STUDIES ON THE EXPRESSION OF VARIOUS SINGED ALLELES IN *DROSOPHILA MELANOGASTER*

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RECENT work on a set of alleles that pleiotropically affect both setal morphology and oogenesis has provided a complex of data that have not been systematically analyzed. These alleles constitute the sex-linked mutation *singed*. In 1951, IVES and NOYES demonstrated 0.04 percent crossing over between *sn*³ and *sn*^{50k}. Genes such as these which are physically close together, similar in function, and yet separable by crossing over are called pseudoalleles. Position pseudoallelism (LEWIS 1942) was demonstrated among the *singed* mutants by HEXTER (1955) who showed that *sn*³ +/+ *sn*¹ flies displayed a *singed* phenotype while *sn*³ *sn*¹/++ flies were wild type in appearance. Subsequently he demonstrated three loci in the *sn* complex which he preliminarily characterized (HEXTER 1957).

Twenty-eight *singed* mutations have been reported since MOHR's first record (1922), but at present only 11 are available, and these were used in the following study. These stocks and progeny of the possible combinations were examined for bristle morphology, fertility, and the anatomy of the female reproductive tract. The classes of phenotypes were analyzed; this phenotypic array of gene effect was then used together with crossover data to provide a model for the loci involved.

MATERIAL AND METHODS

The *Drosophila melanogaster* stocks used in this investigation were supplied through the kindness of Drs. R. C. KING, W. HEXTER, I. I. OSTER, and D. F. POULSON. The history of the *Drosophila melanogaster* stocks utilized and their source is provided in Tables 1 and 2.

The flies in this study were grown on medium (corn meal-Karo-agar mixture) at 25°C in half-pint bottles kept in a refrigerated incubator. Observations of the setal morphology of 25 males and 25 females of each of the available *sn* mutants were made with stereoscopic microscope. Bristles were scored as "gnarled" if the anterior and/or posterior scutellar setae were knotted together, "kinky" if they were thickened or bent. Hairs were scored as "kinky" if any bending or kinking

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TABLE 1
Singed mutants

Singed mutant	Reproductive ability of homozygote	Discoverer	Date first observed	Occurrence	Stock maintained as	Source
<i>sn</i> ¹	sterile	MOHR*	Oct. 5, 1918	Spontaneous	<i>sn</i> ¹ /M5	Amherst College
<i>sn</i> ²	fertile	BRIDGES*	Dec. 2, 1919	Spontaneous	<i>sn</i> ²	Amherst College
<i>sn</i> ³	fertile	MOHR*	July 11, 1922	Spontaneous	<i>sn</i> ³	Amherst College
<i>sn</i> ⁴	fertile	BRIDGES*	Jan. 26, 1930	Spontaneous	<i>sn</i> ⁴	Amherst College
<i>sn</i> ^{34e}	fertile	DUNCAN*	May 20, 1934	Spontaneous	<i>sn</i> ^{34e}	Amherst College
<i>sn</i> ⁵	sterile	BRIDGES*	Feb. 5, 1930	Spontaneous	<i>w sn</i> ⁵ /M5	Amherst College
<i>sn</i> ^{5ok}	sterile	IVES and NOYES†	Nov., 1950	X-ray induced	<i>γ sn</i> ^{5ok} <i>ras</i> ² /M5	Amherst College
<i>sn</i> ^{36a}	sterile	SPENCER*	Jan. 21, 1936	Spontaneous	<i>sn</i> ^{36a} /M5	Cal. Inst. of Tech.
<i>sn</i> ^{x2}	sterile	MULLER‡		X-ray induced	<i>In</i> (1)49 <i>sn</i> ^{x2} /M5	Indiana Univ.
<i>sn</i> ^{55a}	fertile?	HILLMAN§	Jan., 1955	Spontaneous	<i>γ</i> ² <i>w</i> ^a <i>cv sn</i> ⁵⁵ <i>v</i> + <i>f</i>	Yale University
<i>sn</i> ^c	sterile	MULLER‡		Spontaneous	<i>sn</i> ^c / <i>γ Hw In</i> (1)49 <i>m g</i>	Indiana Univ.

* BRIDGES and BREHME 1944.
† IVES and NOYES 1951.
‡ DR. I. I. OSTER (Personal communication).
§ HILLMAN 1958.

