MUTANT ISOALLELES AT THE VERMILION LOCUS IN DROSOPHILA MELANOGASTER

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The term isoalleles has been used to describe allelic genes which when homozygous product phenotypes indistinguishable from one another, but which can be separated from each other by special tests.[1] Wild type isoalleles have been described for the cubitus interruptus[1] and white[2, 3] loci in *Drosophila melanogaster* and for the bobbed locus in *D. hydei*.[4] Similar cases which may be ascribed to isoallelism are the multiple six alleles in Lymantria and Habrobracon and the self-sterility alleles in Nicotiana, Oenothera, etc. The results to be reported herein, viz. isoallelism among vermilion mutants in *D. melanogaster* are of interest because of the known biochemical basis for the action of vermilion mutants. Thus it should be possible to attempt a more exact analysis of the nature of isoallelism.

Mutants at the vermilion locus in *D. melanogaster* are recessive, sex-linked and are characterized phenotypically by the almost complete absence of the brown pigment component of the eyes. From the analysis of the behavior of vermilion mutants in gynandromorphs,[5] of transplantation of optic disks[6] and later of biochemical studies[7, 8] it has been shown that in vermilion mutants a failure occurs in the conversion of tryptophane to kynurenine. This inability to convert tryptophane to kynurenine in vermilion flies results in a block in the synthesis of brown eye pigment. When vermilion eye disks are supplied kynurenine—as in gynandromorphs, in transplants into wild-type hosts, or by feeding or injection of kynurenine to vermilion larvae—brown pigment synthesis occurs. In addition it has been found that brown pigment formation occurs in homozygous vermilion flies made homozygous for certain suppressor mutants.[9] As will be demonstrated here, by using the suppressor mutants it is possible to distinguish between vermilion mutants of independent origin which are otherwise phenotypically inseparable.

The following vermilion mutants were used (mutants which arose spontaneously will be designated by (s), and mutants which arose as a result of x-ray treatment will be designated (X)): v^1(s), v^2(s), v^{36f}(s), v^{48a}(X), v^{51a}(X), v^{51b}(s), v^{51c}(X). The various *v* mutants were tested *inter se*, and it was observed that compound ♀ ♀ carrying any two of the *v* mutants were clearly vermilion in phenotype. Two sex-linked, recessive suppressor mutants were used: su^2-*s*, a suppressor of sable and of vermilion and spontaneous in origin, and su^9-*v*, an x-ray induced suppressor of vermilion. The usual tests showed su^2-*s* and su^9-*v* to be allelic.

To determine the phenotype of the eyes of the various *v* mutants in the presence of the suppressors the following procedure was used. The suppressors are localized at position 0.0 on the X chromosome and are therefore completely linked with the recessive yellow (*y*) body color. The *v* mutants are localized at 33 on the X chromosome and thus very closely linked to raspberry (*ras*), another recessive eye color mutant located at 32.8. The eye colors of *v*, *ras* and *ras v* are all easily separable phenotypically. Stocks of the genotypes su^2-*s*, ras^2v^1 and su^9-*v*, ras^2v^1 were constituted. These carry the y^+ allele of *y*. Since v^1 is suppressed by both suppressors, the aforementioned flies are *ras* v^+ and y^+ in phenotype, and thus *ras* serves to mark the presence of v^1, and y^+ the presence of the suppressor. Stocks of the various *v* mutants marked with the mutant *y* and lacking either suppressor were also made up. By the appropriate crosses, ♀ ♀ of the genotype su^2-*s*, $ras^2v^1/y\ v$ were obtained and these were crossed to sibling ♂ ♂. The male progeny were checked. Single crossovers between su^2-*s* and ras^2

TABLE 1

PHENOTYPES OF *v* ALLELES IN TESTS WITH SUPPRESSOR MUTANTS AND IN GYNANDROMORPHS

	EYE PHENOTYPE		
	HOMOZYGOUS *v* WITH		
v ALLELE	HOMOZYGOUS su^2-*s*	HOMOZYGOUS su^9-*v*	IN GYNANDORMORPHS
v^1	Wild-type	Wild-type	Non-autonomous
v^2	Wild-type	Wild-type	.....
v^{36f}	*v*	*v*	Non-autonomous
v^{48a}	*v*	*v*	.....
v^{51a}	*v*	*v*	Non-autonomous
v^{51b}	*v*	*v*	Non-autonomous
v^{51c}	*v*	*v*	Non-autonomous

result in ♂ ♂ of the genotype *y* ras^2v^1 and su^2-*s*, *v*. Since crossovers between ras^2 and v^1 are relatively rare, and may be disregarded, all ♂ ♂ phenotypically y^+ras^+ must be genotypically su^2-*s*, *v*, and examination of these ♂ ♂ permits the determination of whether the suppressor suppresses the *v* mutant being tested. Since crossing over between su^2-*s* and ras^2 is high, ca. 30%, these ♂ ♂ are abundant. Following recovery of su^2-*s*, *v* ♂ ♂, stocks were constituted in which ♀ ♀ homozygous su^2-*s*, *v* were obtained. Identical tests were made using su^9-*v*, ras^2v^1.

