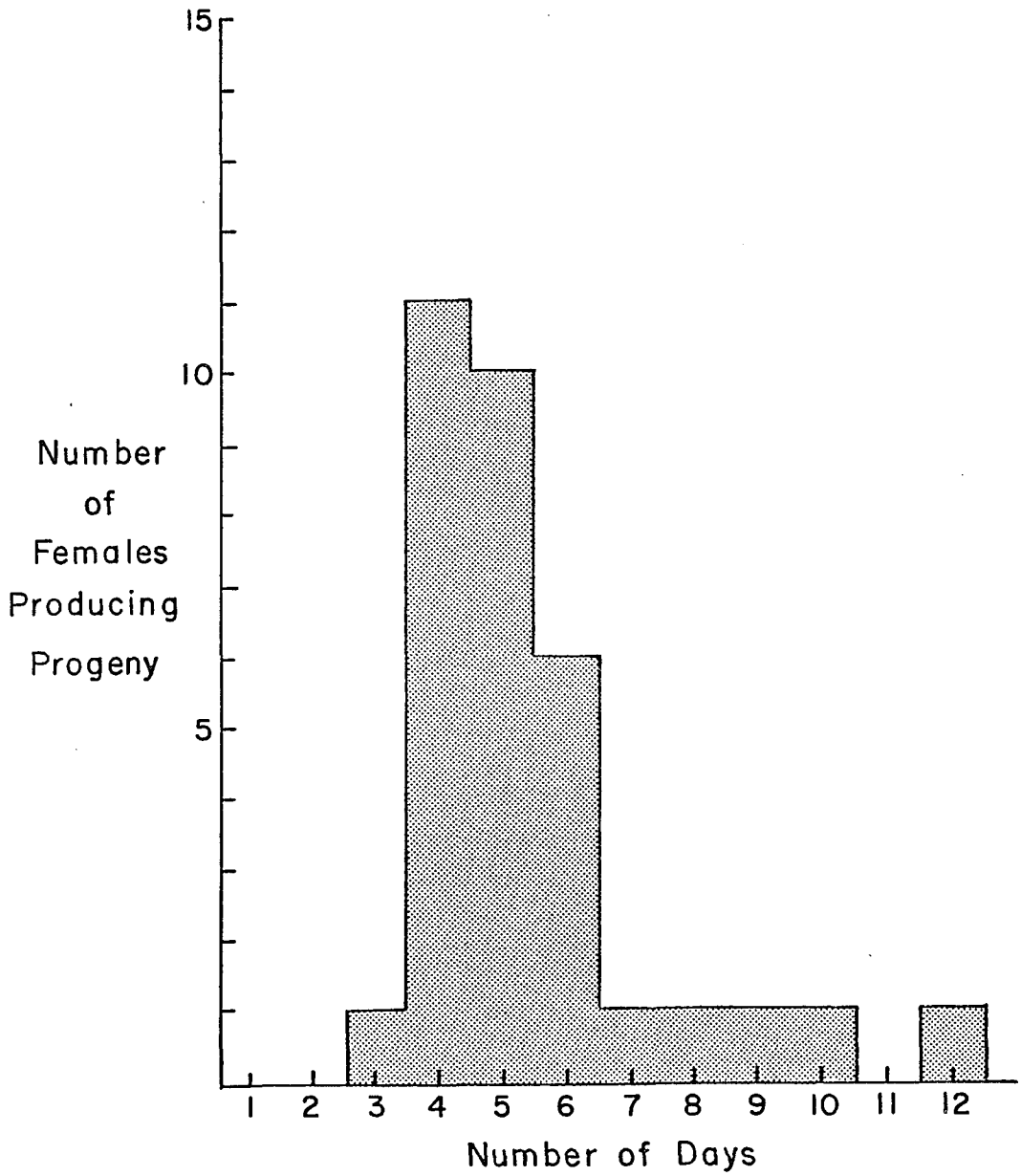

Figure 1. Number of days from first insemination until appearance of progeny from second insemination of 50 test females, SR male used as the first mate.