TABLE 2
Singed double mutants synthesized HEXTER (Amherst College)

Double singed mutant	Fertility of homozygote	Stock
<i>sn</i> ¹ <i>sn</i> ²	sterile	<i>cm ct</i> ⁶ <i>sn</i> ² <i>sn</i> ¹ /M5
<i>sn</i> ¹ <i>sn</i> ³	sterile	<i>cm ct</i> ⁶ <i>sn</i> ³ <i>sn</i> ¹ /M5
<i>sn</i> ¹ <i>sn</i> ⁴	sterile	<i>cm ct</i> ⁶ <i>sn</i> ⁴ <i>sn</i> ¹ /M5
<i>sn</i> ² <i>sn</i> ³	sterile*	<i>ct</i> ⁿ <i>sn</i> ³ <i>sn</i> ² /M5
<i>sn</i> ² <i>sn</i> ³	fertile*	<i>ct</i> ⁿ <i>sn</i> ³ <i>sn</i> ² /M5

* See text.

could be observed. A wild type phenotype of the hairs denotes no visible bending or kinking.

Quadruplicate crosses were made in all the possible permutations of representatives of the three known pseudoallelic loci of the *sn* complex. Reciprocal crosses were made also. The results of these reciprocal crosses indicated no differences and consequently, the data were considered collectively. The morphology of the bristles and hairs of 25 females emerging from each cross was examined, and flies were placed in the categories described above. Flies were classified as fertile or sterile on the basis of the presence or absence of larvae in quadruplicate cultures where six freshly hatched females of the genotype to be tested were crossed to six freshly hatched males. Feulgen-stained whole mounts were made of the ovaries of the various singed mutants. The procedure for the preparation of Feulgen whole mounts was developed from KING, BURNETT, and STALEY (1957).

The stages of oogenesis as described by KING, RUBINSON and SMITH (1956) were utilized in this study.

RESULTS

Classification based on morphology of bristles and hairs

The available *sn* mutants can be classified into three categories on the basis of bristle and hair morphology: Class A—kinky hairs and gnarled bristles (sn^1 , sn^3 , sn^5 , sn^{50k} , sn^1sn^2 , sn^1sn^3 , sn^1sn^4 , sn^2sn^3 , sn^{x2} , and sn^c); Class B—kinky bristles only (sn^2 , sn^4 , and sn^{34e}); Class C—gnarled bristles only (sn^{36a}). These observations support and supplement those of HEXTER (1957). Figure 1 demonstrates the typical appearance of wild type bristles and hairs. Figures 2 and 3 illustrate the typical appearance of the Class A, sn^1 and sn^3 mutants. Figures 4 and 5 illustrate the appearance of two Class B mutants sn^2 and sn^4 . Figure 6 illustrates the phenotype of the Class C mutant sn^{36a} . It is interesting to point out that the double mutant homozygotes sn^1sn^4 , sn^2sn^3 and sn^1sn^2 shown in Figures 7, 8, and 9 respectively, are indistinguishable from one another and from the homozygous Class A mutants.

The *sn* mutants can be classed into four categories if fertility data are used in addition to setal morphology: Class 1—sterile with kinky hairs and gnarled bristles (sn^1 , sn^2 , sn^{50k} , sn^{x2} , sn^c and the double mutants); Class 2—fertile with kinky bristles only (sn^2 , sn^4 , and sn^{34e}); Class 3—fertile with kinky hairs and gnarled bristles (sn^3); and Class 4—sterile with gnarled bristles only (sn^{36a}).

Phenotypic consequences of genotypic combinations of sn mutants

Fertility: It was found that any individual compounded with a fertile *sn* allele on one chromosome was fertile, and that an individual homozygous for any two sterile *sn* alleles was sterile (Table 3). However, individuals homozygous for the double mutant sn^2sn^3 are sterile; whereas individuals homozygous for sn^2 or sn^3 are fertile. The sterility of the sn^2sn^3 stock is puzzling since synthesis of a second sn^2sn^3 stock (HEXTER, personal communication) resulted in a highly fertile double homozygote.

Setal morphology: It was noted (Table 3) that Class 2 bristle and hair morphology is dominant over Class 3 and Class 4 and blends with Class 1. Class 4 bristle and hair morphology is dominant over Class 1 and 3.

Oogenesis in singed mutants

Cytological investigations: Oogenesis was studied in representatives of all members of all four classes of mutants and in representatives of most of the sterile progeny of all crosses among these four classes as listed in Table 3. The number of ovaries examined in each case is given in Table 4.

