inspection approximated that of background. Administration of thyrotrophic hormone greatly increased the number of colloid droplets and they, too, were clearly labeled (Fig. 1*B*). Quantitative estimations of grain counts were made by photographing every available field at a conveniently fixed magnification (× 4000), enlarging the photographs to 10,000 times, and superimposing on the enlargements a standard grid. The results are shown in Table 1. The number of grains per unit area for colloid droplets and follicular colloid is close to the same value in both groups and results in a ratio of colloid-droplet I^{125} activity to follicular colloid activity that differs insignificantly from unity.

These data show clearly that iodinated protein is present within the confines of the thyroid cell in the form of colloid droplets, and that its activity matches that of iodinated thyroglobulin (follicular colloid) under conditions of physiologic and exogenous stimulation by thyrotrophic hormone. It is not possible to account for the corresponding I^{125} activity in both follicular colloid and colloid droplets in these experiments by any mechanism of formation in which newly synthesized thyroglobulin would be used. We would expect newly formed thyroglobulin to be iodinated to a minimum degree under the conditions of this experiment and to result in a ratio of activities much less than unity. The simplest explanation of these findings is that colloid droplets are derived from follicular colloid in the normal thyroid gland and that follicular colloid is rapidly taken into the cell following exogenous hormonal stimulation. Considerable weight must therefore be given to the observations (*4*) suggesting that fragments of follicular colloid are engulfed by cytoplasmic extensions of the thyroid cell and drawn into the interior. Proteolysis of thyroglobulin and release of thyroxine then may ensue.

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Genetic Activity in a Heterochromatic Chromosome Segment of the Tomato

Abstract. *The first example of genetic activity within a heterochromatic region of the tomato is provided by the delimitation of* nv *to the long arm of chromosome 9 by means of the induced deficiency method. A close spatial relationship between* nv *and* ah *was established by deficiencies for* ah *in the same arm and by linkage tests between the two genes.*

As part of our research on the genome of the tomato (*Lycopersicon esculentum*), we have attempted to find suitable genetic markers for chromosome 9 and to identify these with cytological landmarks. By means of the trisomic ratio method, *ah* and *wd* were related to this chromosome (*1*). Standard F_2 tests revealed tight linkages (<3 morgans) between the following pairs of genes: *ah-wd, ah-Tm*$_2$, *nv-Tm*$_2$, *ah-pum,* and *ah-nv*. In view of our interest in the last interval, the following pertinent linkage data are presented. Ten recombinants were yielded in a total of 46,577 F_2 segregants of the trans cross (24,793 + +, 9994 + *nv*, 11,780 *ah* +, 10 *ah nv*), corresponding to a crossover value of 3.2 ± 0.6 morgans. The most critical F_3 tests were obtained from the *ah*/*ah* F_2 segregants: eight proven heterozygous for *nv* and 284 homozygous for the normal allele. From the equation

$$\frac{2p}{1-p} = \frac{8 \times 100}{284}$$

1.4 is estimated for the *ah nv* distance.

More definitive information concerning these loci was then sought by means of the induced deficiency method (*2*). For this purpose individuals homozygous for *ms*$_{17}$, *ah, yv,* and *dl* were crossed with var. Red Cherry, which possesses the normal, dominant alleles for these loci. Mature pollen of the Red Cherry was treated with 5000 r of x-rays (generated at 90 kv, filtered with ½ mm aluminum, and delivered at an intensity of 300 r/min). Emasculated, fertile *nv*/*nv* plants were hybridized with irradiated pollen bearing the normal allele in order to produce *nv* deficiencies. A total of 1079 progeny of the former cross included two that were mutant in phenotype only for *ah* (anthocyanin deficiency) and two, subsequently demonstrated to be haploid, that were mutant for all four marker genes. Of the two *ah* mutants, one showed no cytological abnormality, and the other, 63L744-1, had an interstitial deficiency of the long arm of chromosome 9 (9L). A second interstitial deficiency for the *ah* region in 9L, 63L-1850-1, was effected in the progeny of another cross sired by irradiated pollen. Both deficiencies embraced heterochromatic as well as euchromatic regions (Fig. 1) and delimit the *ah* locus to a region including the distal four knobs of the heterochromatic zone and the proximal fourth of the euchromatic.