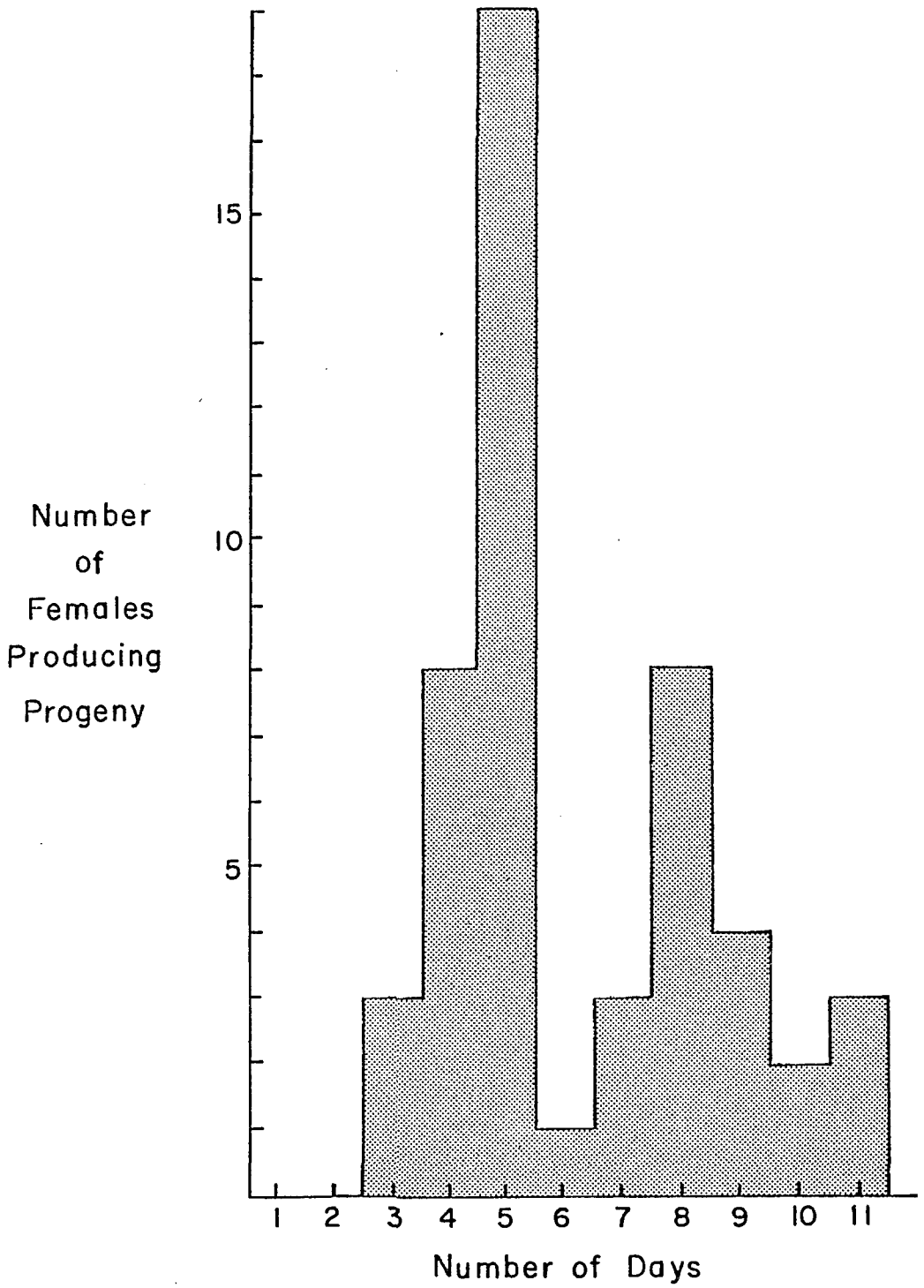

Figure 2. Number of days from first insemination until appearance of progeny from second insemination of 33 test females, ST male used as the first mate.

Table 5. Mean nonreceptive periods of females.

Male	Female	N	$\bar{X}$ (days)
ST	ST/ST	23	5.48±0.39
ST	SR/ST	18	5.78±0.51
SR	ST/ST	19	5.26±0.34
SR	SR/ST	12	4.83±0.37
Totals			
ST		41	5.61±0.31
SR		31	5.10±0.25

karyotype of the female apparently had no effect on the results of this test.

The duration of copulation was timed for 63 of the first matings. The means are to be found in Table 6. The only significant difference in means is in the comparison of SR x SR/ST with SR x ST/ST ($t = 2.605$, $P < 0.02$). As seen in the totals, there were no differences between karyotype among the males or among the females.

For the correlation between copulation time and nonreceptive period, $r = +0.1296$ with 51 females considered. This is nonsignificant.

Table 7 presents the mean number of eggs laid per day by females inseminated by SR or ST males. These means are not significantly different. The number of eggs laid per day is positively correlated with copulation time ($r = +0.3279$, $N = 37$, $P < 0.05$).

Table 8 gives the numbers of adult offspring produced by SR or ST males from the first insemination until offspring from the second insemination began to appear. All of the offspring from females which failed to remate are included. SR males produced the same number of total offspring and twice as many female offspring as were produced by ST males. These offspring were produced over 116 egg-laying days by the SR males and 171 egg-laying days by the ST males. Therefore SR males averaged 9.98 female offspring per day and ST males averaged 4.54 female offspring per day.

Discussion

Policansky (1974) has found that, for flies caught in the field, the percentage of females producing unisexual progenies is about half

Table 6. Duration of copulation.

Male	Female	N	$\bar{X}$ (seconds)
ST	ST/ST	17	212.12±22.67
ST	SR/ST	19	215.32±16.04
SR	ST/ST	14	184.64±13.70
SR	SR/ST	13	260.00±25.48
Totals			
ST		36	213.81±13.45
SR		27	220.93±15.73
	ST/ST	31	199.71±13.90
	SR/ST	32	233.47±14.36

Table 7. Mean eggs laid per day by females inseminated by SR or ST males.

Male	N	$\bar{X}$ eggs/day
SR	24	10.81±0.70
ST	29	9.98±0.81

Table 8. Number of adult offspring produced by SR or ST males in single insemination.

Male	N	$\bar{X}_{♀♀}$	$\bar{X}_{♂♂}$	$\bar{X}$ total
SR	26	44.54±5.03	0.35±0.14	44.88±5.07
ST	35	22.17±2.76	17.89±2.43	40.06±4.95

the percentage of SR males. A similar difference is found in most of the collections described in Table 1 of this paper. Results of a brooding experiment and a multiple-insemination experiment (Policansky, 1974) indicate SR males transfer only about half as many sperm as ST males transfer. Policansky proposed that, compared with an ST inseminated female, an SR inseminated female uses up the smaller quantity of sperm more quickly and is much sooner receptive to another mating. This might explain the disparity between the frequency of SR inseminated females and the frequency of SR males in nature.

Table 5 indicates there is no difference in nonreceptive periods of females inseminated by males of the different genotypes. The bimodality demonstrated in Fig. 2, however, results from the extended nonreceptive periods of several ST inseminated females. There are at least three possible explanations for this bimodality:

1. The ST males vary in their effectiveness in inducing the nonreceptive period in females.
2. The SR males vary in their courtship proficiency.
3. The females vary in their response either to a mating inhibiting factor of ST males or to the courtship of SR males.

Unfortunately, this test does not allow the investigator to distinguish between these possibilities.

It is questionable flies behave in a vial as they would in nature. The conditions of a pair of flies in a vial are very different from those under which courtship takes place at natural mating sites.

Therefore this test has yielded little information about nonreceptive periods of females.

The rate of oviposition and the nonreceptive period may be determined by the amount or nature of the paragonial gland secretion in the seminal fluid. In D. melanogaster this secretion has been found to affect female celibacy (Burnet et al., 1973) and to stimulate oviposition (Leahy, 1966). The quantity of spermatozoa in the spermathecae, however, seems to be a more important factor in maintaining mating inhibition in this species (Manning, 1967).

