TWO NEW MUTANT CHARACTERS ON THE SPERMATHECAE OF THE FEMALES OF *DROSOPHILA MELANOGASTER*. CELL-DEGENERATION AND SUPERNUMERARY SPERMATHECAE.

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INTRODUCTION

In this work two mutant characters have been studied, both affecting the normal development of the spermathecae in the females of *Drosophila melanogaster*. The first character is a hereditary degeneration of the cells of the epithelium of the spermathecae. It was found in January 1921 by Doctor J. L. COLLINS as a mutation in a stock of curved flies which was extracted from a cross, black purple curved×jaunty. The second is the occurrence of the supernumerary spermathecae, a stock being produced in which the females all showed 3 instead of the normal number 2 spermathecae. This mutant was found in the stock which carried the hereditary cell-degeneration. The present investigation was carried out in the Genetics Laboratory of the UNIVERSITY OF CALIFORNIA during the fall semester of 1926 and the spring semester of 1927. I take great pleasure in thanking Professor E. B. BABCOCK for the facilities given me in the Genetics Laboratory and Doctor J. L. COLLINS for the material and for help and suggestions during the course of the work. Acknowledgment is also given to the INTERNATIONAL EDUCATION BOARD for the fellowship granted to me.

THE HEREDITARY CELL-DEGENERATION

Description

As far as it has been possible to observe the cell-degeneration is strictly limited to the epithelium of the spermathecae. The gross appearance is

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1, 2 or 3 black spots on the dorsal side of the posterior part of the abdomen (figure 1). When highly developed it can be seen with the naked eye; if the degeneration is slight, however, the spermathecae are hard to see, even with large magnification and a dissection of the fly is often necessary

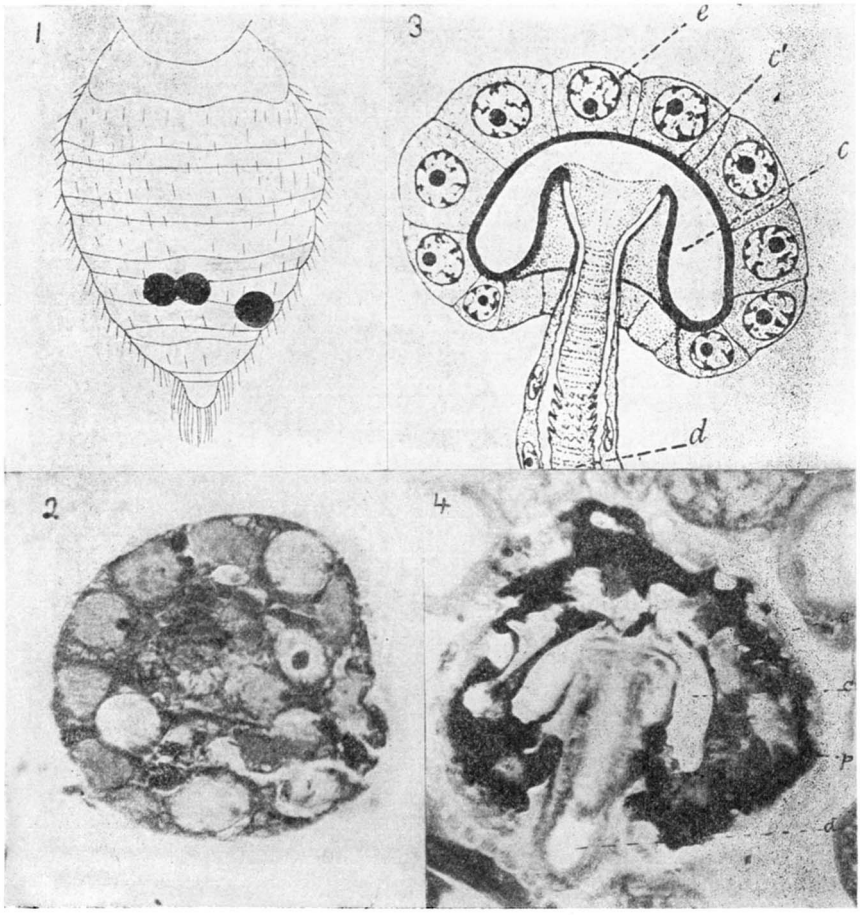

FIGURE 1.—Abdomen of female carrying 3 spermathecae each with a cover of degeneration tissue.

