The sensitivity of crossing over in *C. reinhardi* to ionic environment is consistent with the theory that chromosomes are composed of macromolecular unit particles linked together by divalent ions.

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## *A FURTHER ANALYSIS OF THE FORKED LOCUS IN DROSOPHILA MELANOGASTER*

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One question posed by the discovery of pseudoallelism is whether crossing over may be utilized to define the limits of the gene. Alternatively, this question may be stated as follows: Are pseudoallelic mutants divisible by crossing over into a discontinuous array just as are linked, nonallelic mutants, or do pseudoalleles represent a continuous array of chromosomal sites within which crossing over occurs? The data from *Drosophila* are consistent with the first-stated view. However, the putative cases of pseudoallelism recently described in bacteriophage by Benzer[1] and in *Salmonella typhimurium* by Demerec *et al.*[2] suggest that pseudo-

alleles may represent a continuous array of mutational sites separable by recombination. Previously, an analysis of four independent forked (*f*) bristle mutants in *D. melanogaster* showed that each may be assigned to one of two loci as defined by crossing over.[3] The subsequent data represent a continuation in the analysis of the forked locus designed to determine whether more than two loci occur.

*Crossing-over Tests.*—Six independent *f* mutants were analyzed for recombination. All, when tested to the suppressor of *f* (*su-f*), proved to be unsuppressed. Phenotypically, five are similar to $f^1$, while one, $f^{36a}$, produces an extreme forked phenotype. Two mutants, $f^h$ and $f^{36a}$, arose spontaneously, while the remainder, designated $f^{x1}$, $f^{x2}$, $f^{x3}$, and $f^{x4}$, occurred following X-irradiation.

Crossing-over experiments were carried out as described previously. In order to maximize crossing over, all *f* mutants were inserted into the scute-8 inversion ($In(1)sc^8$), and ♀ ♀ were made heterozygous for an independent *f* mutant, an *f* tester mutant, as well as the autosomal inversions *Cy* and $Ubx^{130}$. Only ♂ progeny were scored. Since crossing over occurs between mutants $f^1$ and $f^{3n}$, they were used as testers for the *f* loci. The X chromosome of each *f* mutant to be tested carried, in addition, the markers *un* (uneven eye) 1 unit to the right and $Bx^2$ (Beadex-2 wing) 2.8 units to the left of the *f*. The $f^1$ tester carried the mutant *B* (Bar eye) 0.3 units to the left of *f*. It should be noted that in $In(1)sc^8$, the $f^1$ locus occurs to the left of $f^{3n}$. If a given *f* mutant has its locus to the right of $f^1$, then recombination types expected on testing to $f^1$ are $Bx^2\ f^+$ and $Bf^{ex}\ un$ (where $f^{ex}$ is extreme *f*, the phenotype concomitant to two *f* pseudoalleles coexisting on the same X chromosome.) If the *f* locus occurs to the left of $f^1$, the expected recombination types are $Bx^2\ f^{ex}$ and $B\ f^+\ un$. Similarly, if the *f* mutant has its locus to the left of $f^{3n}$, the recombination types on testing to $f^{3n}$ are $Bx^2\ f^{ex}$ and $un\ f^+$; however, if the locus occurs to the right of $f^{3n}$, the expected recombination types are $f^{ex}\ un$ and $Bx^2\ f^+$.

Since all six *f* mutants investigated are unsuppressed, they were first tested for crossing over with $f^1$, a suppressible mutant. The results are listed in Table 1.

TABLE 1

TESTS OF *f* MUTANTS FOR RECOMBINATION WITH $f^1$

| ALLELE TESTED | *N* RECOMBINANT ♂♂ $Bx^2\ f^+$ | $B\ f^{ex}\ un$ | *N* ♂♂ |
|---|---|---|---|
| *36a* | 0 | * | 77,130 |
| *h* | 0 | 0 | 33,823 |
| *x1* | 0 | 0 | 45,948 |
| *x2* | 0 | 2 | 14,007 |
| *x3* | 2 | 0 | 16,399 |
| *x4* | 0 | 1 | 11,281 |

* Not readily detectable, since $f^{36a}$ produces an $f^{ex}$ phenotype.

It may be noted that three of the *f* mutants recombined with $f^1$, and, on the basis of the distribution of the marker genes among the recombinants, are localized to the right of $f^1$, presumably allelic to $f^{3n}$.

Three *f* mutants failed to recombine with $f^1$. This failure was interpreted to mean that they are truly allelic to $f^1$. Accordingly, these mutants should recombine with $f^{3n}$. These tests were made, and the results are listed in Table 2.