Gwadz (1972) has proposed that in Aedes aegypti the female's receptivity immediately after mating is switched off by the filling of her bursa with seminal fluid. The female Drosophila actively terminates copulation by kicking the male and withdrawing her ovipositor. If the termination of copulation in Drosophila is brought about by the filling of the sperm storage organs with seminal fluid, the duration of copulation may be indicative of the rate of seminal fluid transfer by the male.

No difference in mean copulation time was found between SR and ST males. The correlation between copulation time and nonreceptive period was nonsignificant. The correlation between copulation time and oviposition rate was positive and significant. Copulation time apparently determines the quantity of a transferred factor which stimulates oviposition. The factor may be paragonial gland secretion, spermatozoa, or both of these. If the quantity of spermatozoa is an important factor in determining nonreceptive period, as it is in D. melanogaster, copulation time may not affect the quantity of spermatozoa transferred.

In that case, paragonial gland secretion probably plays a major role in stimulating oviposition rate in D. pseudoobscura.

It has been suggested by Policansky (personal communication, 1977) that, if SR inseminated females oviposit at the same rate as ST inseminated females, the rate of daughter production would be might higher for the SR inseminated females. For a colonizing species, such a faster rate would give an average time advantage in the search for new breeding sites by the female offspring.

As shown in Table 7, the mean number of eggs laid per day is the same for females inseminated by males of the different genotypes. Since the SR males produced about twice as many female offspring per day as the ST males produced, SR should enjoy the colonizing advantage suggested by Policansky.

Of the 72 females tested, none was found to have mated during the first two days after the first insemination. This contrasts sharply with the results of the female choice test in which second matings within a 24 hour period occurred in at least 5% and perhaps as many as 10% of the cases. In the female choice test each female was presented with two males. It may be that two males are able to stimulate a female to copulate more frequently than one male can.

Larval Competition Tests

Materials and Methods

Test for viability differences. Females which were heterozygous for the SR and the ST X-chromosomes were mated with ST males. The ST X-chromosomes carried either of two recessive marker genes,

vermilion (v) or short vein (sh). Vermilion is located in the left arm of the X, near the centromere. Short vein is near the tip of the right arm, probably within the terminal inversion.

Fifteen heterozygous females and 10 males were placed in each of a series of eight dram vials containing banana, agar, molasses, and yeast medium. After a one or two day egg laying period the females were transferred to a fresh vial. This procedure was continued through five transfers.

Vials which were to have moderate larval crowding contained food to a depth of 1 cm (7.8 cc). The females were allowed to lay eggs for 2 days in these vials (350-500 eggs). Relatively uncrowded vials contained 2 cm of food (15.6 cc). Females were allowed to lay eggs for one day in these vials (100-300 eggs).

Wet conditions were brought about by placing a damp piece of tissue paper in the vial. The paper was moistened each day with a few drops of distilled water from an eye dropper. Dry tissue paper was placed in those vials under dry conditions.

Adults which emerged in these vials were scored each day for sex and eye color or vein length.

2. Test for developmental differences. In two vials set up as described in Part 1, moist tissue paper was pressed against the inside of each vial so that the glass was completely covered. Most of the pupae were formed on the paper. These were transferred to an empty vial each day for three days simply by transferring the paper. Pupae which were formed in the cotton plug were also removed and placed in

the empty vial. The adults which emerged were scored for sex and genotype each day. This test was run at 23° C.

3. Test for fertility differences. From each of three vials set up as described in Part 1, newly-emerged adults were removed daily and placed in a milk bottle (5 cm diameter, 62.5 cc food). This formed a cumulative F_1 population for each vial. Beginning on the fifth day after first emergence, samples of females were taken from each population. These were isolated in fresh vials where they were allowed to lay eggs. They were then discarded. The progenies of these females were checked for the ratio of sexes to determine the genotype of their mating partners. Vials and milk bottles were maintained at 23° C throughout the experiment.

Results

1. Test for viability differences. Table 9 presents the numbers of adult offspring produced by heterozygous females crossed with ST males. There is no known distortion of F_1 genotypic ratios due to meiotic drive in the female; therefore the ratio of ST/ST to SR/ST offspring is expected to be equality, as is the expected ratio of ST to SR offspring. Distortions of these ratios in the F_1 adult generation are indicative of selection operating on one or several stages of development.

Since the eggs were counted in each vial, the egg-to-adult survival rate can be calculated for each set of test conditions. At 23° C, when short is used as the marker gene on the ST chromosome, the percentages of survival were:

Table 9. Numbers of adult offspring produced by SR/ST females mated with ST males.--ST chromosomes contained either the short or vermilion markers.

Conditions	Karyotypes of offspring				
	ST/ST		SR/ST	ST	SR
At 23° C, short used as marker:					
Crowded, dry	1246		1300	1012 *	1127
Crowded, wet	994		1030	935	966
Uncrowded, dry	781	*	603	624	692
Uncrowded, wet	552	*	400	433	422
Totals	3573		3333	3004	3207
Crowded	2240		2330	1947 *	2093
Uncrowded	1333	*	1003	1057	1114
Dry	2027		1903	1636 *	1819
Wet	1546		1430	1368	1388
At 23° C, vermilion used as marker:					
Crowded, dry	830		871	731 *	577
Uncrowded, dry	549		528	503 *	426
Totals	1379		1399	1234	1003
At 15° C, short used as marker:					
Crowded, dry	514	*	1027	526 *	757
Uncrowded, dry	542		498	439	447
Totals	1056		1525	965	1204

* Indicates the flanking numbers are significantly different. Lack of an asterisk indicates no difference. Comparisons between sex and comparisons of totals are not included.

Conditions	%
Crowded, dry	68.5
Crowded, wet	68.5
Uncrowded, dry	79.1
Uncrowded, wet	81.9

Crowding has an effect on survival rate; moisture apparently does not.