Two major types can be recognized: I. Normal oogenesis occurs in all fertile *sn* mutants; II. Abnormal oogenesis occurs in infertile *sn* mutants. The sterile category can be further subdivided on the basis of differences in their oogenesis

FIGURE 1.—The dorsal aspect of the thoracic region of a wild type *Drosophila melanogaster*. Note the bristles (B) (macrochaetae) and hairs (H) (microchaetae). FIGURES 2 and 3.—Class A singed mutants (sn^1 and sn^3). Note the gnarled bristles and kinky hairs. FIGURES 4 and 5.—Two class B mutants, sn^2 and sn^4 . These mutants are typified by having kinky bristles and wild type hairs. FIGURE 6.—The Class C mutant sn^{36a} . Note the gnarled bristles and nonkinky hairs. Note also the hairs are somewhat intermediate to those of the Class A and wild types. FIGURES 7-9.—Three mutants homozygous for two singed alleles, (sn^1sn^4 , sn^2sn^3 , and sn^1sn^2 respectively).

TABLE 3
Crosses of all possible permutations of the three representatives of the singed pseudallellic complex

	Class 1 <i>sn¹</i>	Class 2 <i>sn²</i>	Class 3 <i>sn³</i>	Class 4 <i>sn³sn²</i>	<i>sn¹sn²</i>	<i>sn¹sn³</i>	<i>sn²sn³†</i>
Class 1 <i>sn¹</i>	gnarled b.* kinky h.† Sterile	gnarled b. wild type h. Fertile	gnarled b. kinky h. Fertile	gnarled b. wild type h. Sterile	gnarled b. kinky h. Sterile	gnarled b. kinky h. Sterile	gnarled b. kinky h. Sterile
Class 2 <i>sn²</i>		kinky b. wild type h. Fertile	kinky b. wild type h. Fertile	kinky b. wild type h. Fertile	kinky b. wild type h. Fertile	kinky b. wild type h. Fertile	kinky b. wild type h. Fertile
Class 3 <i>sn³</i>			gnarled b. kinky h. Fertile	gnarled b. wild type h. Fertile	gnarled b. kinky h. Fertile	gnarled b. kinky h. Fertile	gnarled b. kinky h. Fertile
Class 4 <i>sn³sn²</i>				gnarled b. wild type h. Sterile	gnarled b. wild type h. Sterile	gnarled b. wild type h. Sterile	gnarled b. wild type h. Sterile
<i>sn¹sn²</i>					gnarled b. kinky h. Sterile	gnarled b. kinky h. Sterile	gnarled b. kinky h. Sterile
<i>sn¹sn³</i>						gnarled b. kinky h. Sterile	gnarled b. kinky h. Sterile
<i>sn²sn³</i>							gnarled b. kinky h. Sterile

* Bristles.
† Hairs.
‡ Sterile (see text).

TABLE 4

Ovarian preparations

Genotype	Age in hours	Number of preparations	Appearance
sn^1/sn^1	156 ± 12	27	Abnormal
sn^2/sn^2	180 ± 12	20	Normal
sn^3/sn^3	120 ± 12	26	Normal
sn^4/sn^4	144 ± 5	13	Normal
sn^5/sn^5	156 ± 12	9	Abnormal
sn^{34e}/sn^{34e}	120 ± 24	18	Normal
sn^{50k}/sn^{50k}	156 ± 12	14	Abnormal
sn^{36a}/sn^{36a}	varying*	219	Abnormal
sn^3/sn^{36a}	168 ± 10	20	Normal
sn^2/sn^{36a}	156 ± 12	8	Normal
sn^{34e}/sn^{36a}	168 ± 24	13	Normal
sn^4/sn^{36a}	156 ± 12	15	Normal
sn^{36a}/sn^2sn^3	156 ± 12	2	Abnormal
sn^{36a}/sn^1sn^3	156 ± 12	2	Abnormal
sn^{36a}/sn^1	156 ± 12	1	Abnormal
sn^1sn^2/sn^1sn^2	156 ± 12	14	Abnormal
sn^1sn^3/sn^1sn^3	156 ± 12	10	Abnormal
sn^1sn^4/sn^1sn^4	156 ± 12	12	Abnormal
sn^2sn^3/sn^2sn^3	156 ± 12	13	Abnormal
		Appearance	Number
Totals		Normal	122
		Abnormal	104
		Abnormal (sn^{36a})	219
			445

* See Table 6.

