GENETIC STUDIES IN DROSOPHILA PSEUDOOBSCURA

Thesis by

Klaus Mampell

In partial fulfillment of the requirements for the degree of Doctor of Philosophy

California Institute of Technology
Pasadena, California
1943

FOREWORD

Drosophila is one of the preferred organisms for geneticists. D. melanogaster is the most thoroughly investigated species of the genus. Research with this species for over thirty years has resulted in the establishment of a remarkable variety of stocks; this greatly facilitates the work of any geneticist who now chooses this species for his researches. No such stocks exist for most species of Drosophila. It is, of course, more difficult to work with a species where all necessary stocks have to be built up and maintained by the investigator himself. This involves time, labor and luck, and even so, the stocks remain incomplete at best. Most of the D. pseudoobscura stocks used in this work were made up in the course of the research.

The choice of the species was accidental. The laboratory where the author first began Drosophila research had only D. pseudoobscura, race B, available. A chronological account of the work will show how each part of the research described in this thesis presented itself accidentally and unexpectedly. Thus we get a chain of problems whose only connection is the appearance of one

problem in the course of solving another. It is entertaining to begin this chronological account with the day when the author began Drosophila research. On that day a mutation was found: shortest, an extreme allele of short. In the course of mapping this gene new mutant genes were found, and mapping those, others were found, etc. One of these new mutant genes was radius rudimentary, a sex-linked recessive showing in females only. This seemed analogous to the behavior of bobbed in D. melanogaster where a wild type allele in the Y-chromosome prevents the appearance of the character in the males. To test for a like explanation in this case, XO males had to be obtained; there, the Y-chromosome with its dominant allele would be removed, and the recessive character could express itself.

It is known that D. pseudoobscura interracial hybrid females produce an increased proportion of XO males. Mating a radius rudimentary male to a hybrid female homozygous for a sex-linked recessive like vermilion should have resulted in vermilion sons and an occasional male wild type for eye color which would then have resulted from the union of an X-bearing sperm with a no-X-egg; this would have been the desired individual. D. pseudoobscura hybrid females ordinarily are fertile, but these vermilion hybrid females proved to be

sterile. This unexpected phenomenon led to the study described in chapter I. While investigating this problem, one of the race B cultures which was used for marking the race B X-chromosome showed four new sex-linked recessives. This initiated the study described in chapter II. One of the wild type stocks to which the flies were outcrossed was the Seattle strain. Besides the more usual point mutations many mutations suggesting deficiencies began to appear. After a period of confusion about the nature of this phenomenon, it was finally recognized as being due to an independent cause, a gene that had been introduced from the Seattle strain. The study of this case is described in chapter III. Already a new phenomenon has appeared in the study of the last problem. It will furnish another chapter in the future.

Besides the problems mentioned in the foregoing account, literally hundreds of others suggested themselves in the course of the different researches. Of those, many seemed interesting and profitable to be investigated. Obviously, one investigator can only do a limited amount of work, and it is up to his judgment to select out of the multiplicity of problems those which seem to him the most interesting and profitable. Thus, the majority of problems

has to be discarded in favor of a very few. This is regrettable and sometimes painful, but cannot be helped.

The "Female sterility in interracial hybrids of D. pseudoobscura" was selected, for here we have unusually favorable material for the study of speciation. Race A and race B are still closely enough related to show no significant morphological differences, but the hybrid males are completely sterile. As is described in chapter I, the hybridization of some strains of the two races produces sterile females as well. This is the final step in the divergence of the species. No exchange of genes is possible any longer and the evolution of the two will progress independently. The sterility of the females is due to a beautifully complicated mechanism the analysis of which is interesting regardless of the evolutionary implications.

The two other problems deal with mutation. The author's main interest lies in the concepts having to do with the nature of the gene. This is indeed a large field, but any problem dealing with the causes of gene mutation is apt to throw some light on the nature of the gene. The "high mutation frequency in D. pseudoobscura, race B" in chapter II shows how one gene can control the mutation

rate of all other genes. This is probably the mechanism controlling mutation under natural conditions, for irradiation of all kinds, temperature, etc. have been ruled out as significant natural factors. Therefore, the problem is interesting both for questions concerned with evolution and those concerned with the mechanism of gene mutation.

In chapter III "a deficiency inducer in D. pseudoobscura, race B" is described. Here mutations of a different nature are observed. All arguments in favor of studying the former problem can be applied to this one. In addition, an analysis and comparison of the two kinds of mutation, namely; point mutation versus deficieny, may show to what extent chemical or physical changes can account for either.

The author is indebted to those members of the Biology Department who have contributed suggestions and criticism concerned with various phases of these studies.

CHAPTER I

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 27, No. 7, pp. 337–341. July, 1941

FEMALE STERILITY IN INTERRACIAL HYBRIDS OF DROSOPHILA PSEUDOÖBSCURA

BY KLAUS MAMPELL

WM. G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated May 27, 1941

Male sterility is a regular occurrence in the hybrids of race *A* and race *B* of *D. pseudoöbscura*.[1] A case of female sterility in the same hybrids has been described recently.[2] The genetic analysis of this sterility will here be extended, and new data concerning the map of the *X*-chromosome will be given.

The salivary chromosomes of the *A B* hybrid female show two inversions in the *X*-chromosome, one in the right, one in the left arm. The *XR* inversion has now been established genetically. In race *A* the genetical map shows the genes: compressed, sepia, ascute, javelin (slender) in the order given.[3] The *X*-chromosome of race *B*, based on the map already known[4] and on additional data, shows the order: compressed, ascute, sepia, javelin (table 1). A comparison of the maps shows that the breakpoints of the inversion lie to the right of compressed and to the left of javelin, respectively.

Included in the map are some genes which seem to have no counterpart in either race *A* or *D. melanogaster*. Filiform is a good dominant viable in male and homozygous female; the macrochaetae are long and slender with tapering tips. The lethal plexus kills the males in the late pupal stage; when occasional males emerge their wings show a plexus-like effect; they are very weak and die soon after emergence. The mutant radius rudimentary is expressed in the female only. The suppression of the effect in the male is not due to the influence of the *Y*-chromosome (tested in *XO* males). The expression in the female is rather variable and is influenced by environmental conditions; in flies raised at a temperature of 15°C. very

marked plexus effects are added to the rudimentary condition of the radius; at 25°C. the character usually overlaps wild type.

In race *A*, Gottschewski[2] has described two genes, lm_1 and lm_2, which have a certain lethal action in an environment of hybrid cytoplasm. In race *B* there are two corresponding genes which have a similar action. The symbols for these genes, proposed here, are: lm_1A and lm_2A for race *A*, lm_1B and lm_2B for race *B*.

