PRELIMINARY CHARACTERIZATION OF "SEX RATIO" AND REDISCOVERY AND REINTERPRETATION OF "MALE SEX RATIO" IN *DROSOPHILA AFFINIS*[1]

ROBERT A. VOELKER[2]

Department of Biology, University of Oregon, Eugene, Oregon 97403

Manuscript received July 12, 1971
Revised copy received December 20, 1971

ABSTRACT

In *D. affinis* "sex ratio" (*sr*), a form of meiotic drive characterized by the production of mostly or only female progeny by certain males, is associated with two different *X* chromosome sequences, XS-I XL-II and XS-II XL-IV. The behavior of the two sequences differed, depending on the *Y* chromosome constitution, being either Y_L or 0. Males with sequence XS-II XL-IV and Y_L produced progenies with nearly normal sex ratios; males with the same *X* chromosome sequence but in the absence of a *Y* chromosome in some cases gave progenies with nearly normal sex ratios but in other cases gave progenies which tended toward phenotypic *sr*. Males with sequence XS-I XL-II and Y_L gave progenies which were characteristically *sr* (0.97–0.98 females); in the absence of a *Y* chromosome males with this sequence produced progenies which were virtually all-male. This latter finding is presumably identical to NOVITSKI's (1947) "male sex ratio" (*msr*). The interpretation offered here attributes *msr* to an interaction between *sr* sequence XS-I XL-II and the 0 condition. A general consideration of the available data on *sr* in *D. affinis* is presented.

IN various species of Drosophila there exists a form of meiotic drive known as "sex ratio," which is characterized by the production of only or mostly female progeny by a male whose *X* chromosome carries certain genetic factors. "Sex ratio" (abbreviated hereafter as *sr*) has been observed in *D. obscura* (GERSHENSON 1928), *D. athabasca, azteca, affinis, pseudoobscura* and *persimilis* (STURTEVANT and DOBZHANSKY 1936) and *D. subobscura* (JUNGEN 1968) in the subgenus Sophophora and in *D. paramelanica* (STALKER 1961) and possibly *D. sulfurigaster albostrigata* (WILSON *et al.* 1969; unpublished) in the subgenus Drosophila. Recent work with the electron microscope suggests that the basis for *sr* in *D. pseudoobscura* is some type of spermiogenic failure, with potential *Y*-bearing sperm apparently aborting before the mature sperm bundle stage (POLICANSKY and ELLISON 1970). Whether this *modus operandi* of *sr* exists in all the above species must await further studies, since only *D. pseudoobscura* has been examined.

In *D. affinis* the phenomenon of *sr* exhibits several interesting features. First, the expression of *sr* is extremely variable. STURTEVANT (1940) commented that

[1] This study was supported by NSF Postdoctoral Grant 40027 to the author.
[2] Present address: Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607.

"males carrying it often give essentially normal sex ratios, yet descendents carrying the same *X* may give large families with few or no sons." The genetic basis of this variable expression has not been investigated. Second, NOVITSKI (1947) described a "male sex ratio" (denoted MSR by NOVITSKI, but here called *msr*) in *D. affinis*, characterized by the production of largely or only male progeny by males carrying the appropriate genetic constitution. He attributed *msr* to an interaction between *sr* and homozygosis for a certain autosomal recessive allele.

This paper reports the results of new studies of the genetics and cytology of *sr* in *D. affinis*. During the course of these studies *msr* was rediscovered and reexamined; a new interpretation of the genetic basis of this phenomenon is preseated.

MATERIALS AND METHODS

Since the stocks of *D. affinis* with which all earlier work had been done were either lost or discarded, it was necessary to use newly established mutant and *sr* strains. Two laboratory stocks used were *br rs jv* and *sc*, with the symbols representing the following mutants: *br*—bristle (VOELKER 1968); *jv*—javelin (new name for *sn*, VOELKER 1968); *rs*—rose, an apricot-like eye color; and *sc*—scute, absence of all four scutellar bristles. The loci of *sc* and *br* are in the left arm of the *X* chromosome (element *A* of STURTEVANT and NOVITSKI 1941), while the loci of *jv* and *rs* are located in the right arm of the *X* chromosome (element *D* of STURTEVANT and NOVITSKI 1941). The salivary gland chromosome sequences were determined by mating *sr* or mutant males to females of a Nebraska National Forest, Halsey, Nebraska strain which was homozygous for the standard sequence (ST) in all chromosomes (STONE 1967).