Two pertinent facts are evident. First, the *f* mutants which failed to recombine with $f^1$ all recombined with $f^{3n}$. Consistent with the distribution of the marker

genes to the recombinants, the *f* mutants are localized to the left of $f^{3n}$, thereby fulfilling the interpretation, noted above, that they are allelic to $f^1$. Second, no evidence has been uncovered in either test for the existence of *f* loci to the left of $f^1$ and to the right of $f^{3n}$. It would be peculiarly fortuitous for both *f* testers selected to represent the extreme recombinational limits of the loci. Similarly, no evidence is available which points to the occurrence of *f* loci between $f^1$ and $f^{3n}$. While too much significance cannot be placed on the frequency of recombination, it is perti-

TABLE 2

TESTS OF *f* MUTANTS FOR RECOMBINATION WITH $f^{3n}$

| ALLELE TESTED | N RECOMBINANT ♂♂ $unf^+$ | $Bx^2f^{ex}$ | N ♂♂ |
|---|---|---|---|
| *36a* | 1 | * | 29,201 |
| *h* | 4 | 0 | 24,290 |
| *x1* | 3 | 2 | 43,928 |

* Not readily detectable, since $f^{36a}$ produces an $f^{ex}$ phenotype.

nent, nonetheless, to note that the data are consistent. Thus the recombination frequency manifested by mutants crossing over with $f^1$ was 5/41,687, or about 1/8,000, while with mutants crossing over with $f^{3n}$ the frequency was 11/97,419, or about 1/9,000. (The number of recombinants between $f^{36a}$ and $f^{3n}$ was doubled, since only the $f^+$ class could be detected.) The totality of the recombination data militate for the interpretation that the six *f* mutants studied here and the four analyzed previously occur in either one of two *f* loci, with five alleles occurring at each locus.

It should be noted that these observations invalidate the hypothesis proposed earlier that separation of the *f* pseudoalleles by crossing over parallels their separation based on their phenotypic response to the suppressor of *f*. Thus suppressed and unsuppressed mutants now prove to be allelic.

*Mutation Analysis.*—A detailed study of the data reported by Benzer and by Demerec *et al.* leads to the conclusion that essentially each independent mutant analyzed represents a mutation at a separate locus. Thus, in the case of phage, almost every new mutant recombines with all other mutants, and in the case of *Salmonella* each mutant is transduced by nearly all other mutants. This is the basis for the interpretation of a continuous array of pseudoallelic loci. The following represents an attempt to determine from the analysis of induced mutants whether more than two *f* loci are indicated. The rationale of the analysis is predicated on the following observations. First, in all cases observed, the phenotypic consequence of two *f* pseudoalleles coexisting on the same X chromosome is an unsuppressed, $f^{ex}$ phenotype. Second, X-ray-induced mutants producing an $f^1$ type or medium-*f* phenotype in general behave as single-locus mutants. The four X-ray-induced mutants reported here support this statement. Third, most X-ray-induced mutants are of the $f^1$ type. Fourth, X-ray mutants are of the unsuppressed type. This is borne out by the observation that, of 12 X-ray *f* mutants tested to *su-f*, all were unsuppressed.

From a study of *f* mutants induced in a chromosome already carrying a *f* mutant, contrary results are predictable, depending upon the nature of the *f* locus. The detection of induced mutants is facilitated by irradiating *f su-f* individuals. Induced mutants will occur as either $f^1$ or $f^{ex}$ phenotypes among *f su-f* sibs whose phenotype approaches wild type. Following X-irradiation of *f su-f*, if a multi-

plicity of $f$ loci occur, $f^1$-type mutations will occur primarily at loci independent of $f^1$ and will be manifested as $f^{ex}$ phenotypes. If only two loci occur, two consequences of $f^1$-type mutants are expected. If mutation occurs at the $f^{3n}$ locus, the resultant individuals will be $f^{ex}$ types. If mutation occurs at the $f^1$ locus, $f^1$ will mutate to an unsuppressed type which will be manifested as an $f^1$ type unaffected by *su-f*. In summary, following irradiation of *f su-f*, if a multiplicity of loci occur, induced mutants will occur as $f^{ex}$ types, and $f^1$-type mutants will be rare; but if two loci occur, $f^1$ types will occur as frequently as $f^{ex}$ types.

