SONLESS, A SEX-RATIO ANOMALY IN *DROSOPHILA MELANOGASTER* RESULTING FROM A GENE-CYTOPLASM INTERACTION¹

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THE mode of sex determination in *Drosophila melanogaster* leads to a Mendelian expectation of a 1:1 sex ratio. However, in *Drosophila* numerous deviations from this expectation have resulted from a wide variety of mechanisms. These fall into three main categories: aberrant meiotic segregation, gametic selection and zygotic selection.

Cases involving aberrant sex-chromosome segregation have been reported by STURTEVANT and DOBZHANSKY (1936), NOVITSKI (1947), SANDLER and HIRAI-ZUMI (1959), ERICKSON (1965), YANDERS (1965), HANKS (1965, 1969) and FAULHABER (1967). Gametic selection was reported initially by GERSHENSON (1928). Either mechanism can distort the primary sex ratio at fertilization and barring a compensatory mortality during the larval or pupal stages, result subsequently in an abnormal sex ratio among the emerging adults.

The third mechanism, zygotic selection, may act selectively against either male or female zygotes any time between fertilization and adult emergence. Aberrations belonging to this last category have been attributed to cytoplasmically transmitted infectious particles (MAGNI 1953, 1954; CAVALCANTI, FALCÃO and CASTRO 1957; MALOGOLOWKIN 1958; POULSON and COUNCE 1959; POULSON and MALOGOLOWKIN 1959; POULSON and SAKAGUCHI 1961) as well as to nuclear genes (REDFIELD 1926; BELL 1954).

This paper describes the action of "sonless," a sex-ratio anomaly in *D. melanogaster* which results from zygotic selection. The condition is unique in that it is chromosomally transmitted but results from an interaction of the maternal cytoplasm with a nuclear gene.

MATERIALS AND METHODS

Description of anomaly: The "sonless" sex-ratio anomaly of *Drosophila melanogaster* was first noted by PARÉ (1964). Subsequent study (COLAIANNE 1969) has revealed that the condition is inherited as a sex-linked recessive gene (sonless; *snl*) located on chromosome 1 at approximately 56.1, near and to the left of the forked (*f*) locus. The frequency of males among the progeny of *snl*/*snl* females averages less than 1% regardless of the male parent's genotype, while *snl*/+ females produce normal sex ratios. The *snl*/*snl* females, whether from *snl*/*snl* or *snl*/+ mothers, are phenotypically wild type with the aberrant sex ratio among progeny being the only manifestation of their genotype. Males of the *snl*/Y genotype are also phenotypically wild type

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and they produce progeny with normal sex ratios when mated to females other than *snl/snl*. While the expression of the anomaly is the same at 28°C, 23°C, and 18°C, it can be modified to a small but significant degree by the genetic background.

Culturing conditions and techniques: Single-pair matings were used exclusively, and progeny were reared in half-pint bottles containing a standard cornmeal-agar medium. All cultures were maintained at 23°C and 70% relative humidity. For studies of hatchability, consecutive 24-hr egg collections were taken in half-pint bottles containing a button of banana-charcoal medium on the bottle caps as the sole source of food. After the number of eggs in each 24-hr collection had been recorded, the caps were incubated in individual Petri dishes under standard conditions for 48 hr. Each cap was then observed under 10× magnification for the number of eggs which had hatched (signified by empty egg cases) and for the number of larvae present (referred to as "48-hr larvae.") Finally, the contents from each cap were transferred to individual culture bottles containing standard medium and were reared under standard conditions. Adults emerging from each culture were sexed and counted. The notation used throughout to describe genotypes is that of LINDSLEY and GRELL (1968).

RESULTS

Time of gene action: An initial experiment was designed to investigate the possible sources of the sex-ratio distortion and to determine the stage in the life cycle at which *snl* operates. Four consecutive 24-hr egg collections were taken from each of 15 single-pair matings: 10 of the type *snl*+/*snl*+ × +*B*/*Y* and 5 of the type ++/++ × +*B*/*Y*. The latter matings acted as a control since the females (C3) had a genetic background similar to that of the *snl/snl* females.

The results from these matings are summarized in Table 1. In considering

TABLE 1
*Viability at various stages in the life cycle of offspring of snl/snl females
as compared with that for control females*

Ratios of various stages	Observed offspring viability by type of females			
	+/+		<i>snl/snl</i>	
	Ratio	Percent	Ratio	Percent
Adults/eggs laid	297/366	81.1	322/703	45.8*
Adult females/eggs laid	153/366	41.8	305/703	43.4
Eggs hatched/eggs laid	346/366	94.5	440/703	62.6*
48-hr larvae/eggs hatched	338/346	97.7	361/440	82.0*
Adults/48-hr larvae	297/338	87.9	322/361	89.2

* Probability of $\chi^2 < .001$ (Expected numbers of survivors *vs.* nonsurvivors at each stage for contrast with observed numbers of offspring of *snl/snl* females were determined from the corresponding observed frequencies for +/+ females.)

adults/eggs laid, it can be seen that the viability ratio for progeny of *snl/snl* females was nearly half that for +/+ females, yet the viability of adult female offspring was about the same for the two groups. This significant result is inconsistent with gametic selection or aberrant segregation since these hypotheses would predict an increase in the percentage of adult females from *snl/snl* mothers.