into subcategories IIa and IIb. Class IIa is represented only by the sn^{36a} mutant. KING and BURNETT (1957) reported several abnormalities of the 168-hour ovarian tissue in sn^{36a} flies. These observations have been verified and supplemented (BENDER and KING 1958) and show that nurse cell nuclei of the chambers past stage 4 contain abnormally large plasmosomes. (It is possible that the total amount of plasmosomal material remains unchanged, but that one large plasmosome is formed instead of several smaller ones). Further the Feulgen-positive material is abnormally distributed and reduced in quantity (Figure 10). Fusion

FIGURES 10-12.—Photographs of Feulgen-stained whole mounts of ovarian tissue. FIGURE 10.—Stage 7 and 9 follicles of a 168-hour sn^{36a} mutant. Notice the peripheral distribution of Feulgen-positive material and large plasmosome (P) in the trophocyte nucleus. A columnar follicular epithelium (F) surrounds the oocyte (O). The cytoplasm of a large, posteriorly located trophocyte (T) in the stage 9 follicle is indicated. FIGURE 11.—Several ovarioles of a 168-hour sn^{36a} mutant. Many of the chambers are wider than long (W). Pycnotic nurse cell nuclei (Py) are apparent. FIGURE 12.—A stage 13 chamber from a 156-hour sn^1 ovary demonstrating the blunted micropylar cone (M), shortened dorsal filament (D), and chorion (C). Note the vacuolated and nonhomogeneous appearance of the yolk.

of adjacent chambers in 168-hour *sn^{36a}* ovarian tissue occurs rarely, but the incidence of fusion increases with age. The volumes of the nuclei of the nurse cells are markedly increased in the mutant chambers. Chambers corresponding in volume to stage 6 and 7 contain nuclei equivalent in volume to those characteristic of stages 8–10. Oogenesis apparently ceases at stage 13. This is a slightly arbitrary statement, since the distinction between stage 13 and stage 14 in wild type flies is largely based on different stages in the development of the chorion, micropylar apparatus, and dorsal appendages. In *sn* mutants the development of these structures is so abnormal that they are no longer suitable for identifying stages.

Examination of the ovarian tissue of the sterile categories of *sn* mutants demonstrates that the method of classification of the chambers developed by KING, RUBINSON and SMITH (1956) cannot be applied readily to stages 11 through 14. Increase in the volume of yolk is inhibited to a greater extent than is formation of the dorsal appendages. Consequently, dorsal appendages which normally appear at stage 13 are present at a stage which would be classified as 11 with respect to volume of yolk. This observation demonstrates the rather autonomous behavior of the dorsal appendages which develop at the appropriate time independently of the volume of ooplasm.

Ovaries of 96- to 168-hour flies appear quite similar. Several ovarioles of a 168-hour *sn^{36a}* mutant are shown in Figure 11. Many chambers are rectangular with the long axis perpendicular to the long axis of the ovariole, whereas the wild type chamber is elongated in the direction of the axis of the ovariole thereby confirming earlier observations of KING and BURNETT (1957). Pycnotic nurse cell nuclei are quite noticeable in degenerating chambers. Oogenesis has usually advanced no farther than stage 11. Degeneration of chambers often occurs before this stage is reached.

Class IIb contains all other sterile *sn* mutants studied. All members of this group show similar cytological abnormalities. Again, the shape of the developing chambers in optical section often is rectangular with the long axis perpendicular to the long axis of the ovariole. The younger chambers appear to have been subjected to compression. Such a compression would be expected if early stages in oogenesis proceed at a normal rate, whereas later stages are retarded. Fully developed eggs of normal appearance are never present. The most advanced stage attained was 13, but these eggs were markedly abnormal. Dorsal filaments (as in *sn^{36a}*) were twisted and malformed, appearing shortened and thickened (Figure 12). The micropylar cone, when visible, seemed shorter and wider than in wild type eggs (Figure 12). Perhaps abnormalities of the micropyle prevent fertilization. The chorion forms late and seldom completely surrounds the anterior regions of the developing oocyte. MOHR (1922) reported that *sn¹/sn¹* females lay approximately the same number of eggs as heterozygous controls. He further noted that eggs of homozygous flies are short, blunt and wrinkled, and that their dorsal filaments are small and blunt and are inserted at a more central position than in eggs of wild type flies. MOHR's observations were corroborated by this study. In addition, eggs deposited by the sterile class were amorphous and thus differed strik-

ingly from the firm, plump, and well-formed eggs of fertile heterozygotes. Abnormalities of the chorion, at least in part, accounted for these differences. Yolk is often abnormally vacuolated in contrast to the homogeneous yolk of fertile heterozygotes. The occasional necrotic appearance of the yolk of the most developed chambers could result from their retention within the ovariole for abnormally long periods of time. Sterility in this class of mutants could well be a consequence of aberrant yolk and/or inability of sperm to penetrate the malformed micropyle.

