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On cell lethals in *Drosophila**

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Abstract. Somatic crossing over in flies heterozygous for a dominant Minute mutant ^{may} result in two cells, homozygous for wild type and Minute, respectively. If both cells took part in development a twin spot of two genotypes, different from the rest of the body, would result. Two hundred wild type spots were found none of which was accompanied by an adjacent twin spot homozygous for Minute. The absence of these spots shows that their cell lethality is not overcome by transport, including diffusion, across a few cells of gene dependent material from the neighboring cells which carry one or two wild type alleles of Minute.

Genetic mosaics provide opportunities to study the problem of transfer of gene controlled material from cells of one genotype to those of another. If no such transfer occurs or if it remains insufficient to result in observable effects the cells along the boundary between the two genotypes will behave autonomously, i.e. reflecting only their own genotype. If sufficient transfer does occur the cells of a deficient genotype would be "fed" by neighboring cells of a nondeficient genotype and thus behave in a nonautonomous manner. In *Drosophila melanogaster* both autonomous and nonautonomous behavior of different genotypes is known. Autonomy seems to be the rule in *Drosophila* but most of the evidence recorded so far does not exclude the possibility that a nonautonomous border zone may be present at the junction of two autonomous regions. Nonautonomy may be based on substances circulating in the insect. For instance, kynurenine, the "v⁺" substance, permits formation of normal eye color in genetically vermilion patches of eyes mosaic for vermilion and nonvermilion genotypes.

In other instances of nonautonomy cell-to-cell transfer of diffusing materials has been described as in the sex specific pigmentation of the terminal abdominal segments where male tissue influences the coloration of adjoining female tissue and where not-yellow female tissue influences the coloration of adjoining yellow male tissue.¹

Similar cases of nonautonomy in respect with pigmentation of mosaics for eye colors have been described in the wasp *Habrobracon*.² In the plant *Delphinium* mosaic pigmentation of flower colors due to mutation result in purple spots on a rose background but a "halo" of cells with intermediate pigmentation separates the mutant cell or cells from the phenotypically nonmutant ones.³ The halo represents a ring of nonmutant cells one cell wide into which pigment or pigment precursor substance has diffused from the mutant cells. Similar cases occur in mosaic maize kernels.

In *Drosophila* a special kind of mosaicism involving recessive X-linked lethals has been studied by Demerec.^{4, 5} The question was asked whether such lethals which do not permit survival of whole individuals are also lethal to single cells or small patches of cells occurring in individuals which are heterozygous for the recessives and therefore viable. The studies showed that some lethals are compatible with survival of cell patches but that others act not only as lethals for individuals but also as lethals for hemi- or homozygous lethal bearing cells. Among the latter lethals were two which belong to the peculiar class of Minute mutants. Minute loci exist on all chromosomes of *Drosophila*. Mutants at these loci produce smaller and finer bristles on the body surface of heterozygotes than wild type and cause a significant slow-down in the development of the individual. In hemi- and homozygotes the Minutes act as lethals. Many Minute mutants have been shown cytologically to be chromosomal deficiencies. According to Schultz⁶ the similarities in phenotype of different Minutes indicate a basic similarity of an underlying "Minute reaction". It has been suggested by

Atwood that the Minute loci are concerned with the coding for the different transfer RNAs and that the variety of Minutes produce their similar effects by reduced rates of t-RNA dependent protein synthesis.⁷ In view of this hypothesis it seemed proper to reinvestigate the cell lethality of Minutes with emphasis on the question whether cells homozygous for a Minute can survive if close to cells that are heterozygous or homozygous for the normal, not-Minute allele.