The *sr X* chromosomes were isolated from natural populations by mating individual wild-caught males to several *br rs jv* females. The F_1 sex ratio of these matings was counted and those containing mostly or only females were kept as *sr* stock lines. Since homozygous *sr* lines are difficult or impossible to maintain, all stocks of *sr* were heterozygous for *sr* and the *br rs jv* chromosome. As will be shown later, the sequence carrying *br rs jv* differs from the *sr* sequences by several inversions and served as an effective crossover suppressor.

Natural populations in three localities provided the *sr X* chromosomes used in these studies. Together with the abbreviations used to represent them, they are: NJE—Englewood, New Jersey (flies collected by DR. MAX LEVITAN); NLI—Lincoln, Nebraska (flies collected by DR. DWIGHT D. MILLER); and TAU—Austin, Texas (flies collected by the author). The large *Y* chromosome (Y_L) used in the genetic tests of *sr* was isolated from a strain of *D. affinis* from Chadron State Park, Nebraska. Males lacking a *Y* chromosome were descended from an *X*/0 strain which was initially established from a wild-caught female collected at Weeping Water, Nebraska. Both of these strains were established and provided by DWIGHT D. MILLER, University of Nebraska, Lincoln, Nebraska.

In all experiments the flies were cultured in small vials containing cornmeal-agar medium. The experiments were conducted at room temperature (23–24°C) and all anesthesizing was done with carbon dioxide.

PROCEDURES AND RESULTS

Isolation of sr X *chromosomes:* As shown in Table 1, seven different *sr X* chromosomes were isolated from natural populations. Column 2 contains the k_{φ} (proportion of progeny that are female) values of the *sr* chromosomes when they were initially recovered. They range from a low of 0.78 (TAU-3) to a high of 1.00 (NJE-13 and NLI-82). Subsequent to isolation the *sr* chromosomes were kept in laboratory stocks which were heterozygous for *sr* and the *br rs jv* chromosome for varying lengths of time. Later when detailed studies of the *sr X* chromo-

TABLE 1

Initial and reisolation k_{φ} values and salivary gland chromosome sequences of sr *stocks.*
Numbers in parentheses indicate the number of progeny counted

Stock*	Initial k_{φ}	Reisolated k_{φ}	Salivary gland *X* chromosome sequence
TAU-3	0.78 (87)	1.00 (48)	XS-I XL-II
TAU-9	0.82 (58)	0.88 (42)	XS-II XL-IV
TAU-13	0.99 (102)	1.00 (29)	XS-I XL-II
TAU-15	0.99 (98)	1.00 (62)	XS-II XL-IV
NJE-13	1.00 (36)	0.96 (67)	XS-I II
NLI-54	0.90 (19)	0.78 (40)	XS-II XL-IV
NLI-82	1.00 (21)	1.00 (81)	XS-II XL-IV

* Abbreviations used: TAU—Austin, Texas, NJE—Englewood, New Jersey, NLI—Lincoln, Nebraska.

somes were to be carried out, *sr* was reisolated from each of the strains. The k_{φ} values when the *sr* chromosomes were reisolated are given in Table 1, column 3. For each strain a number of males was tested and the male exhibiting the highest k_{φ} value was used to found the "reisolated" line. The *Y* chromosomes carried by the males when the *sr*'s were reisolated were the same as that carried by the original wild-caught male in the strains where the initial k_{φ} value was less then unity; in the strains where the initial k_{φ} value was unity, the *Y* chromosome was that carried by the *br rs jv* line.

Salivary gland chromosome cytology: In a survey of inversion polymorphism in natural populations of *D. affinis*, STONE (1967) found three and five different sequences, respectively, in the short and long arms of the *X* chromosome. To determine whether *sr* occurred in a number of these sequences or was restricted to a single sequence, the salivary gland chromosome sequence of each newly established *sr X* chromosome was determined. In each case the original wild-caught *sr* male or one of its descendents carrying *sr* was mated to females homozygous for a non-*sr* sequence designated as Standard (ST) by STONE. The salivary gland chromosomes of a number of F_1 female larvae were examined. The results (Table 1, column 4) show that two different sequences, XS-I XL-II and XS-II XL-IV. carry *sr*. In these salivary gland chromosome sequence designations the symbols and sequence numbering follow the work of STONE (1967), with XS and XL indicating the short and long arms, respectively, of the *X* chromosome and with the Roman numerals denoting different sequences in each arm. Each of the *sr* sequences was found both at Austin, Texas and at Lincoln, Nebraska. The lone Englewood, New Jersey strain contained the XS-I XL-II sequence.