Several interesting contrasts between oogenesis in class IIa and IIb can be made. Approximately two percent of the total chambers observed in the 144-hour *sn*^{36a} ovaries are degenerating as indicated by the presence of pycnotic nuclei. This represents more than a ninefold decrease from the number of degenerating chambers observed in class IIb ovarian tissue of similar age. As expected there is a significantly larger percentage of younger chambers in *sn*^{36a} ovaries.

Comparative studies of stage distribution between the two sterile categories and the wild type (Table 5) display several other interesting differences. Note that the *sn*^{36a} ovary has a higher percentage of chambers in all the stages of development (except the most mature chamber present, stage 13) than does the *sn*¹ mutant. Further, the percentage of chambers in yolk-forming stages (8–13) is greater in the *sn*^{36a} ovary than the *sn*¹ ovary (58% *vs.* 38%). Obviously vitellogenesis is retarded in the *sn*^{36a} ovary. A retardation would account also for the fact that the modal yolk stage of the IIa group is 13 while that of the IIb group is 11. This retardation of vitellogenesis is markedly shown in the IIa group by

TABLE 5

Stage distribution in sn^{36a}, *sn*¹ and wild type ovarian tissue of similar age

Stage	<i>sn</i> ^{36a} 144±6 hours (II a)			<i>sn</i> ¹ 156±4 hours (II b)			+ type 168 hours	
	n=27			n=27			n=10*	
	Percent	±(s _x)%		Percent	±(s _x)%		Percent	±(s _x)%
No yolk	1	9.11	0.04	8.18	0.06		13.59	0.83
	2	10.79	0.04	6.60	0.06		11.12	1.32
	3	9.90	0.03	1.99	0.03		10.13	1.10
	4	10.52	0.04	8.81	0.07		6.49	0.00
	5	0.80	0.01	0.21	0.01		3.19	0.55
	6	10.70	0.04	8.28	0.05		13.10	3.36
	7	10.34	0.04	7.44	0.06		12.88	1.54
Yolk forming	8	9.28	0.04	8.28	0.07		6.60	0.88
	9	4.16	0.02	1.36	0.02		3.96	0.38
	10	3.45	0.03	1.05	0.01		4.62	0.93
	11	16.00	0.05	2.41	0.02		0.44	0.22
Yolk formed	12	1.41	0.02	0.73	0.01		0.66	0.33
	13	3.54	0.09	44.65	1.22		0.22	0.22
	14		...		...		12.99	1.76
Total percent		99.99		100.00			99.99	

* KING (1957).

comparisons with IIb where the volume of yolky ooplasm is calculated in the two groups; whereas the volume of yolky ooplasm per female is $14 \times 10^7 \mu^3$ in *sn*^{36a} females (both stocks have 15–16 ovarioles per ovary). Chamber volumes are calculated from the formula $v = 0.5236 w^2 l$, where w = maximum width and l = maximum length (KING, RUBINSON and SMITH 1956).

It would thus seem that the abnormalities displayed by the two classes of mutants are closely related, differing mainly in the degree. The one possible exception is the abnormal behavior of the nurse cell nuclei of the IIa group. However, it is possible that this too will turn out in the course of further investigations to be a more drastic reflection of a similar effect. Cytochemical studies with the light microscope of sections of the yolk of these two groups and among members of the IIb category could be expected to provide further contrasts.

Stage distribution in sn^{36a} *as a function of time:* A study was made of the stage distribution of the developing chambers in ovaries of *sn*^{36a} homozygotes of varying ages. These data are summarized in Table 6 and compared with information for wild type ovaries of various ages in Figure 13. It appears that development can proceed to stage 13 by 144 hours, and that by 432 hours the majority of chambers have reached this developmental stage where their maturation is impaired. The percentage of chambers in stage 5 is considerably reduced in comparison with wild type flies, whereas stages 8 and 11 are significantly higher than in the latter. The high percentage of chambers in stage 13 of the 432-hour flies is reflected by the tandem arrangement of two or more of these follicles per ovariole.