FIGURE 2.—Cross-section of the epithelial layer of a spermatheca at an early stage of degeneration. No nuclei are found in the epithelial cells which are filled with a brown pigment. Microphotograph Magnification ×800 diam. 5μ. Haematoxylin.

FIGURE 3.—Longitudinal section of a normal spermatheca; c=cavity; c^1=cuticle; d=duct; e=epithelium. Photograph after drawing of NONIDEZ (1920, p. 215).

FIGURE 4.—Longitudinal section of a highly degenerated spermatheca. Brown pigment covers most of the epithelium in which all cellular structure is lost. Microphotograph Magnification ×800 diam. 5μ. Haematoxylin. c=cavity; d=duct; e=epithelium; p=pigment.

to make a safe decision. The spermathecae are 2 (or 3) mushroom-shaped bodies connected with the uterus by narrow ducts and serving as storage organs for the sperm. Their terminal cavity is surrounded by a brown cuticle, secreted by the epithelium forming the walls of the organ (figure 3).

As the degeneration proceeds there is a deposition of dark brown pigment in the epithelial cells. Figure 2 shows a cross-section of the epithelial layer at an early stage of pigment formation. The cell structure is still retained, but the nuclei are gone and the cells are filled with a brown pigment. Later on the cell structure is completely gone and the brown pigment forms a complete bag around the spermathecae. A section through such a spermatheca is shown in figure 4.

The deposition of pigment seems often to be checked at an early stage and the gross appearance is then a few brown granules scattered throughout the epithelial layer, a type which I have called d_g-*a* for sake of convenience as contrasted with d_g-*b* in which the pigment forms a complete bag around the spermathecae, and is visible as conspicuous black spots such as shown in figure 1. There seem to have been present in the stock modifiers, reducing the pigment deposition, for by selection, the percentage of d_g-*b* females was considerably raised.

However, even after 15 generations of selective inbreeding both types occur, showing that they may appear as modifications of the same genotype. The males of the d_g stock are normal as far as has been observed, but no dissection of males has been made. The data given below on development and inheritance are therefore concerned with the females only.

Development and variability

The degeneration has never been observed in larvae or pupae and indeed not in newly hatched flies. Observations were made on 42 virgin females which were isolated at the pupal stage (throwing out the males as soon as possible after emergence). The females were examined as often as possible during the first 72 hours; visible pigment did not develop until 24 hours after emergence and developed in some cases as late as 72 hours after emergence. Classification of d_g *versus* non-d_g flies can therefore be safely made only on females at least 3 days old; a circumstance which complicates the genetic analysis considerably. By counting the flies twice a day, for instance, one could run pure cultures of the mutant stock, finding only a single individual with degenerated spermathecae, now and then. If only sufficiently-old females are examined, the degeneration frequency in pure cultures is now 100 percent.

There is still variation in the degree of degeneration and a few experiments were carried out to determine the influence of external factors. Breeding at 24° and 31° C did not give any difference in the degree of degeneration, nor did a variation in the moisture of the cultures have any effect. To test the effect of the amount of food, vials were made up with 5cc banana-agar food in each and in these were placed 50, 100, 150 or 200 eggs from cultures of the mutant stock. The results are given in table 1 under 2 groups (1) 50-100 eggs—(2) 150-200 eggs. The degree of cell-degeneration is expressed by the relative proportion of d_g-a and d_g-b flies.

TABLE 1

Effect of amount of food upon cell-degeneration.

NUMBER OF BOTTLES	NUMBER OF EGGS PER BOTTLE	AVERAGE NUMBER OF FLIES	d_g-a	d_g-b
12	50–100	18.42	44	52
6	150–200	42.80	38	105

Although the numbers are small, there is a clear indication of increasing degeneration with decreasing amount of food.

It is not likely that the degeneration is due to infection or some other external cause as it segregates out in Mendelian ratios. The sex-ratio in the mutant stock is normal, which shows that the females with the abnormal spermathecae are equal in viability to the males which are normal. The viability of the d_g classes in relation to the non-d_g classes in segregating populations is discussed under the treatment of the results from the backcrosses.

The fertility of the females is good. It has not been possible to ascertain whether the sperm can enter an abnormal spermatheca, and whether such a spermatheca can again give off sperm for fertilization, but this seems likely in view of the good fertility of the females. It was thought that fertilization was perhaps necessary for the cell-degeneration, as the development does not begin till after the time when the females have usually been fertilized in mixed cultures. The foregoing experiment with isolation of virgin females showed that the cells degenerate in virgin females as well.