Figure 1 is a diagrammatic representation of the salivary gland chromosome sequences used in this study. At the top is the Standard (ST) sequence. Sequence XS-I XL-II differs from ST by a medium-sized median single inversion in the short arm and a large median single inversion in the long arm. Sequence XS-II XL-IV differs from ST by a double overlapping inversion in the short arm and a double included inversion in the long arm. The smaller of the overlapping in-

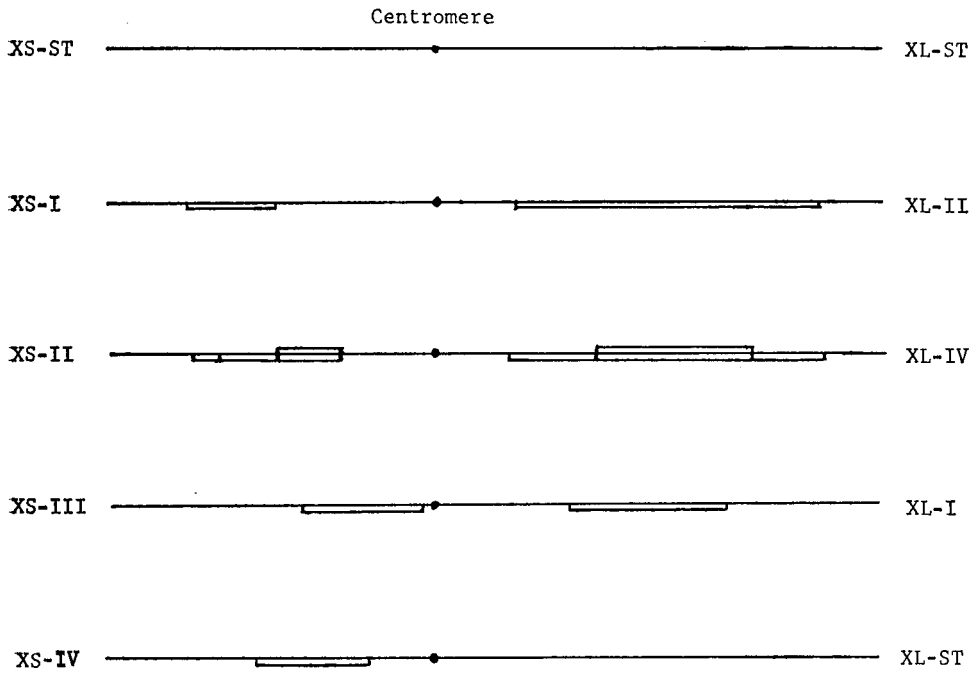

FIGURE 1.—Diagrammatic representation of salivary gland *X* chromosome sequences. Legend: —— Standard Sequence (ST), ▭ Singly Inverted Region, ▭ Doubly Inverted Region. Vertical lines indicate breakpoints of inversions.

versions in the short arm is the same inversion by which XS-I differs from XS-ST. The larger of the two included inversions in the long arm is nearly the same size as the inversion by which XL-II differs from XL-ST, but careful examination shows that they clearly have different breakpoints and are, therefore, not identical. Sequence XS-III XL-I is the sequence which carries the mutants *br rs jv*. It differs from ST by a subbasal single inversion in XS and a median single inversion in XL. The inversion by which XL-I differs from ST is the same as the smaller included inversion of XL-IV. The last sequence shown is XS-IV XL-ST; it is the sequence which carries *sc* and differs from XS-ST by a medium-sized submedian single inversion. XS-III and XS-IV are the only inversions used in this study which were not observed by STONE in natural populations; they were apparently induced by X-ray treatment simultaneously with the mutants *br* and *sc*, respectively.