The results of these tests are listed in table 1. It will be noted that the *v* mutants fall into two classes: those that are suppressed (collectively designated as v^s) in which the *v* phenotype is modified to wild-type in the presence of either suppressor mutant, and those that are unsuppressed (collectively designated as v^u) in which the *v* phenotype is unaltered in the presence of either suppressor mutant. The phenotypic separation of the *v* mutants in the presence of the suppressors is unequivocal. Phenotypes

of the ♀ ♀ agreed without exception with that of the ♂ ♂ for each *v* mutant tested. Among the v^u mutants are those of both spontaneous and x-ray origin. These results allow certain assumptions to be made regarding the nature of the action of *v* mutants. It may be assumed that in the v^s mutants, the presence of the suppressor allows the conversion of tryptophane to kynurenine to take place. Once this metabolic block is released, brown pigment synthesis proceeds. Conversely, it may be assumed that in the case of the v^u mutants the suppressor is ineffective in promoting the conversion of tryptophane to kynurenine. There is evidence from Neurospora that suppressor mutants release specific biochemical blocks.[10]

Proceeding from these assumptions, it is necessary to provide an explanation for the failure of the suppressor to act in the presence of the v^u mutants. One hypothesis which suggests itself is that the v^u mutants lead to a complete inactivation of the brown pigment synthesis system. In such a scheme the suppressors might foster the conversion of tryptophane to kynurenine but v^u individuals cannot utilize the kynurenine or any other component of the brown eye pigment mechanism. Since it is known that v^1 eyes develop non-autonomously in gynandromorphs[5] the hypothesis can be tested by observing the behavior of v^u mutants in gynandromorphs. If the total inactivation mechanism operates then it would be predicted that v^u mutants would be autonomous. Gynandromorphs involving v^u mutants were obtained from the following cross: ♀ $y\ sn^3\ v^u$ X ♂ X^{c2} (ring X chromosome carrying $y^+sn^+v^+$). The use of the X^{c2} chromosome apparently increases the occurrence of gynandromorphs.[11] F_1 progeny were examined and numerous gynandromorphs were obtained. Study of the gynandromorphs showed that the genotypically *v* eye was modified phenotypically to wild type. Thus the v^u mutants behave non-autonomously. These observations eliminate the possibility of a total inactivation mechanism being involved.

An alternative hypothesis stems from more recent information on the mechanism of the conversion of tryptophane to kynurenine. By a most elegant series of experiments, it was demonstrated that in rat liver the step-wise conversion of tryptophane to kynurenine involves two intermediate compounds of which only the second, formylkynurenine, has been identified.[12, 13] These findings suggest that perhaps the difference between v^s and v^u mutants rests in their effect on different steps in the conversion of tryptophane to kynurenine. Thus v^s mutants might block the conversion of tryptophane to formylkynurenine and v^u mutants might block the conversion of formylkynurenine to kynurenine. Accordingly the suppressors, which might be assumed to act in releasing only a specific biochemical block, would foster the conversion of tryptophane to formylkynurenine and thereby suppress v^s mutants but could not suppress v^u mutants which are blocked biochemically at a different step.

To test this hypothesis experiments were conducted in which formylkynurenine and kynurenine were fed to v^u and v^s mutants. Three *v* mutants, v^1, v^{36f} and v^{51c} were used. All three mutants were combined with the recessive, autosomal eye color mutant brown (*bw*) such that all *v; bw* homozygotes had eyes essentially white in appearance. Ten larvae of each genotype of the age 72–96 hrs. after oviposition were added to 2 ml. of regular corn meal-molasses-agar medium supplemented with either 1 mg. *l*-kynurenine sulfate or 1 mg. formyl-*dl*-kynurenine. Larvae grown on unsupplemented medium served as controls. In addition, control and test media were supplemented with 50 μg. of streptomycin in order to eliminate the possibility of bacterial conversion of tryptophane to kynurenine. Tests were run in duplicate at 22–24°C. The results are tabulated in table 2. It is evident that both formylkynurenine and kynurenine act as precursors for brown pigment in Drosophila. No difference was noted in the level of brown pigment formed by flies fed either compound, nor was any

TABLE 2

EFFECT OF FEEDING KYNURENINE AND FORMYLKYNURENINE ON THE EYE COLOR OF *v* MUTANTS, *D. melanogaster* AND *v* AND *cd* MUTANTS, *D. virilis* (SEE TEXT FOR DETAILS OF METHOD)

