Table 1. Effect of darkness or far-red light in converting *Marsilea* sporelings to either water or land forms. After treatment with continuous far-red light or darkness, the sporelings were transferred to continuous fluorescent light. The results for dark-treated sporelings are averages of two experiments.

Time (days)	Cultures (No.)	Percentage	
		Water forms	Land forms
Sporelings treated with darkness			
1	15	100	0
4	16	89	11
7	14	71	29
9	20	40	60
11	16	11	89
25	18	0	100
Sporelings treated with far-red light			
1	10	60	40
4	10	20	80
7	10	0	100
9	10	0	100
11	10	0	100
25	10	0	100

the plant was given red light (as supplied by the fluorescent lighting) it never developed into either the land or the water form.

Plants were placed in continuous darkness or under a continuous far-red light (4600 erg/cm² sec) for a varying number of days and then were placed under continuous fluorescent lighting for the remainder of the 47-day period. As the length of time in darkness or under far-red light increased, more of the etiolated plants developed as the land form upon return to red light (see Fig. 1*B*). Apparently etiolation results in some change such that the plants are converted from the water form to the land form.

Table 1 shows that far-red light is more effective in accomplishing this conversion than darkness. If 1 percent sucrose or ⅕ the normal concentration of minerals are used in the medium, much more darkness or far-red light is required to convert the plants. In the case of 1 percent sucrose darkness is not effective at all, and the plants remain in the water form.

According to James and Birge (*7*), light energy above 7000 Å may be rapidly absorbed by lake water. Thus lake water could act as a barrier to far-red radiation, and water forms of aquatic plants would then be growing in the absence of far-red light. Aquatic plants growing in shallow water or on land would be exposed to far-red radiation and would develop as land forms. Other environmental factors have some effect on this conversion (*8*).

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5. Light energy was measured with a large-area bolometer (Kurlbaum type) calibrated against a standard tungsten lamp (U.S. National Bureau of Standards). The far-red source consisted of two 25-watt incandescent bulbs whose radiation was filtered through 7 cm of water and six layers each of red and blue cellophane.
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Mammalian X-Chromosome Action: Inactivation Limited in Spread and in Region of Origin

Abstract. *In its simplest form the hypothesis of the single-active-X chromosome does not explain variegated-type position effects in the mouse. Inactivity appears not to involve one entire X chromosome; furthermore, even those parts of the chromosome that can change to an inactive state spread inactivation not to the entire attached piece of autosome, but along a gradient to limited distances.*

Variegated-type (V-type) position effects from X-autosome translocations in the mouse have played a major role in development of the hypothesis of the single-active-X chromosome. Recent results from seven such translocations will be presented to show that this hypothesis is not valid in its simplest form (*1*).

V-type position effects were first reported by us in 1959 (*2*). Now, eight stocks of independent origin exist—seven of these (some radiation-induced, some spontaneous) at our laboratory (*1–5*) and one (induced with tri-ethylene melamine) in Edinburgh (*6*). All eight carry X-autosome translocations, one involving linkage-group (L.G.) VIII and the remainder L.G. I. We have explained the variegated phenotype by concluding that when an autosomal gene is transferred to the vicinity of portions of the X chromosome its action is suppressed in some cells of the body, thus allowing, in these cells, expression of a recessive allele present on the intact autosome. Evidence for this conclusion has been presented (*4*), particularly the proof that variegation is not due to any permanent change in the autosomal gene and the demonstration that the translocation does indeed involve the X chromosome. Cytological studies at mitotic metaphase indicate that all of the translocations are unequal. Meiotic metaphase studies are not possible since degeneration of spermatogenic elements occurs in pachytene (*4*).

