ALPHA AND BETA LOCI IN DROSOPHILA

C. AUERBACH AND BARNET WOOLF

Institute of Animal Genetics, Edinburgh

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IN a recent paper (1959a) FAHMY and FAHMY claim that the *Drosophila* genome consists of "two classes of genes, which differ in their stability and manner of response to mutagens."

These they call alpha and beta loci. They further claim to have established a differential response to mutagens within the beta, but not within the alpha, class, and they consider this "the most interesting recent development in mutagenesis studies." It is worth-while to examine the evidence brought forward in favour of these claims.

MATERIAL

All mutants used in the FAHMYs' investigation were sex-linked.

The 43 *alpha* mutants are said to have been "previously detected in radiation mutagenesis work." This, however, is true for only 27 of them, and of these 24 have also occurred spontaneously. The remaining 16, according to BRIDGES and BREHME (1944), have only occurred spontaneously.

The 63 mutants used were chosen from several hundred "new mutants" at "hitherto undescribed loci which have mutated in our laboratory mainly by chemical means." The following among them do not fulfill these conditions of choice.

small-wing, of which three occurrences are listed in BRIDGES and BREHME, one by radiation;

Turned-up, which is now admitted by the FAHMYs to have occurred before;

scute^{fahmy} at 0.0, reported to be a deficiency of bands 1B1-2, viable in hemizygotes and inviable in homozygotes, and giving a near-normal phenotype in heterozygotes with scute;

notchoid, a viable allele of Notch, as is easily verified by crossing it to a Notch deficiency;

bronzy, an allele of the radiation induced maroon-like (GLASSMAN 1959). Phenotypically, bronzy is subject to a maternal effect: hemizygotes and homozygotes are wild-type when the mother was so. In old cultures, the maternal effect disappears. In Muller-5 tests (the source of all beta mutants) bronzy must therefore always have seemed to occur as a gonadic mosaic; curiously enough, this

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has not been mentioned or observed by the FAHMYS. The maternal effect will be found to play a rôle in the interpretation of the experimental data.

It is true that the FAHMYS never make it quite clear whether they restrict the term beta mutations to those that occur at new loci, or whether they include new alleles at known loci. In the latter case, notchoid, scute^{fahmy}, and bronzy might qualify as beta mutants. The possibility of allele rather than locus specificity in response to mutagens is, indeed, interesting. It seems to find support in a recent preliminary communication by the FAHMYS (1959b) in which they intimate that different cistrons of the rudimentary locus respond differently to mutagens. The rudimentary locus is one of the alpha loci chosen for the experiments under discussion. If allele specificity were used for classification, an unknown proportion of mutations at this locus should, therefore, be put into the beta class together with bronzy and notchoid; the same presumably applies to the other alpha loci. Since classification into alpha and beta *alleles* is not generally possible, it cannot legitimately be applied to the few cases in which chemical treatment produced alleles with new phenotypical features.

In the discussion of the FAHMYS' paper, more criteria are added to the definition of beta loci.

(1) Beta mutants "are on the whole markedly more pleiotropic in their phenotypic manifestations" than alpha loci. In this respect, the ten beta loci chosen for extended studies are atypical; for with the exception of *Oce*, none is reported to have pleiotropic effects (FAHMY and FAHMY 1958a).

(2) Many beta mutants "are slow in development and, therefore, late in eclosion and also have reduced viability and/or fertility." Again, the ten chosen beta loci are atypical, for viability and fertility are stated to be "good" or "excellent" in all of them, and delayed emergence is noted for only one (*tc*) (FAHMY and FAHMY 1958a).

(3) Beta loci are more "radioresistant" than alpha loci. Proof of this statement forms the main subject of the present investigation and will be examined below. It is not clear how important it is for identification of beta loci. The mutant small-wing was not promoted to the alpha-class when its occurrence after irradiation was pointed out to the FAHMYS. Conversely, when smalloid, a beta mutant, appeared even more frequently after X-radiation than after chemical treatment, it was called a "radiation responsive locus which has been missed by earlier workers." Whether this turns it into an alpha locus is not clear; it remains included in the calculations on the first set of tests, but is removed from the second tests.

