SOME CYTOGENETIC FEATURES OF THE GENOME IN DIPLOID PLANT SPECIES*

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For the honor of participating in this Symposium I am deeply grateful, particularly since I am celebrating the 30-year anniversary of my first visit here, which was a priceless opportunity to visit with LEWIS STADLER, to witness his enthusiasm for mutation research, and to try to absorb some of his erudition.

Diploid plant species were selected as my topic for several reasons. Firstly, the genomes of these species are the basic units of plant evolution. Secondly, by definition, only their genomes permit extensive cytogenetic analysis. Finally, I am more at home with them; it would be presumptuous of me to wander into the complexities of polyploidy.

We shall be concerned here principally with features of the cytological and linkage maps and with attempts to understand them in relation to their differences, similarities, and origins. For the overdose of tomato (Lycopersicon esculentum), to which I shall subject you, I offer no apologies; nevertheless, reference will be made to other pertinent species and even to the realms of Drosophila and Neurospora research.

The qualifications for such comparisons are met by rather few plant species. It is essential that the linkage maps be reasonably well populated, that the distribution of euchromatin vs. heterochromatin be known, that a reasonable number of genetic loci be positioned on the cytological map, and, reciprocally, that the location of key cytological landmarks be approximated on the linkage map. At the present time only tomato, maize (Zea mays), and, to a limited extent, barley (Hordeum vulgare) qualify. I have inquired into the current status of Antirrhinum, Arabidopsis, Pisum, Datura, and other genera, but have been disappointed in the lack of critical information, especially in the relations between cytological and linkage maps. Fortunately the aforementioned three species represent widely divergent patterns of chromosome organization--strong regional differentiation of euchromatin vs. heterochromatin in tomato, lack of such differentiation in barley, and an intermediate condition in maize. The tomato pattern of well-defined blocks of heterochromatin flanking the centromere in each chromosome arm exists in many plant genera (RICK and KHUSH 1969). It may, in fact, be the most widespread condition in angiosperms.

The intragenomic spatial relations that form the substance of this paper are ascertained by standard cytogenetic techniques, which can be exemplified by our research on the tomato (summarized by RICK and KHUSH 1969). Primary trisomics aided in the early stages by assigning genes to their respective chromosomes and were particularly useful in finally achieving markers for the poorly endowed chromosome 12. Secondary, tertiary, telo- and compensating trisomics, whose

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extra chromosomes are modified mainly by breakage and reunion at the centromere, offer the added advantage of assigning loci to their respective chromosome arms. The double-iso compensating trisomic is unique in permitting simultaneous identification of the chromosome as well as of the respective arm. The most precise positioning has been accomplished by radiation-induced deficiencies. Various combinations of these tests enabled us to restrict the site of the centromere to a relatively small portion of the linkage maps. With this informational framework, further mapping has been achieved most efficiently by straightforward linkage analysis. For this purpose we have exploited strategically situated markers on each arm of the complement, assembling in tester stocks pairs of such markers for each chromosome. The first progeny tests with such marker stocks are, consequently, three-point tests; the first assay thereby not only detects linkage, but usually approximates the site of the locus. For regions that we suspect our markers do not screen effectively (1S, distal 4L, 12L), we resort to tests with the appropriate trisomics. The effectiveness of such procedures for screening the genome is revealed by our results in locating a series of X-ray-induced mutations produced by STUBBE (1957, 1958, 1959, 1963, 1964): of 115 mutants whose linkage relations were sought, 94 have been located, 15 are tentatively located, and only 6 have not been located.

The status of the tomato genome in respect to the aspects being considered here was first evaluated in 1959 (RICK 1959), but conclusions were severely restricted by the lack of critical data at that time. I shall try here to reevaluate the genome in the light of the accumulated information and to make relevant comparisons with other eukaryotes.

DISTRIBUTION OF GENES AMONG CHROMOSOMES

TOMATO. The most recently revised linkage map of the tomato (Fig. 1) shows the approximate distribution of 233 genes. Since 168 (72%) of them were situated by our group at Davis, we are familiar with their idiosyncrasies and details of their localization. This summary includes mutant genes of both spontaneous and radiation (almost entirely X-ray) induced origin but does not include multiple alleles at known loci.