TABLE 6
Stage distribution as a function of age of sn^{36a} *ovarian tissue*

		A		B		C		D		E	
		12 hrs.		42± 18 hrs.		96± 6 hrs.		144± 6 hrs.		432± 6 hrs.	
Stage*	Percent	± (s _x) %	Percent	± (s _x) %	Percent	± (s _x) %	Percent	± (s _x) %	Percent	± (s _x) %	
No yolk	1	15.38	2.85	11.32	0.16	9.30	0.99	9.11	0.04	6.83	0.06
	2	15.38	2.85	11.01	0.06	10.85	0.97	10.79	0.04	9.02	0.06
	3	15.38	2.85	13.21	0.17	12.40	1.05	9.90	0.03	9.02	0.07
	4	3.08	0.51	12.58	0.14	10.08	0.92	10.52	0.04	9.88	0.07
	5	1.54	0.51	0.51	0.03	0.78	0.19	0.80	0.01	0.37	0.01
	6	33.85	5.96	12.26	0.16	9.30	0.84	10.70	0.04	10.61	0.08
	7	15.38	2.24	11.64	0.12	11.63	0.87	10.34	0.04	12.68	0.08
Yolk forming	8		...	11.32	0.24	15.50	0.89	9.28	0.03	5.37	0.04
	9		...	7.55	0.02	6.20	0.55	4.16	0.02	2.20	0.04
	10		...	4.72	0.09	2.33	0.19	3.45	0.03	0.60	0.01
Yolk formed	11		...	1.26	0.06	11.63	0.67	16.00	0.05	0.98	0.02
	12		...	0.94	0.03		...	1.41	0.02		...
	13		...	1.57	0.06		...	3.54	0.09	32.44	0.22
	14		...		...		...		...		...
Total percent		99.99	...	100.01	...	100.00	...	100.00	...	100.00	...
Number of examined ovaries		6		17		4		27		21	

* Stage as percent of total number of chambers.

FIGURE 13.—A histogram showing the percent of the total number of *sn*^{36a} chambers in any stage of development in ovaries from flies of different ages. Comparisons may be drawn with information for wild type ovaries of various ages superimposed on the histogram in line-graph form.

The most advanced oocyte in each ovariole was recorded, and on the basis of these data it is possible to select the modal oldest stage (underlined in Table 7). The modal oldest stages are plotted in Figure 14 and are compared with similar information for wild type flies presented by KING and WOLFSBERG (1957). It is evident that oocyte development in the *sn*^{36a} homozygote lags considerably behind that of the wild type, and that oogenesis is never brought to completion (stage 14). No stage 14 chambers were observed in more than 100 *sn*^{36a}/*sn*^{36a} ovaries of varying ages.

Comparative studies of stage distribution as a function of age on the IIa group and wild type indicate that the rate of oogenesis is decreased, especially in the yolk-forming stages. This finding is further substantiated when the amount of yolk/fly, calculated for the class IIa category for flies of various ages, is compared with similar information for wild type flies (Figure 15).

Examination of Figure 15 shows that the amount of yolk in the ovaries of group IIa flies of advancing age generally remains lower than that of wild type flies of identical age. The total amount of yolk produced by the wild type and *sn*^{36a} females differs even more significantly, however, since wild type females under optimal conditions lay daily 20 times as many eggs (60 *vs.* 3) as do *sn*^{36a} females.

TABLE 7

Stage distribution as a function of age of sn^{36a} ovarian tissue

		A		B		C		D		E	
Stage*		12 hrs.	$\pm (s_x)\%$	42 hrs.	$\pm (s_x)\%$	96 hrs.	$\pm (s_x)\%$	144 hrs.	$\pm (s_x)\%$	432 hrs.	$\pm (s_x)\%$
No yolk	1										
	2									0.60	0.01
	3	19.51	5.50	1.77	0.08	2.63	0.66	0.90	0.01	1.21	0.06
	4	2.44	8.09	3.54	0.13	5.26	1.31	0.30	0.01	2.41	0.02
	5			1.77	0.02			0.30	0.01		
	6	53.66	9.39	7.08	0.28			3.89	0.02	2.41	0.12
	7	24.40	3.54	12.39	0.25	2.63	0.66	4.79	0.06	9.04	0.18
Yolk forming	8			26.55	0.65	26.32	1.68	11.68	0.06	8.43	0.68
	9			20.35	0.27	18.42	2.01	7.49	0.04	5.42	0.05
	10			15.04	0.28	7.90	0.66	7.19	0.05	1.81	0.15
	11			3.54	0.51	36.84	1.68	46.41	0.32	2.41	0.54
Yolk formed	12			3.54	0.09			3.89	0.02		
	13			4.42	0.19			13.17	0.30	66.27	0.34
	14										
Total		100.01		99.99		100.00		100.01		100.01	
Number of examined ovaries		6		17		4		27		21	

* Stage as percent of total number of the oldest (most posterior) chambers in the ovariole.

