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PHENOTYPIC VARIATION AND PSEUDO-ALLELISM AT THE FORKED LOCUS IN DROSOPHILA MELANOGASTER

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In previous reports[1, 2] it was demonstrated that, by use of a suppressor mutant, the otherwise phenotypically inseparable vermilion (v) eye-color mutants of *Drosophila melanogaster* could be separated into two classes—suppressed and unsuppressed. Moreover, pseudo-allelism is indicated, since recombination was demonstrated to occur between suppressed and unsuppressed v mutants. The description of a suppressor of the recessive, sex-linked forked bristle (f) mutants[3] plus the fact that f mutants recur frequently suggested that the observations made for the v mutants be extended to the f mutants.

Four independent f mutants, spontaneous in origin, have been used. These are f^1, f^3, f^{3n}, and f^5. All are characterized phenotypically by causing a gnarling or twisting of the bristles. The f^5 mutant differs from the others in that the microchaetes as well as the macrochaetes are forked. In the tests with the sex-linked suppressor of forked (su-f), the following mutants (with map locations) were used: f (56.7); Bar eye, B (57.0); carnation eye color, car (62.5); and su-f (64.0). Since f^1 is suppressed by su-f, a stock of the genotype f^1 B su-f was obtained. Tests for the suppression of any independent f mutant, designated f^x, were made by first obtaining ♂♂ of the genotype f^x car. These ♂♂ were crossed to ♀♀ f^1 B su-f, thereby producing ♀♀ of the genotype $f^1\ B + su\text{-}f/f^x + car +$. The ♂ progeny of these heterozygous ♀♀ were scored. Those ♂ progeny phenotypically B^+ car^+ must be genotypically f^x su-f, and their bristle phenotype was compared with that of their f^x *car* sibs to see if the suppressor affected the forked phenotype. Among the four mutants, f^5 and f^4 are suppressed, while f^3 and f^{3n} are unsuppressed. Thus two types of f mutants are indicated—suppressed and unsuppressed.

Since the f mutants can be classified into two groups by use of *su-f*, tests were made to detect the occurrence of recombination between suppressed and unsuppressed mutants. In the initial tests attached-X ♀ ♀ were used, since, as noted previously,[2, 4] the occurrence of reciprocal crossover between pseudo-alleles in such ♀ ♀ permits the recovery of the chromosome carrying both pseudo-allelic mutants as well as the chromosome carrying both wild-type alleles together in the same ♀. Using the mutants f^1, f^{3n}, and r (rudimentary wing) 2.2 units to the left, and B 0.3 units to the right, of the forked locus as markers, attached-X ♀ ♀ of the genotype $r\ f^1\ B\ +/f^{3n}\ +\ car$ were constituted and tested. The results of these tests are listed in Table 1. It may be noted that two classes of f^+ ♀ ♀ were recovered: nonrudimentary and heterozygous B ($f^+\ B/+$), and rudimentary and heterozygous B ($r f^+ B/+$).

TABLE 1

CROSSING-OVER BETWEEN *f* PSEUDO-ALLES IN ATTACHED-X ♀ ♀

EXP. No.	GENOTYPE ATTACHED-X ♀ ♀	PHENOTYPE EXCEPTIONAL ♀ ♀ $f^+\ B/+$	$r\ f^+\ B/+$	TOTAL ♀ ♀
1	$r\ f^1\ B/f^{3n}$ *car*	1	..	13,552
2	$r\ f^1\ B/f^{3n}$ *car; Cy/+; Ubx*^{130}/+	1	..	44,872
3	$r\ f^1\ B/f^{3n}$ *car; Cy/+; In(3)T, Mé/+*	..	1	40,033
4	$r\ f^5\ B/f^{3n}$ *car; Cy/+; In(3)T, Mé/+*	1	..	18,969