More than one X chromosome has to be present for the expression of variegation (*3*). Thus, translocation heterozygotes without an additional X chromosome (XY males or XO females) are nonvariegated (*2–5*); conversely XXY males with the translocation are variegated (*6, 7*). This evidence, considered in conjunction with various cytological results in other mammals—namely, the single nature of the sex chromatin, the "n-1" rule for sex chromatin, and the difference between the two X's both in morphology at late prophase and in time of DNA synthesis—led us to suggest (*8*) that genic balance required the action of one X, leaving additional X chromosomes (or X-chromosome regions) to assume a heteropycnotic state; and that, in this state, they were able to exert a position effect on transposed autosomal genes. This idea of alternate states of X chromosomes is in keeping with Cooper's postulate that "heterochromatin" and "euchromatin" do not reflect underlying structural peculiarities, but portray temporal states of behavior related to the functioning of genes (*9*). Lyon later suggested that one or the other X, at random, becomes entirely inactive early in embryonic development, and she explained the position effects by assuming that the translocated autosome behaves completely like the X to which it is attached (*10*), as shown in Fig. 1*A*.

According to Lyon's explanation of the position effects, all of the recessives on the standard autosome should (on the average) be expressed in half the cells of the body. Thus for any given autosomal locus, variegation characteristics should be independent of the position of the X and autosomal break-

points. Our new data show that this is not the case. On the basis of these results, we regard the position effects in terms of gradients of inactivation. Furthermore, these proceed from only certain parts or part of the X (see Fig. 1*B*).

Since, in all seven of our X-linked translocations, there is free recombination between the transposed autosome and its intact homologue—in contrast to Cattanach's translocation where such crossovers do not occur (*6*)—it has been possible to map the autosomal breakpoints (*11*). Tentative positions of the autosomal rearrangement points and estimates of the amount of fur showing the recessive phenotype at a number of loci, for each of the rearrangements, are shown in Fig. 2.

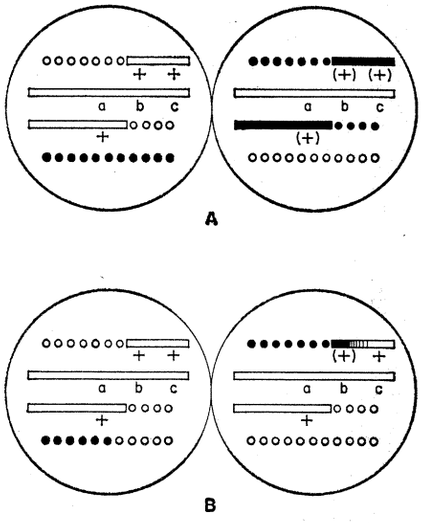

Fig. 1. Diagrammatic representation of V-type position effect from X-autosome translocation according to (*A*) the hypothesis of a single-active-*X* chromosome in its simplest form (Lyon) and (*B*) the hypothesis developed in this paper. Dotted line, X chromosome; unbroken line, autosome; black portions, inactive regions; *a, b, c,* generalized symbols for recessives on autosome; +, active wild-type alleles; (+), inactive wild-type alleles. In parts *A* and *B*, each of the two cells represents the alternate condition in approximately half the cells of the body, with activity of one or the other X chromosome fixed at random. In (*A*) when the standard X is inactive (left cell), the translocated X is active; therefore the translocated autosome is active and the recessives on the standard autosome are not expressed; alternatively (right cell), when the translocated X is inactive, the translocated autosome is inactivated, and the recessives on the intact autosome are expressed. Expression of recessives in only half the cells of the body leads to variegated phenotype. On this hypothesis, variegation at any given locus is independent of position of rearrangement points. (*B*) depicts the hypothesis based on our finding that variegation is *not* independent of rearrangement points. We postulate that only part of the X can become inactive and that inactivity spreads along a gradient. Thus, there are two possible causes of nonvariegation of an autosomal gene: (i) attachment to a noninactivating portion of X (see *a*-locus in the figure) and (ii) excessive distance from an inactivating portion of X (see *c*-locus).

The amount of variegation for any given locus is not at all the same in different rearrangements of the same two chromosomes (L.G.I and the X). At the *p*-locus there appears to be some negative correlation between distance of breakpoint and amount of variegation, which suggests a gradient of inactivation. A gradient is also strongly indicated by a spreading effect that occurs in the case of R3, R5, and R6. Thus, animals of the type R3(++)/$c^{ch}p$ have areas of c/c^{ch} and occasionally of $cp/c^{ch}p$ (near-white) but not of p/p, while animals of types (++)R5/$c^{ch}p$ or (++)R6/$c^{ch}p$ have areas of p/p and $cp/c^{ch}p$ but not of c/c^{ch}. A spreading effect has also been found by Cattanach (*6*).