Effect of the Y *chromosome on* sr: While appropriate crosses were being performed to put each *sr X* chromosome in combination with a specific *Y* chromosome or the 0 condition (*X/0* males are fertile in *D. affinis*; VOELKER 1967; VOELKER and KOJIMA 1971), it was observed that males which should have sired progenies with high frequencies of females in some cases gave progenies with nearly normal sex ratios and in other cases gave progenies which consisted of nearly all males. To determine whether these observations were repeatable, the *sr X* chromosomes were tested in the presence of a large *Y* chromosome (Y_L) or

the 0 condition. Ten of each type of male (X^{sr}/Y_L or $X^{sr}/0$) were individually mated to 3–5 aged virgin females. Each male genotype was mated in separate experiments to *br rs jv* females and to *sc* females to test for a possible female effect. After six to eight days the parents were removed and discarded. The results of these crosses are given in Table 2.

Throughout the series of crosses there is no evidence of a female effect. The control sequence XS-III XL-I gave a $\bar{k}_{\varphi}$ of approximately 0.50 both with Y_L and without a Y. This is not true for *sr* sequence XS-I XL-II which in all cases yielded $\bar{k}_{\varphi}$'s of 0.97–0.98 with Y_L and $\bar{k}_{\varphi}$'s of 0.00 when the Y chromosome was absent. This reversal is probably the "male sex ratio" (*msr*) found by NOVITSKY (1947). With the XS-II XL-IV *sr* chromosome both the presence of Y_L and the absence of a Y chromosome reduced the $\bar{k}_{\varphi}$ below the values when these chromosomes were isolated and reisolated, but in no case was there a reversal of the effect.

Males carrying either *sr* chromosome without a Y chromosome showed a significant reduction in the proportion of males which were fertile and in number of progeny produced compared with comparable males carrying a Y chromosome. The reduction in number of progeny bore no obvious relationship to the difference in $\bar{k}_{\varphi}$ values. The controls showed no evidence of a reduced number of progeny when a Y was not present.

Further data on msr: To determine whether the low fertility of *msr* males observed here had a similar basis as that of the *msr* males reported by NOVITSKI (1947), sperm production, sperm motility and sperm transfer were studied. Since the TAU-3, TAU-13 and NJE-13 *msr* males gave very similar results, only males of the constitution NJE-13/0 were examined. In a series of 90 pair matings with *sc* females, 34 were fertile and 56 were sterile or produced fewer than ten progeny per vial after prolonged culture. Males and females from 17 of the latter 56 matings were examined. In all cases the males were found to contain quantities of sperm similar to the numbers found in normal males with a Y chromosome; however, relatively few of the sperm appeared as motile as sperm in a normal male. Fourteen of the seventeen females bore few to many motile sperm in the ventral receptacle and/or spermathecae; however, only four of the seventeen produced even a few progeny.

DISCUSSION

In all the species mentioned in the introduction, with the exceptions of *D. obscura* and *D. sulfurigaster albostrigata* where data are not available, *sr* chromosomes differ by multiple inversions from non-*sr* sequences (STURTEVANT and DOBZHANSKY 1936; MILLER 1971; DOBZHANSKY and SOCOLOV 1939; JUNGEN 1968; STALKER 1961). In *D. affinis sr* is borne by two different chromosome sequences, XS-I XL-II and XS-II XL-IV, both of which differ by one or more simple inversions or complexes of inversions from the non-*sr* sequences. As has been suggested by others, these inversions may prevent recombination between *sr* and non-*sr* sequences, thereby keeping intact those alleles or groups of alleles controlling *sr*.

TABLE 2

The effect of Y_L *and* 0 *on the expression of* sr *in the two* X *chromosome sequences*