The occurrence of the f^+ ♀ ♀ as a consequence of crossing-over between f^{3n} and f^1 can be explained by assuming that the mutants are pseudo-allelic, with f^{3n} occupying a position to the left of f^1. In Figure 1 the genotype of the parental ♀ ♀ is diagramed. In addition, the consequences of reciprocal and nonreciprocal exchanges occurring between f^{3n} and f^1 are listed. It may be noted that the phenotypically $r\ f^+\ B/+$ ♀ recovered is in agreement with expectation for a nonreciprocal exchange. That the two ♀ ♀ phenotypically $f^+\ B/+$ were derived from reciprocal exchanges was demonstrated from the results of progeny testing these ♀ ♀. The results of the progeny tests are listed in Table 2. In addition to the parental phenotypic class, two nonparental phenotypic classes were recovered as a consequence of crossing-over to the right of the B locus. One class comprised those ♀ ♀ homozygous B with an f phenotype designated as f^{ex}, i.e., extreme f, in which the microchaetes as well as the macrochaetes are forked. This represents a phenotype more extreme than that of either f^1 or f^{3n}. The second class was made up of ♀ ♀ $r\ f^+\ B^+$ in phenotype. On the basis of these progeny tests, it can be concluded that the ♀ ♀ $f^+\ B/+$ possessed one X chromosome of the genotype $r\ f^+\ B^+$ and the homologous X of the genotype $r^+ f^{3n} f^1\ B$. These results fulfill expectation for a reciprocal exchange between f^{3n} and f^1.

That the chromosome designated $f^{ex}\ B$ did in fact carry both f^{3n} and f^1 was proved in the following manner. Detachments of the X chromosomes of attached-X ♀ ♀ homozygous $f^{ex}\ B$ were obtained. On testing with the *su-f*, it was observed that f^{ex} is unsuppressed. Furthermore, ♀ ♀ $r\ f^{ex}\ B/+++$ and heterozygous for the autosomal inversions *Cy* and *In(3)T, Mé* were obtained. If f^{ex} represents f^{3n} and f^1 coupled on the same X chromosome, then from these ♀ ♀ it should be possible, by virtue of crossing-over between f^{3n} and f^1, to recover, among their ♂ progeny, ♂♂ $r\,f^{3n}$ and f^1B. Among 16,416 ♂ progeny, 2 ♂♂ $r\,f^{3n}$ and 3 ♂♂ $f^1\,B$ were obtained, demonstrating that the f^{ex} represents f^{3n} and f^1 coexisting on the same X chromosomes. Further, these results demonstrate that f^{3n} and f^1 are pseudo-alleles.

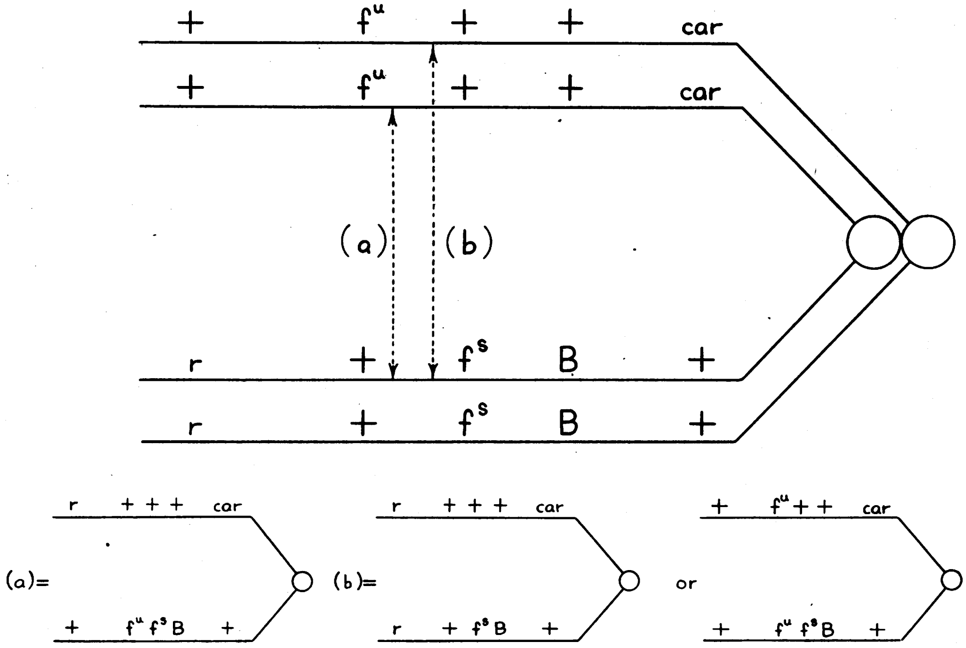

FIG. 1.—Scheme for detecting crossing-over between f pseudo-alleles in attached-X ♀♀. Upper diagram represents genotype of attached-X ♀♀ with f^s = suppressible and f^u = unsuppressible forked mutants. The reciprocal crossover is labeled (*a*), the nonreciprocal (*b*). The corresponding crossover products shown below are designated (*a*) and (*b*).

