mutants, skin color was unexpectedly found to be under the control of the crest derivatives. The inheritance of skin color is still unclear but, judging from the wide spectrum of hues and intensities, the genetic mechanism is probably complex.

Figures 1–6 illustrate representative effects observed. Figure 1 illustrates the two mutant types, burnsi (left) and kandiyohi (right), joined in gill parabiosis. Figure 2 is a flank parabiosis of a normal (right) and burnsi (left), in which the right-hand neural crests were exchanged. Note the appearance of normal spots on the right side of the burnsi animal and their absence in the normal. In this case considerable lateral mixing probably occurred in the flank regions supplying the hind legs. Not clear from the photo is a pronounced color difference. The burnsi is brown while the normal is green except in the region where the spotting is reversed. Here the burnsi is green while the normal is brown. The color differences blend laterally but not longitudinally with the host colors, again indicating lateral mixing following closure of the neural folds. Not evident in dorsal view, the two inner forelegs are spotted, the outer spotless. The remaining pictures are left, or host (*A* in Figs. 3–6), and right, or donor (*B* in Figs. 3–6), views of the same animal which has received right flank crest from the genotype indicated. Figure 3 shows a normal host which received crest and blood island from a kandiyohi donor. Notice the kandiyohi mottlings are limited largely to the right or donor side. Figure 4 shows the reciprocal animal, a kandiyohi frog which received crest and blood island from the normal in Fig. 3. Compare especially the flank and forelimbs. Figure 5 is a normal host which received right crest and blood island from a double mutant donor (kandiyohi-burnsi). In this case very little mixing occurred as the effect is largely unilateral. Figure 6 shows a kandiyohi gill parabiont which had received double mutant right crest. The animals spontaneously separated after metamorphosis. The effect here is largely limited to the hind limbs, which are spotted in the host view and spotless in the donor view. The reciprocal animal, not pictured, shows the reciprocal effects. Notice in the left view the absence of a well-defined tympanic membrane. This is characteristic of the separated gill parabionts and is the only visible defect marking the region of original attachment.

I conclude from these observations that the burnsi and kandiyohi loci, as well as those genes responsible for color differences, all act through modifications in the pigment-forming elements themselves, rather than in the skin substrate through which these cells aggregate and differentiate to determine the adult phenotype (*8*).

JOHN DAVISON
Department of Zoology, Louisiana State University, Baton Rouge 3

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Sex Chromatin Patterns and the Lyon Hypothesis

Abstract. *Polyploid human cells in culture often have paired sex chromatin masses. They are neither chance juxtapositions of randomly distributed X chromosomes nor manifestations of a propensity for pairing. The pairs are characteristic of the first polyploid interphase. They are thought to reflect the persistent differentiation of X chromosomes, as proposed in the Lyon hypothesis.*

Many investigators have noticed that (presumably) tetraploid cells arising from diploid, diplo-X human cells in culture have two sex chromatin masses (SCM's) instead of the single one found in the diploid state. Some published photographs of such cells (*1*) have the feature in common of depicting the two SCM's close together. These pairwise arrangements are found whether the SCM's are on the lateral margin of the flattened nuclei or not. If one considers, for the sake of simplicity, only those nuclei in which both SCM's are on the lateral margin, the nonrandom nature of the paired arrangements can easily be demonstrated. Each such nucleus can be characterized by a ratio relating the shorter segment of the perimeter separating the two SCM's (= *D*) to the total perimeter (= *P*). If the SCM's are distributed about the perimeter at random with regard to one another, then all values of *D/P* are equally likely. I determined *D/P* as follows: cultures of diploid, diplo-X human cells derived from the skin were grown on cover slips (*2*). The cells were fixed in absolute ethanol (3 parts) and glacial acetic acid (1 part), treated with 1*N* HCl (60°C for 11 to 12 minutes), and stained with the Feulgen reaction. The cover slips were scanned systematically and nuclei having two peripheral SCM's in reasonably sharp, simultaneous focus were photographed as encountered. The negative images were projected and traced. From the tracings, *D* and *P* were determined with a map measurer.