At 15° C, twice as many SR/ST adults as ST/ST adults emerged in the crowded, dry conditions. The difference in survival rate in uncrowded, dry vials is not significant.

SR males were superior to ST males in crowded, dry conditions at 15° C. This advantage was found at 23° C but it is much greater at the lower temperature. In uncrowded vials the viabilities of SR and ST males were the same.

Tests involving the vermilion marker gene on the ST chromosome were carried out at 23° C only. No significant differences were found among the females in crowded and uncrowded vials, although the directions of the differences were the same as those found when short was used as a marker. Surprisingly, ST males carrying vermilion were superior to SR males in crowded and uncrowded conditions. This is the reverse of the results among the males when short was the marker.

2. Test for developmental differences. Table 10 presents the mean number of days required to eclose, from the day the first adult emerged in the same vial. These means are derived from the same F_1 generation which was scored in the test for viability differences. Therefore, the sample sizes for eclosion time are given in Table 9.

The SR males were slower than ST males in eclosion in every set of conditions under which the test was conducted. This was true

Table 10. Mean number of days to eclosion.--Day one is the day of the first adult emergence in each vial. Sample sizes are given in Table 6.

Conditions	Karyotypes of emerging adults			
	ST/ST	SR/ST	ST	SR
At 23° C, short used as marker:				
Crowded, dry	2.734±0.054 *	3.511±0.053	3.730±0.058 *	4.405±0.057
Crowded, wet	3.541±0.059 *	3.838±0.058	3.891±0.064 *	4.312±0.062
Uncrowded, dry	3.330±0.098	3.483±0.122	3.978±0.122 *	4.358±0.107
Uncrowded, wet	2.467±0.062 *	2.725±0.083	3.074±0.077 *	3.439±0.081
At 23° C, vermilion used as marker:				
Total adults	4.251±0.064	4.200±0.066	4.343±0.065 *	4.573±0.065
At 15° C, short used as marker:				
Crowded, dry	5.604±0.129 *	6.655±0.086	6.520±0.120 *	7.572±0.101
Uncrowded, dry	2.871±0.069	2.924±0.077	3.634±0.079 *	4.409±0.085

* Indicates the flanking means are significantly different. Lack of an asterisk indicates no difference. Comparisons between sexes are not included.

whether the vermilion or short gene was used for the marker on the ST chromosome.

Of 83 vials tested at 23° C, ST males outnumbered SR males on the first day of emergence in 70 vials. The first day produced 425 ST males and 197 SR males, summed over all vials.

At 15° C, ST males were the majority on the first day of emergence in 17 out of 18 vials. For all vials on the first day of emergence the total number of ST males was 70, the total number of SR males 8.

The SR/ST females were slower to eclose than ST/ST females under most of the conditions when short was used as the marker gene. Only under uncrowded, dry conditions at both temperatures were SR/ST females eclosing at the same rate at ST/ST females. When vermilion was used as the marker there was no difference in eclosion rate between the karyotypes.

In 58 of 83 vials tested at 23° C, ST/ST females were the majority on the first day of emergence. The total number of ST/ST females on the first day was 798 for all vials, compared with 552 SR/ST females.

In 11 of 18 vials tested at 15° C, ST/ST outnumbered SR/ST females on the first day of emergence. Total ST/ST females in all vials on the first day was 80; total SR/ST females was 68.

The results of the test which was designed to determine whether developmental differences occurred during larval or during the pupal stage are presented in Tables 11 and 12. Pupae which were segregated

Table 11. Numbers of adult offspring emerging from pupae which were removed from vials daily.

Day of emergence	1st Day pupae				2nd Day pupae				3rd Day pupae			
	ST/ST	SR/ST	ST	SR	ST/ST	SR/ST	ST	SR	ST/ST	SR/ST	ST	SR
1	3	1	1	1	63	54	30	10	20	26	8	8
2	47	8	28	1	13	31	26	61	10	17	8	21
3	27	13	44	5	0	0	1	3	1	0	1	15
4	0	1	8	5	1	0	1	2	--	--	--	--
Totals	77	23	81	12	77	85	58	76	31	43	17	44

Table 12. Ratios of percentages of females which are SR/ST and males which are SR for each emergence day compared with total percentages.--Data are from Table 8.

Day of emergence	1st Day pupae (N)	2nd Day pupae (N)	3rd Day pupae (N)
Females			
% SR/ST of females for emergence day X ÷ % SR/ST of total females:			
1	*	0.880 (117)	0.973 (46)
2	0.632 (55)	1.343 (44)	1.084 (27)
3	1.413 (40)	--	--
Males			
% SR of males for emergence day X ÷ % SR of total males:			
1	*	0.441 (40)	0.693 (16)
2	0.267 (29)	1.236 (87)	1.004 (29)
3	0.791 (49)	*	1.300 (16)
4	2.981 (13)	*	--

* Ratios for samples smaller than five adults are not included.

as they pupated yielded differing karyotype ratios dependent upon the day of pupation.

The percentages of karyotypes within each sex were:

Genotype	1st day pupae	2nd day pupae	3rd day pupae
ST/ST	77.0%	47.5%	41.9%
ST	87.1%	43.3%	29.9%

These data indicate larval developmental differences between the karyotypes.

To determine whether there were pupal developmental differences, the number of emerging adults was recorded for each group of pupae each day. Table 12 presents these data, each datum expressed as a ratio of the percentage of a given karyotype emerging on a given day to the percentage of that karyotype in the totals of the pupae for that day.

For the females the ratio given compares the percentage of the SR/ST karyotype among females for a given emergence day with the percentage of SR/ST among the total females of the segregated group. For the males the ratio compares the percentages of SR among males.

This ratio allows a comparison of the observed percentage of a karyotype with an expected percentage. The larger the value, the greater the proportion of SR/ST or SR flies in the emergence totals for a specific day.