The alternative hypothesis that "sonless" is acting after fertilization is further

supported by the results in the third and fourth rows of Table 1 which correspond to embryonic and early larval viability, respectively. Both cases demonstrate a significant reduction in the viability of offspring from *snl/snl* females, with the greater decrease occurring previous to hatching. Apparently male zygotes are killed selectively during these stages. Zygotic viability between the 48-hr larval stage and adult emergence is presented in the bottom row and is about equal for the two types of offspring. Probably the majority of male offspring of *snl/snl* females have died in earlier stages, hence few sons are present for subsequent challenge. However, it is also plausible that the effect of the "sonless" condition decreases during development and that once a male zygote escapes the *snl/snl* egg cytoplasm he soon becomes "immune" to the effect of *snl*.

Phenomenon of exceptional males: Progeny testing of *snl/snl* females frequently used mates of the genotype *B/Y*. Under normal sex-linked inheritance, any sons surviving from such matings should appear wild type (*snl+/Y*) and the daughters should have heterozygous Bar eyes (*snl+ / +B*). While all daughters conformed to this expectation, surviving sons were of two types, wild type and Bar eye, with the latter type predominating.

In an attempt to relate this unexpectedly high frequency of exceptional sons to the action of *snl*, four types of matings were made as listed in Table 2. (Excep-

TABLE 2

Observed frequency of normal and exceptional male offspring from various matings, as determined by eye type

Mating*	Number of matings	Number of offspring			
		Males		Females	
		Normal (wild-type)	Exceptional (<i>B</i> or <i>w^a</i>)	Normal (<i>B/+</i> or <i>w^a/+</i>)	Exceptional (wild-type)
1. ♀ <i>snl+/snl+</i> × ♂ <i>B/Y</i>	30	0	2	1422	0
2. ♀ <i>snl+/snl</i> × ♂ <i>w^a/Y</i>	30	3	11	2472	not observed
3. ♀ <i>+/+/+</i> (C3) × ♂ <i>B/Y</i>	20	1454	4	1669	0
4. ♀ <i>+/+/+</i> (P1) × ♂ <i>B/Y</i>	30	749	0	802	0

* Females for crosses 1, 2 and 3 had the same genetic background (C3) and were unrelated to *+/+/+* (P1).

tional males receiving a paternal X chromosome as a consequence of abnormal segregation were initially observed by BRIDGES (1916) but in the relatively low frequency of 1/1200. He also observed exceptional females in a frequency of about 1/2500.) Three significant findings are evident from Table 2. First, sons are very rare among the offspring of *snl/snl* females and those which emerge are largely of the exceptional type. Secondly, exceptional male offspring are not the exclusive property of the *snl/snl* females (Crosses 1 and 2), but are also observed among the progeny of *+/+/+* (C3) females (Cross 3) which possess a genetic background similar to the *snl* stock. On the other hand, no exceptional males are observed among the offspring of the unrelated *+/+/+* (P1) females of Cross 4. Thus, the observation of exceptional sons is not an integral part of *snl* expression

but is related to the genetic background. Thirdly, exceptional males occur with equal frequency among the progeny of *snl/snl* and *+/+* (C3) females. (Data in addition to those reported for Crosses 1 and 3 of Table 2 indicate that both frequencies are about 1/500.) Hence, exceptional sons of *snl/snl* females apparently emerge unaffected by the "sonless condition" while sons resulting from normal segregation seldom survive. Of further interest is the fact that no "exceptional" type daughters are observed from any of the matings listed in Table 2.

The genetic markers used in the crosses of Table 2 clearly show that the exceptional males had received a paternal *X* chromosome and all proved sterile on test mating. These two facts suggest that the exceptional males had an *X/0* sex-chromosome composition, but to substantiate this conclusion a testcross was made utilizing a genetically marked *Y* chromosome. The results from thirty single-pair matings are summarized in Table 3. The only sons observed among the resulting

TABLE 3

Further test for the presence of Y chromosome in exceptional sons of snl/snl females

Mating	Number of offspring			
	Males		Females	
	Bar	Exceptional* (<i>su-w^aw^a</i>)	Wild	Exceptional (<i>Bar/+</i>)
♀ <i>snl/snl</i> × ♂ <i>B^sY/γ² su-w^aw^a</i>	0	7	3439	0

* Eyes non-Bar with a brownish color resulting from the combination of apricot (*w^a*) and suppressor of apricot (*su-w^a*).

offspring were exceptional in that they received a paternal *X* chromosome. Furthermore, the round (non-Bar) eyes revealed that these males lacked a paternal *Y* chromosome. The possibility that these exceptional sons had received a maternal *Y* chromosome from *X/X/Y* females is exceedingly remote in view of the absence of heterozygous Bar eye daughters among the offspring (chromosome segregation in *X/X/Y* females is such that at least a few *X/X/B^sY* daughters would be expected.)