Since f^5 is also suppressed by *su-f*, tests for recombination between f^{3n} and f^5 were made. Attached-X ♀♀ of the genotype $r\,f^5\,B\,+/+\,f^{3n}\,+\,car$ were obtained and tested. The results are listed in Table 1. From these tests, one $f^+\,B/+$ ♀, derived from a reciprocal exchange between f^{3n} and f^5, was obtained. This conclusion was supported by the progeny test of the $f^+\,B/+$ ♀, the results of which are included in Table 2. Therefore, the $f^+\,B/+$ ♀ obtained was genotypically $r + + +/+ f^{3n} f^5 B$. Since f^5 produces an extreme f phenotype, demonstration of the recovery of the $f^{3n} f^5\,B$ genotype followed the detachment of the X chromosomes of attached-X ♀♀ phenotypically $f^{ex}\,B$. On testing $f^{ex}\,B$ to *su-f*, it was found that

TABLE 2

PROGENY TESTS OF ♀♀ $^+B/+$

PHENOTYPE ♀ PROGENY	SOURCE OF ♀♀ Exp. 1	Exp. 2	Exp. 4
$f^+\,B/+$	78	207	40
$f^{ex}\,B$	6	7	1
$r\,f^+\,B^+$	7	4	2

f^{ex} is unsuppressed. Since f^5 is suppressed, the recovery of f^{ex} as unsuppressed follows if f^{3n} and f^5 coexist on the same X chromosome.

The crossing-over results listed in Table 1 indicate that recombination between the *f* pseudo-alleles is extremely rare and precludes the routine testing of recombination between independently occurring mutants by use of attached-X ♀♀. An attempt was made to obviate this difficulty in the following manner. The *f* locus is proximal to the centromere of the X chromosome. A centromere effect on crossing-over has been demonstrated,[5] i.e., crossing-over between loci proximal to the centromere is increased when they are moved distal to the centromere and vice versa. Accordingly, a study of crossing-over between *f* pseudo-alleles was made where the loci are distal to the centromere. For this purpose, the scute-8 (sc^8) inversion, which extends from a point near the distal tip to the heterochromatin proximal to the centromere, was selected. In sc^8 the *f* locus is shifted nearly the entire length of the X chromosome away from the centromere. For the crossing-over tests the following stocks were constituted: a tester stock of the genotype $sc^8\ B\ f^1$; Cy, Ubx^{130}/Xa; and stocks of the unsuppressed mutants of the genotypes $sc^8\ Bx^2\ f^{3n}\ un$ and $sc^8\ Bx^2\ f^3\ un$. (Bx^2 = Beadex wing, *un* = uneven eye, 2.4 and 1 unit, respectively, on each side of the *f* locus.)

Crossing-over tests were made with ♀♀ heterozygous for the *f* mutants and the autosomal inversions *Cy* and Ubx^{130}. Only ♂ progeny were scored. It may be noted that the products expected if recombination occurs between the *f* pseudo-alleles are the following: ♂♂ $sc^8\ Bx^2\ f^+$ and $sc^8\ B\ f^1\ f^{3n}\ un$ or $sc^8\ B\ f^1\ f^3\ un$, the latter genotypes producing a f^{ex} phenotype. The results of these crossing-over tests are listed in Table 3. It may be noted that recombination between the pseudo-alleles was obtained in each case with the markers distributed as predicted. The results also suggest that by using sc^8 an increase in the recombination frequency between the pseudo-alleles has been effected. These tests demonstrate that f^3 and f^{3n} are allelic.