The ratios for SR/ST and SR increase with day of emergence. This is true whichever day of pupation is represented. Therefore, developmental differences occur in the pupal stage.

3. Test for differences in mating success. The results of the test to determine the effect of differences in developmental times

upon early mating success are represented in Table 13. The data have been derived from three vials. Percentages and numbers are cumulative for the F_1 populations of adults from these vials.

The percentage of males which are SR is low through the second day but it is near 50% thereafter. Females sampled from the populations on the third day were mostly inseminated by ST males. The percentages of SR inseminations increase gradually through the eighth day, and are consistently much lower than the percentages of SR among the males for the same days. This indicates slow development is detrimental to SR males in regard to mating success in these populations.

The slow development of SR/ST females may be responsible for a high percentage of unmated heterokaryotypic females in these samples. Of 92 ST/ST females, 11 (12.0%) were unmated. Of 134 SR/ST females, 24 (17.9%) were unmated.

Discussion

The results of the field studies indicate that selection may influence fluctuations in SR frequencies. Three of the factors which are likely to provide selective pressure resulting in seasonal and habitat differences in SR frequencies have been examined through laboratory tests. These factors are crowding, temperature, and moisture. They have been studied with regard to their effects upon larval survival and developmental rates.

The intensity of larval density effects on D. pseudoobscura populations in nature is unknown because little is known about natural breeding sites. The larval substrate must be different at the various

Table 13. Percentages of SR males and SR inseminations in F_1 generation accumulated from first day of emergence.

Days	Cumulative males		Cumulative inseminated females	
	% SR	N	% SR	N
0	0.0	1	--	--
1	0.0	4	--	--
2	28.0	50	--	--
3	48.3	178	9.7	31
4	50.7	268	13.9	79
5	52.0	327	15.6	135
6	54.4	366	18.3	191
7	53.4	395	18.4	223
8	53.8	437	21.4	262

Numbers in column headed "Days" represent the numbers of days from the first emergence.

elevations of the Santa Catalina Mountains because the vegetation is different. Variation of substrates is likely to result in variation of density effects.

Sokoloff (1966) has suggested that D. pseudoobscura does not normally experience intense competition. He has found that wild-caught flies are comparable in size to flies reared under mildly crowded conditions in the laboratory. Intense competition is not necessary for the manifestation of density effects, however. Therefore, the present tests were designed to produce degrees of crowding resulting in flies of about the same size as wild-caught flies.

Birch (1955) has demonstrated that the different responses of third chromosome inversions to crowding could explain the seasonal changes in inversion frequencies at Pinon Flat in the San Jacinto Mountains. When flies were subjected to crowded conditions in cages, the Standard (ST) gene arrangement was superior to Chiricahua (CH). When flies were uncrowded CH was slightly superior. In nature ST is most frequent in the autumn and early spring, when population size appears to be stable. CH reaches its peak in June during a period of rapid population expansion (Dobzhansky, 1947).

The data of Epling et al. (1957) from Pinon Flat collections show a strong correlation between the frequencies of CH on the third chromosome and SR on the X chromosome ($r = +0.7175$, $P < 0.02$). This suggests the genes within the SR arrangement may influence competitive ability.

In regard to egg-to-adult survival, the SR arrangement is superior to ST under crowded conditions in the laboratory tests reported

here. This is contrary to expectations based upon the evidence above. With the survival rate of ST/ST flies taken to be equal to one, the relative survival rates for heterokaryotypes are 1.04 in crowded vials and 0.81 in uncrowded vials at 23° C. At 15° C the rate in crowded vials was 2.00; in uncrowded vials the rate was 0.92. Among males the results varied with the ST chromosome used.

These differences in survival rates indicate that temperature and crowding interact to determine the strength of selection. In natural populations the interaction is probably even more pronounced because temperature will influence the quality and number of breeding sites.

If density of adults at baited traps is indicative of density of larvae within breeding sites, larval crowding must be most intense in the summer in the Santa Catalina population. Large numbers of adults are attracted to baits at Mt. Bigelow from late May until late August. This period may present the greatest amount of larval competition. As seen in Table 1, the frequencies of SR are generally high during this season.

In nature the frequency of SR is probably influenced by both temperature and larval density. Since temperature changes may be more abrupt than changes in larval density, temperature is likely to be more important in bringing about short term fluctuations in SR frequency.

Humidity has been found to influence differentially the survival rates of pupae homokaryotypic for various third chromosome gene arrangements (Heuts, 1947). At 25° C CH/CH was the most viable

karyotype in 100% humidity, ST/ST was superior in 92%, and AR/AR was superior in 0%. In the tests reported here larvae were not stressed by desiccation. The greater moisture provided in some of the vials did not affect survival rates nor developmental times. This does not mean that moisture is unimportant in determining SR frequencies, but it is unimportant within the narrow range of moisture contents used in this experiment.

Possession of the SR arrangement results in a slow development in the larval and pupal stages in both sexes. Consequently ST/ST females and ST males should reach reproductive age more quickly than flies with the SR inversions.

In regard to the innate capacity for increase of females, the oviposition schedule at different ages is more important than the total lifetime egg production (Ohba, 1967; Lewontin, 1965). The sooner the eggs are laid, the greater the female's contribution to the capacity for increase of the population. For males, early maturation will generally lead to a greater contribution but this may be diminished by characteristics of sperm transfer and storage such as sperm displacement.

Early reproduction should confer a selective advantage within a colonizing species such as D. pseudoobscura (Lewontin, 1965). In this species colonization may occur during much of the year, especially in the mountain populations within the Sonoran Desert since these seasonally change their habitats.

From the developmental differences in the laboratory tests, the frequency of the ST gene arrangement is expected to be highest during

the latter part of the transitional seasons, spring and autumn. The field data do not support this prediction. SR is increasing in frequency during these periods. The developmental differences may be strictly a laboratory phenomenon.