Two experiments were performed to test the hypotheses. In one experiment, $f^+$ ♂♂, in the second, *f B su-f* ♂♂, were treated with an X-ray dose of 4,000 r, crossed to attached-X ♀♀, and the ♂ progeny scored for *f* mutants. The results of these experiments are tabulated in Table 3. All mutants listed were tested and

TABLE 3

INDUCTION OF *f* MUTANTS BY X-IRRADIATION

| GENOTYPE ♂♂ IRRADIATED | *N* NEW MUTANTS $f^1$ Type | $f^{ex}$ Type | *N* ♂♂ |
|---|---|---|---|
| $f^+$ | 4 | 2 | 36,651 |
| $f^1$ *B su f* | 3 | 2 | 22,721 |

proved not to be singed bristle mutants which produce a phenotype simulating forked, and in the case of *f B su-f* irradiations were tested and proved not to be reversions of *su-f*. While the data are not voluminous, they point to several conclusions. First, X-irradiation of $f^+$ indicates that primarily $f^1$-type mutants are induced. Second, from the irradiation of *f B su-f* as many, if not more, unsuppressed $f^1$-type mutants were recovered as $f^{ex}$ type, an observation not consistent with the hypothesis of a multiplicity of *f* loci. Since the recombination analysis showed that unsuppressed and suppressed *f* mutants can be alleles, the recovery of unsuppressed medium-*f* mutants following irradiation of $f^1$ *B su-f* is interpreted to mean that $f^1$ has mutated to an unsuppressed allele. Therefore, the irradiation analysis failed to uncover any convincing evidence for a multiplicity of *f* loci and is consistent with the crossing-over data.

*Discussion.*—Taken at face value, the data from *Drosophila* appear to be at variance with the observations made in bacteriophage and *Salmonella*. If it is assumed that the mechanism of recombination in *Drosophila* is identical with that in bacteriophage and with transduction in bacteria, although the available data are not completely compelling on this point, the question may be raised whether an objective comparison is possible at this time. The conclusion that phenotypic alleles are in reality pseudoalleles is based upon the observation that single crossovers occur between these alleles, and the complementary crossover products, wild type and the two mutants coexisting on the same chromosome, are recovered. In the case of the observations in bacteriophage and *Salmonella* the data presented thus far indicate that only "wild-type" progeny have been recovered. Thus the existence of pseudoallelism in these organisms has not been unequivocally demonstrated. Until such a demonstration is made, a comparison of observations must be considered to be conjecture. The recent observations of M. B. Mitchell[4] of so-called "gene conversion" in *Neurospora* and the apparent general occurrence of this phenomenon in microörganisms emphasize the need for detecting both single crossover products before an interpretation of pseudoallelism is warranted. It is

noteworthy to point out that, in the case of the forked pseudoalleles, the Mitchell phenomenon has been ruled out by the recovery simultaneously in attached-X ♀ ♀ of the complementary products of crossing over between the pseudoalleles.[3]

Contrariwise, it may be argued that in the case of the *f* mutants reported here the failure to uncover more than two loci is related directly to the limited number of mutants tested *inter se*. It should be noted that the recombination data presented demonstrate that no *f* loci occur outside the limits of $f^1$ and $f^{3n}$ and that no loci occur between the two tester mutants. These conclusions are augmented by observations with the lozenge (*lz*) mutants, where three loci are indicated.[5] Each of the 18 *lz* mutants studied can be assigned, on the basis of recombination, to only one of the three loci, and no additional loci are indicated from the results of extensive *inter se* crossing-over tests.

It might be argued further that the nature of the tester mutants precluded the detection of additional loci. If both $f^1$ and $f^{3n}$ are associated with minute rearrangements which delimited crossing over so that it could occur only between these mutants, only two loci could be uncovered. There is good reason to believe that neither $f^{3n}$ nor $f^1$ is associated with any rearrangements. Their cytology is normal. Both mutants have been observed to undergo spontaneous back-mutation to $f^+$ at characteristic rates, a fact which controverts their association with chromosomal aberration.

It is possible to reconcile the observations in *Drosophila* with those of microörganisms by assuming that two different orders of magnitude are being compared. It may be reasoned that within each *f* locus a number of contiguous, mutational sites occur which, in their linkage relations, are equivalent to the loci described for bacteriophage and *Salmonella*. This viewpoint implies that the locus of the microorganisms is dimensionally equivalent to subdivisions of the loci of *Drosophila* and other higher organisms. The detection of subloci in *Drosophila* would be based on the recovery of recombinations at a rate comparable to that in bacteriophage and bacteria. Experimentation based on rates of recombination of this magnitude is impractical in *Drosophila*.

*Summary.*—(1) A continuation of the analysis of pseudoallelism at the forked locus in *D. melanogaster* is presented. (2) Crossing over results with ten *f* mutants, as well as mutation experiments at the forked locus, failed to uncover more than two forked loci. (3) These results are discussed in relation to observations on pseudoallelism reported for microörganisms.

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