The best available frame of reference for comparison of the number of loci per chromosome is the length of the euchromatic portion of the respective chromosome at pachytene. Heterochromatin is disregarded because, as in other genetically well investigated diploids, it appears to be inactive in respect to a near absence of marker genes, reduced recombination, and negligible effect on viability. When such a comparison is made (Table 1) and tested by goodness-of-fit, the corresponding χ^2 (35.61, 11 d.f.) is highly significant. The distributions (Fig. 2) reveal that the largest share of the deviation from random distribution is contributed by the high observed number on chromosome 11 and low numbers for chromosomes 3 and 12. When loci of spontaneous mutations are compared, the differences from expected values are also significant; for radiation-induced mutations, the fit is reasonably good (Table 1); nevertheless, the observed values for these three chromosomes deviate in the same direction as they do in the spontaneous group.

A comparison between chromosomes 11 and 12 merits attention because they are remarkably similar in respect to (1) cytological length, (2) such other cytological details as localization of heterochromatin and freedom from knobs in the euchromatin, and (3) degree of essentiality to viability. In regard to the last point, the tomato sporophyte can endure losses of either 11 or 12 (but of no other chromosome) in the simple monosomic condition. Neither

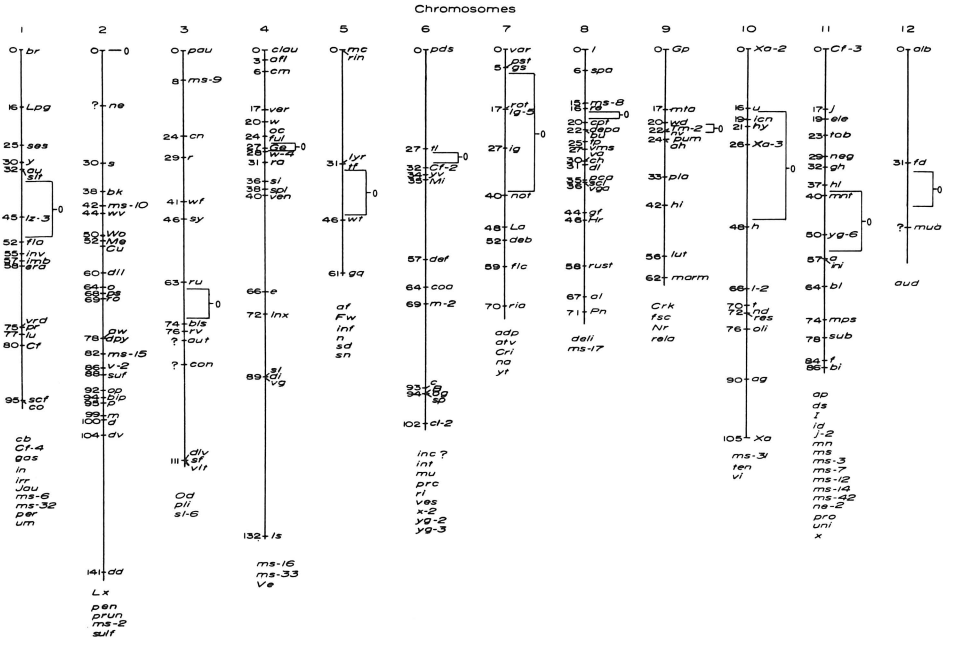

Figure 1. Revised linkage map of the tomato based on the summary in Report of the Tomato Genetics Cooperative No. 18 (1968) and additional information in subsequent TGC Reports.

Table 1. Distribution of mapped genes of the tomato: comparison with expected values.

	Chromosome												Total	X^2 obs. vs. exp.
	1	2	3	4	5	6	7	8	9	10	11	12		
Proportion of euchromatic length †	.149	.123	.120	.095	.068	.096	.067	.072	.062	.049	.058	.041		
Total genes††	27	29	17	22	12	22	15	22	15	16	32	4	233	35.61**
Spontaneous mutations	12	21	9	12	8	17	7	15	8	13	22	2	142	38.23**
Induced mutations	17	11	9	11	5	10	10	10	8	4	11	2	108	7.14

† Chromosome lengths at pachytene stage according to Barton (1950).

†† Values do not all equate to sums of spontaneous and induced mutations because both kinds of alleles are known for certain (17) loci.