Of special interest are the cytological features of an *nv* deficiency, 63L1878-1, yielded by *nv* hybridization. Three plants in a total progeny of 1734 showed the *nv* phenotype (patterned chlorophyll deficiency): one haploid, another with no detectable cytological aberration, and the third—a plant with vigor and phenotype comparable with that of nondeficient *nv*—, the aforementioned deficiency heterozygote. The deficiency in this last individual also proved to be interstitial, but with the deleted portion limited entirely to the heterochromatic zone of 9L. The consistent appearance in more than a score of cells of good preparations left no doubt that the treated chromosome of 63L1878-1 is clearly deficient for most of the internal heterochromatin of 9L, but still retains the proximal knob, the two distal ones, and the large euchromatic gap, which is so diagnostic for this chromosome (Figs. 2–4). Pairing is disrupted solely for the deficient region in Fig. 4 and for an additional distal region in Fig. 2, while heterochromatic knobs seem to be nonhomologously associated within the loop in Fig. 3. A somewhat shorter interstitial deficiency for 9L was previously reported (*3*), but it was not related genetically to the corresponding linkage map. The extent of each of the three deficiencies reported here is indicated diagramatically in Fig. 6.

The gene *nv* is the first marker in

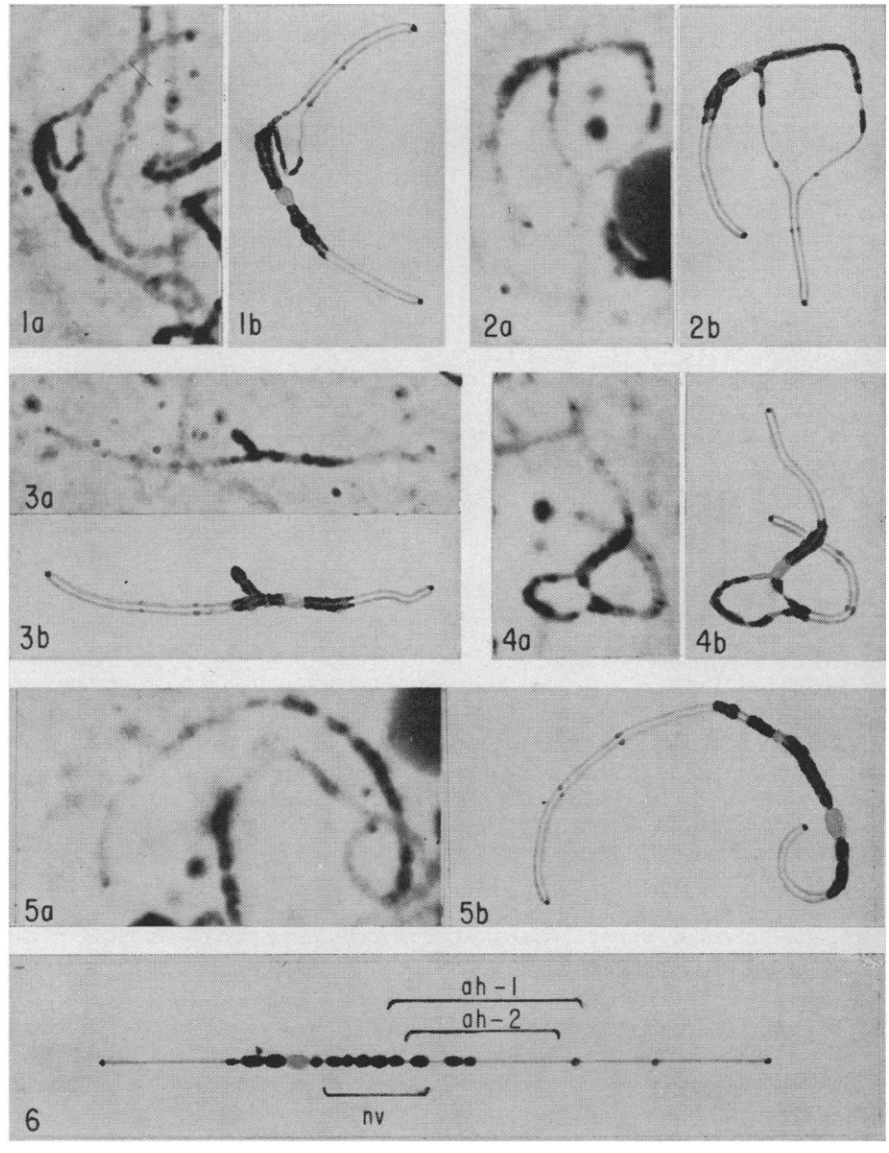
our experience with the tomato to be located unequivocally within a heterochromatic region. To date we have analyzed a total of 44 deficiencies for 15 genes by the induced deficiency method. Of these genes, 12 have been localized to euchromatin, and for two the data do not discriminate between the two kinds of chromatin (as in the case of the two *ah* deficiencies reported here). For the following considerations, nevertheless, genes of the indeterminate group are probably also euchromatic. It can be safely assumed from the well-established fact *(3–5)* of vastly greater breakage in heterochromatin than in euchromatin of the tomato that interstitial deficiencies would result more frequently from paired breaks within heterochromatin than any others. Also, since heterochromatic losses generally affect viability less, their recovery would be favored over that of other kinds of interstitial deficiencies. It therefore appears unlikely that any genes so far tested and residing in heterochromatin would not have been detected by intraheterochromatic losses; consequently genes that we have detected by deficiencies of both kinds of chromatin probably have their loci in euchromatin.