(4) Beta loci "are on the whole less responsive to mutagens than the alpha class." This is claimed to be shown in the present publication. Even if it were true for the particular sample used in these experiments, it can hardly be maintained as a general statement. Many of the mutants listed in BRIDGES and BREHME have been reported only once, and even in the experiments by the FAHMYS many beta mutants occurred more often than rare alpha ones.

(5) The great majority of beta loci respond differentially to various chemical mutagens. This, too, is claimed to be shown in the present publication and will be

discussed below. It may be said here that even if the results justified this claim—and they do not—heterogeneity in respect to mutagens has been tested only for a whole *group* of beta loci; whether any single beta locus shows differential response to mutagens has not been tested and would, indeed, be difficult to establish.

Altogether, the definition of beta loci is hazy and arbitrary in the extreme. The only criterion that is fulfilled by all beta mutants used in the present experiments is that they have been found by the FAHMYS in tests with chemicals and that, at the time of their discovery, the FAHMYS believed they had not occurred before.

METHODS

The data used in the first part of the FAHMYS' investigation were obtained in Muller-5 tests for sex-linked lethals; in the second part, specific-loci tests were carried out.

Muller-5 tests: These were carried out in the usual way. Visible mutants were scored in the F_2 cultures; in order to reduce personal bias to a minimum, scoring was done exclusively by M. J. FAHMY. The method of scoring has never been fully described. Scoring, under a low-power binocular, of unetherized cultures, as practiced by mutation workers in general and by the FAHMYS originally (1955), can hardly be a reliable method for the detection of the many mutants that are listed as "not easily classified" (FAHMY and FAHMY 1958). Moreover, scoring of cultures "rather early after emergence" (FAHMY and FAHMY 1955) would not have detected bronzy or any of the late-emerging mutants, unless these are at the same time semilethal. The latter class was detected because the FAHMYS, in contrast to most mutation workers, preserve all suspected lethal cultures until no more flies emerge. A remark on p. 1157 (FAHMY and FAHMY 1959a) throws light on the technique employed for scoring. It is stated there that the early experiments with TEM (triethylenemelamine) and the carboxylic mustards could not be used as sources of beta loci because they had been undertaken "before the accumulation of the beta loci." Since these same experiments were considered unobjectionable sources of alpha loci, this indicates a change in scoring technique between the early experiments and the later ones. If this is so, it explains why the ratio of visible to lethal mutations was unusually high in all recent experiments by the FAHMYS, but not in their early ones.

The Muller-5 tests that served as sources of alpha and beta mutants were carried out with different, unspecified doses. It is assumed that the proportion of different types of mutants is not affected by dose. This is probably a correct guess; its validity is, however, not improved by the statement that "there is no evidence for a threshold effect for particular loci". Evidence of this type would, indeed, be difficult to obtain, and this has never been attempted. The mutants were also produced in germ cells of different, unspecified stages. Here, the assumption that this can play no rôle in creating an apparent specificity seems much less justified. It is entirely conceivable that mutations in early spermatogonia may tend to be different in kind from those induced in spermatozoa. It might, e.g.,

not be an accident that three out of four mutations to slim occurred among the 25 beta mutants induced by CB1506 (chloroethyl methanesulphonate), which acts almost exclusively on spermatogonia.

Specific loci tests: Wild-type males were treated with either X-rays or an amino-acid mustard (AAM) and mated to females that were homozygous or heterozygous for genes at loci whose mutability after these two treatments was to be tested. The F_1 flies were examined for mutant phenotypes. Mutants were submitted to further tests in order to distinguish between lethal and viable ones. The former were attributed to deficiencies. Cytological checks were, apparently, not carried out, so that the possibility of lethal point mutations (such as have, e.g., occurred quite frequently at the cut locus) is neglected.