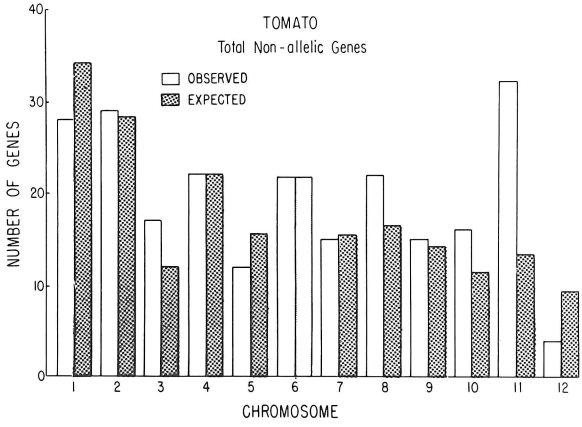

Figure 2. Frequency distributions of nonallelic genes among the tomato chromosomes. Expected numbers based on proportional length of euchromatin in the pachytene chromosomes according to BARTON (1950).

deficiency is transmissible because it is not tolerated by the gametophytes. Thus, no cytological information can account for the large discrepancy between the present share of marker genes on chromosomes 11 and 12. The techniques used for linkage detection have not favored chromosome 11; in fact, our extensive use of triplo-12 in screening new mutants excludes bias in favor of chromosome 11.

MAIZE. The same comparisons are made with maize, for it is the only other higher plant in which the chromosomes are sufficiently mapped and the pachytene lengths known. My unfamiliarity with the history of linkage studies in this species limits the extent of comparisons made, but I believe the following points are valid. The summary in Fig. 3 was derived from NEUFFER et al. (1968). Since the proportion of detectable heterochromatin in maize is negligible, expected values are based on the proportion of total pachytene lengths. For the 214 mapped genes, the distribution differs significantly from expected (χ^2 = 24.021, 9 d.f.). Two chromosomes--no. 8 and 10--contribute most of the discrepancy, the former being deficient and the latter overloaded with loci. Since the summary by EMERSON et al. (1936) reported two markers for 8 and three for 10, neither ostensibly enjoyed much historical advantage in acquiring new linkages. The presence of the useful and intensively studied R locus on 10 might account for its excess of markers, and certain inadequacies of markers for the paucity on 8.

DISTRIBUTION OF GENES WITHIN CHROMOSOMES

TOMATO. The tomato map (Fig. 1) reveals several interesting intrachromosomal distributional features. First, a tendency is observed for genes to concentrate in closely congregated groups; secondly, a regional pattern of the clustering can be detected for most of the complement.

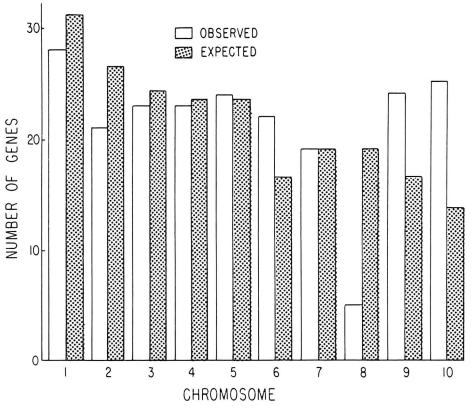

Figure 3. Frequency distributions of nonallelic genes among the maize chromosomes. Expected numbers based on proportional length of the pachytene chromosomes according to NEUFFER et al. (1968).

Clustering is evident in the following examples of the better populated chromosomes. Thus, in chromosome 4, 53% (10/19) of the located genes are restricted to 17% (23/132 units, in the proximal region) of the map length; in chromosome 8, 50% (10/20) are similarly limited to 23% of the map (16/71 units). A curious relationship is observed in loci of several spontaneous dominant mutants on chromosome 2. Of the 233 genes now assigned to chromosomes, only 14 are spontaneous dominants (omitting interspecific derivatives); of these 14, 4 are situated on chromosome 2; only six have been mapped, and of these, three are on chromosome 2; furthermore, the three--Cu, Me, and Wo--each with completely different phenotypes, are located within a few crossover units of each other. Although this relationship has been known since 1959, it has not been diluted by the threefold increase in genes meanwhile mapped.