X chromosome sequence	Male parent	*br rs jv* ♀♀ parents			*sc* ♀♀ parents		
		$\bar{k}_{♀}$	♂♂ Fertile / ♂♂ Tested	Mean progeny per fertile ♂	$\bar{k}_{♀}$	♂♂ Fertile / ♂♂ Tested	Mean progeny per fertile ♂
XS-III XL-I	*br rs jv*/0	0.46 ± 0.06	20/20	118 ± 44	0.47 ± 0.08	19/20	105 ± 29
(control)	*br rs jv*/Y_L	0.50 ± 0.06	20/20	123 ± 40	0.50 ± 0.05	19/20	113 ± 45
	TAU-3/0	0.00	6/10	73 ± 29	0.00	9/10	61 ± 57
	TAU-3/Y_L	0.97 ± 0.01	10/10	173 ± 34	0.97 ± 0.02	10/10	115 ± 32
XS-I XL-II	TAU-13/0	0.00*	6/10	60 ± 45	0.00	7/10	34 ± 36
(sex ratio)	TAU-13/Y_L	0.98 ± 0.02	9/9	157 ± 46	0.97 ± 0.03	10/10	141 ± 38
	NJE-13/0	0.00*	8/10	59 ± 50	0.00	6/10	28 ± 26
	NJE-13/Y_L	0.98 ± 0.01	10/10	167 ± 42	0.98 ± 0.02	10/10	117 ± 30
	TAU-9/0	0.68 ± 0.11	9/10	87 ± 47	0.69 ± 0.15	10/10	86 ± 51
	TAU-9/Y_L	0.56 ± 0.04	10/10	116 ± 38	0.57 ± 0.08	10/10	139 ± 36
	TAU-15/0	0.44 ± 0.09	8/10	91 ± 43	0.46 ± 0.06	10/10	48 ± 32
XS-II XL-IV	TAU-15/Y_L	0.52 ± 0.02	10/10	117 ± 30	0.54 ± 0.03	10/10	118 ± 45
(sex ratio)	NLI-54/0	0.54 ± 0.08	8/10	62 ± 39	0.54 ± 0.03	7/10	104 ± 26
	NLI-54/Y_L	0.56 ± 0.05	10/10	120 ± 20	0.54 ± 0.06	10/10	142 ± 27
	NLI-82/0	0.82 ± 0.10	6/10	22 ± 15	0.95 ± 0.08	3/10	20 ± 20
	NLI-82/Y_L	0.58 ± 0.05	10/10	116 ± 36	0.55 ± 0.09	10/10	92 ± 51

* A single female was found among the progeny of one of these males.

In this study and in other unpublished cytological investigations of *D. affinis*, *sr* has never been observed in the absence of either XL-II or XL-IV, while XS-I and XL-I (the included inversion of sequence XL-IV) have on numerous occasions been observed separate from *sr*. This suggests that the genetic factor(s) controlling *sr* may lie somewhere within or adjacent to the large inversions of XL-II and XL-IV. That *sr* should be located in the long arm of the X chromosome is not unexpected. STURTEVANT (1940) had inferred its existence in XL. In addition, XL of *affiinis* is element *D* (STURTEVANT and NOVITSKI 1941), the same element which contains *sr* in *D. pseudoobscura* and *persimilis* (STURTEVANT and DOBZHANSKY 1936).

The ability of the *Y* chromosome to modify *sr* in *D. affinis* is rather remarkable. The availability data indicate that Y^L suppresses *sr* when combined with sequence XS-II XL-IV ($\bar{k}_{\varphi} = 0.52$–0.58) but not when combined with sequence XS-I XL-II ($\bar{k}_{\varphi} = 0.97$–0.98). These observations are similar to those described by STALKER (1961) in *D. paramelanica* where northern and southern types of *sr*'s and *Y* chromosomes exist; southern type *Y* chromosomes suppress northern type *sr*'s, but all other combinations of *sr's* and *Y* chromosomes are phenotypically *sr* in the absence of suppression by the minor autosomal system.

In the absence of a *Y* chromosome sequence XS-II XL-IV exhibits no uniform behavior. The $\bar{k}_{\varphi}$ values vary from 0.44 to 0.95, being consistent within the same strain but different in different strains. On the other hand, sequence XS-I XL-II in the absence of a *Y* gave consistent $\bar{k}_{\varphi}$ values of 0.00. Thus, the type of *Y* chrosome present, including the absence of a *Y*, contributes to the variable expression of *sr* and may well have been responsible for the variable expression of *sr* reported by STURTEVANT (1940) and NOVITSKI (1947). Previous investigations have shown the *Y* chromosome in *D. affinis* to be extremely variable in size and shape (MILLER and STONE 1962; MILLER and ROY 1964; MILLER personal communication); perhaps *Y* chromosomes other than the above may confer additional variability on one or both *sr* sequences.