Wallace (1948) and Anderson (1968) have described polymorphic laboratory populations in which the ST gene arrangement became fixed or reached frequencies near fixation within a few generations. If early maturation is favored under the laboratory conditions, developmental differences between karyotypes could explain these results. The test of differences in mating success demonstrates the advantage ST males have in an expanding population.

I have maintained ST and SR chromosomes together in small populations in vials at 23° C for over a year (at least 20 generations). SR has stabilized at 10 to 15% in these populations. The flies are not transferred to fresh vials until few noneclosed pupae remain. This apparently minimizes the advantages of the ST/ST and ST flies.

Female Choice Test

Materials and Methods

Newly-emerged SR males were paired with newly-emerged ST males and each pair was placed in an eight dram vial with a five-day-old virgin female. The vial contained banana, agar, molasses, and yeast medium. After 24 hours the males were transferred, without anesthesia, to a fresh vial containing a virgin female of the same age and genotype as the first female. This procedure was continued until the males had been introduced to 6 virgin females. The thorax length of each male was

measured before the fly was discarded. Thirty-six male pairs were used with each of two genotypes of females -- ST/ST and SR/ST. Vials were kept at 23° C with periodic light exposure.

After the males had been removed from each vial, the female was retained and allowed to lay eggs over a twelve day period. She was then discarded. Emerging F_1 adults were scored daily for sex. The karyotype of the inseminating P_1 male was inferred from the F_1 sex ratio.

SR inseminations are defined as those which produce at least 95% female offspring. Inseminations producing less than 60% females are recorded as ST. Intermediate female percentages are regarded to be ambiguous unless the daily sex ratios change abruptly. Such a change is considered to be evidence of sperm displacement by a second insemination. Sperm mixing would lead to equivocal interpretations of the sex ratios. The data from two matings are excluded because they are ambiguous.

Discussion

The results of the larval competition tests indicated the possessor of the SR chromosome has a longer larval and pupal developmental period than individuals which are homokaryotypic or hemikaryotypic for the ST gene order. The female choice test was designed to determine whether this developmental difference affects the sexual maturation rate of the adult male.

Since immature males are usually unsuccessful in courtship, males which fail to mate early in adulthood may be considered sexually immature, provided they are successful in mating on a later day.

SR males, which are slow to develop, make up some of the lost time by maturing rapidly as adults. They were five times as successful in mating on the first day after emergence as were the ST males. In the larval competition tests about twice as many ST as SR males emerged on the first day at 23° C. More than eight times as many ST as SR males emerged on the first day at 15° C. Apparently, adult maturation rate differences are sufficient to give SR males an advantage at 23° C.

Because the female choice test was carried out at 23° C, adult maturation rates at 15° C are unknown. SR mating success would have to be greater than it was at 23° C in order to overcome the advantage ST has in first day emergence numbers. Results from the test for mating success of the F_1 generation indicate ST males inseminate many more females than would be expected from their relative frequency for the first several days after the first emergence of a population. The larvae and pupae of this generation were reared at 15° C. The developmental time advantage of ST males at 15° C is probably too great to be overcome by a faster rate of adult sexual maturation of SR males.

Beckenbach (1978) has found that 20 hour old ST males participated in twice as many fertile matings as did the SR males. His results differed from those presented in this paper, perhaps because the males tested were not competing in courtship or because the X-chromosomes had different genic contents from those of the X-chromosomes in the female choice test.

There is evidence from the female choice test that ST/ST females show some preferences for male karyotype. More tests are required to determine the nature of these preferences.

Results

Table 14 shows the numbers of inseminations by each karyotype of male under the conditions of female choice. Within 24 hours of emergence, SR males were much more successful than ST males at inseminating the females ($X^2 = 14.23$, $P < 0.005$).

Neither karyotype of male showed an advantage when the males were older than 24 hours. The totals for the six days indicate the heterokaryotypic females have no preference for a specific karyotype of male. The SR males were preferred by the ST/ST females, however ($X^2 = 5.82$, $P < 0.025$).

Double inseminations could be detected only when they involved both males. On the assumption that the sperm of the second inseminating male is utilized first (Beckenbach, 1975), the first inseminating male of double inseminations could be ascertained. The results of 18 double inseminations were:

	SR 1st	ST 1st
SR/ST	8	1
ST/ST	7	2
Totals	15	3

The SR male was usually the first inseminator of double inseminations. Perhaps the SR males are less able than ST males to prevent remating by the female during the 24 hour period.

Table 14. Numbers of single inseminations occurring when females were given a choice of mates.

	1st day		2nd day		3rd day	
	SR	ST	SR	ST	SR	ST
SR/ST	16	4	15	14	15	16
ST/ST	10	1	16	12	21	11
Totals	26	5	31	26	36	27

	4th day		5th day		6th day	
	SR	ST	SR	ST	SR	ST
SR/ST	12	22	13	19	16	16
ST/ST	16	16	22	11	13	16
Totals	28	38	35	30	29	32

	Totals	
	SR	ST
SR/ST	87	91
ST/ST	98	67
	185	158

When the mating success of each male on successive days is considered, repeated successes are more frequent than failures to repeat, 138 to 113. The numbers of successful males of specific karyotype on day X, given the karyotype of the successful male of the previous day are:

for crosses with SR/ST females --

		Successful male	
		SR	ST
successful ♂ , previous day	SR	26	37
	ST	33	31

for crosses with ST/ST females --

		Successful male	
		SR	ST
successful ♂ , previous day	SR	51	25
	ST	18	30

The SR male is quite likely to repeat its success if it is courting ST/ST females. Given an SR success on the previous day, SR males have a 67% probability of repeating. This differs significantly from the 50% probability expected if success were independent of male karyotype ($X^2 = 8.89$, $P < 0.005$).

The SR and ST males were reared under similar conditions. The means and variances of the thorax lengths for the two kinds of males were the same, so size differences are probably not responsible for these differences in mating success.

CONCLUSIONS

The frequency of SR in a population is increased by selective factors resulting from the "sex-ratio" trait. This is counteracted by selection operating against the SR chromosome. Little is known about the nature of the factors involved or the strengths of the selective forces in wild populations. This study has shed some light on these problems.