Scoring in these experiments was apparently not done exclusively by M. J. FAHMY, and indeed the experiments were carried out on such a large scale that it would hardly have been possible for one person to score them unaidedly. It is, however, regrettable that this was so; for while specific-loci tests for easily classifiable mutants can safely be carried out by several workers in cooperation, this is not so in the present case. One of the ten beta mutants used, concave wing, is listed in DIS (1958a) with the remark "not easily classified". For three others, ruddle, copper, and bronzy (all slight eye colour deviations towards a brown tinge) trickiness of classification is implied by the remark "best classification in newly emerged flies". The mutant smalloid is given Rank 2 in spite of good viability and fertility, presumably because in crowded cultures it is difficult to distinguish a genotypically small from an undernourished fly.

For a specific-loci test to provide valid data on mutagen specificity, certain general conditions have to be fulfilled. These are:

(1) The two kinds of treatment must be of comparable strength. In *Drosophila*, this can be achieved by choosing treatments that yield comparable frequencies of sex-linked lethals.

(2) Culture conditions must be strictly standardized both within and between series. This is particularly necessary when late-emerging mutants (like *tc*) are scored.

(3) Care must be taken to avoid—or correct for—bunching and germinal selection, which may occur when premeiotic cells have been treated.

(4) The same loci must be tested with both mutagens.

(5) The scale of the experiments should be chosen in advance so as to give either comparable numbers of progeny after each treatment or a larger number after the treatment expected to yield fewer mutants.

Of these prerequisites, only (1) has definitely been taken care of by the FAHMYS. Indeed, they have carried accuracy to an extraordinary degree. In every experiment with AAM, an aliquot of flies was tested for sex-linked lethals, and the dose-equivalent was calculated in units of r. Since three percent sex-linked lethals are roughly equivalent to 1000r, this implies that the determination of lethal frequencies in these tests was accurate within less than .01 percent. In actual fact, it is doubtful whether any of the dose measurements—for radiation as well as chemical treatment—is accurate within 1000r. Progeny for the lethal

tests was sampled during the nine days following treatment. During this period, the frequency of X-ray induced lethals varies by a factor of two or more (AUERBACH 1954); for AAM FAHMY and FAHMY (1958b) have shown the existence of a similar brood difference. If precautions were taken to minimize this variation, they are not described.

(2) Whether or not precautions were taken to standardize culture conditions, they are not described. In particular, the authors do not explain what steps were taken to avoid as far as possible fluctuations of population density between bottles belonging to different experiments, or to different series of the same experiment.

(3) Errors due to bunching or germinal selection are said to have been avoided by testing only progeny obtained during the first nine days after treatment because "under our experimental conditions" samples of spermatozoa collected during this period are presumed to stem entirely from treated postmeiotic stages. One wonders what these experimental conditions were; they are not described. In their previous investigations on brood patterns, the FAHMYs have adhered to a standard breeding procedure, giving each male one new female every three days. It is possible that their claim is justified for this slow-breeding procedure, although experiments recently carried out in this Institute cast doubt on it (REDDI, in press). In any case, it seems unbelievable that this procedure should have been used in experiments of the magnitude described here. If it was not used, where is the proof that progeny produced during the later days of sampling did not come from treated meiotic or premeiotic cells?

The remaining conditions were definitely not fulfilled. This can be inferred from Table 1, in which Tables 4 and 5 of the FAHMYs' (1959a) paper have been rearranged in such a way that the actual experiments can be reconstructed. In particular, this table makes it possible to infer what strains of females were used as testers. While it is just conceivable that two X-ray experiments should have been carried out with exactly the same dose, it is inconceivable that two experiments with AAM should have given exactly the same dose equivalent. It appears that the following tester chromosomes were used:

I. *sc ct wy g f* (X-rays 2990r)

II. *ccw tc sl bz* (X-rays 2990r; AAM 2563 "r")

Presumably, the females in the radiation test were heterozygous for I and II. If so, this rules out bronzy as scorable mutant, since it would be masked by the maternal effect of the phenotypically wild-type P_1 females. It is just possible that, instead, two homozygous tester stocks were used in the two X-ray experiments with 2990r. This is, in fact, suggested by the different numbers of progeny counted. Even so, however, bronzy will have to be disqualified because it could not be scored in the experiment with AAM, in which the tester females definitely were heterozygous (see below).