Heterochromatic regions of tomato chromosomes have hitherto been considered void of active macro genes (*5*). Maize, the only other extensively investigated plant species, cannot be compared because heterochromatin does not appear to be localized on its chromosomes. The evidence in animal species points to little or no genetic activity in heterochromatin. The few genes—*bb, bt, ci, in, lt, ltd, p, stw*—in the heterochromatin of *Drosophila melanogaster* contrast with the hundreds of genes known for euchromatin of that species (*6*).

Although our evidence reveals that *nv* exists in a heterochromatic zone, it does not prove that the locus itself is heterochromatic. Such zones of the tomato consist of dark-staining knobs interspersed with faint-staining portions detected either visibly or by differential contraction rates (*7*). The long arm of chromosome 9 is no exception, for at least two such regions are consistently seen there (Figs. 1–5). Cytological factual proof of the delimitation of any gene to heterochromatin would be difficult for the tomato or any other organism.

Attention should be called to the lack of agreement concerning the identification of heterochromatin in the tomato. Although the majority (*4, 5, 8*) consider the dark-staining regions of the tomato pachytene chromosomes to be heterochromatic, others (*7, 9*) disagree, maintaining correctly that they differ in their allocyclic staining reactions from heterochromatin according to Heitz' original concept (*10*). But a broader concept of heterochromatin has become accepted among plant cytologists and this concept includes the proximal regions of deeper staining capacity in many angiosperm species (*11*). We are convinced that these regions are equivalent to heterochromatin in a strict sense in animal species because: (i) they tend to be centric, (ii) they are subject to much lower crossover rates, (iii) they differ markedly from other chromatin in their exceedingly different staining capacity and in sensitivity to breakage by x-ray, and (iv) the same region may differ in size without affecting phenotype or viability—for example, the remarkably varied configurations of the short arm of chromosome 2 (*12*).

The delimitation of *nv* to the heterochromatic zone of 9L and of *ah* to its proximity suggests an explanation



Figs. 1–5. Tomato pachytene chromosomes. In each pair of figures *a* is a photomicrograph and *b* is an interpretive drawing. The large, internal, faint-staining body of each bivalent is the centromere, × 2000. Figs. 1–4. Interstitial deficiencies of chromosome 9: Fig. 1, 63L744-1, deficiency for *ah*; Figs. 2–4, 63L1878-1, deficiency for *nv*. Pairing is disrupted solely for the deficient region in Fig. 4 and for an additional, distal region in Fig. 2; heterochromatic parts seem to be nonhomologously associated in Fig. 3. Fig. 5, normal chromosome 9. Fig. 6, drawing of chromosome 9, indicating the extent of three deficiencies, 63L1850-1 being represented by *ah*-1, 63L744-1 by *ah*-2, and 63L1878-1 by *nv*.

for the unusual circumstance that the only genes thus far located in this region—*ah, nv, pum, Tm*$_2$, and *wd*—are all tightly linked. The low crossover rate generally characteristic of heterochromatin is probably responsible.

Our study constitutes another example of the unique advantages offered by the tomato for relating chromosome structure with genetic activity.

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Irreparable Mutations and Ethionine Resistance in Neurospora

Abstract. *Some ethionine-resistant mutants of* Neurospora crassa *are temperature-sensitive, in that they fail to grow in the upper temperature range at which wild type* Neurospora *grow best. Two of these mutants have lost an indispensable function since at elevated temperatures they are unable to grow on a variety of complex media.*

Horowitz and Leupold (*1*) examined the frequency of microbial mutants which have lost an indispensable function—one that cannot be circumvented by furnishing the organism in question with any given nutrient or combination of nutrients. Since such mutations are by definition lethal, it might be thought that such strains would defy isolation and investigation. Horowitz first pointed out that conditional lethals, which apparently grow normally at low temperatures, but which fail to grow on either simple or complex media at higher temperatures, provide an opportunity to estimate the frequency of such mutants. Since that time, a number of ingenious studies of irreparable mutants have been conducted (*2*). For obvious reasons, however, little is known of the biochemical lesion associated with any given temperature-conditional lethal mutant.