An exact mathematical treatment of the clustering tendency does not seem to be warranted now for reasons vested in the technique of linkage detection. Most of the associations represented in Fig. 1 were first sensed by standard F_2 tests of new mutants against standard markers, both recessive in the great majority of combinations. Now, for closely linked genes, this test is highly efficient in detecting linkage, but its efficacy for measuring linkage intensity is proportional to the square of the distance. Thus, as the distance approaches zero, the chances for union of two crossover gametes become remote and the size of the population required to recover at least one such product, impracticably large. For such tests in our laboratory, usually no more than 2,000 individuals are grown, corresponding approximately to a fiducial limit of 10 crossover units as the maximum distance between two genes that appear to be completely linked, although the majority of such intervals will prove to be smaller. On the basis of these considerations, the frequently used testers should tend to acquire clusters of newly placed genes at the same loci. Such a tendency is indeed observed for the testers a, ah, ful, and tf. On the other hand, inseparables have not yet been found in tests with the equally used markers d, e, hl, and yv. Furthermore, intensive studies at a few loci have recovered crossovers and thereby

have revealed that such clusters as those surrounding c and dl are real and not artifacts of the detection system. For distances of 10 to 40 units the F_2 method is reliable for detection and useful for estimating distances, particularly if used in three-point tests. That several large "silent" regions, notably those in the vicinity of d, e, and h, have continued unmarked despite testing of many new genes lends additional support to the notion that the apparent non-randomness is real. A bona fide non-randomness has been revealed in Drosophila by ELSTON and GLASSMAN (1967).

The second aspect of intrachromosomal distribution relates to the patterning of non-randomness. A tendency toward clustering near the centromere in the tomato map is evident in most of the chromosomes. For pooling information between chromosomes to test this tendency, the distances from centromeres can be treated as real or proportional (adjusting all arm lengths to 100%). In order to compensate for their overloading effect, terminal markers were deleted from half the arms. Pooling the absolute values suffers the disadvantage that each arm has a different map length; on the other hand, while solving this problem, proportional distances in certain respects lack biological meaning. Both methods are employed here as appropriate to answer various questions.

The following procedure was adopted for absolute distances. Map distances from the centromere were plotted for the first 30 units, arms of shorter length being disregarded. Also omitted were those chromosomes for which the centromere is not reasonably well delimited, as well as chromosome 2 for reasons to be presented below. Thirteen of the 23 euchromatic arms meet these criteria, the remainder, except for 2L, have similar distribution patterns. The summation (Figure 4) reveals a proximal aggregation, as well as the

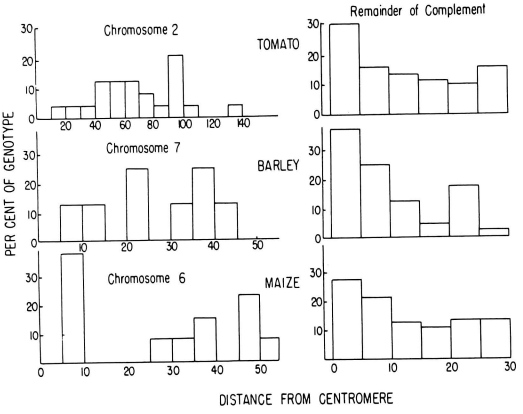

Figure 4. Comparison of gene distribution between nucleolar chromosomes vs. the remainder of the complement in tomato, barley, and maize. Distance from centromere in cross-over units. For distributions on the right side, frequencies were pooled for non-nucleolar chromosomes mapped to at least 30 units. The barley nucleolar chromosome no. 6 is omitted because only four of its genes have been mapped. Tomato data from text; barley data from NILAN (1964); maize data from NEUFFER et al. (1968).

opposite tendency in chromosome 2, whose centromere is subterminal and short arm bears the only nucleolar organizer of the complement.

COMPARISON WITH OTHER EUKARYOTES. Similar patterns are revealed in barley and maize (Figure 4). Barley possesses nucleolar organizers on chromosomes 6 and 7, but the linkage map is adequately populated only for the latter. The maize nucleolar chromosome, no. 6, shows an anomalous concentration (five genes) in the subproximal interval, but none have been mapped in the first, third, fourth, and fifth intervals; in respect to its having the bulk of its markers in the central or distal region, the nucleolar chromosome of maize resembles those of barley and tomato. In such small groups of mapped genes, sampling deviations might account for the observed aberrant distributions in one species, but hardly so in all three.