Certain backcrosses of hybrid females were found to produce no or very few offspring. An example of certain mass matings will illustrate this: 30 males of a wild race *A* strain (Boise 1) were mated to 20 hybrid females with a vermilion sepia *X*-chromosome from the *B* father, an eosin vermilion *X*-chromosome from the *A* mother. Since the hybrid females were homozygous for vermilion, all normal male offspring should have carried that gene. The total offspring of these 20 females consisted of 47 wild-type

TABLE 1

THE *X*-CHROMOSOME OF RACE *B*

beaded	0.0	rose	38.9
scutellar	24.9	bubble	53.3
yellow	24.9	fused	54.0
Notch		compressed	
white	31.3	ascute	55.7
lm_1B*		scarlet	76.1
singed	32.7	lm_2B*	
vermilion	34.5	sepia	105.8
Filiform	34.6	radius rudimentary	128.2
lethal plexus	34.8	javelin	133.0
dusky	35.3	short	138.6
forked	35.8		

* The position of the interracial lethals is uncertain.

females and 15 wild-type males. These patroclinous males could not have inherited their *X*-chromosome from the mother, but must have resulted from the union of an *X*-bearing sperm and a no-*X* egg. This no-*X* egg can arise through primary non-disjunction or, as seems more likely, through crossing-over within the inversions, a four strand double crossover producing only dicentric and acentric *X*-chromosomes.

Gottschewski pointed out that the lethals are not completely dominant in the backcross females; in many cases "rare survivors" appear. The 47 females from the above cross belong in that category.

When the backcross males were w^e *v* (*A*) or *v se* (*B*), no offspring at all were produced; this is expected, since the *XO* males too, in this case, would inherit the lethals. In a third type of cross: a hybrid female free of the

lethals, backcrossed to a male carrying them should produce males and no or few females; the data bear out this expectation.

From these typical examples it may be concluded that both the w^e v (A) X and the v se (B) X must have carried lethal genes which were completely innocuous intraracially; also, they did not affect the F_1 hybrid generation where they would still be in an environment of race A or race B cytoplasm. It is the hybrid cytoplasm, then, which allows these genes to act as lethals.

Two genes must be concerned in the production of the lethal effect as shown previously by Gottschewski. A race A fly which is known to carry the lethals and which has the proper marker genes in the X is mated to a race B fly which is known to be free of the lethals. The resulting hybrid female, when backcrossed, gives a majority of wild-type sons. Those sons carrying any race A marker genes prove to be the result of crossing-over with the race B X-chromosome. No sons appear which have intact the section between the white region and the XR inverted region. Those males survive in which the two regions have become separated by crossing-over. Since crossovers in both directions survive, lm_1A in the white region and lm_2A in the XR inverted region must be together to produce the lethal effect; individually they no longer act as lethals. The reciprocal mating shows a corresponding situation in race B; lm_1B and lm_2B lie in the same respective regions and act in the same manner as the race A lethals.

The maternal effect by which these interracial lethals act was investigated by the experiment shown in table 2. A race B wild-type male is mated to a race A female which carries in the X-chromosome an inversion across the spindle attachment region associated with a dominant effect, short scutellum (Ss); this X-chromosome is lethal to the male. The autosomes are marked as follows: chromosome II carries Bare (Ba) in an x-ray induced inversion; chromosome III carries Blade (Bl) in standard sequence; chromosome IV carries Curly (Cy) in an x-ray induced inversion; chromosome V cannot be marked. In the hybrid female crossing-over should be suppressed effectively and the identity of racial origin of each chromosome be preserved, since in addition to the inversions mentioned above, there are present two interracial inversions in X, one in II. Crossing-over is suppressed least effectively in III, since some crossing-over does occur between Standard and Klamath, the common race B sequence. Ss Ba Bl Cy hybrid females are backcrossed to w^e v males of race A; the w^e v strain carries the race A interracial lethals. From this cross only few female offspring are obtained; they consist of the 16 different phenotypes given in table 2. Each phenotype individually is backcrossed to w^e v. All classes with the Ss marker are fertile; all classes without the Ss marker are sterile. All the fertile females are homozygous for the race A X-chromosome; all sterile females are racially heterozygous for X. The race A interracial lethals act only in cytoplasm (eggs) produced by a female

TABLE 2

$+(B)\ \sigma^{\prime}\ x\ Ss\ Ba\ Bl\ Cy\ (A)\ \varphi$

$w^{e}\ v\ (A)\ \sigma^{\prime}\ x\ \underline{Ss}\ \underline{Ba}\ \underline{Bl}\ \underline{Cy}\ \varphi\ x\ v\ dy\ (B)\ \sigma^{\prime}$

$+\ +\ +\ +$

$w^{e}\ v\ (A)\ \sigma^{\prime}\ X$

$\underline{w^{e}\ v\ (A)}$	$\underline{+(A)}$	$\underline{+(A)}$	$\underline{+(A)}$	
Ss	Ba	Bl	Cy	$= f$
$+$	$+$	$+$	$+$	$= s$
Ss	$+$	$+$	$+$	$= f$
$+$	Ba	Bl	Cy	$= s$
Ss	Ba	$+$	$+$	$= f$
$+$	$+$	Bl	Cy	$= s$
Ss	Ba	Bl	$+$	$= f$
$+$	$+$	$+$	Cy	$= s$
Ss	$+$	Bl	Cy	$= f$
$+$	Ba	$+$	$+$	$= s$
Ss	$+$	$+$	Cy	$= f$
$+$	Ba	Bl	$+$	$= s$
Ss	Ba	$+$	Cy	$= f$
$+$	$+$	Bl	$+$	$= s$
Ss	$+$	Bl	$+$	$= f$
$+$	Ba	$+$	Cy	$= s$

$v\ dy\ (B)\ \sigma^{\prime}\ X$

$\underline{v\ dy\ (B)}$	$\underline{+(B)}$	$\underline{+(B)}$	$\underline{+(B)}$	
Ss	Ba	Bl	Cy	$= *(s)$
$+$	$+$	$+$	$+$	$= f(13)$
Ss	$+$	$+$	$+$	$= f(5)$
$+$	Ba	Bl	Cy	$= *(s)$
Ss	Ba	$+$	$+$	$= f(13)$
$+$	$+$	Bl	Cy	$= s(.5)$
Ss	Ba	Bl	$+$	$= *(f)$
$+$	$+$	$+$	Cy	$= s(3)$
Ss	$+$	Bl	Cy	$= s(0)$
$+$	Ba	$+$	$+$	$= f(11)$
Ss	$+$	$+$	Cy	$= s(0)$
$+$	Ba	Bl	$+$	$= *(f)$
Ss	Ba	$+$	Cy	$= s(1)$
$+$	$+$	Bl	$+$	$= f(6)$
Ss	$+$	Bl	$+$	$= *(f)$
$+$	Ba	$+$	Cy	$= s(0)$

In the 16 classes of backcross females all marked chromosomes are race A, all $+$ chromosomes are race B. In the $w^{e}\ v$ backcross, s = sterile, f = fertile. In the $v\ dy$ backcross, s = no or few $v\ dy$ females produced, f = $v\ dy$ females produced, * = class not obtained, symbol in parenthesis refers to expectation. Numbers in parenthesis indicate average $v\ dy$ ♀ offspring per mother.

racially heterozygous for the *X*-chromosome. The autosomes have little or no influence.