Inheritance

The character is recessive: when d_g females are outcrossed to males of other stocks the F_1 females have never shown cell-degeneration. At the time the character was found some work was done with it and a stock had been built up which contained besides d_g the 2 second-chromosome mutants, curved wings (*c*, locus 75.5) (BRIDGES AND STURTEVANT

1914) and cinnabar eyes (c_n, locus 57.5) (CLAUSEN 1924). This work had shown that the mutant was probably due to a factor in the second chromosome. To get some data on this according to the standard Star Dichaete method, a c_n d_g c female was outcrossed to a Star Dichaete (*SD*) male and F_1 *SD* males backcrossed to females of the c_n d_g c stock. It was found, however, that Star in this cross was constantly overlapping the normal type; the characteristic roughness of Star eyes was often unseen but, as was found later, the Star flies could be picked out by the smaller size of the eyes. The first backcross gave $28 d_g$: 216 non-d_g. It was suspected, however, that some of the flies classified as non-d_g were genetically d_g, which later experiments proved to be true. The low percentage of d_g flies was probably due to the fact that classification had been made on too young individuals.

After selection for extreme type of degeneration had been carried on for some generations, females and males from the d_g stock were outcrossed to wild males and females, and F_1 individuals from each kind of cross were mated together in 2 mass cultures and 2 single pair cultures. The results of the F_2 are given in table 2.

TABLE 2

Female offspring from the mating of F_1 individuals heterozygous for c_n d_g c.

CULTURE NUMBER	+++	$c_n d_g c$	$+ d_g c$	c_n ++	++ c	c_n + c
35	65	14	1	9		3
36	39	7	3			1
Total	104	21	4	9		4
F_2 mass	155	50	3	5	4	13
Total F_2	259	71	7	14	4	17

In this, as in the following tables the symbols standing for the various mutant genes and + for their corresponding wild-type allelomorphs are used to designate the phenotype of the classes. Thus, +++ is used to designate individuals which are normal as regards the 3 mutant characters, cinnabar eyes, d_g and curved wings; the c_n d_g c class comprises the individuals which show all 3 mutant characters; whereas the $+ d_g$ c class includes the individuals which show d_g and curved wings, but not cinnabar eyes. The ratio of non-d_g:d_g flies in the F_2 is 270:102. On the basis of one factor difference the expected numbers would be 279:93; so the results agree with the assumption that cell-degeneration depends on a single recessive factor. It is clear that this factor is carried both by males and females although it expresses itself phenotypically only in the latter. As

to the location of this factor, the results show that it is located in the second chromosome, somewhere near curved. To determine the locus of d_g females heterozygous for c_n d_g c were backcrossed to males homozygous for c_n d_g c.

The results from this cross are listed in table 3.

TABLE 3

Female offspring from backcrossing females heterozygous for c_n d_g c to c_n d_g c males.

CULTURE NUMBER	+++	c_n d_g c	+ d_g c	c_n ++	c_n d_g +	++ c	c_n + c	+ d_g +	TOTAL
52	52	12	1	12					77
53	63	34	2	4			2		105
54	24	20	1	3			3		51
55	13	7		1					21
56	63	34	6	8					111
58	22	14	2	7			3		48
59	22	12	4	3			1	2	44
60	34	28	3	8		1		1	75
61	39	20	5	3	2		1		70
88	48	34	5	7		1	7		102
89	54	30	3	4		1			92
90	61	71	11	8		1		1	153
91	112	135	18	16			1		282
92	59	62	9	4		1	1		136
Total	666	513	70	88	2	5	19	4	1367

From the results it is clear that the factor d_g is very near to c. In the class c_n+c there is an excess of individuals as compared with the corresponding crossover-class +d_g+. This is probably due to the fact that some of these individuals are really c_n d_g c in which the abnormality has failed to develop. If we compute the crossover value d_g—c only on the basis of the crossover-classes showing degenerated spermathecae, we find a value of 1.0 percent. The order of the genes and the relation between single and double crossing over will be discussed later, taking into account data from all the backcrosses.