The frequency distribution of *D/P* is shown in Fig. 1 (solid bars). It is not a random distribution ($\chi^2 = 180$; $P \ll .001$) but exhibits a marked predominance of nuclei with closely associated SCM's. The same sort of analysis was made with the nuclei of cultured triplo-X cells (*3*), which also have two SCM's but in the diploid state. In this case, the frequency distribution of *D/P* (Fig. 1, dotted bars) does not significantly differ from randomness ($\chi^2 = 4.40$; $0.80 < P < 0.90$). It shows that there is no *general* tendency for two SCM's of the same nucleus to be associated and that pairwise arrangements

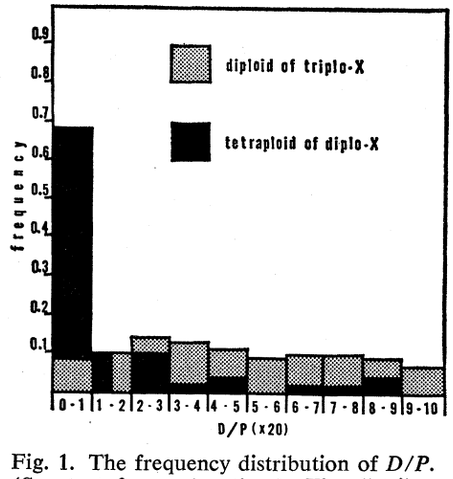

Fig. 1. The frequency distribution of *D/P*. (See text for explanation.) The distributions are based on 100 diploid nuclei of triplo-X origin and 50 tetraploid nuclei of diplo-X origin.

are a special feature of certain tetraploid nuclei. This conclusion was most vividly bolstered subsequently by the observation of mutiple pairs of SCM's in tetraploid nuclei of triplo-X origin and in nuclei of still higher ploidy (Fig. 2). The ploidy numbers indicated are based on the assumption that the nuclei are in the G2 (post-replication) stage and on measurements of the amount of material stained with the Feulgen reaction for all nuclei except number 5, which is presumed to be 16-ploid.

The numerical relations between the number of X chromosomes (x), the ploidy of the nucleus (p) and the number of SCM's (B) displayed in Fig. 2 are in accord with the general relation proposed by Harnden (*4*): $B = x - (p/2)$.

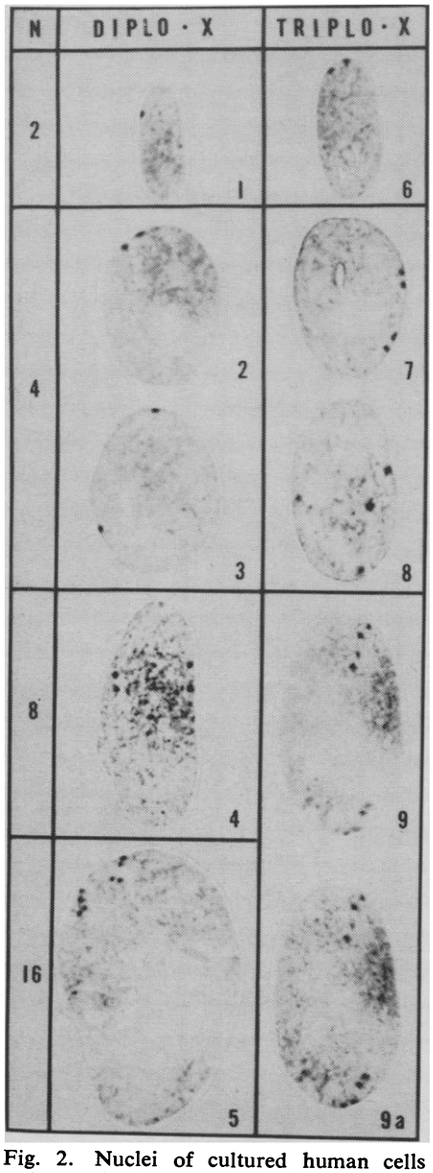

Fig. 2. Nuclei of cultured human cells stained with the Feulgen reaction. The cells were of diplo-X or triplo-X origin. *N* indicates the presumed state of ploidy and, excepting nucleus 5, is based on determinations of Feulgen-stain intensity made with a microdensitometer. Nuclei 9 and 9a are two focal levels of the same nucleus. (About ×900)