Methods. The two Minutes studied were $\underline{M}(1)_{\underline{o}}^{\text{Sp}}$ and $\underline{M}(1)_{\underline{n}}$ located on the X chromosome at 56.6 and 62.7, respectively. They will be jointly referred to as \underline{M} , and individually as $\underline{M-o}$ and $\underline{M-n}$. Females of the constitution $\underline{y} \underline{sn}^3 \underline{M}/+++$ or, in some cases, $\underline{y} \underline{f}^5 \underline{M}/+++$ have Minute macrochaetae and are otherwise normal. (The gene symbol \underline{y} stands for yellow body and chaetae coloration, locus 0.0; \underline{sn}^3 for singed chaetae, locus 21.0; \underline{f}^5 for forked chaetae, locus 56.7. These three mutants are recessives and X-linked. In the following pages the superscript of \underline{sn} will be omitted. The discussion will be in terms of \underline{sn} only but would equally apply to the \underline{f} locus.)

Single somatic crossing over to the right of the \underline{M} locus may result in two cells one of which is homozygous $+++ / +++$, the other $\underline{y} \underline{sn} \underline{M} / \underline{y} \underline{sn} \underline{M}$. The former cell and its descendants will result in a patch which if it contains a macrochaeta will be recognizable as of not-Minute phenotype, the latter cell and its descendants if viable will result in a $\underline{y} \underline{sn}$ Minute patch. A similar situation would exist if double crossing over simultaneously to the left of \underline{sn} and to the right of \underline{M} should occur. In this case, the twin of the resulting not-Minute spot if viable would have the phenotype $\underline{sn} \underline{M}$.

The finding of mosaics was based on inspection of the 11 pairs of macrochaetae ("bristles") on the main dorsal thoracic segment of the adult fly, the mesonotum, and the posterior sternopleural bristle. Whenever a not-Minute spot was found its discovery was followed by a critical search for a twin spot, however small, having macro- or microchaetae of $\underline{y} \underline{sn}$ or \underline{sn}

phenotype (the M phenotype of a y sn or sn spot would only be recognizable in patches which include one or more macrochaetae). The somatic crossing over process responsible for the appearance of spots occurred either spontaneously at low frequency or was induced by X-raying larvae of ages 24 - 48 and 48 - 72 hours after deposition as eggs. All but 15 of the females bearing a not-Minute spot were homozygous for the autosomal recessives se and h. The latter gene, h, hairy, leads to the appearance of microchaetae in certain regions which are bare in not-h flies. This permits scoring of setae in these regions for the y sn phenotype. The presence of se, for sepia eye color was incidental.

Results and Discussion. Seventy-five spots of not-Minute phenotype were found in the M-o series and 125 spots in the M-n series. In every case the spot failed to be accompanied by an adjacent spot of y sn or sn phenotype. This agrees with the earlier work of Bridges⁸, Demerec^{4, 5} and Stern⁹. Since the absence of a y sn or sn spot next to a not-Minute spot applied to all mosaics and regardless of X-ray treatment or nontreatment of larvae of the two age groups the 200 cases will be considered as essentially forming a single class.

Before analysing the nonoccurrence of twin spots it should be pointed out that somatic crossing over as observed by phenotype of macrochaetae in these experiments occurred nearly exclusively to the right of the M locus involved. Had crossing over to the left of the M locus occurred it would have resulted in spots heterozygous for M of the phenotypes y M, y sn M and sn M but no y M and y sn M spots and only two y M spots were found. On the contrary, crossing over involving the region to the right of the M locus would have resulted in not-M spots two hundred of which were found. It is most likely that the great majority of these not-M spots were the result of single crossing over to the right of M. This conclusion is based on the expectation that if double crossing over occurs it should result not only in not-M spots but also in y M and y sn M spots. As pointed out above the

latter were absent in our sample; and the former, occurring twice only, were likely the result of single crossovers to the left of M.