TABLE 3

CROSSING-OVER BETWEEN *f* PSEUDO-ALLELES IN *In(1)* sc^8 ♀♀

		PHENOTYPE EXCEPTIONAL ♂♂		TOTAL
	GENOTYPE ♀♀	$sc^8\ B\ fex\ un$	$sc^8\ Bx^2\ f^+$	♂♂
1.	$sc^8\ B\ f^1/sc^8\ Bx^2\ f^{3n}\ un;\ Cy/+;\ Ubx^{130}/+$	3	0	16,440
2.	$sc^8\ B\ f^1/sc^8\ Bx^2\ f^3\ un;\ Cy/+;\ Ubx^{130}/+$	0	2	6,045

Discussion.—The foregoing analysis of four *f* mutants of *D. melanogaster* parallels in all essential details the earlier analyses made of the *v* eye-color mutants. Thus, by use of a suppressor, otherwise phenotypically similar *f* mutants have been separated into two classes, suppressible and unsuppressible. Morever, pseudo-allelism is indicated, since recombination occurred between the suppressed and unsuppressed mutants with all four mutants tested. Furthermore, there is evidence to indicate that cytologically the forked loci are associated with a doublet in the salivary gland chromosomes, a cytological feature which appears to be characteristic of pseudo-allelism.[6, 2] The forked locus has been assigned to region 15A1-5 of the salivary gland X chromosome.[7] Within this region, a doublet consisting of bands 15A1-2 occurs and, by analogy with the other instances of pseudo-allelism, represents, in all probability, the *f* loci.

The bearing that pseudo-allelism has on the gene concept has been discussed in detail elsewhere.[8] It will suffice here to point out that the *f* pseudo-alleles represent another instance where functional disparity is indicated, thereby rendering the existence of pseudo-alleles as parts of a larger "physiological" gene as unlikely.

Summary.—(1) By the use of a suppressor mutant, the forked bristle mutants of *D. melanogaster* can be classified into suppressible and unsuppressible mutants. (2) Pseudo-allelism is indicated, since recombination between suppressible and unsuppressible mutants has been demonstrated. (3) The significance of these results in relation to the gene concept is briefly considered.

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CURVILINEAR CLUSTER SETS OF ARBITRARY FUNCTIONS

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We first prove a theorem concerning the structure of an arbitrary set of points in the unit circle $|z| < 1$ of the complex plane. Then we establish a property of the cluster sets of an arbitrary complex-valued function, defined in $|z| < 1$, along curves in $|z| < 1$ terminating in points of $|z| = 1$. Finally, we indicate several applications—in particular, to meromorphic functions in $|z| < 1$.

Let $K = \{z:|z| = 1\}$ and $D = \{z:|z| < 1\}$. We shall always understand θ to be restricted to the range $0 \leqq \theta < 2\pi$. By an *arc at* $e^{i\theta}$ we shall mean a Jordan arc in D having one end point at $e^{i\theta}$ and the other end point (regarded as belonging to the arc in question) at a point in D. The symbol P_θ will stand for the radius at $e^{i\theta}$: $\arg z = \theta$, $0 \leqq |z| < 1$. We shall denote the Riemann sphere by R and the closure of $M \subseteqq R$ by $\bar{M}$. The function $f = f(z)$ will be supposed to be defined and single-valued in D and to assume values on R; as will be seen, we could take f to be more general, but we choose this as perhaps the most interesting case to which our results concerning cluster sets apply. We shall have occasion below to restrict f to be continuous, and we shall mean continuous in the extended sense, so that, e.g., a meromorphic function is to be regarded as continuous at a pole.

If Γ is an arc at $e^{i\theta}$, the cluster set of f at $e^{i\theta}$ along Γ, denoted by $C(f, \Gamma)$, is defined to be the set of all points $w \in R$ with the property that there exists a sequence of points $\{z_n\}$ on Γ for which $z_n \to e^{i\theta}$ and $f(z_n) \to w$ as $n \to \infty$. It follows readily that $C(f, \Gamma)$ is a nonempty closed set on R. If f is assumed to be continuous in D, then it is well known that $C(f, \Gamma)$ is a subcontinuum (possibly a single point) of R.