When *Ss Ba Bl Cy* hybrid females are backcrossed to *v dy* (*B*) males which carry the race *B* interracial lethals, a somewhat different picture presents itself. Of the 16 different phenotypes only eleven have been obtained; all of these produce some offspring. In deciding which chromosome combinations are responsible for the lethality of the lm_1B lm_2B complex the male offspring are neglected, first, because the viability is decreased by certain *Y*-chromosomes,[5] an uncontrolled factor, and, second, because the results would be obscured by independent sex-linked lethals whose mutation rate is increased by hybridization.[6] The number of *v dy* female offspring per mother is used for the determination of the action of the interracial lethals, since only this kind of progeny is comparable in all classes. In table 2 it can be seen that none of the classes with the *Cy* marker show as many *v dy* females per mother as any of the classes without the *Cy* marker. It seems, therefore, that the race *B* interracial lethals act in cytoplasm (eggs) produced by a female racially heterozygous for chromosome IV. It must be pointed out, however, that the results are not nearly as clear as those for the race *A* lethals; caution must be used in accepting chromosome IV as the only one responsible, until further evidence is obtained.

With these interracial lethals, dominant in hybrid cytoplasm, an effective isolating mechanism is accomplished, since no or very few offspring are obtained from hybrid females which are backcrossed to any kind of males. The individuals carrying the lethals die in the egg or early larval stage. Whether or not this mechanism in fact is employed in nature will become evident from an analysis of wild populations which is now in progress.

Summary.—1. The break-points of the *XR* interracial inversion lie to the right of compressed and to the left of javelin, respectively.

2. The genetic isolation of race *A* and race *B* has progressed an additional step in the hybrids of certain strains where the females produce no or very few offspring. This is accomplished through interracial lethal genes connected with a maternal effect.

[1] Lancefield, D. E., *Z. i. A. V.*, **52**, 287–317 (1929).
[2] Gottschewski, G., *Ibid.*, **78**, 338–398 (1940).
[3] Sturtevant, A. H., and C. C. Tan, *Jour. Genet.*, **34**, 415–432 (1937).
[4] Beers, C. V., *Genetics*, **22**, 577–586 (1937).
[5] Sturtevant, A. H., *Proc. Nat. Acad. Sci.*, **23**, 360–362 (1937).
[6] Sturtevant, A. H., *Ibid.*, **25**, 308–310 (1939).

CHAPTER II

High Mutation Frequency in Drosophila pseudoobscura, Race B.

by Klaus Mampell

Wm. G. Kerckhoff Laboratories of the Biological Sciences,

California Institute of Technology.

Variations in the mutation rate may be due to a number of factors in the external or internal environment of the organism. We can control many of these factors; others are unknown and appear spontaneously; they may persist for some time and then disappear again. In some cases this may be due to a selection of proper genic modifiers of the mutation rate. Modifying genes have been made responsible for the control of the mutation rate, since the mutation rate can be altered by hybridization; such a case has been described for D. pseudo-obscura[1]. Several investigators have observed cases of high mutation frequencies in D. melanogaster[2,3,4]; other cases of the same nature are more doubtful. D. pseudoobscura, race B, has furnished another example exhibiting the sudden rise of mutation frequency, to be recorded in this paper.

The phenomenon first appeared in the progeny of a pair mating, where four sex-linked mutants were observed. On out-crossing flies from this culture, mutations occurred again to an unexpected degree. The number of mutations per pair mating is quite variable. Some of the most striking cultures are recorded in Table 1. In each case we deal with the progeny of only one pair mating. The average yield per culture is 242 flies; this number is based on counts of the progenies of 758 pair matings chosen at random; even in the largest progenies there

Table 1.

Culture #	Type of mutation	# of mutations
original	bd, y, ri, (spread wings)	4
1045	v, Aw, L, (Abnormal)	4
1176	Bd^{ct}, dx, dy(m), Sc	4
1671	dy(m), L, Vg, (Abnormal)	4
1691	bd, sct, tt, Cy, (wing-mosaic)	5
1697	bd, sct, com, asc, Sc, (Rough eye)	6
2654	bd, dy(m), bbl, Aw, (rough eye)	5
2676	sct, w, sn(f), Aw, tt	5
2708	dy(m), tt, Tho, (Rough eye)	4
2936	bd, sct, Vg, (Rough eye)	4
2971	bd, com, H^{A}, Sb, Vg	5
a177	dy(m), com, asc, jv, dow, (rough eye)	6

are less than 500 flies per bottle.

The mutations evidently may occur at any time during the development of the germ line or of the somatic line. Mutated individuals of a certain type may occur in numbers varying from one to fifty percent of the offspring of a pair mating; in somatic mutations patches of varying sizes have been observed. There seems to be no preference as to the time of mutation. The numbers of mutations recorded in this paper refer to separate occurrences of mutation, not to numbers of individuals showing new mutant characters.

In our study only "visible" sex-linked recessives are used as indicators of the high mutation rate, because they are detected in the immediate offspring of a female with the abnormal rate. Autosomal recessives should not be used, because the probability of their detection depends on the mating system used, and even then it is not certain at what time and in which individual they have arisen. Dominants are disregarded to avoid confusion with another mechanism which is responsible for inducing mutations of the defiency type; we shall refer to this later. Although the high mutation rate is observed in males also, and although dominants and autosomal recessives are produced by the present mechanism, it is for the above reasons that we restrict our observations to visible sex-linked recessives arising in females.

The most likely cause of a phenomenon of this nature is a genic one. It has proved difficult, however, to demonstrate that a gene is responsible for the increase in the mutation rate. We deal here with a quantitative difference between the normal and

the mutating strains; therefore, large numbers are required to make the data meaningful.

The normal spontaneous mutation rate for visible sex-linked recessives in Drosophila pseudoobscura, race B, is 0.01%. This number is based on the occurrence of four sex-linked recessives in 46,804 flies, from cultures preceding the time of the first observation of the high mutation rate, plus those from the pure Seattle strain.

In determining the abnormal mutation rate we shall consider only those cultures which have given at least one sex-linked recessive, since this is the only indication of the presence of the "Mutator" gene in the mother. Thus, we obtain a mutation rate per culture showing mutations, rather than a mutation rate per total number of flies. The higher the total mutation rate, the greater will be the coincidence of several sex-linked recessives in one culture, remembering that we always deal with the offspring of one female per culture and that the average number of offspring is the same for wild type females and Mutator female With a normal mutation rate of 0.01% for sex-linked recessives it is obvious that two mutations would occur by chance in the same culture so rarely that the mutation rate per culture showing mutations is practically 1.00. Thus, from Seattle females seven mutations were obtained in seven cultures (rate = 1.00); from Morro females five mutations were obtained in five cultures (rate = 1.00). But from selected females 160 mutations were obtained in 102 cultures (rate = 1.57). The coincidence of mutations is directly related to the total mutation rate, so

that by knowing one we can determine the other. Due to the small numbers, which are always a disadvantage in a study of this sort, the errors may be considerable; the calculations are also based on the assumption of a constant number of flies per bottle, which further decreases the accuracy of the result. Therefore, the rates which will be presented must be taken as approximations; but we are interested in getting some idea of the mutation frequency in flies heterozygous or homozygous for the Mutator gene. In general, the estimates are probably conservative, since the normal spontaneous mutation rate is included in the abnormal rate, which would tend to lower the coincidence.