III. *v* (X-rays, 3207r; AAM 2563 "r")

In the AAM experiment with 2563 "r", chromosomes II and III must have been combined in heterozygotes.

IV. *brc Oce cop* (X-rays 3834r)

V. *omm* (X-rays 3834r; AAM 3125 "r")

TABLE 1

Data of Tables 4 and 5 in the paper by FAHMY and FAHMY rearranged for the sake of better comparison

Locus α β	Dose (r)	X-rays		Mutants		Dose equivalent "r"	Mustard		Mutants	
		Chromo- somes	No.	Leth.	Viable		Chromo- somes	No.	Leth.	Viable
<i>sc</i>	2990	I/I*	38,352	2	4					
<i>ct</i>	2990	I/I*	38,352	6	1					
<i>wy</i>	2990	I/I*	38,352	7	4					
<i>g</i>	2990	I/I*	38,352	6	5					
<i>f</i>	2990	I/I*	38,352	1	3					
<i>ccw</i>	2990	II/II*	44,071	2	0	2563	II/III	87,547	0	1
<i>tc</i>	2990	II/II*	44,071	0	0	2563	II/III	48,977	0	1
<i>sl</i>	2990	II/II*	44,071	0	0	2563	II/III	74,253	0	6
<i>bz</i>	2990	II/II*	44,071	8	0	2563	II/III	47,036	1	2
<i>v</i>	3207	III/X	89,324	2	4	2563	II/III	129,831	2	3
<i>up</i>	3207	III/X	61,422	2	0					
<i>omm</i>	3834	IV/V	28,626	2	0					
<i>brc</i>	3834	IV/V	28,626	0	0	3152	IV/VI	67,445	0	1
<i>Oce</i>	3834	IV/V	28,626	2	0	3152	IV/VI	67,445	1	1
<i>cop</i>	3834	IV/V	28,626	0	0	3152	IV/VI	67,445	2	1
<i>nd</i>	4266	VI/VI	34,664	0	0	3152	IV/VI	67,445	12	1
<i>smd</i>	4266	VI/VI	22,278	1	8	3152	IV/VI	67,445	1	8
<i>rud</i>	3418	VII/VIII	47,272	2	0	3465	VIII/VIII	44,051	0	4
<i>sma</i>	3418	VII/VIII	47,272	0	0					
<i>Tu</i>	3418	VII/VIII	47,272	0	0					
<i>car</i>	3418	VII/VIII	66,978	7	0					
<i>mo</i>	2678	IX/IX	58,833	2	0					
<i>mel</i>	2777	XI/XI	32,867	0	0					

* or I/II.

Chromosomes IV and V were used in heterozygous conditions for the X-ray test with 3834r. In the AAM test with 3125 "r", chromosome V was used in heterozygous condition with

VI. *nd smd* (AAM 3125 "r")

VII. *sma Tu car* (X-rays 3418r)

VIII. *rud* (X-rays 3418r; AAM 3465 "r")

Chromosomes VII and VIII were used in heterozygous condition for the X-ray test with 3418r. Chromosome VIII by itself was used for the AAM test with 3465 "r".

IX. *mo* (X-ray 2678r), presumably used in homozygous condition.

X. *up* (X-rays 3207r), used in females X/III.

XI. *mel* (X-rays, 2777r), presumably used in homozygous condition.

The table shows a number of very peculiar features.

(1) Well over 100,000 flies were scored in experiments in which the tester females were homozygous for a singly marked chromosome, and another 350,000 in experiments in which the tester females were heterozygous for such a chromosome. It is difficult to imagine why such an inefficient technique was chosen.

(2) No tester was used in both X-ray and AAM experiments; thus no locus was scored in the same genetical background after treatment with both agents.

(3) While seven alpha loci were tested with X-rays, only one of them was tested with AAM. It is difficult to imagine why the other six loci were tested at all.