A strong argument against the interpretation illustrated in Fig. 1*A* is the complete failure, in certain cases, of the recessive to be expressed. This is found for *wi* (whirler) and *m* (misty) in the case of R1, and for *c* in the case of R2 (Fig. 2). The first two of these do not provide such critical evidence as the third. Thus, *wi* affects behavior, and it is possible that expression of the recessive in only half of the cells controlling this character is not sufficient to produce the mutant phenotype. And in the case of *m*, although it affects coat color, it is not certain (though probable) that gene action is local; in any case, *m* is far from the breakpoint. However, the evidence is critical in the case of failure of *c*-variegation in R2. Here, the breakpoint is extremely close to the locus (crossing over < 0.3 percent), and the locus is clearly capable of showing strong variegation, as indicated by five other translocations that have breakpoints on either side of *c*, some at a considerable distance. Clearly, nonvariegation offers strong argument against the interpretation shown in Fig. 1*A*, unless the translocation is either nonreciprocal or multiple.

To explain the result on the basis of nonreciprocal translocation, one must assume that portions of L.G.I distal to R2 (that is to the right, as shown

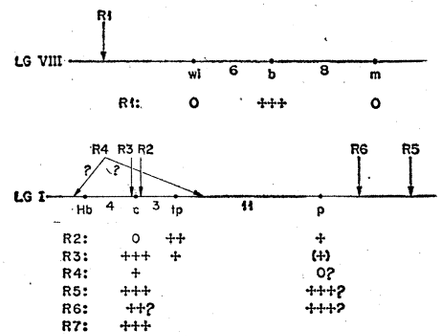

Fig. 2. Autosomal breakpoints (R1-R6) for X-autosome translocations studied at Oak Ridge and amount of variegation produced by each rearrangement at a number of autosomal loci. Variegation was graded visually on a scale where +++ equals about 50 percent of fur (on the average) of recessive phenotype, and (+) equals almost imperceptible lightening of fur. Breakpoint for R7 has not yet been mapped.

in Fig. 2) have become attached to the X, while no portion of X has taken their place. (The order in L.G.I would have to be centromere-*c*-*p*.) If there were only one break in L.G.I, the translocation would be so unequal as to be cytologically quite striking. Actually, translocation R2 is cytologically much less unequal than R3, in which the autosomal breakpoint is very close to that of R2. Nonreciprocal translocation, with two breaks in the autosome, such that only a short section (containing *tp* and *p* loci) is transposed to the X (with no part of X taking their place), is unlikely since it would reduce recombination between the transposed piece and the standard autosome; this appears not to be the case. Multiple translocation, for example of type X;1, 1;2, 2;X, is improbable since it would reduce the proportion of balanced segregants much more than appears to be the case from litter-size determination. It has, therefore, been concluded that the reason there is no *c*-variegation in R2 is that the portion of autosome bearing the *c*-locus has become attached to a noninactivating part of the X (*1*). Thus, the two translocated chromosomes are of this general type: (i) part of L.G.I including *c*-locus, plus noninactivating part of X; and (ii) part of the X including an inactivating region, plus part of L.G.I including *tp* and *p* loci.

The finding that part of the X has no ability to inactivate translocated autosomal loci suggests that this or other parts may themselves never

become inactive. This may explain the failure of certain sex-linked genes, for example *sf* in the mouse, to fit the hypothesis of the single-active-X.

Because of the evidence for gradients of inactivation and evidence that part or parts of the X are noninactivating, we have interpreted the V-type position effects as shown in Fig. 1*B*. While it is true that X-inactivity can occur only when there is more than one X present, this inactivity does not involve one entire X. Furthermore, those parts that can change to an inactive state spread inactivation not to the entire attached piece of autosome, but along a gradient to limited, though occasionally long, distances. Thus, on the simple interpretation of V-type position effect by the single-active-X hypothesis (Fig. 1*A*), all of the recessives in the intact autosome should be expressed. According to our interpretation (Fig. 1*B*), on the other hand, there are two reasons why a recessive might not be expressed: (i) it may be attached to a noninactivating part of the X (for example, *a*-locus in Fig. 1*B*); or (ii), although attached to an inactivating part of X, it may be too distant from it (for example, *c*-locus in Fig. 1*B*).