Females resulting from a mating of a selected fly to wild type, regardless of the direction in which the cross is made, give a rate of 1.47 (100 mutations in 68 bottles giving mutations This is the rate obtained from flies heterozygous for the Mutator gene. Assuming a Poisson distribution, that is, a population homogeneous for a factor increasing the mutation rate, a result like this would be obtained, if the rate of mutations per total number of flies were 0.34%. Thus, the normal spontaneous mutation process is increased thirty-four times with one dose of the Mutator gene.

If flies can be homozygous for the Mutator gene, we might expect a higher mutation rate from females that have resulted from a mating of both selected male and female; or, conversely, only if flies resulting from such a mating give more mutations, can we know that homozygous flies are produced and that they have a higher mutation rate than the heterozygous flies. Females resulting from such a mating, give a rate of 1.76 (60

mutations in 34 cultures giving mutations). That is the rate obtained from females both of whose parents were probably heterozygous for the Mutator gene. From that mating we should expect one fourth of the offspring to be homozygous for the Mutator gene one half heterozygous and one fourth wild type.

Let m be the expected mean frequency of occurrence of a mutation in the population of subsamples. Since m is known to be equal to .0034 x 242 for cultures resulting from heterozygous females, and equal to .0001 x 242 for cultures resulting from homozygous wild type females, the relative frequencies of subsamples with one mutation, two mutations, etc. are known for these two groups. Let k be the number of cultures resulting from homozygous Mutator females; then there are $k\frac{m}{e^m}$ cultures with one mutation in this group, 2k(.3615) cultures with one mutation when the mother was heterozygous, and k(.0234) cultures with one mutation when the mother was wild type; similarly for cultures with two, three, etc. mutations. We equate the theoretical total of cultures with one mutation to the observed number with one mutation, and do likewise for the cultures with two, three, etc. mutations; we obtain the following six equations:

$$k\frac{m}{e^m} + 2k(.3615) + k(.0234) = 21$$

$$k\left(\frac{m^2}{2e^m} + .2979\right) = 7$$

$$k\left(\frac{m^3}{6e^m} + .0960\right) = 2$$

$$k\left(\frac{m^4}{24e^m} + .0168\right) = 2$$

$$k\left(\frac{m^5}{120e^m} + .0028\right) = 1$$

$$k\left(\frac{m^6}{720e^m} + .0004\right) = 1$$

The number of mutations in the homozygous group is equal to km, in the heterozygous group it is equal to 2k(242)(.0034), and in the wild type group it is equal to k(242)(.0001); the total number of mutations obtained was 60; therefore, we find $km + 2k(242)(.0034) + k(242)(.0001) = 60$, or, $k = \frac{60}{1.67 + m}$. We can now determine the most probable value of m in the usual manner [5]. We find m = 1.703. Thus, the mutation rate of females homozygous for the Mutator is 0.70%, approximately twice that of the heterozygotes. This is a linear increase with the dosage. In this connection, it is interesting that the gene dotted in maize produces an exponential increase with the dosage as concerns mutations of the "a" gene [6].

The offspring of heterozygous flies should have a total mutation rate of 0.17% if the above calculations are correct, since now half the flies are heterozygous and half are wild type; therefore the mutation rate should be one half of 0.34%. We actually obtain 7 mutations in 4679 flies which is a rate of 0.15%; this is in good agreement with the expectation.

The crosses designed to locate the Mutator gene are shown in Table 2. All the females shown in the table should be heterozygous for the Mutator gene except those with the wild type chromosome whose homologue is responsible for carrying the Mutator gene; from these females we should expect a rate of 1.00 whereas all the others should give an average of 1.47. Thus, chromosome II would seem to be the carrier of the Mutator gene.

Table 2.

selected Stubble(II) or Scute(III) or Curly(IV) x Morro or Seattle

Stubble or Scute or Curly ♂♂ x Morro or Seattle ♀♀
Morro or Seattle

		# of mutations	in cultures showing mutations	rate
selected II		23	14	1.64
wild type II		4	4	1.00
selected III	♀♀	12	9	1.33
wild type III		6	4	1.50
selected IV		15	8	1.86
wild type IV		15	11	1.36

This, however, must be taken cum grano salis; not only are the numbers uncomfortably small for this kind of a test, but the dot chromosome which cannot be marked was not controlled. The conclusion, therefore, is tentative.

Table 3 shows the maps of the chromosomes; the map of the X-chromosome is based partially on the one published previously[7]. Not all of the mutants shown on the maps can be assumed to have been induced by the Mutator gene here under discussion; there is another mechanism at work in the same cultures which induces an extraordinarily high number of mutations of the deficiency type, mainly Smoky, Notch, and Minutes. Sometimes it is difficult to tell the two apart, because in some cases they have nearly the same effect. Description of the other mechanism will have to wait until a later date; it is believed that the sex-linked and autosomal recessives and many of the dominants have been induced by the Mutator gene here under consideration.

Since tests for allelism were not generally applied, the number of occurrences of a certain mutation can be given only for those loci where identification by phenotype is not likely to lead to confusion with other mutants. The asterisks indicate that the mutation has occurred at least once, but the nature of the mutant does not permit identification by inspection. Thus, dusky and miniature cannot be distinguished phenotypically; although many mutations of this type have appeared, it cannot be stated how many of each. The bar signifies that the mutant has not been observed.

The mutants were given the names of the D, melanogaster mutants which they resemble most closely. Some cannot readily be

Table 3.

X-chromosome		
lozenge (lz)	0.0	*
almondex (amx)	3.6	*
beaded (bd)	31.1	50
Minute^{4} (M^{4})	35.5	*
Hairless^{A} (H^{A})	54.1	*
scutellar (sct)	61.9	39
yellow (y)	61.9	6
prune (pn)		2
deltex (dx)	64.7	3
Notch (N)		24
white (w)	69.6	3
lm$_{1}$B		-
singed (sn)	71.0	*
vermilion (v)	72.8	*
Filiform (Ff)	72.9	*
lethal plexus (l^{p})	73.1	-
Fused^{2} (Fu^{2})	73.5	*
dusky (dy)	73.6	*
miniature (m)	73.6	*
forked (f)	74.1	*
rose (rs)	77.2	-
tilt (tt)	83.7	11
bubble (bbl)	91.6	*
fused^{1} (fu^{1})	92.3	*
compressed (com)		5
ascute (asc)	98.9	10
Abnormal wing (Aw)	99.8	7
Curvoid (Cur)	106.2	*
scarlet (st)	119.3	*
lm$_{2}$B		-
sepia (se)	149.0	-
radius rudimentary (rr)	171.4	-
javelin (jv)	176.2	*
short (s)	181.8	3
radius incompletus (ri)		*
shiny (shi)		*
tiny bristle (tb)		*
condensed (con)		*
roughex (rux)		*
notchy (ny)		*
downy (dow)		*
diminutive (dm)		*
small bristle (sbr)		*
ruby (rb)		*
approximated (app)		*
twisted (tw)		*

II. chromosome		
Smoky (Sm)	0.0	40
Thorax (Tho)	5.1	2
Stubble (Sb)	6.1	10
Hairless^{E} (H^{E})	14.9	*
bithorax (bx)	39.2	-
glass (gl)	42.1	*
cinnabar (cn)	54.7	-
Minute^{8} (M^{8})		*
Minute^{14} (M^{14})		*
Mutator (Mu)	?	