(4) Only ten beta loci were tested with both mutagens. Again, it is difficult to imagine why the other six were tested at all. Unfortunately, the ten loci that were tested with both mutagens include not only bronzy which cannot be used, but also small-wing and notchoid which, by the original definition, are not beta loci (see under "Material").

(5) Throughout, smaller samples were used in the X-ray tests than in the chemical tests, although *ex hypothesi* the latter were expected to produce more mutants.

(6) Within one and the same test, the numbers scored often varied between chromosomes and even between loci on the same chromosome. This is the most peculiar feature of this peculiar table; it may throw some light on the method of scoring. The following discrepancies are found: *X-rays, 2990r*: 38,000 flies scored for the chromosome with the alpha loci, and 44,000 for the chromosome with the beta loci. This, as said before, may mean that two separate tests were done with the same dose. *X-rays, 4266r*: 35,000 chromosomes scored for *nd*, 22,000 for *smd*, both on the same chromosome. An explanation that comes to mind is that scoring of *smd* was discontinued after several mutations had been obtained at this beta locus.

X-rays, 3418r: 47,000 flies for the chromosome with the beta loci, 67,000 flies for the alpha locus *car* on the opposite chromosome, presumably, tests for *car* were continued until some mutations had turned up.

AAM, 2563"r": Here, every one of the five loci was tested on a different scale. For the beta loci, all on the same chromosome, the numbers counted ranged from 47,000 for *bz* to 87,000 for *ccw*. This suggests that scoring for any one beta locus was continued until it had mutated at least once. With *ccw*, this may have happened so late in the test that almost twice as many flies had to be scored as for *bz*. By far the largest numbers of flies were scored for *v*, on the opposite chromosome, an alpha locus expected to respond equally well to chemical treatment as to radiation.

Taken together, these data are strong presumptive evidence for the assumption that the number of flies scored depended on the locus to be tested. For most loci tested with X-rays, numbers from 30,000 to 40,000 were considered adequate and were adhered to. For beta loci in experiments with AAM, and for a few alpha loci in either series scoring appears to have been continued until the appearance of the first mutant or mutants. If this is the correct explanation of the remarkable data, it raises a difficult statistical problem; for it means that a comparison has to be carried out between experiments in which frequencies were determined by two different procedures. Obviously, the FAHMYS have disregarded this difficulty in their statistical treatment. Since, as will be shown, their findings are in any case without statistical significance, this additional source of error may be dis-

regarded; but it will have to be avoided or corrected for in any future tests of the same nature.

RESULTS

Tests of heterogeneity in Tables 1 and 2: In the FAHMYS' paper, Table 1 gives the numbers of mutations observed at 43 so-called alpha loci in response to treatment with X-rays and seven specified chemical agencies. The total of 549 mutations is distributed over the 344 cells, an average of about 1.6 occurrences per cell. Their Table 2 lists the results at 63 so-called beta loci of treatment by X-rays and five chemical agencies. The total for the 378 cells is 275 mutations, averaging about 0.73 per cell. They claim that Table 1 shows no significant heterogeneity, and that this proves that "the over-all relative mutability at the 'classical' or alpha loci, therefore, does not vary with the mutagen". For their Table 2 they claim significant heterogeneity, concluding that "the relative response of the beta loci does differ under the various mutagens".

On the plea that cell expectations are very small, the FAHMYS do not use the standard χ^2 test of heterogeneity. Nevertheless, it is worthwhile to carry out such tests on these tables, since it is generally accepted that the effect of small expectations is to inflate the χ^2 values, so that while a significant result must be treated with caution, a verdict of nonsignificance can be confidently accepted. We have therefore computed the χ^2 values and the corresponding P values by use of the Wilson-Hilferty formula. The results are

	D.F.	χ^2	P
Table 1	294	317.58	0.174
Table 2	310	347.99	0.072

Allowing for the fact that the χ^2 values are probably inflated because of the small expectations, this effect being much the greater for Table 2, there seems to be no statistically demonstrable heterogeneity in either table.