FIGURE 14.—A graph of modal oldest stage in ovary plotted as a function of age in hours.

FIGURE 15.—Volume of yolk ooplasm $\times (10^7 \mu^3)$ per female plotted as a function of age for *sn*^{36A} and wild type flies. (Data for 1-, 2-, 4-, and 7-day wild type flies from KING [1957]).

Also the *sn* stocks have 20 percent more ovarioles (31 *vs.* 25), thus the wild type data should be adjusted upwards an apporportional amount to render the groups comparable. (There were generally seven chambers/ovariole in the 144- to 168-hour *sn*¹, *sn*^{36A} and wild type stocks).

The major particulate constituents of the insect yolk, protein and lipid yolk bodies, are probably of trophocyte origin in *Drosophila* (HSU 1953; KING and DEVINE 1958; KING and BURNETT 1958). In light of this evidence it seems reasonable to suppose that the trophocytes of the sterile singed mutants are unable to carry out their normal role in vitellogenesis. The abnormal appearance of the nurse cell nuclei in *sn*^{36A} mutants strengthens this assumption.

To summarize, cytological evidence suggests that vitellogenesis is retarded in ovaries of the sterile singed mutants, whereas preyolk chambers develop normally. Vitellogenesis appears most drastically retarded in *sn*^{36A}. Studies of the stage distribution of developing chambers in *sn*¹ and *sn*^{36A} flies and comparative values for the amount of yolk ooplasm produced by these two groups once again indicate that vitellogenesis is more drastically affected in the *sn*^{36A} group. Studies of stage distribution as a function of age in the *sn*^{36A} group and in wild type flies showed that the rate of vitellogenesis in the *sn*^{36A} group is markedly decreased and that oogenesis is never brought to completion.

DISCUSSION

Since the *sn* mutants constitute a pseudoallelic series, it is of value to examine the available information concerning the phenotypic effects and spatial configurations of this series with the view of developing an hypothesis which could explain the action of this complex upon fertility and upon setal morphology. HEXTER (1957) divided the mutants into three phenotypic groups on the basis of data on bristle-hair morphology and fertility. Each group of mutants was considered to occupy a specific locus. The present study suggests, instead, the existence of four phenotypic groups on the basis of setal morphology since the *sn^{sea}* mutant appears to stand apart from the others. Further, three different phenotypic categories can be distinguished on the basis of cytological examination of ovarian tissue and progeny tests.

A chromosomal model can be developed which takes into consideration all pertinent information currently available for the *sn* complex. This model is illustrated by Figure 16. The model incorporates three main loci within the *sn* complex. All three loci are assumed to affect setal morphology (b of Figure 16) by the production of an unknown factor (substance y); only the first and third loci affect fertility by production of another factor (substance x). These products may or may not be related. The presence of a singed mutant either partially or completely inactivates the locus in question.

The locus proximal to the centromere is occupied by mutants *sn¹* and *sn^{50k}*. It is suggested that this locus is actually composed of two subloci (b and f) possibly separable by crossing over (BURDETTE 1949). The middle locus is occupied by mutants *sn²* and *sn⁴*. The locus farthest from the centromere is occupied by the *sn³* and *sn^{sea}* mutants and resembles the first locus in that it is composed of two subloci (b and f), probably separable by crossing over. It is hypothesized that the *sn^{sea}* mutant causes greater inactivation (perhaps even complete inactivation) of the fertility factor than the *sn³* mutant and an incomplete inactivation of the bristle-hair factor; whereas the converse holds for the *sn³* mutant.

Such a proposal can possibly be tested experimentally. Mutants of the *sn^{sea}* group can be subjected to irradiation in an attempt to further inactivate the setal factor. If mutants can be recovered showing the bristle-hair morphology of the class 3 group (*sn³*) and having modified oogenesis similar to the class 4 group

FIGURE 16.—A proposed model of the singed complex.