III. chromosome		
Abbreviated (Abb)	0.0	*
Lobe (L)	29.2	*
Scute (Sc)	50.5	*
Vestigial (Vg)	50.5	*
gap (gp)		*
orange (or)		*
Minute^{5} (M^{5})		*
Minute^{6} (M^{6})		*
Minute^{7} (M^{7})		*
Minute^{10} (M^{10})		*
Minute^{12} (M^{12})		*
Minute^{15} (M^{15})		*

IV. chromosome		
Minute^{3} (M^{3})	0.0	*
Scutellum diminished (Sd)	32.9	*
Hairless^{B} (H^{B})	57.2	*
Curly (Cy)	69.7	*
Dachsoid (Dsd)		*
net (net)		*
dachs (d)		*
Large wing cells (Lwc)		*
Enhancer-glass (E-gl)		*
Minute^{1} (M^{1})		*
Minute^{2} (M^{2})		*
Minute^{9} (M^{9})		*
Minute^{11} (M^{11})		*
Minute^{13} (M^{13})		*

compared. Hairless^{A} is a dominant, lethal to the male. Both prune alleles always show a distinct mottlingeffect. The deltex alleles are female sterile and semilethal to the male. Abnormal wing has large wing cells giving the wing a coarse appearance; delta or net type of venation is common; it is usually lethal to the male. In shiny the chitin has a polished appearance. Thorax shows a longitudinal median groove of the thorax as the most striking characteristic. HairlessE rarely survives when homozygous; in the homozygous state it shows extreme aristopedia effects; the heterozygous flies sometimes have the aristae reduced or absent; the legs also are sometimes reduced. Vestigial takes off some bristles along the costa when heterozygous. Scutellum diminished affects the size of the scutellum which may be practically absent in extreme cases. Hairless^{B} resembles heterozygous Hairless^{E}. Dachsoid shortens the legs and roughens the eyes. Large wing cells resembles Abnormal wing in its phenotypic effect. Enhancer-glass allows heterozygous glass to express a rough eye.

In addition to the mutants shown in the maps there has occurred a large number of mutants which were either inviable or sterile, lost or discarded before being mapped, or which have not yet been mapped. The total number of observed mutations is approximately two thousand.

Aside from the evolutionary significande of Mutator genes it is interesting to speculate about their mode of action. From the nature of the mutations which are induced by this mechanism it seems obvious that we are dealing with true point mutations. Mutator genes probably act through a chemical medium. Chemicals are the least satisfactory agents of the more important ones

which have been investigated as to their faculty of inducing mutations. It is possible that Mutator genes interfere with the proper reproduction of genes and that by learning the nature of this mechanism we may know more about the chemistry of gene reproduction. Mutator genes rather than producing certain substances might be responsible for the lack of a substance necessary for growth and reproduction of genes in general. There is no good chance at this time to learn much about Mutator genes except the result of their activity, unless the substance involved were in the nature of a hormone; this is unlikely, since intranuclear processes are concerned. It must be left to future research further to illuminate this problem.

The author is indebted to Professor A.H.Sturtevant for suggestions and criticism and to Mrs. Harriet Bonner for the application of the mathematical treatment to the data.

Summary.

1. Drosophila pseudoobscura, race B, has furnished certain stocks with an abnormally high mutation rate.

2. The mutations may occur at any time during the development of the germ line or of the somatic line.

3. The phenomenon is due to a dominant "Mutator" gene which increases the normal spontaneous rate about thirty-four times when heterozygous, and about seventy times when homozygous; this represents a linear increase in the mutation rate with the dosage of the Mutator gene.

4. The Mutator probably is linked to the second chromosome.

5. Atotal of approximately two thousand mutations was observed; some of the mutations were located.

[1]Sturtevant,A.H., P.N.A.S. 25: 308-310 (1939).

[2]Demerec,M., Genetics 22: 469-478 (1937).

[3]Tiniakov,G., C.R.Doklady Acad.Sci.U.R.S.S. 22: 609-612 (1939).

[4]Neel,J.V., Genetics 27: 519-536 (1942).

[5]Whittaker and Robinson, The Calculus of observations,
Blackie & son: 214 (1924).

[6]Rhoades,M.M.,Cold Spring Harbor Symp.Quant.Biol.9:138-142(1941).

[7]Mampell,K., P.N.A.S. 27: 337-341 (1941).

CHAPTER III

A DEFICIENCY INDUCER IN DROSOPHILA PSEUDOOBSCURA, RACE B

by

Klaus Mampell

Wm.G.Kerckhoff Laboratories of the Biological Sciences,

California Institute of Technology

INTRODUCTION

Recently a case was described where a Mutator was responsible for a great increase in the appearance of point mutations (Mampell 1943). In the present investigation a gene induces mutations of the deficiency type such as Smoky, Notch and Minutes; a high percentage of mosaics is common. In Drosophila and Zea genes have been described which by various mechanisms induce gross mutations. The present phenomenon is unlike any of the others, although many of the effects are the same. For instance: The "sticky" gene in maize alters the cell physiology; mitotic and meiotic abnormalities are common; both chromosome aberrations and mutations are induced (Beadle 1937). Chromosome elimination occurs in the eggs laid by D. simulans females homozygous for the claret gene (Sturtevant 1929). Minute-n in D. melanogaster produces patches in which the Minute carrying X-chromosome is frequently eliminated (Bridges 1925a).

Somatic crossing-over leading to small mosaic areas is accentuated by certain minutes (Stern, 1936). Mosaics of the haploid-diploid type were observed in certain stocks of D. pseudoobscura, race A (Crew and Lamy 1938). The mechanism by which the "Deficiency Inducer" acts is still obscure, but some of the results of its activity can now be presented.

METHODS

As with the Mutator stocks, in the present study we deal only with cultures that are the result of a pair mating. To avoid confusion with the Mutator, only Minutes, Smoky and Notch and all kinds of mosaics are taken as indicators of the presence of the Deficiency Inducer. All other kinds of mutant individuals are neglected. This is necessary, because the Mutator and the Deficiency Inducer must be supposed frequently to exist in the same individual, but we are able to tell them apart by the kind of change they induce. Most of the mosaics appear to be bilateral; it is not necessary, however, to assume that other mosaics are less common. Bilateral mosaics are more easily detected, and the flies were not carefully examined for possible small patches of mutated tissue. Practically all the Minute mosaics recorded were mutated at least for half the thorax. Other patterns have been observed, but are not usually recorded.