The FAHMYS do not give χ^2 figures, but use a test which they claim "is far less disturbed by small numbers in the individual cells than χ^2 ." This is the Ψ test of SMITH (1951, 1952), which they say yielded a heterogeneity P of 0.41 for Table 1 and 0.02 for Table 2. They do not make it clear which of the several possible variants of the test they used, but we have confirmed that it yields P values of the magnitude they claim, subject to one all-important proviso. The author of the test does *not* state that it is undisturbed by small expectations. On the contrary, he gives a suggested method for correcting for continuity, remarking that it probably over-corrects. When this correction is applied to Table 2, the P for heterogeneity changes from 0.02 to 0.99999999974, which means in effect that the Ψ test with SMITH's suggested correction breaks down. This is not surprising, since the correction consists in ignoring altogether deviations from expectation which are less than 0.5, and subtracting 0.5 from all other deviations; but 81 percent of the expectations in Table 2 are less than unity, and 56 percent are less than one half. The only reasonable conclusion is that heterogeneity in Table 2 has not been demonstrated.

Two very important points of principle arise. The FAHMYS explicitly claim that the inability of the test to demonstrate significant heterogeneity in Table 1 is a proof that the null hypothesis is true. This of course is completely unjustified. Of their 43 loci in Table 1, 19 mutated less than eight times, and seven mutated only twice. It would be a powerful statistical test indeed that could prove that these loci would react with equal proportionate frequency to eight different mutagenic agencies. In general, the null hypothesis of complete identity of behaviour can never be established. All that one can say, on adequately numerous observations, is that heterogeneity, if it exists, is unlikely to exceed certain confidence bounds. On the data of Table 1, these bounds are so wide as to be biologically meaningless.

Even if significant heterogeneity had been found in Table 2, this could not be taken as proof of differential susceptibility to mutagens until all other possible sources of heterogeneity had been examined and eliminated. Heterogeneity would arise from any departure in the experimental conditions from the strictest uniformity. In work with chemical mutagens, as in all pharmacological trials, it is quite common to find that experiments replicated as closely as possible will give heterogeneous results; and it is only when differences between treatments are significantly greater than those between replicates that an authentic effect can be inferred. It is not legitimate to rely on the inner conviction that one's technique is absolutely perfect. Even if further more extensive data should provide clear evidence of heterogeneity at different loci, this could not be accepted as evidence of differential specificity to mutagens until the requisite internal checks have been applied.

Tests of heterogeneity on Table 3: Table 3 of the FAHMYS is a 2×6 contingency table giving the number of mutations observed at the alpha and the beta loci under the action of X-rays and five different chemical mutagens. A χ^2 test for heterogeneity gives 18.8 for 5 degrees of freedom, which is highly significant. There is however a serious source of bias in their figures. The alpha loci were already known, and mutations produced by any agent, whether X-rays or chemicals, were presumably scored impartially as they occurred. But the beta loci, by definition, were those first identified by the FAHMYS as mutating under a chemical treatment. Every beta locus is therefore loaded with at least one mutation of chemical origin. Any mutation due to X-rays that occurred before the chemical mutation was observed would either not have been noticed, or would have disqualified the locus from inclusion in the beta category. There will therefore be a spurious excess of chemically induced mutations in the beta group as compared with the alpha group. The effect of this can be seen if Table 3 is summarized as a 2×2 table. This would read

	alpha loci	beta loci	
X-rays	57	19	76
Chemicals	403	256	659
	460	212	735

This gives a heterogeneity χ^2 of 5.58, significant beyond the 2.5 percent level. But the beta loci-chemicals figure of 256 is artificially inflated. For a true correction, one would need access to all the experimental records. But a fairly good adjustment can be made by using the method of correcting for "rare ascertainment" in human pedigree studies, namely by deducting the event which brought the locus into the record, using subsequent mutations as an unbiased sample of its behaviour. One therefore has to diminish the figure 256 by the 63 identifying chemically induced mutations. The revised table now reads

	alpha loci	beta loci	
X-rays	57	19	76
Chemicals	403	193	596
	460	212	672

This gives a heterogeneity of 1.70, which is clearly not significant.