(*sn^{36a}*), this hypothesis will be strengthened. The reciprocal approach, namely irradiation of the *sn^s* mutant and subsequent study of the ovarian tissue, is too laborious since this would necessitate investigation of the ovarian tissue of every female tested. By assuming the presence of a separable sublocus for fertility within the *sn^s-sn^{36a}* locus (Figure 16), it is not only possible to develop an explanation for the unexpected intermediate phenotypes observed when *sn^t* and *sn^{36a}* mutants were examined in crossover experiments (HEXTER, unpublished), but to establish the relative positions for the setal and fertility moieties. In recombination experiments, expected wild type individuals from *sn^{36a}-sn^t* crossovers were not completely wild type, and the presumed double mutant *sn^{36a}-sn^t* was more nearly wild type than singed. Such a finding is what one might expect if the crossover occurred between the *sn^{36a}* sublocus for setal morphology and the sublocus for fertility (Figure 17) and if the resulting chromosomal segment of (A) pro-

duces more of a singed effect than the resulting chromosomal segment of (B). Both segments of (A) and (B) are affected by the type of neighboring fertility factor since they are distinguishable from *sn^s* and *sn^{36a}*. The two chromosomes resulting from recombination (A and B) could be tested for fertility. If the progeny have arisen as a result of crossover between the setal sublocus and the fertility sublocus as considered in Figure 17, then (A) the singed product should be fertile, whereas (B) the nearly wild type product should be highly infertile. However, if the fertility factor of the *sn^s-sn^{36a}* sublocus lies to the left, then a crossover between these factors could be expected to produce an infertile, more nearly singed product

FIGURE 17.—Diagram of an hypothetical crossover between the fertility and the bristle-hair subloci of the *sn^{36a}* locus (developed from the model shown in Figure 16). The presence of a singed mutant is indicated by a "*sn*" in the appropriate position in the model with the bristle (b) or fertility (f) sublocus indicated by a superscript. Conversely, a wild type allele is indicated with a "+" sign. The products of such a crossover are indicated as "A" and "B". "A" is predicted to have mildly singed bristles and hairs and to be fertile; whereas "B" has only the fertility sublocus of the *sn^{36a}* locus and is expected to be nearly wild type but infertile.

with features of oogenesis comparable to those of the sn^{36a} group. In either case such findings would provide strong evidence of a separable fertility sublocus within the third main locus of the singed complex.

SUMMARY

1. Fourteen singed stocks were examined with respect to setal morphology and fertility. These stocks could be subdivided into four categories: (1) sterile, with kinky hairs and gnarled bristles— sn^1 , sn^5 , sn^{50k} , sn^1sn^2 , sn^1sn^3 , sn^1sn^4 , sn^2sn^3 , sn^{x2} , and sn^c ; (2) fertile, with kinky bristles only— sn^2 , sn^4 , and sn^{34e} ; (3) fertile, with kinky hairs and gnarled bristles— sn^3 ; and (4) sterile, with gnarled bristles only— sn^{36a} .

2. The phenotypic consequences of the genotypic combinations of the four sn classes were examined with respect to: (A) Fertility—any individual compounded with a fertile sn allele on one chromosome was fertile, and any individual homozygous for any two sterile sn alleles was sterile and (B) Setal morphology—Class 2 bristle and hair morphology is dominant over Class 3 and Class 4, and blends with Class 1. Class 4 bristle and hair morphology is dominant over Class 1 and 3.

3. Two major types could be recognized utilizing a study of oogenesis in the sn mutants. I. Normal oogenesis occurs in all fertile sn mutants. II. Abnormal oogenesis occurs in infertile mutants. The sterile category can be further subdivided into IIa and IIb with class IIa being represented only by the sn^{36a} mutant. Class IIb contains all of the sterile sn mutants studied except sn^{36a} . Cytological evidence suggests that vitellogenesis is retarded in ovaries of the sterile singed mutants; whereas preovulatory chambers develop normally. Vitellogenesis appears more drastically retarded in the sn^{36a} group. Studies of the stage distribution of developing chambers in 156-hour sn^{36a} and sn^1 flies and comparative values for the volume of yolky ooplasm produced by these two groups once again indicate that vitellogenesis is more drastically affected in sn^{36a} flies. Studies of stage distribution as a function of age in sn^{36a} and in wild type flies showed that the rate of vitellogenesis in the sn^{36a} class is markedly decreased and that oogenesis is never brought to completion.

A model is described which attempts to explain the action of this complex locus upon fertility and upon bristle and hair morphology. This model contains three subunits, all portions of which deal with bristle-hair development. Two of the subunits are in turn divided into a bristle and fertility moiety. A separable fertility factor is hypothesized in connection with the subunit associated with the sn^3 and sn^{36a} mutants.

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