THE APPEARANCE OF THE PHENOMENON

The Deficiency Inducer was found in the Seattle strain which had been suspected for some time of producing an unusually high number of Minutes and various mosaics. The number of mutations per pair mating is variable according to the genetic constitution of the individuals and the chance coincidence of the random distribution. Some of the cultures with a particularly high frequency are recorded in Table 1. In each case we deal with the progeny of one pair mating. The average yield per culture is 235 flies; this number is based on counts of the progenies of 851 pair matings chosen at random. Even in the largest progenies there are less than 500 flies per culture. This is not ture, however, for lines with particularly high frequencies. There, the average number of flies per culture goes down to 96. This number is based on 41 pair matings yielding 3920 flies in a line considered homozygous for the Deficiency Inducer. As will be explained later, it is probable that an appreciable number of dominant lethals are induced. This may account for the lower yield in the latter series.

Only cultures with five or more mutations have been recorded in Table 1. Those with less than five are, of course, much more numerous. The numbers must be taken

TABLE 1.

Culture No.	Type of mutation								No. of mutations
	M	Sm	N	other dominants	Minute mosaic	Smoky mosaic	Notch mosaic	other mosaics	
1079	+	-	-	-	6	-	-	1	8
1270	+	-	-	-	7	-	-	4	12
1342	+	-	-	-	5	-	-	9	15
1415	-	-	-	-	3	-	-	2	5
1453	-	-	-	-	4	-	-	1	5
1457	+	-	-	-	2	-	-	2	5
1593	-	-	-	-	2	-	-	4	6
2070	+	-	-	-	2	-	-	6	9
2221	+	-	-	-	3	-	-	1	5
2228	-	-	-	-	4	-	-	2	6
2238	-	-	-	-	4	-	-	1	5
2528	+	-	-	-	2	-	-	2	5
a287	+	-	-	3	-	-	1	-	5
a666	+	-	-	-	-	-	-	4	5
a677	+	+	-	-	3	1	-	2	8
a683	+	-	+	-	3	-	-	1	6
a758	+	-	-	-	4	-	-	-	5
b 85	-	-	-	1	3	-	-	2	6
b 92	+	-	-	-	5	-	-	2	8
c 71	+	-	-	-	4	1	-	1	7
c117	+	-	-	-	4	-	-	3	8
c242	+	-	-	-	2	-	-	2	5
c245	+	-	-	2	2	2	-	4	11
c253	-	-	-	-	2	-	-	3	5
e143	+	-	-	-	4	1	-	-	6
e217	-	-	-	-	4	1	-	2	7

\+ = at least one individual of that nature observed.

\- = no individual of that nature observed.

Numbers refer to number of mutations, not number of individuals showing new mutant characters, although in the case of mosaics, the two coincide.

as a minimum, since only clear cases are included. There may have been different Minutes in some cultures, but since they are usually difficult to tell apart phenotypically, only one Minute is recorded; in the case of the mosaics, those with missing parts of the anatomy were neglected, although they probably represent dominant lethal mutations.

There is no doubt that the mosaics represent real mutations, since in some cases they affect the germ line and are then inherited as single factors. Although no cytological investigation has been made, it is probable that we usually deal with deficiencies, since Notch, Smoky (Delta of D. melanogaster) and Minutes are commonly found to be due to deficiencies. Whether these represent actual deficiencies or an inactivation of a series of genes is unknown, but the former is more probable.

In order to explain the deficiency type of mutation, we might postulate faulty chromosome division as the cause for their origin. Incomplete reduplication of the chromosome would lead to a large deficiency probably lethal to the individual or to a smaller one which may permit the individual to survive.

With this in mind we can understand one reason for the predominance of mosaics. The mutation evidently occurs at any time during the development.

Mutated individuals of a certain type may occur in varying numbers in the offspring of a pair mating; there seems to be no preference as to the time of mutation in the germ line or the somatic line since mutated patches of varying sizes have been observed. One of the reasons for the predominance of mosaics is the possibility that, due to the gross kind of mutation, those individuals survive more often which have some wild type tissue. Most of the gross mutations are lethal to the individual, and though they occur at any time, they are detected only when they occur late enough to affect only part of the fly. Thus, dominant lethals are sometimes detected when they happen to be linked to a dominant marker gene and when they arise early in the development of the germ line. On outcrossing such an individual to an unrelated fly the ratio of the dominant marker to wild type would be upset; such a case has been observed at least once. A male heterozygous for Stubble was outcrossed to a wild type female. There were seven Stubble flies and 358 wild type flies in the offspring; the Stubble flies had tumors and were weak. Flies frequently emerge with various parts of their anatomy missing. This could be interpreted as the result of the action of dominant lethals arising in somatic tissue and accounting for the missing parts usually of the thorax such as legs, wings

and halteres. Obviously, if the dominant lethal occurred somewhat earlier, the whole fly would be missing, and the mutation would not be discovered.

The second reason for the apparent predominance of mosaics is the fact that only one Minute was recorded per culture regardless of the actual number of Minute individuals appearing in that culture. Since the Minutes are usually much alike phenotypically, no attempt was made to distinguish between them. Only through linkage tests would it be safe to conclude how many different Minutes there may have been in one culture. This was not attempted. There are only three phenotypes which were used as an indication of the Deficiency Inducer: Notch, Smoky and Minutes. In a line with a particularly high rate we should find some cultures with more than one of the three phenotypes represented. Since this is the case (a677 and a683 in Table 1), it is safe to assume that in those cultures we sometimes deal with different Minutes rather than with several individuals showing the same Minute.

A third reason can be given. As will be discussed later, it is possible that cytoplasm which has been preconditioned by the Deficiency Inducer will induce deficiencies. If this is so, homozygous wild type individuals may yet have deficiencies induced by the action of the cytoplasm, but all of these individuals would be mosaics, since the deficiencies could arise only somatically by this mechanism.

Certain Minute mosaics serve as an indication that deficiencies of varying extent are produced. When a Stubble female was outcrossed to a wild type male, a mosaic occurred in the offspring which was Minute for half the thorax without indication of Stubble, whereas the unmutated half was Stubble. This is interpreted as a deficiency including the Stubble locus and by removing the gene, it would also remove its phenotypic effect. In a similar case, when a Stubble Hairless^{E} female was outcrossed to a wild type male, a mosaic female in the offspring was Stubble Hairless^{E} on the right side whereas the mutated left had an abnormal eye and wing, but was wild type for bristles. This probably means that a deficiency arose long enough to include both the Stubble and Hairless^{E} loci, thus removing their phenotypic effects. Similarly, in two instances, a Stubble female produced a female whose one half was Stubble, while the other half was wild type for bristles, but had a smaller eye and wing. The latter three cases may also be explained on the same basis as the two following ones, namely: haploidy for part of the tissue.