When the X-ray row is deleted from the FAHMYS' Table 3, there is still apparently significant heterogeneity between the alpha and the beta loci with respect to the five kinds of chemical treatment; and the FAHMYS give a separate test purporting to show significant heterogeneity in the 2×2 table comprising rows 2 and 3 of their table, comparing the amino-acid mustards and the sulphonates. But there are 63 occurrences to be subtracted from the beta loci column, and until one knows by how much each of the cell entries need to be adjusted, it is not possible to draw any conclusions from the figures. It therefore would appear that no significant heterogeneity has been demonstrated between the totals for the alpha and the beta loci.

Specific loci tests. The Poisson distribution (Tables 4, 5, 6, 7) of the FAHMYS' paper: It would be tedious to explore all the misconceptions embedded in the short section on the Poisson distribution and the tests in Table 6 of the paper. Mention of two outstanding ones will suffice. The FAHMYS do not make clear what question they think that their tests are answering. Actually, their tests, if they have any validity at all, are testing the hypothesis that the *absolute* mutation rates at the various loci are identical. Of course, the disparate row totals in Table 1 and Table 2, as well as the whole mass of genetical experience, prove that they are not. Yet the FAHMYS seem pleased that their tests on seven loci, based on a total of 31 deficiencies and 21 visible mutations, appear to indicate that there is no significant departure from Poisson expectations in each of the two categories. The only conclusion that could be drawn, at best, is that their data are too sparse to reveal even the most elementary and best authenticated differences between loci.

There is however no warrant for drawing even this conclusion. The Poisson distribution deals with discrete events, and can be applied only to whole numbers. But the FAHMYS do not analyze whole numbers. They take the mutation rates per 10^8 chromosome r , rounded off to the nearest integer, and compare the variance of the rates with their mean. In the first row of their Table 6, for example, the mean mutation rate is 3.29 and its variance is 4.24, not significantly greater than the mean. But the use of 10^8 chromosome r is purely arbitrary. If they had

taken the rate per 10^9 chromosome r, for example, the mean would have been 32.9, and the variance 424.0, indicating dispersion tremendously greater than the Poisson expectation. Actually the χ^2 tests already considered are the most appropriate tests of conformation to Poisson expectation.

To these fallacies of the statistical treatment has to be added a further one, discussed above: the neglect of the statistical problem how to deal with frequencies that have been obtained in different ways for different series.

It remains to discuss the claim that certain loci are "refractory to X-rays," to the extent that the chromosome regions including these loci cannot be broken by X-rays. The eight "refractory" loci are *brc*, *nd*, *sma*, *cop*, *tc*, *sl*, *Tu*, *mel*. They will be considered in turn.

brc lies at 0.0, i.e. in the same region as scute. Two scute deficiencies were produced in the X-ray experiments; many more have been produced by other workers. It is impossible to claim that this region is refractory to X-ray breakage, unless one postulates that its response to radiation in spermatozoa depends on whether the ovum subsequently to be fertilized carries an alpha or beta locus at 0.0. Since homozygotes for *brc* are reported to be viable, zygotic inviability cannot be invoked as explanation.

nd is an allele of Notch and can be uncovered by Notch deficiencies. Twelve such deficiencies were, indeed, obtained in the experiments with AAM. Surprisingly, none occurred among 35,000 irradiated chromosomes. Even so, the fact that this region is easily broken by irradiation is too well established to be overthrown by this one negative result.

cop 67,000 chemically treated chromosomes contained two deficiencies (or lethal mutations) for this locus; 29,000 irradiated chromosomes contained none. This is hardly evidence for a specifically refractory attitude towards radiation.

tc No deficiency in 49,000 chemically treated and 44,000 irradiated chromosomes.

sl No deficiency in 74,000 chemically treated and 44,000 irradiated chromosomes.

Neither of these two loci can, therefore, be said to be specifically refractory to X-rays.

The last three "refractory" loci, *sma*, *Tu*, and *mel*, were tested only with X-rays and are therefore useless for comparison with AAM.

It is evident that there is no reason for believing that any one of these eight loci possesses the peculiar ability of endowing its chromosomal environment with resistance to X-ray breakage.