A reasonable explanation of this rule, and of the pairwise associations of SCM's in polyploid nuclei, can be based on the persistent differentiation of homologous X chromosomes in mammalian, somatic cells, as proposed by Lyon (*5*). In terms of sex chromatin formation, the proposal asserts that the sister chromatids of chromosomes that form SCM's also form SCM's in successive interphases. One might expect sister chromatids to lie close together during the first interphase following their origin but preceding their separation by mitosis, leading to the appearance of paired SCM's. One might also expect that the pairs would tend to disperse during cell divisions following that interphase. Observations up to now agree with these expectations. When a few individuals cells from cultures of either diplo-X or triplo-X origin are allowed to proliferate in feeder layers of XO or XY cells (lacking SCM's) they form clones, which can be located by scanning with the microscope after they are stained. Occasional small clones of tetraploid cells are found and the SCM's in these cells are not obviously paired (nuclei 3 and 8 of Fig. 2). When a single tetraploid is found in a clone of diploid cells, the SCM's are paired, indicating that such arrangements are indeed characteristic of the first polyploid interphase. However, the relation, as replicas, of SCM's found in sister cells may occasionally be discerned even in subsequent tetraploid interphases. Figure 3 illustrates sister tetraploid nuclei, of triplo-X origin, in a feeder layer of diploid, XO cells. Each nucleus had four distinct SCM's. No other tetraploids of at least 100 examined had the same characteristic disposition of SCM's. The chance of finding, at random, two such nuclei side by side is so small that another explanation seems reasonable: the spatial relations of sister chromatids were not greatly disturbed during and following mitosis and the sister chromatids of the chromosomes that formed SCM's in the parent cell did so again after their distribution into both daughter cells.

Not all modes of polyploid formation would be expected to result in the appearance of paired SCM's. Without going into the details in this report, it seems that the formation of diplochromosomes by two rounds of chromosome replication without an intervening mitosis could lead to the appearance of paired arrangements. Diplochromosomes are occasionally found in metaphase configurations of cultured human cells. However, it might be questioned that the members of the pairs would be as distinctly separated as in nuclei 2, 7, and 8 of Fig. 2 unless the diplochromosomes fell apart during interphase, forming two separate chromosomes that then remained close together. The main point is that, without specifying the mode of polyploid formation, it is most likely that the members of each pair of SCM's are related as replicas. This, and the appearance of nuclei such as those depicted in Fig. 3, add to the accumulating evidence that the functional differentiation of X chromosomes in human somatic cells is indeed persistent (*6*).

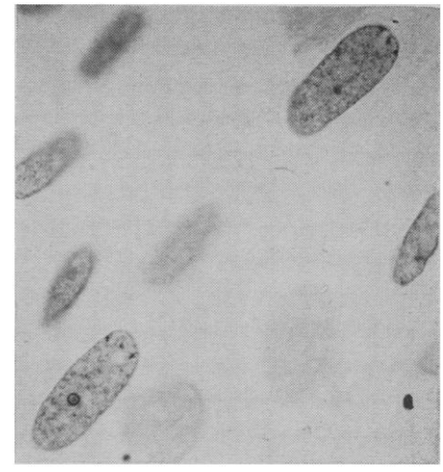

Fig. 3. Sister, tetraploid nuclei of triplo-X origin in a feeder layer of diploid, XO cells. The nuclei were stained with the Feulgen reaction. The black circle indicates the location of a sex chromatin mass that was out of focus. (About ×535)

ROBERT DEMARS
Department of Medical Genetics, University of Wisconsin, Madison 6

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