There are two examples for this kind of mosaic. In one cases a wild type male was mated to a female homozygous for seven sex-linked mutant genes: bd^{ct} sct dy rs st se s. In the progeny, one mosaic was wild type female on one side and bd^{ct} sct dy rs st se s female on

the other side. Similarly, a bd^{ct} sct dy rs st se s male was mated to a female heterozygous for wild type and bd^{ct} sct dy rs st se s. In the progeny a mosaic occurred which was wild type female on the right half and bd^{ct} sct dy rs st se s female on the left half. Since the side showing the sex-linked recessives was female in both cases, it could not have arisen merely by elimination of an X-chromosome, for then, that part would have been male. Although in both cases other explanations are possible, it could both times be a matter of an entire chromosome set failing to divide. Then the part revealing the sex-linked recessives would be completely haploid and, therefore, normal female. In the first case the male set, in the second case the female set could have lagged one division. Haploid-diploid mosaics have been described also in D. melanogaster where they were first observed (Bridges 1925b), and in D. miranda-D. pseudoobscura hybrids (MacKnight, 1937)

Gynandromorphism has also been observed. It could likewise be explained on the basis of an entire chromosome failing to divide.

THE CAUSE OF THE PHENOMENON

The most likely cause of a phenomenon of this nature is a genic one, but it has proved difficult, so far, to make a gene responsible for the phenomenon. However, unless clear evidence to the contrary is obtained, we shall take the genic cause for granted.

Little is known about the location of the Deficiency Inducer gene. So far, the only chromosomes which can be excluded are Y, X and II; there is then purely negative evidence. When Stubble is mated to Morro, and the Stubble sons are again outcrossed to Morro females, the wild type offspring are homozygous for wild type second chromosomes. On outcrossing these flies to Morro again, fourteen mutations are obtained in nine cultures showing mutations. Therefore, chromosome II is excluded, and from the nature of the cross it is evident that at the same time we exclude Y and X.

THE EXPRESSION OF THE DEFICIENCY INDUCER

In race B the normal spontaneous mutation rate for Minutes, Smoky, Notch and various mosaics is so low that no percentage can be given. In 30819 flies from cultures that had never been crossed to the Seattle strain, only one Minute has been observed. When a Minute is observed in a culture, this is taken as an indication of the presence of the Deficiency Inducer in the parent. We are interested in knowing the mutation rate of males and females heterozygous or homozygous for the Deficiency Inducer. In order to determine the percentage of mosaics among flies heterozygous for the Deficiency Inducer, we count the number of mosaics in cultures that have a Minute, Smoky or Notch, and that have resulted from a mating of a selected fly to

wild type (Morro in this case). The appearance of one of these mutants indicates that the selected parent carried the Deficiency Inducer. We know that this parent is heterozygous for the Deficiency Inducer, because it has resulted from a mating of a selected fly outcrossed to Morro. Mosaics in this case are supposed to have resulted from a mutation in the zygote at the earliest. From heterozygous male outcrosses nineteen cultures with Minutes were obtained. Multiplying this number by the average number of offspring per culture we obtain a total of 4465 flies half of which will carry the Deficiency Inducer. Among these 2233 heterozygous Deficiency Inducer flies there are three mosaics or 0.13%. From the same kind of female outcross, we obtain fourteen such cultures or 3290 flies half of which are heterozygous. There are here eight mosaics in 1645 heterozygous Deficiency Inducer flies or a rate of 0.49%. The chi square test shows such a difference to occur by random sampling with a probability of about 5%. There are thus almost four times as many mosaics when the heterozygous parent was female than there are with the male as the heterozygous parent. We deal then probably with a maternal effect, that is, the Deficiency Inducer gene is more effective in cytoplasm that has been preconditioned by the Deficiency Inducer gene than in wild type cytoplasm.

From a certain mating (cl17 in Table 1) seven mosaics were obtained in 265 flies. This culture may be homozygous

for the Deficiency Inducer gene. Some inbred lines were established from this culture. Among a total of 3920 descendants there were 62 mosaics or a rate of 1.58%. If it is true that a maternal effect is connected with the Deficiency Inducer, we should expect the following rates: one dose in wild type cytoplasm, (this gives a rate of 0.13% as was learned from the male outcross), no Deficiency Inducer, one dose and two doses of it in heterozygous cytoplasm; no Deficiency Inducer, one dose and two doses of it in homozygous cytoplasm. All of those we can tell only by carefully marking the Deficiency Inducer. This has not been accomplished so far. We do know that the combination of no Deficiency Inducer and one dose of it in heterozygous cytoplasm gives a rate of 0.49%, but we do not know which proportion of this rate is represented in either group.

We now determine the relative numbers of male and female mosaics. A total of 459 mosaics is considered here. Of those, 219 are males and 240 are females. Among the females there are 12 Notch mosaics which cannot occur in males, since Notch is lethal to the male. Subtracting this number from the total number of female mosaics, we get 228, or a nearly equal number of male and female mosaics. Since there usually is a slight excess of females in the cultures, the numbers are certainly not significantly different. We should, however, expect a higher number of Minute mosaics in females than in males, since

a sex-linked Minute would probably be lethal to the male. Indeed, there are 95 male Minute mosaics and 135 female Minute mosaics, yet, the total number of mosaics is approximately the same in the two sexes. This might mean that the Deficiency Inducer affects the males more readily than the females. They would have an excess of mutations in the autosomes to make up for the excess of sex-linked dominants the females can reveal. Thus, there are fifteen male Smoky mosaics and eleven female Smoky mosaics, but, since the numbers are small, the difference may not be significant. The other possibility is that the Deficiency Inducer is responsible for sex-linked recessives which would show in males, but, of course, not in female mosaics. This is the more probable explanation, especially since mosaicism for a condition strongly suggesting beaded has been observed ten times in males, but never in females. Also, mosaicism for conditions indicating such sex-linked recessives as dusky, tilt, white and yellow has occurred in males, but never in females. This would mean that recessives are induced as well, but it may be that they are of a different nature than ordinary recessives. Perhaps we deal here with very small deficiencies including just one locus.

If we assume, then, that the mutability affects both sexes equally, we must come to the conclusion that the mechanism induces an approximately equal number of recessives and dominants, but since dominants seem to be in the

minority in the ordinary spontaneous mutation process, it would induce more dominants proportionally. This is contrary to the observations with the Mutator, which also induces recessives and dominants, but seems to do so preserving the proportions of the normal spontaneous mutation process.

THE DISTRIBUTION OF THE MUTATIONS

The Minutes seem to be distributed at random. Only the X-chromosome shows relatively fewer Minutes, but this can be explained on purely technical grounds. Since Minute in the X is almost always lethal to the male, we would automatically discriminate against sex-linked Minutes by employing the technique usually used in allocating mutants to chromosomes, namely, male outcrosses. In a random sample of 85 new Minutes, 34 were males and 51 were females. The excess of females indicates that sex-linked Minutes occur with proportionate frequency. The same conclusion was derived from the mosaics. We assume, then, that the Deficiency Inducer affects the chromosomes at random. Table 2 shows Smoky, Notch and the Minutes as far as they have been located.