GENOTYPE	PHENOTYPE		
	CONTROL	KYNURENINE	FORMYLKYNURENINE
v^1;*bw*	0[a]	++	++
v^{36f};*bw*	0	++	++
v^{51c};*bw*	0	++	++
v^{40d};*es*	0	+	+
cd;*es*	0	+	+

[a] Estimate of amount of brown eye pigment formed where 0 represents no pigment and ++++ represents the apparent pigmentation of homozygous *bw*.

difference noted among the three *v* mutants tested. If the v^s and v^u mutants act by blocking tryptophane metabolism, then the blocks must come prior to the formation of formylkynurenine. It has been shown previously that the v^1 mutant accumulates tryptophane.[14] Since it has been demonstrated that there is an unknown intermediate compound formed between tryptophane and formylkynurenine,[12, 13] it might be postulated that v^s mutants block tryptophane metabolism between tryptophane and the intermediate and v^u mutants block between the intermediate compound and formylkynurenine. It would then follow that the suppressor mutants release the block between tryptophane and the intermediate compound. The precise chemical nature of the intermediate compound is not clear, although there is strong evidence that it is not alpha oxytryptophane.[15] Consequently the obvious tests cannot be performed.

An additional hypothesis to explain the action of the suppressor might postulate that the suppressor directs the metabolism of tryptophane along

a route not involving the formation of formylkynurenine. Such a hypothesis would presume further that the alternate pathway occurs only in v^s mutants. Evidence to date has not elucidated an alternative pathway,[15] and such a hypothesis appears unlikely.

The observation that formylkynurenine acts as a precursor for both v^s and v^u suggested that similar tests be made on two mutants in *D. virilis*, vermilion (*v*), sex-linked and recessive and cardinal (*cd*), autosomal and recessive. It has been reported that both mutants are non-autonomous when transplanted to wild-type hosts and when larvae of both are fed kynurenine.[16] Tests were performed in the manner noted above using the *v* allele, v^{40d} and *cd*. Both v^{40d} and *cd* were first made homozygous for the recessive eye color mutant eosinoid (*es*), the homologue of *bw* of *D. melanogaster*. The results as listed in table 2 demonstrate that formylkynurenine acts as a precursor to brown pigment in *D. virilis* and that the block in pigment synthesis in both mutants occurs prior to the formation of formylkynurenine. No difference was observed in the level of brown pigment formed by flies fed either compound nor was any difference observed between the two mutants studied.

The results reported demonstrate that in *D. virilis* formylkynurenine functions as a precursor for two separate mutants, v^{40d} and *cd*, while in *D. melanogaster* for only *v* mutants. There is evidence strongly indicating homology between the *v* mutants of the two species[17] but thus far no homolog of *cd* has been uncovered in *D. melanogaster*. It is tempting to postulate that the *v* mutants in *D. melanogaster* are not truly allelic, but rather the v^s and v^u mutants represent a case of close linkage (pseudoallelism) much like that reported for the mutants Star and asteroid[18] and the lozenge mutants[19] in *D. melanogaster*. It would follow from such a postulate that one class of *v* mutants (perhaps v^u) of *D. melanogaster* is homologous to *v* of *D. virilis*, while the other (perhaps v^s) is homologous to *cd* of *D. virilis*. This explanation can remain for the present only as a postulate. The validity of a linkage hypothesis can be established only by demonstrating crossing over between v^s and v^u mutants. Experiments to test the possibility of close linkage are now in progress.

Summary.—(1) The use of suppressor mutants makes possible the classification of *v* mutants in *D. melanogaster* into two types: suppressed and unsuppressed. (2) Formylkynurenine is utilized as a precursor for brown eye pigment in Drosophila. (3) The mutants investigated, *v* in *D. melanogaster* and *v* and *cd* in *D. virilis* appear to be blocked biochemically between tryptophane and formylkynurenine.

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THE PARACOMPACTNESS OF THE WEAK SIMPLICIAL COMPLEX

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The main purpose of this paper is to prove that a simplicial complex taken in the weak topology,[1] is a paracompact T_2 space.[2] Apart from this result, the method involves some new considerations which are expected to prove of wide application.

We assume throughout that $K = \bigcup K^n$, where K^n is the n dimensional skeleton. If U and V are collections of open sets we define the concept: V is a partial refinement of U, by requiring every element of V to be contained in some element of U. We write $St(s, U)$ for *Star* (s, U). The symbol I or I_a (where a may be any script) *invariably* denotes the unit interval. We use both $\tilde{A}$ and $-A$ to denote complements so $B - A = B \cap \tilde{A}$. The topology giving the smaller open sets is the *finer* topology. In the weak (*or w*) topology for a simplicial complex K, open sets are those with relatively open intersections with each closed (Euclidean) simplex of K.

Denote the generic vertex of K by a and the totality by A. We use π in the dual sense of a finite subset of A constituting *the vertex set* of the open simplex $\sigma(\pi)$ of K, and for the *barycenter* of this simplex. The context will