Field studies have shown that the frequencies of SR can differ considerably over short time periods and between nearby habitats. These differences may be explained by habitat preferences and balancing selection such as cyclic selection and annidation.

The effects which various conditions have upon the frequency of SR in natural and laboratory populations are summarized in Table 15. An examination of some of the selective factors which might be involved indicates that temperature is very important. SR is favored in cooler temperatures. Larval density also influences SR frequency but the importance of this factor in natural populations is debatable.

Developmental time differences between karyotypes are probably affecting SR frequencies quite strongly. D. pseudoobscura is a colonizing species through much of the year. Therefore, differences in rates of reproduction are likely to provide much stronger selection than are differences in total numbers of offspring. The individuals possessing the ST chromosome as homokaryotypes or hemikaryotypes have a great advantage in rate of development.

Table 15. A summary of the effects which various conditions have upon the frequency of SR in natural and laboratory populations.--(+) indicates the factor should increase the frequency, (-) indicates the factor should decrease it.

Conditions	Effect
Natural populations	
High temperatures	- *
High population density	+
Laboratory populations	
High temperatures	-
Larval crowding	+
Colonization potential	
Maturation time	-
Meiotic drive mechanism	+

* Effect not found on north-facing slopes.

Equilibria are maintained in populations because of the balance between the selective factors described above and the rapid rate of production of female offspring as well as greater numbers of females produced by the SR male.

The right arm of the X-chromosome may be considered a supergene, with little recombination occurring in heterokaryotypes. The genes along the length of the chromosome are probably "adjusted" to one another. Selection for a specific epistatic combination may lead to very high fitness in certain environments. Recombination between "good" superalleles might break up cooperating genes and lead to reduced fitness. Wasserman and Koepfer (1977) found that within a single locality there exists only one supergene of each third chromosome gene order. The supergenes from different localities may be quite different genetically, however (Dobzhansky and Pavlovsky, 1958). The X-chromosomes tested in the present study are probably unlike those of other populations and the results described here may be unique to these chromosomes.

There are marked differences between the alleles associated with the SR and ST inversions (Prakash, 1974). These may be due to selection but can also be explained as the result of random events (Policansky and Zouros, 1977). The larval competition tests described in this paper indicated there are many genetic differences which appear to be subject to selection.

Further investigations of the "sex-ratio" equilibria should include studies of mating preferences under various conditions of stress and various proportions of the sexes and the X-chromosome arrangements.

More information is needed on the natural history of the species. Little is known of the breeding sites nor the distribution of adults in nature. Cyclic changes in population density and structure would be a valuable area of study.

Many aspects of the reproductive biology of D. pseudoobscura are still mysteries, in spite of the recent work by Policansky and Beckenbach. Information concerning sperm maturation rates, sperm transfer and storage, and oviposition patterns is needed to better evaluate the forces acting upon SR in nature.

LITERATURE CITED

Anderson, W. W. 1968. Elimination of the sex-ratio X chromosome in experimental populations of Drosophila pseudoobscura. Drosophila Inform. Serv. 43: 110.

Anderson, W. W. 1974. Frequent multiple insemination in a natural population of Drosophila pseudoobscura. Am. Nat. 109: 233-235.

Barker, J. S. F. 1958. Simulation of genetic systems by automatic digital computers. Australian J. Biol. Sci. 11: 613-625.

Beckenbach, A. T. 1975. Population genetics of the "sex-ratio" trait in Drosophila pseudoobscura. Ph.D. thesis. Univ. of Calif., Riverside, Calif. 91pp.

Beckenbach, A. T. 1978. The "sex ratio" trait in Drosophila pseudoobscura: fertility relations of males and meiotic drive. Am. Nat. 112: 97-117.

Birch, L. C. 1955. Selection in Drosophila pseudoobscura in relation to crowding. Evolution. 9: 389-399.

Burnet, B., K. Connolly, M. Kearney and R. Cook. 1973. Effects of male paragonial gland secretion on sexual receptivity and courtship behavior of female Drosophila melanogaster. J. Insect Physiol. 19: 2421-2431.

Cobbs, G. 1977. Multiple insemination and male sexual selection in natural populations of D. pseudoobscura. Am. Nat. 111: 641-656.

Crumpacker, D. W., and J. S. Williams. 1973. Density, dispersion, and population structure in Drosophila pseudoobscura. Ecol. Monogr. 43: 499-538.

Darlington, C. D., and Th. Dobzhansky. 1942. Temperature and "sex ratio" in Drosophila pseudoobscura. Proc. Natl. Acad. Sci. USA 28: 45-47.

Dobzhansky, Th. 1943. Genetics and natural populations. IX. Temporal changes in the composition of populations of Drosophila pseudoobscura. Genetics 28: 162-186.

Dobzhansky, Th. 1947. Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution 1: 1-16.

Dobzhansky, Th. 1958. The genetic changes in populations of Drosophila pseudoobscura in the American Southwest. Evolution 12: 385-401.

Dobzhansky, Th. and C. Epling. 1944. Contributions to the genetics, taxonomy, and ecology of Drosophila pseudoobscura and its relatives. Carnegie Inst. Wash. Publ. 554: 1-183.

Dobzhansky, Th., A. M. Holz and B. Spassky. 1942. Genetics of natural populations. VIII. Concealed variability in the second and fourth chromosomes of Drosophila pseudoobscura and its bearing on the problem of heterosis. Genetics. 27: 463-490.

Dobzhansky, Th. and O. Pavlovsky. 1958. Interracial hybridization and breakdown of coadapted gene complexes in Drosophila paulistorum and Drosophila willistoni. Proc. Natl. Acad. Sci. USA 44: 622-629.

Dobzhansky, Th. and A. H. Sturtevant. 1938. Inversions in the chromosomes of Drosophila pseudoobscura. Genetics 13: 28-64.