There is in this cross a deficiency of 23 percent in the class c_n d_g c which may be due to a lower viability of the triple mutant type as compared with the wild-type. To get correct crossover values in cases with a notably lowered viability, one has to make *balanced viability crosses*, that is, different combinations of factors are used in crossing. Besides the cross in table 3 one further backcross was made: c_n++/+d_g c ♀ ♀ × c_n d_g c ♂ ♂. To obtain females of this constitution c_n d_g c females were outcrossed to wild males and an F_2 was raised. F_2 d_g c individuals were mated together

and F_3 d_g c females were mated to males of cinnabar stock. Wild-type females from this cross, with the constitution $c_n++/+d_g\ c$ were mated to $c_n\ d_g\ c$ males. The results from these matings are given in table 4.

In this cross there is no deficiency of the d_g classes in relation to the non-d_g classes; the same holds true for the F_2 (table 2) and there is no

TABLE 4

Female offspring from backcrossing $c_n++/+d_g\ c$ females to $c_n\ d_g\ c$ males.

CULTURE NUMBER	$+d_g\ c$	c_n++	$c_n\ d_g\ c$	$+++$	c_n+c	$+d_g+$	$c_n\ d_g+$	$++c$	TOTAL
81	23	19	5		2			1	50
82	26	31	4	4			1		66
83	17	26	6	4	2			2	57
84	48	35	7	3					93
85	32	23	5	2			1		63
86	13	9	1	3	1				27
87	30	25	7	4	1				67
Total	189	168	35	20	6		2	3	423

significant deviation in another later backcross (table 5). It is probable, therefore, that the large deficiency in the first backcross (table 3) is not due wholly to lower viability of the d_g class, but is rather due to the fact that the females had to be kept for many days, and it was noted that there was sometimes a heavy loss of females during this time. In the later experiments this was avoided through a better method of keeping the females. The general impression is that the d_g stock is of good viability, although the $c_n\ d_g\ c$ type is not equal to the wild-type, which is not an unexpected condition in any stock containing 3 mutant genes. The d_g flies in table 4 give a crossover value $d_g - c$ 0.9 percent.

The next step in the location of d_g was to find a gene nearer to c and d_g than c_n; such a gene is Lobe^2 (L^2 locus 72.0) a dominant factor which has the effect of reducing the eyes. A Lobe^2 male was mated to a $d_g\ c$ female and F_1 Lobe$_2$ females were mated to $d_g\ c$ males. The results of this backcross are given in table 5.

The crossover value between d_g and c, basing the calculation only on the d_g classes is 1.2 percent.

The data do not allow a safe decision as to whether the genic order is $c_n\ d_g\ c$ or $c_n\ c\ d_g$, though the evidence is slightly in favor of the order $c_n\ d_g\ c$. There seems to have been in the experiment a low percentage of crossing over between c_n and c, 11.9 and 15.5 as compared with the standard value 18.0. There is further a high percentage of double crossing over. Although the amount of crossing over is thus somewhat abnormal

it is safe to conclude that d_g lies within about one unit to the right or left of curved (75.5 ± 1.0).

TABLE 5

Female offspring from backcrossing L^2++/+d_g c females to d_g c males.

CULTURE NUMBER	L^2++	+d_g c	+++	$L^2 d_g$ c	L^2+c	+d_g+	$L^2 d_g$+	++c	TOTAL
66	15	16					1		32
67	66	41	5					3	115
68	55	55	4					1	115
69	65	54	2	3	1			1	126
70	37	25	1		1		1	1	66
71	44	47	1	1		1		1	95
72	52	57	2	3	2			3	119
73	31	26	1	2	1	1			62
Total	365	321	16	9	5	2	2	10	730

TABLE 6

A summary of the 3 backcrosses, including only the d_g classes, on which basis the computations of the crossover percentages are made.

COMBINATIONS	--	+-	-+	++	TOTAL	PERCENTAGE CROSSING OVER						
						c_n–d_g	d_g–c	c_n–c	double c_n–d_g–c	L^2–d_g	L^2+c	double L^2–d_g–c
c_n d_g c/+++	513	70	2	4	589	11.9	1.0	12.2	0.6			
c_n++/+d_g c	189	35	2	0	226	15.5	0.9	16.4	0.0			
L^2++/+d_g c	321	9	2	2	332		1.2		0.6	3.3	3.9	0.6

THE HEREDITARY BASIS FOR SUPERNUMERARY SPERMATHECAE

During the study of inheritance of the cell-degeneration it was necessary to dissect a large number of females. Some of the cultures were found to have many females with three spermathecae instead of two, which is the normal number.