If the earlier reported *msr* and that reported here are to be considered identical. it should be possible to reconcile the earlier results with the hypothesis offered here. NOVITSKI (1947) attributed *msr* to an interaction between *sr* and homozygosis for a recessive allele (*a*) on autosome B, while the interpretation presented here attributes *msr* to an interaction between *sr* sequence XS-I XL-II and the absence of a *Y* chromosome. That the genetic basis of *msr* is the presence of *sr* sequence XS-I XL-II in combination with the 0 condition is confirmed by the crosses used to derive *msr* males. Numerous crosses have been made with *sr* sequence XS-I XL-II during these and previous unpublished experiments. In such crosses *msr* males are always produced when sequence XS-I XL-II is put in combination with the 0 condition, whether the source of the 0 condition is from any of various *X/0* inbred or outbred strains. On the other hand, combination of sequence XS-I XL-II with *Y* chromosomes from any of various inbred or outbred *X/Y* strains never yielded the *msr* condition. Thus, while the autosomes have not been followed as NOVITSKI attempted to do, the nature of the crosses used to

derive *msr* males present very strong evidence that the interpretation presented here is indeed correct. In the following, the earlier results are reinterpreted in light of the explanation offered here:

(1) NOVITSKI (1947, p. 528) states, "If a female of the constitution *w m/sr* is outcrossed to certain unrelated strains, as *asc st* or *jt net*, the F_1 *sr* males invariably show the typical 'sex ratio' effect. MSR males can be found among the *sr* males of the F_2." He does not indicate that *msr* males constitute approximately one-fourth the F_2 *sr* males (as would be expected in the case of an autosomal recessive), but only that "MSR males may be found among the *sr* males of the F_2." If, instead of the above interpretation, it is assumed that the F_2 *msr* males were $X^{sr}/0$, they could be produced in two ways. First they could arise by fusion of nullo-*X* eggs and X^{sr}-bearing sperm from the "typically 'sex ratio' " F_1 males. Nullo-*X* eggs due to primary nondisjunction and/or *X* chromosome loss would be expected to occur in low frequency in females heterozygous for the *sr X* chromosome and non-*sr X* chromosomes which lacked one or both of the inversions borne by the *sr* sequence (STURTEVANT and BEADLE 1936). Second, $X^{sr}/0$ males could have been produced by the fusion of normal X^{sr}-bearing eggs and nullo-*X* or nullo-*Y* sperm; such sperm apparently constitute a substantial proportion of the non-*X*-bearing sperm produced by *sr* males since in at least some species (STURTEVANT and DOBZHANSKY 1936; STALKER 1961; VOELKER and KOJIMA 1971) sons of "sex ratio" males are sterile due to the absence of a *Y* chromosome. NOVITSKI states that secondary nondisjunction was not observed although it could for the most part have been detected due to the nature of the crosses and mutant markers used; the primary nondisjunction and/or chromosome loss suggested in the above crosses could not have been detected by observation of the progeny classes.

(2) NOVITSKI's interpretation that some of his mutant strains were heterozygous or homozygous for the autosomal allele *a* may be explained by suggesting that these mutant strains contained *X/0* and *X/Y* males (heterozygous for *a*) or only *X/0* males (homozygous for *a*).

(3) NOVITSKI was unable to synthesize *msr* when a *Y* chromosome was forcibly retained because of *Y*-autosome translocations; he considered the possible involvement of a *Y* chromosome but dismissed it as being unlikely. The finding is, however, consistent with the absence of a *Y* chromosome in *msr*.

(4) NOVITSKI reported that cytological analyses of spermatogonial metaphases of three progeny of *msr* males revealed that two lacked a *Y* chromosome and one possessed a *Y* chromosome. The absence of a *Y* is consistent with the explanation offered here. The presence of a *Y* in the third male may be explained by suggesting that a) there really was a *Y* present and that it was received from the female parent by secondary non-disjunction (considered unlikely by NOVITSKI); or b) an error was made in identification of the *Y* chromosome in the spermatogonial metaphase and that really no *Y* was present. The supposition that a *Y* chromosome was necessary for male fertility in most or all Drosophila species may have contributed to an incorrect analysis.

(5) The characteristic sterility or low fertility of *msr* males was especially

noteworthy in NOVITSKI's work and in the present study. The observations of dissected females which had been mated to *msr* males but produced no or very few progeny are very similar in both reports.