DISCUSSION

Geneticists must welcome the paper by FAHMY and FAHMY (1959a), which for the first time presents data on the specific effects of chemical mutagens in *Drosophila*, so long proclaimed by its authors. Unfortunately, it does not bring sufficient data, nor does it describe details of experimental procedure that are indispensable for critical appraisal of the evidence. Faults of both omission and

commission in the presentation suggest that the procedure was inadequate in many respects.

The setting-up of two mutually exclusive classes of loci, differing in mutability under the influence of various mutagens, is gratuitous, unsupported by evidence, and an obstacle to further research. The FAHMYS, in the discussion of their paper, admit that their present classification of gene loci is "neither rigid nor final," but they claim to have "clearly shown the reality of such a classification." Our examination of their data has shown this claim to be unjustified. Their criteria of classification are manifold and have been used inconsistently, the only indispensable criterion of beta mutants being discovery by the FAHMYS. In particular, we have shown that the FAHMYS have failed to establish the two alleged main criteria of beta loci: low mutational response to radiation and marked differential response to various mutagens. The statistical tests purporting to establish these beta properties have been shown to be faulty. Even if this had not been so, the data for statistical treatment had been collected in such a way that in no case could the results have been interpreted in terms of differential mutagen sensitivity at the tested loci.

This critique must not be taken as implying that there is no differential susceptibility to mutagens at different loci of *Drosophila*. On general biological grounds, including the references given by the FAHMYS in their introductory section, one would assume that such differences may exist. The data presented by the FAHMYS do not disprove this possibility, even though they fail to support it. Clear evidence for mutagen specificity in *Drosophila* will not be easy to obtain because of the necessarily very limited scale of the experiments. A hypothesis regarding the causes of possible specificity might reduce the required scale. Thus, the fairly well established fact (FAHMY and FAHMY 1956; BELITZ 1957; FAHMY and FAHMY 1959a) that certain chemical mutagens tend to produce mutations in the proximal region of the X chromosome might be taken as a possible starting point. If, as this observation suggests, there is a regional specificity of mutagenic response, autosomal mutations, too, might tend to occur in heterochromatic or else centromere-near regions after treatment with triethylenemelamine or amino-acid mustards. The influence of the centromere might be studied by following the suggestion, made several years ago by Dr. MULLER, that an inverted X chromosome should be used for treatment. Should regional specificity become sufficiently confirmed, more effective specific loci tests than those used so far might be designed by choosing loci within the preferred regions. Perhaps it is not accidental that in the very large-scale tests on the *car* locus no *bona fide* mutation occurred after irradiation, although two had occurred in a, presumably, very much smaller sample of chemically treated chromosomes used for Muller-5 tests.

Regional specificity is, in any case, the more likely explanation for those cases in which a locus as a whole seems to respond better to one mutagen than another; for *Drosophila* loci, like those of microorganisms, are presumably complex and contain subloci with different mutational potentialities. From this point of view, the occurrence of unusual alleles at known loci, such as notchoid or scute^{fahmy} is of special interest. One way of testing specificity at this level would be to de-

termine induced frequencies of reverse mutations in a series of pseudoalleles. A different method, which appears to have been taken up by the FAHMYs (1959b), consists of dividing all induced mutants at one locus into groups by tests of recombination and complementation, in the hope of finding group differences in mutagen specificity.

We should be glad if our critique would clear the way for a really rewarding approach to the interesting problem of mutagen specificity in *Drosophila*. The more plausible and congenial a hypothesis, the more necessary it is to be content with nothing less than water-tight reasoning from adequate and unbiased observation.

SUMMARY

This paper presents a detailed examination of the claim (FAHMY and FAHMY 1959a) that the *Drosophila* genome consists of two classes of loci, differing in mutational capabilities. It has been concluded that the biological techniques used were inadequate for an unbiased test of this hypothesis, and that the statistical techniques contained various fallacies and did not, in fact, prove what they were supposed to have proved. It is stressed that this conclusion does not exclude the possibility of mutagen specificity in *Drosophila*, but that different experiments will be required for proving it.

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