DISCUSSION

If our assumption that the cytoplasm plays a role in the production of these mutations is correct, it would seem that we may deal with a greater viscosity of the cytoplasm

TABLE 2

X-chromosome			II. chromosome		
Minute[4]	35.5		Smoky	0.0	(48)*
Notch		(25)*	Minute[8]		
Minute[17]			Minute[14]		
Minute[19]			Minute[16]		
			Minute[18]		

III. chromosome	IV. chromosome	
Minute[5]	Minute[3]	0.0
Minute[6]	Minute[1]	
Minute[7]	Minute[2]	
Minute[10]	Minute[9]	
Minute[12]	Minute[11]	
Minute[15]	Minute[13]	
Minute[20]		

* Numbers in parenthesis refer to number of occurrences.

which thus would tend to break the chromosomes. In that case it would not be explained why we do not observe inversions and translocations. The same would hold, if we were dealing with a spindle abnormality. Here, likewise, we should expect aberrations of all kinds. It is necessary to make a number of crossing-over tests in order to discover inversions genetically, but translocations should be discovered readily. In the great majority of male outcrosses the autosomes were marked with dominants. Any translocation affecting them would have been discovered. There is only one single case suggesting a translocation, and that one is between homologous chromosomes. A male which was Smoky over Stubble was outcrossed to wild type. In the offspring, besides the expected Smoky and Stubble flies, Smoky Stubble and recessive Stubble occurred as well. If the breakpoints were close and in the vicinity of the Stubble locus, we might get a small duplication in one chromosome and a small deficiency in the other. This is the only suggestion of anything resembling a translocation.

It is because of the fact that apparent deficiencies are the only abnormalities produced that the author prefers the hypothesis that a failure for the chromosomes to be perfectly reduplicated accounts for the deficiencies. In the discussion of the Mutator gene it was postulated that the Mutator may be responssible for the lack of a substance necessary for the proper growth and reproduction of genes.

It is indeed intriguing to think that the Deficiency Inducer might be responsible for the lack of a substance necessary for the proper growth and reproduction of the connecting fibers linking the genes together. We could then understand why it could induce mutations of a much grosser nature than the Mutator, since the area involved could be much more extensive. Anything from one locus to the entire chromosome set could be and evidently is affected. Together these two genes would control one of the most original and vital processes that are the property of chromosomes and genes.

SUMMARY

1. A gene is described which induces mutations of the deficiency type, such as Notch, Smoky and all kinds of Minutes and mosaics.
2. The "Deficiency Inducer" acts in males and females, but a maternal effect seems to be connected with the difference in rates, since heterozygous females produce more mosaics than heterozygous males.
3. The Deficiency Inducer is not in Y, X, or II.
4. The mutations are distributed in random fashion over the chromosomes.
5. The possibility that the Deficiency Inducer has to do with chromosome growth and reproduction is discussed.

LITERATURE

Beadle, F. W., 1937. Chromosome Aberration and Gene Mutation in Sticky Chromosome Plants of Zea mays. Cytologia, Fujii Jubelee Volume: 43-56

Bridges, C. B., 1925a Elimination of chromosomes due to a mutant (Minute-n) in Drosophila melanogaster. Proc. Nat.Acad. Sci. 11:701-706.

Bridges, C. B., 1925b. Haploidy in Drosophila melanogaster. Proc. Nat. Acad. Sci. 11:706-710.

Crew, F. A. E. and R. Lamy, 1938. Mosaicism in Drosophila pseudoobscura. J. Genet. 37:211-228.

MacKnight, R. H., 1937. Haploid Ovarian Tissue in Drosophila. Proc. Nat. Acad. Sci. 23:440-443.

Mampell, K., 1943. High Mutation Frequency in Drosophila pseudoobscura, Race B. Proc. Nat. Acad. Sci. 29:

Stern, C., 1936. Somatic crossing-over and segregation in Drosophila melanogaster. Genetics 21:625-730.

Sturtevant, A. H., 1929. The claret mutant type of Drosophila simulans: a study of chromosome elimination and of cell-lineage. Zeitschr.f.wiss.Zool. 135:323-356.

CONCLUSION

Every research must remain, more or less, incomplete. There is practically no end to the questions that have a bearing on any problem. Is it necessary to answer all questions? Again, the investigator must use his own judgment. It is as important to know where to stop as it is to know how to proceed with the research. It is easy to lose sight of the real aim and to follow blindly the many alleys which lead nowhere in particular. Often it is helpful to terminate the research temporarily, and to view it from a distance. Then, the big question-marks are still visible whereas all the innumerable little ones have faded away. It is these big questions which we must aim to solve.

In the case of the "female sterility" the genetic analysis could be extended, but the important question: " Is this mechanism employed in wild populations?", demands an answer in preference to all others. It is necessary to test populations of race A and race B from regions where they overlap and from those where they never come in contact for the frequency of the "lm" genes. We know now how the genetic isolation of these species can be accomplished. After the study of wild populations we shall know whether some populations have

already become so isolated. This research may profitably be continued in the future.

The "high mutation frequency" research requires for its continuance the maintenance of the stocks, which in itself constitutes a problem. The few investigators who have worked on problems of a similar nature can appreciate the difficulty. The inevitable fate of these stocks has been their loss. Studies of this sort are carried on at a great disadvantage to the investigator. He never knows whether the next step can be reached, for the phenomenon might have vanished in the meantime. Mutator genes are notorious in this respect, and the author does not know at this time, if the Mutator still exists in the cultures. Many times before the phenomenon had apparently disappeared, only to reappear periodically. Thus, the uncertainty makes the research extremely difficult. However, it is still planned to solve many of the important problems connected with this work. We now know the effect of the Mutator. It is doubtful that the most important question: "How does the Mutator act?", can be answered in the near future.

With the " Deficiency Inducer" the same difficulties exist. Work with this problem is being pursued actively at this time. It must be pointed out again that a non-genic explanation has not been excluded. There are

reasons for suspecting an infectious agent as the direct or indirect cause of the deficiencies. Epidemic-like waves of deficiencies of the same nature as those described in Chapter III, have persistently appeared in other stocks of race B, in certain stocks of race A, and in D. melanogaster. If we substitute "organism" for "gene", "mild and heavy dose" for "heterozygous and homozygous", the facts could be explained just as consistently on that basis. The possibility of a contagious disease is not too fantastic to be at least considered. If one can show that the cause is linked to a definite chromosome, it can safely be called a gene. Work is in progress now to establish this. The solution may be expected soon. In the meantime, the Deficiency Inducer will tentatively be considered a gene. The next important phase of the work is the cytological investigation. Not only will this throw some light on the nature of the "deficiencies" that are induced, but we may also find out whether or not the cytoplasm per se is affected. This leads to the important question: "How does the Deficiency Inducer act?" As with the Mutator, the answer should not be expected soon, if it can be given at all. In both cases transplantation and injection experiments are planned to test for the possibility of diffusible substances; particularly in the case

of the Deficiency Inducer where a cytoplasmic effect seems to exist, those experiments may yield some results.

It was pointed out in the foreword that new problems, completely independent of those discussed, arise constantly. Some of these may be preferred in the future. The author does not want to predict which problem will be solved next.