(6) NOVITSKI's inability to resynthesize the *msr* after his earlier stocks were lost may have been due to his having used *sr* sequence XS-II XL-IV, which in these studies never gave rise to *msr*.

Thus, having considered the evidence presented by NOVITSKI and that presented here, it appears that both relate to the same *msr* phenomenon. Nearly all of NOVITSKI's data can be readily reinterpreted in a manner consistent with the genetic basis of *msr* suggested here. Only his report of the presence of a *Y* chromosome in a son of a *msr* male appears inconsistent; however, it too can be explained in terms of misidentification of a chromosome.

An interesting speculation concerns the reason for the occurrence of *msr* only in *D. affinis*. Thus far, *D. affinis* and *D. narragansett* are the only two known species whose males regularly possess a *Y* chromosome but which are *X/0* fertile (VOELKER and KOJIMA 1971). *D. affinis* is the only known species where both *X/0* male fertility and *sr* occur; since *sr* has not been reported in *D. narragansett*, *msr* may be expected not to occur in that species.

The author expresses his appreciation to the following persons for providing flies used in this study: DR. MAX LEVITAN, Mt. Sinai School of Medicine, New York, N.Y. and DR. DWIGHT D. MILLER, University of Nebraska, Lincoln, Neb. Special thanks are also due to DR. EDWARD NOVITSKI for providing laboratory facilities and sponsoring the author during the post-doctoral fellowship tenure.

LITERATURE CITED

DOBZHANSKY, TH. and D. SOCOLOV, 1939 Structure and variation of the chromosomes of *Drosophila azteca*. J. Heredity **30**: 3–19.

GERSHENSON, S., 1928 A new sex ratio abnormality in *Drosophila obscura*. Genetics **13**: 488–507.

JUNGEN, H., 1968 "Sex ratio" in natürlichen Populationen von *Drosophila subobscura*. Jahresber. Schweiz. Ges. Vererbforsch. **28**: 52–57.

MILLER, D. D., 1971 On the identity of the "sex ratio" *X* chromosome of "eastern" *Drosophila athabasca*. Drosophila Inform. Ser. **46**: 95.

MILLER, D. D. and L. E. STONE, 1962 A reinvestigation of karyotype in *Drosophila affinis* and related species. J. Heredity **53**: 12–24.

MILLER, D. D. and R. ROY, 1964 Further study of variation of the *Y* chromosome of *D. affinis* subgroup species. Drosophila Inform. Serv. **39**: 117.

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

POLICANSKY, E. and J. ELLISON, 1970 "Sex ratio" in *Drosophila pseudoobscura*: Spermiogenic failure. Science **169**: 888–889.

STALKER, H. D., 1961 The genetic systems modifying meiotic drive in *Drosophila paramelanica*. Genetics **46**: 177–202.

STONE, L. E., 1967 Investigation of salivary gland chromosome variation in *Drosophila affinis*. A doctoral dissertation. University of Nebraska, Lincoln, Nebraska.

STURTEVANT, A. H., 1940 Genetic data on *Drosophila affinis* with a discussion of the relationships in the subgenus Sophophora. Genetics **25**: 337–353.

STURTEVANT, A. H. and E. NOVITSKI, 1941 The homologies of the chromosome elements in the genus Drosophila. Genetics **26**: 517–541.

STURTEVANT, A. H. and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 544–604.

STURTEVANT, A. H. and TH. DOBZHANSKY, 1936 Geographical distribution and cytology of "sex ratio" in *Drosophila pseudoobscura* and related species. Genetics **21**: 473–490.

VOELKER, R. A., 1967 Further studies on the genetics of *Drosophila affinis*. Genetics **56**: 593.

——, 1968 Drosophila species—New mutants, Report of R. A. VOELKER. Drosophila Inform. Serv. **43**: 78.

VOELKER, R. A. and K. KOJIMA, 1971 Fertility and fitness of *X*0 males in Drosophila. I. Qualitative study. Evolution **25**: 119–128.

WILSON, F. D., M. R. WHEELER, M. HARGET and M. KAMBYSELLIS, 1969 Cytogenetic relations in the *Drosophila nasuta* subgroup of the *immigrans* group of species. Univ. Texas Publ. **6918**: 208–253.