Figure 1*B* may be an oversimplification. Thus, there could be more than one inactivating and more than one noninactivating part of the X. Furthermore, there could be such differentiation within the inactivating part or parts that some regions are more potent than others.

It should also be pointed out that the results make it likely that inactivation can "flow" in both directions. Thus the *p* locus is inactivated by a "flow" to the right (Fig. 2) in the case of R2, R3, and R4, but by a "flow" to the left in the case of R5 and R6.

On the interpretation of Fig. 1*B*, rearrangements R3, R5, and R6 would involve breaks within the inactivating part of the X; and R2, a break within the noninactivating part. There are preliminary indications that R4 may also involve a break in a noninactivating part. Mapping of the X-chromosome breakpoints, which is now in progress, will be of great interest in connection with the interpretations developed in this paper.

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* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

5 April 1963

Analysis of Variance of the Composition of a Migmatite

Abstract. *To determine the variation of composition in a Front Range migmatite, an analysis of variance (nested design) was run on field measurements. The regional variation is too small to be considered significant, but the areal variation (between outcrops) is large enough to warrant comparison with some other measurable variable such as country rock composition.*

Migmatites, a mixture of granite and previously existing metamorphic rock, are a widespread phenomenon in plutonic terrains. This intimate association between the two rock types provides an opportunity for testing hypotheses of the origin and emplacement of granite (*1*).

The granite fraction of a migmatite may (model A) orginate *in situ* by anatexis (partial melting) of the country rock, or it may (model B) originate elsewhere and be emplaced in the country rock. (In this case the accessible portion of the migmatite is a chemically open system.) In model B two submodels may arise: (model B_1) the emplacement of the granite may leave the composition of the country rock essentially unchanged, or (model B_2) the granite material may permeate the country rock and make it more granitic.

These three hypotheses may admit of nonintuitive statistical tests: in model A the regression of some expression of the chemical composition of the country rock—say, silica percentage—upon the percentage of granite in the migmatite should have a negative slope and a high coefficient of determination (or correlation coefficient). In model B_1 a very low correlation should exist between the variables, and in model B_2 a positive slope and a high coefficient of determination should exist.

The first requirement for this study is a body of data previously nonexistent in geological literature: enough random measurements of the proportions of the end members to determine the composition and distribution of variation in a migmatite.

Our original target population (*2*) was the Idaho Springs gneiss in the Poudre River canyon, Larimer County, Colorado, along a 40-mile stretch from the Paleozoic overlap to the Home moraine (26 miles airline distance). It later became necessary to modify our target population, as we discuss below. In this area large, fresh exposures are abundant. The country rock portion of the migmatite consists of middle and upper almandine-amphibolite facies pelites and quartzofeldspathic rocks with minor amphibolites. A detailed study of the composition of and variation within the country rock is presently in progress. Data on this variation and heterogeneity may require extensive modification of the simple regression models proposed above.

An outcrop was defined as 100 feet of continuous, relatively fresh exposure (measured perpendicular to the foliation). The 112 outcrops (our sampled population) were grouped into five segments of 23 each (except the last, which had only 20). Seven outcrops were chosen by random number table from each segment. At each outcrop the distribution of granite (*G*) and country rock (*M*) was measured by steel tape along four 10-foot lines with random starts. Each 10-foot measurement is considered one sample. Two random specimens of country rock and one of granite were collected at each line for composition determination.

Large tabular bodies of granite were of different appearance from the small irregular masses of granite which make up the migmatite proper. Consequently, all such bodies greater than 12 inches thick were excluded as probably representing a separate population or cycle of emplacement. This treatment seems justified. Records were kept of the size and locations of these larger bodies; when these measurements are included in the calculations of composition, the distribution becomes apparently nonparametric.