There is one type of not-M spot which requires special attention. It would be the result of double crossovers between sn and M and to the right of M which may lead to not-M spots whose twin if viable would have ++M phenotype. This phenotype would not be distinguishable from that of the prevailing phenotype of the M individuals. The type of double crossover which would result in these twin constitutions must be at most very rare since it would also result in y sn M spots which, as stated, were not found. It is therefore concluded that the specific type of double crossover was either very rare or did not occur in our sample. Therefore, absence of a y sn M or sn M twin to a not-Minute spot will be attributed basically to cell lethality of M/M and not to lack of recognition of a M/M spot on a M/+ individual.

The shift of mitotic as compared to meiotic crossing over mainly to the region proximal to the M loci has been found in some but not all earlier work. In our present experiments it was not due to the presence of an undiscovered inversion or other crossing over suppressing situation as shown by tests of meiotic crossing over in females of the same constitution as those yielding the mitotic crossover spots.

Absence of y sn M or sn M spots next to some +++ spots is not unexpected even if homozygosity for M were not cell lethal. Two cells of complementary genotypes will form phenotypic twin spots only if the descendants of both cells form a sufficiently large patch covering at least one chaeta each. Therefore, on the whole, the frequency of identifiable twin spots should increase with larger size of the not-Minute spot. It is therefore instructive to list the 200 not-Minute spots according to their size as expressed by numbers of macrochaetae included (Table I). There were 89 single bristle spots whose chance of having a twin partner would

have been low even in case of the partner having cell viability. A low though increasing chance of finding twin spots would still be expected for the 48 not-Minute spots covering 2 and 3 bristles. But the chances of twin spots would be high in most of the 63 spots which covered 4 or more bristles. Thus, the complete absence of y sn M or sn M spots is due to intrinsic nonviability of the genotype even in very small cell patches. Furthermore the cell lethality of homozygous Minute is not sufficiently counteracted by diffusion or transport of not-Minute material from the heterozygous M/+ cells which constitute most of the body of the spot-bearing fly, or from the homozygous not-Minute cells which lie adjacent to the M/M cells.

If homozygosity for M were not cell lethal then y sn M or sn M patches should have occurred without an accompanying +++ patch as a result of accidentally restricted development of the initial normal cell of the two crossover sister cells. The absence of any y sn M or sn M patches independent of the existence of +++ patches adds to the evidence from the 200 +++ patches that homozygous M cells do not persist.

The macro- and microchaete of the mesonotum of *Drosophila* are separated from one another by cells which lack chaetae and instead have a hair-like outgrowth, a "trichome", which is not homologous with a chaeta. It is easy by means of counting the number of trichomes situated between two chaetae, to obtain approximate data concerning the number of cells which separate them. Counts of cells located in between four selected macrochaetae, the anterior supra-alar, anterior and posterior dorsocentral and the anterior scutellar, and the nearest microchaeta yielded a range of from 1 to 7 cells with the majority of distances covered by 3 to 5 cells (n = 25). A transport mechanism, including diffusion, for a normal not-Minute product from +/M or +/+ into M/M tissue would thus have to pass through a very few cells only before the product reaches a cell which

is able to differentiate into a bristle and to become competent to respond to the transported material. Indeed the distance in terms of cell numbers is much lower than recent estimates for the extent of embryonic fields which seem often to involve distances of less than 50 cells¹⁰. The absence of an effect of $+/\underline{M}$ and $+/+$ tissue to permit survival of $\underline{M}/\underline{M}$ cells suggests that the \underline{M}^+ -dependent product (i.e., perhaps, t-RNA) is not easily transported from one cell to another.

In the experiments reported here the autonomy of cell lethality results in elimination of cells. In another context, autonomy of differentiation in mosaic individuals was found to permit cells in spots of new genotypes to form structures such as sex comb teeth or sections of transverse rows of bristles independently of the prevailing genotype.^{11, 12} Communication between cells is a necessary aspect of development but cellular isolation also is required.

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Table 1 The number of macrochaetae in 200 not-Minute spots.

Number of macrochaetae	1	2	3	4	5	6	7	8	9	10	11
Number of spots	89	33	15	16	12	10	10	4	7	2	2

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