Therefore the above results establish the fact that the exceptional sons of *snl/snl* females have a paternal *X* chromosome and lack a *Y* chromosome, verifying their *X/0* composition.

Importance of Y chromosome: Since the exceptional males proved to be *X/0*, the question arose as to why they should predominate among the sons of *snl/snl* females and apparently be unaffected, while exceptional *X/X/Y* daughters were never observed (see Tables 2 and 3). It appeared that progeny having a *Y* chromosome were at a decided disadvantage regardless of their sex. This in turn suggested that the presence of the *Y* chromosome might be the factor responsible for the differential nature of the *snl* lethality, its presence being the necessary catalyst for death.

Special *X-Y* chromosomal combinations (LINDSLEY and GRELL 1968) provided

an effective genetic tool to check this hypothesis. Twenty single-pair matings were made of the following type:

$$snl/snl \times ywf Y^L Y^s/0$$

The male parents used had attached-XY chromosomes with no free Y, and because of the aberrant segregation pattern of the X and Y under these conditions the vast majority of female zygotes formed were X/X/Y, and the majority of male zygotes were X/0, (the X in this case being maternal in origin.) If the Y chromosome hypothesis were correct, one would expect the X/X/Y daughters from the *snl/snl* females to die and the X/0 sons to survive, consequently, most of the offspring would be male. The actual results were in sharp contradiction, 1749 daughters were observed but no sons. Obviously, the X/0 genotype *per se* did not impart any advantage to sons, nor did the presence of the Y chromosome in X/X/Y daughters impart any detectable disadvantage. However, it should be noted that the X/0 zygotes formed in this case received maternal X chromosomes carrying *snl*. In contrast, the exceptional X/0 males observed in previous "sonless testcrosses" received paternal X chromosomes carrying the normal allele of *snl*.

DISCUSSION

The appearance of exceptional or X/0 sons among the progeny of *snl/snl* females provided an important key to the understanding of *snl* action, yet the nature of their origin is uncertain. Nondisjunction is an unlikely source since it should yield equal frequencies of X/0 and X/X/Y offspring, but the latter were never observed except in special matings involving attached-XY chromosomes. A more plausible hypothesis suggests meiotic lagging or systemic elimination of the X chromosome from some egg nuclei. Fertilization of such ova by X bearing sperm would result in X/0 sons.

Regardless of their origin, however, these exceptional males have revealed an important feature of the "sonless" condition. An X/0 son of an *snl/snl* female is unaffected by the condition's lethal action if it receives an X chromosome carrying the normal allele, yet it is subject to the lethality if it receives an X carrying *snl*. Thus, two requirements predispose lethality for male offspring. First, the female parent must be homozygous for *snl*. Secondly, the son must carry *snl*, either as *snl/Y* or *snl/0*. Consequently, the "sonless" genetic anomaly results from an interaction between the maternal cytoplasm and the genotype of the male zygote. Since "sonless" is inherited distinctly as a sex-linked recessive gene (no evidence for infectious cytoplasmic particles) this interaction appears quite unique among sex-ratio anomalies.

It is our hypothesis that *snl* inhibits the synthesis of some substance (or substances), possibly a hormone, necessary in male ontogeny. This leads to a deficiency in the cytoplasm of eggs from *snl/snl* females. Normal male zygotes (*snl/Y*) of such females cannot obtain enough of the essential substance to survive. However, exceptional sons possessing the normal allele of *snl* are able to synthesize the deficient substance during embryogenesis and develop normally.

Since the eggs from *snl/+* and *+/+* females support the normal development

of *snl/Y* sons, this substance is apparently essential only during early development. All evidence indicates that female zygotes are unaffected by the cytoplasmic deficiency, thus suggesting that *snl* controls a developmental pathway unique to male differentiation.

SUMMARY

The "sonless" sex-ratio anomaly in *D. melanogaster* is inherited as a sex-linked, recessive gene (*snl*, 1-56.1). The frequency of male offspring among the progeny of homozygous (*snl/snl*) females is less than 1% regardless of the male parent's genotype. Heterozygous (*snl/+*) females and hemizygous (*snl/Y*) males have normal progeny sex ratios, and both can transmit the gene to subsequent progeny. —Viability studies of offspring from *snl/snl* females indicated that *snl/Y* sons die during the embryonic or early larval stages, while exceptional sons (*X/0*) carrying the normal allele of *snl* (i.e., *+/0*) escape the lethal action. Thus, two requirements predispose lethality for male zygotes. First, the female parent must be homozygous for *snl*. Secondly, the male offspring must carry the mutant gene. Consequently, "sonless" is unique in that it is chromosomally transmitted yet results from an interaction of maternal cytoplasm with the nuclear gene.—It is hypothesized that *snl* inhibits the synthesis of a substance (or substances) essential for male ontogeny. Exceptional sons possessing the normal allele of *snl* are able to synthesize the deficient substance during embryogenesis and overcome the maternal deficiency of eggs from *snl/snl* females. Furthermore, a sufficient amount of the requisite substance is in eggs from *snl/+* females to provide for the normal development of *snl/Y* sons.

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