In the present study, wild type, ethionine-sensitive conidia of *Neurospora crassa* (mating type *a* derived from Em 5297*a*) were suspended in water and irradiated with a germicidal lamp until about 95 percent were killed; then portions were plated on agar containing Fries' minimal salts (*3*), sucrose (1.5 percent), and L-ethionine (50 μg/ml). The plates were incubated at 24°C until centers of ethionine-resistant growth appeared. To assure the independent origin of the various strains, we picked only one growth center from each plate. To assure homokaryosis of the isolates, we transferred them to the crossing medium of Westergaard and Mitchell (*4*) supplemented with ethionine as already described. After 6 days' incubation at 24°C, the resulting protoperithecia were fertilized with wild type conidia of strain STA-4*A*. We then plated, at low density, random ascospores from each cross onto Fries-sucrose-ethionine agar and induced the spores to germinate by the usual heat-shock treatment. After 12 hours' incubation at 24°C, we isolated ethionine-resistant sporelings. Of 18 original isolates, 16 passed through such a cross successfully and were deemed to be true mutants.

Linear growth rates were measured by the method of Ryan *et al.* (*5*), with comparisons being made between growth on solid Fries' minimal medium containing 1.5 percent sucrose and 1.5 percent agar, with or without L-ethionine (50 μg/ml). All strains were tested on both media at 25° and 38°C. The parental wild type strain grew 20 to 30 percent more rapidly at 38° than at 25°C. Strain STA-4*A* showed similar behavior. In the 16 mutants, the degree of resistance to ethionine at 24°C, expressed as the ratio of growth rate with ethionine to the growth rate without ethionine, ranged from 0.12 to 0.99, with an average value of 0.68. The parental strains had a value of less than 0.02 at both temperatures.

Of the 16 mutants, eight resembled wild type in growing more rapidly at 38° than at 25°C on minimal medium; one grew at an identical rate at both temperatures, and seven grew more slowly or not at all at 38°C. One of the latter, henceforth referred to as *r-eth-1*, was chosen for further study on the basis of its high temperature sensitivity, relatively high ethionine resistance (0.82), good fertility in crosses, and abundant conidiation. The steady-state growth rates of wild type and *r-eth-1* on Fries-sucrose medium at several temperatures are shown in Table 1. It will be noted that the growth rate of the mutant drops sharply between 35.0° and 36.5°C, suggesting that a macromolecule with a large Arrhenius energy of inactivation is being denatured. The failure of *r-eth-1* to grow at elevated temperature was not relieved to any measurable degree by supplementation of Fries' minimal medium with methionine or ethionine at a variety of concentrations, nor by supplementation with the following complex nutrients at a concentration of 2 percent: Difco Bacto-peptone, Bacto-tryptone, yeast extract, malt extract, nutrient broth, and acid-hydrolyzed casein. None of the complex media inhibited growth of the mutant at 24°C or of wild type at either temperature.

Table 1. Growth rates of wild type and *r-eth-1* on Fries' minimal medium at various temperatures.

Strain	Growth rate (mm/hr)			
	24.0°C	35.0°C	36.5°C	38.0°C
Wild type	3.4	5.0	5.1	4.5
r-eth-1	3.0	2.4	0.0	0.0

In order to test the possibility that *r-eth-1* contained separate mutations for ethionine resistance and temperature sensitivity, which were only fortuitously related, we outcrossed the strain to wild type (*r-eth-1*a × STA-4-*A*). Of a total of 248 cultures from random spores, 112 grew at 38°C when tested on spot plates, and 136 failed to grow. This ratio is not significantly different from the expected one-to-one segregation. Of these cultures, we tested 180 for resistance to ethionine. All the temperature-sensitive cultures were ethionine-resistant, and vice versa. As a more sensitive test for segregation, larger numbers of random spores were germinated on ethionine plates, which were then incubated at 38°C. Among 1681 germinated spores examined, we found no ethionine-resistant, temperature-permissive segregant. This, coupled with the independent isolation of a