The form and function of the spermathecae has been described by NONIDEZ (1920), while STURTEVANT (1926) has examined the intraspecific variability as to number of spermathecae. He examined specimens from wild stocks of *D. melanogaster*, *D. simulans*, F_1 hybrids between these two, *D. funebris*, *D. imigrans*, *D. busckii*, *D. repleta* and found two specimens with three spermathecae, all the others having two. The number of spermathecae thus seems to be a constant character and in the cases examined the species belonging to the same genus show the same number of spermathecae.

STURTEVANT (1926) describes a stock of *D. melanogaster* which showed a higher percentage of flies with three spermathecae and from which he

established a race in which from 25 to 75 percent of the females had three spermathecae. The genetic analysis has so far indicated that at least two factors are involved in the production of the extra spermatheca in this race.

In our d_g stock most of the individuals with three spermathecae had three of equal size; however, in some cases one was smaller than the other two. In individuals with two spermathecae one was sometimes larger than the other and often constricted. Occasional flies with four spermathecae were also found.

During the course of the investigation of d_g selection for three spermathecae was also carried on by always choosing flies from the cultures showing the highest percentage of three spermathecae. In a short time a stock was obtained which appeared to be homozygous for this new character. Two cultures counted at this time gave 205 with three spermathecae to 10 with two spermathecae. This stock was continued for several generations by brother-sister mating with no attention paid to the number of spermathecae. Then two cultures were again counted, giving 293 with three spermathecae to 4 with two spermathecae, which is 98.6 percent with three spermathecae.

The apparent ease with which a homozygous race was established indicated that the genetic basis for this character was perhaps simpler than in the case reported by STURTEVANT. It was, therefore, decided to attempt to determine the genetic basis for the inheritance of three spermathecae in this stock. The determination of the number of spermathecae was always made by dissecting the flies, a fact which in itself limits the number of flies which can be examined. Females from the three spermathecae (s_p^3) race were crossed with Star Dichaete (SD) and the F_1 SD ♂ ♂ were backcrossed to s_p^3 ♀ ♀, producing the following classes and numbers of female progeny.

$SD+$	SDs_p^3	$S++$	$S+s_p^3$	$+D+$	$+Ds_p^3$	$+++$	$++s_p^3$	total
81	—	78	1	45	2	66	32	305

The flies that have S or D or both do not develop s_p^3 (with the exception of 3 flies). This, therefore, indicates that there are in the second and third chromosomes recessive genes that contribute to the production of the third spermathecae. Since only a portion of the non-Star and non-Dichaete individuals show three spermathecae there must also be segregation in one other pair of chromosomes. If there were no other genes involved in the production of s_p^3 than those in the second and third chromosomes, then all the non-Star non-Dichaete flies should be s_p^3.

The segregation observed in the non-Star non-Dichaete flies cannot be due to a gene in the X-chromosome, because all the females of this culture have the X-chromosomes from the s_p^3 stock. Therefore, the segregation in non-Star non-Dichaete flies is probably due to a recessive gene in the IV-chromosome (table 7). A definite proof of the existence of such a gene in the fourth chromosome can only be delivered through linkage tests with a fourth chromosome mutant. This would require additional mutant stocks and additional time neither of which was available.

TABLE 7

Comparison of observed and calculated results on the basis that s_p^3 is due to the simultaneous action of three recessive genes, one in each of the II, III and IV chromosomes.

CHARACTER	*SD*	*S*	*D*	+	s_p^3	TOTAL
Observed numbers	81	78	45	66	35	305
Calculated numbers	*76*	*76*	*76*	*38*	*38*	*304*

The deviation from the 1:1 ratio in the Dichaete and normal classes cannot readily be explained, but it is probably due at least in part to difficulties in recognition of characters. The culture also involved curved, a wing character brought in by the d_g stock, which may have in some way interfered with classification. However, if we consider the segregation of s_p^3 and non-s_p^3 on a three chromosome (II, III and IV) basis the correspondence of observed and calculated numbers is very good.

Observed 270.0 non s_p^3 to 35.0 s_p^3
Calculated 266.7 non s_p^3 to 38.1 s_p^3

These results do not exclude the possibility of essential genes for s_p^3 being in the X-chromosomes; for these flies were all homozygous for these chromosomes. That the sex chromosomes are not involved, however, is indicated by the results of another backcross in which F_1 ♀ ♀ were used.

TABLE 8

Results of crossing ♀ ♀ of the constitution $\frac{+\ c_n\ d_g\ c\ s_p^3}{S++++}\ \frac{+s_p^3}{D+}$ *with* $\frac{+\ c_n\ d_g\ c\ s_p^3}{+\ c_n d_g\ c\ s_p^3}\ \frac{+s_p^3}{+s_p^3}\ \frac{s_p^3}{s_p^3}$ ♂♂.