Edwards, A. W. F. 1961. The population genetics of "sex ratio" in Drosophila pseudoobscura. Heredity 16: 291-304.

Epling, C., D. Mitchell and R. H. Mattoni. 1957. The relation of an inversion system to recombination in wild populations. Evolution 11: 225-247.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford: Clarenden Press. 272pp.

Gershenson, S. 1928. A new sex ratio abnormality in Drosophila obscura. Genetics 13: 488-507.

Giesel, J. T. 1972. Sex ratio, rate of evolution, and environmental heterogeneity. Am. Nat. 106: 380-387.

Gwadz, R. W. 1972. Neuro-hormonal regulation of sexual receptivity in female Aedes aegypti. J. Insect Physiol. 18: 259-266.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156: 477-488.

Heuts, M. J. 1947. Influence of humidity on the survival of different chromosomal types in Drosophila pseudoobscura. Proc. Natl. Acad. Sci. USA 33: 210-213.

Jungen, H. 1967. Abnormal sex-ratio linked with inverted gene sequence, in populations of D. subobscura. Drosophila Inform. Serv. 42: 109.

Kolman, W. 1960. The mechanism of natural selection for the sex ratio. Am. Nat. 94: 373-377.

Leahy, M. G. 1966. Egg deposition in D. melanogaster increased by transplant of male paragonia. Drosophila Inform. Serv. 41: 145.

Lefevre, G. and U. B. Jonsson. 1962. Sperm transfer, storage, displacement and utilization in Drosophila melanogaster. Genetics 47: 1719-1736.

Lewontin, R. C. 1965. Selection for colonizing ability. In: The Genetics of Colonizing Species, ed. H. G. Baker, G. L. Stebbins, pp. 77-94. New York: Academic.

Manning, A. 1967. Control of sexual receptivity in female Drosophila. Anim. Behav. 15: 239-250.

Morgan, T. H., C. B. Bridges and A. H. Sturtevant. 1925. The genetics of Drosophila. Bibliogr. Genetica 2: 1-262.

Novitski, E. 1947. Genetic analysis of an anomalous sex ratio condition in Drosophila affinis. Genetics 32: 526-534.

Novitski, E., W. J. Peacock and J. Engel. 1965. Cytological basis of "sex ratio" in Drosophila pseudoobscura. Science 148: 516-517.

Ohba, S. 1967. Chromosomal polymorphism and capacity for increase under near optimal conditions. Heredity 22: 169-185.

Patton, J. L., W. B. Heed and C. Lowe. 1966. Inversion frequency analysis of Drosophila pseudoobscura in the Santa Catalina Mountains, Arizona. J. Ariz. Acad. Sci. 41: 105-117.

Policansky, D. 1974. "Sex ratio", meiotic drive, and group selection in Drosophila pseudoobscura. Am. Nat. 108: 75-90.

Policansky, D. and B. B. Dempsey. 1978. Modifiers and "Sex ratio" in Drosophila pseudoobscura. Evolution 32: 922-924.

Policansky, D. and J. Ellison. 1970. "Sex-ratio" in Drosophila pseudoobscura: spermiogenic failure. Science 169: 888-889.

Policansky, D. and E. Zouros. 1977. Gene differences between the Sex Ratio and Standard gene arrangements of the X chromosome in Drosophila persimilis. Genetics 85: 507-511.

Powell, J. R., Th. Dobzhansky, J. Hook and H. Wistrand. 1976. Genetics of natural populations. XLIII. Further studies on rates of dispersal of Drosophila pseudoobscura and its relatives. Genetics 82: 493-506.

Prakash, S. 1974. 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. Genetics 77: 795-804.

Robertson, F. W. 1957. Studies in quantitative inheritance. X. Genetic variation of ovary size in Drosophila. J. Genet. 55: 410-427.

Sandler, L. and E. Novitski. 1957. Meiotic drive as an evolutionary force. Am. Nat. 91: 105-110.

Shaw, R. F. 1959. Equilibrium for the sex ratio factor in Drosophila pseudoobscura. Am. Nat. 93: 385-386.

Shaw, R. F. and J. D. Mohler. 1953. The selective advantage of the sex ratio. Am. Nat. 87: 337-342.

Sokoloff, A. 1966. Morphological variation in natural and experimental populations of Drosophila pseudoobscura and Drosophila persimilis. Evolution 20: 49-71.

Spieth, P. T. 1974. Theoretical considerations of unequal sex ratios. Am. Nat. 108: 837-849.

Stalker, H. D. 1961. The genetic systems modifying meiotic drive in Drosophila paramelanica. Genetics 46: 177-202.

Sturtevant, A. H. and Th. Dobzhansky. 1936. Geographical distribution and cytology of "sex-ratio" in Drosophila pseudoobscura and related species. Genetics 21: 473-490.

Taylor, C. E. and J. R. Powell. 1977. Microgeographic differentiation of chromosomal and enzyme polymorphisms in Drosophila persimilis. Genetics 85: 681-695.

Thomson, G. J. and M. W. Feldman. 1975. Population genetics of modifiers of meiotic drive: IV. On the evolution of sex ratio distortion. Theoret. Popul. Biol. 5: 155-162.

Trivers, R. L. and D. E. Willard. 1973. Natural selction of parental ability to vary the sex ratio of offspring. Science 179: 90-92.

Verner, J. 1965. Selection for sex ratio. Am. Nat. 99: 419-421.

Wallace, B. 1948. Studies on "sex-ratio" in Drosophila pseudoobscura. I. Selection and "sex-ratio". Evolution 2: 189-218.

Wallace, B. 1969. Topics in Population Genetics. New York: Norton. 481pp.

Wasserman, M. and H. R. Koepfer. 1977. Fitness of karyotypes in Drosophila pseudoobscura. Genetics 79: 113-126.

Zimmering, S., L. Sandler and B. Nicoletti. 1970. Mechanisms of meiotic drive. Ann. Rev. Genet. 4: 409-439.