Classes	*SD*	*S*	$c_n D$	$c_n c$	$c_n c s_p^3$	*D*	Sc_n	*Sc*	Total
Number	29	13	35	22	12	20	3	1	
Classes	$S c_n D$	+	c_n	c	s_p^3	$D s_p^3$	$c_n D s_p^3$	$SD s_p^3$	
Number	1	13	5	1	1	1	1	2	160

A ♀ from a cinnabar- d_g- curved- s_p^3 stock was crossed with a SD♂ and F_1 SD ♀ ♀ backcrossed to males of the c_n- d_g- c- s_p^3 race. Three backcross cultures gave the results in table 8. In this table curved-Dichaete flies are listed with the Dichaete.

If we consider the ratio of s_p^3 to non-s_p^3 we get, as before, evidence of a segregation in only 3 chromosomes.

Observed 143 non-s_p^3:17 s_p^3
Calculated 140 non-s_p^3:20 s_p^3

The male and female backcrosses thus give similar results showing that the sex chromosomes do not carry genes essential for the s_p^3 character. The conclusion is reached, therefore that the extra spermathecae in this race is dependent upon recessive genes in the second, third and fourth chromosomes. The appearance of Star s_p^3 and Dichaete s_p^3 flies may mean that in some cases the third spermathecae can be produced when either the second or third chromosome genes are in a heterozygous condition if the other two sets of genes are in a homozygous condition.

No definite evidence has been obtained relative to the linear location of any of the three s_p^3 genes. On the suggestion of DR. STURTEVANT females from cultures of SD from the stock used in this analysis were dissected to determine whether any of them carried 3 spermathecae. Two single pair cultures and one mass culture gave 73 SD:39 $S+$females all with 2 spermathecae; no $+D$ and $++$females. A probable explanation of this result is that a lethal has arisen by mutation in the second chromosome allelomorphic to the S gene, thus producing a balanced lethal stock breeding true for S. A culture, segregating for S, gave the result 12 SD:8 $S+$:7$+D$:1$++$females, all with 2 spermathecae. In all 140 females were examined, all had 2 spermathecae. It is not likely, therefore, that there are in the SD stock used in the analysis of the supernumerary spermathecae any genes which would interfere with the analysis.

As has been mentioned, the number of spermathecae, is a fairly constant character within a species, and as a rule uniform for all the species of a genus. It is, therefore, of interest to find such a character arising as a mutation in one species, giving rise to a race breeding true for a number of spermathecae, different from that which is characteristic of the Drosphilidae.

The genetic basis of this character provides an interesting side light on the problem of organic evolution. Against the theory of evolution through the selection of small independent mutations, it is urged as a main difficulty, that complex organs which must depend upon the interaction of many genes cannot be thought to arise through a series of quite indepen-

dent random mutations. So far all evidence indicates random mutation in *D. melanogaster*. It is reasonable to assume that it is the case here. Three mutations have occurred, each of which apparently has no influence upon development, but which together interact to cause the development of a complex organ, an extra spermatheca. This tends to show that the criticism of the theory, that mutations form the basis of organic evolution, may not have the importance often attached to it.

SUMMARY

1. In this work is studied a hereditary cell-degeneration on the spermathecae of the females of *Drosophila melanogaster*. It arose as a mutation in a stock of curved flies and has been proved to be dependent on one single recessive factor located in the second chromosome near curved 75.5 ± 1.0.

2. The abnormality appears only in adult females some time after emergence and consists in a deposition of a dark brown pigment in the epithelial layer of the spermathecae; in highly degenerated spermathecae the pigment forms a complete bag around the spermathecae, and is visible as conspicuous black spots on the abdomen of the females.

3. In pure stock nearly all the females show the cell-degeneration, but the degree of degeneration is variable and has been shown to be influenced by the amount of food in the cultures, such that with a smaller amount of food there is higher degree of degeneration.

4. The viability and fertility of the d_g females are good.

5. During the cell-degeneration investigations a stock was established which was homozygous for the new mutant character, supernumerary spermathecae, that is, 3 instead of the normal number 2.

6. A genetic analysis showed this character to be dependent upon recessive factors in the second, third and possibly fourth chromosomes.

7. It is pointed out that this character has an interesting bearing on the problem of organic evolution.

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