Distributions of Drosophila markers plotted along the linkage map were prepared from the recent summary by LINDSLEY and GRELL (1968) (Figure 5). Again, the clustering tendency is witnessed in

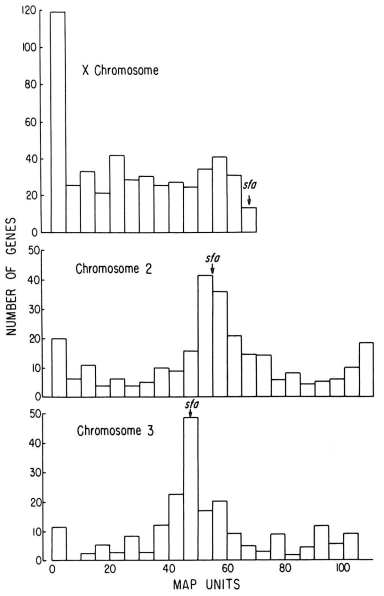

Figure 5. Frequency distributions of genes along chromosome map of Drosophila melanogaster. Data from LINDSLEY and GRELL (1968).

the proximal regions, but only in the autosomes, not in the X (nucleolar) chromosome. The distributions also tend to peak at the ends of the arms--a trend not yet evident in the plant maps.

Attention should also be called to the linkage map of Neurospora crassa, the most intensively mapped fungal species. According to the complete survey by ESSER and KUENEN (1967), most of the maps show a tendency toward denser gene populations near the centromeres. The trend is unmistakable in the most thoroughly investigated group I: over 60% of its loci are restricted to the centric third in the most

recently published map (PERKINS et al. 1969). This aggregation in group I might conceivably be an artifact of the linkage detection system, since the highly useful mating type locus is situated near this region; nevertheless, the aggregation occurs entirely to the left of this locus--that is, in the direction of the centromere. Exceptional to this pattern is group V, in which mutant loci are aggregated at the center of the long arm. Predictably, PHILLIPS (1967) and BARRY and PERKINS (1969) discovered that V belongs to the nucleolar chromosome, no. 2, the organizer and satellite situated on the short arm and all the known genes on the long arm. The consistency with which this pattern of gene distribution is found in all well-investigated plants and animals suggests that it might be universal among eukaryotes.

Thus, a hitherto unnoticed feature has been revealed for nucleolar chromosomes. It has been known for some time (HEITZ 1931, GATES 1939) that the nucleolus organizer is situated at a constriction proximal to a sattelite, usually on a short arm and frequently on the sex chromosome. To these unique features can now be added a recombination pattern in the long arm that differs from that of the other chromosomes.

When treated proportionately, the pooled tomato data show the same trend toward centric accumulation. The number of genes in the proximal fifth being 31, and for the next fifths the values are, consecutively, 23, 17, 12, and 15. The share of the genes in the proximal half, 71 vs. 36 in the distal half, deviates significantly from 1:1 ($X^2 = 10.8^{**}$).

RELATIONSHIPS WITH CYTOLOGICAL MAPS. Thus far our comparisons have been restricted to linkage maps. Relative distances on the cytological maps are pertinent, but it must be realized that the spatial relations of the pachytene or salivary maps are not necessarily those of the chromosomes at the time of crossing over; they only provide the best available approximations. The tomato maps provide few positions useful for this analysis; only in 8L can proximal and more distal regions be satisfactorily compared: bu has been positioned very close to the juncture between euchromatin and heterochromatin, dl rather accurately placed close and proximal to the only knob of that arm, and al in a subterminal section. Adopting midpoints for these localizations, the bu-dl interval comprises 34% of the bu-al cytological distance, whereas the linkage map distance of the former is only 13% of the latter. Thus the only comparison afforded by the tomato shows the cytological map to be disproportionately long in the proximal region for a typical metacentric chromosome. STRINGAM (1968) has determined spatial relations on the cytological map of the second (nucleolar) chromosome by means of translocation breakpoints. The linkage and cytological maps roughly agree; unfortunately, the absence of markers within 30 map units of the centromere prevents analysis of this proximal region.

Better data are available in maize: reference points have been provided for chromosomes 5, 6, and 7 by PHILLIPS' (1969) study of interchange breakpoints. The relationships in the metacentrics 5 and 7 agree with those of tomato chromosome 8. Thus, the bt-ae interval, proximal to the centromere on 5L represents 23% of the linkage distance from bt to yg, but occupies roughly 37% of the pachytene chromosome length of the same region. Likewise, the v_5-ra interval, including proximal portions of both 7S and 7L, represents 6% of the linkage distance from o_2 to bd, but cytologically covers 22% of that distance. For 6L, on the other hand, the first testable proximal interval y-pl is roughly equivalent in respect to both linkage and cytological lengths; the lack of markers close to the centromere precludes comparisons in that region.

Such non-linear relationships are familiar in the intensively studied Drosophila. According to Figure 6 prepared from LINDSLEY

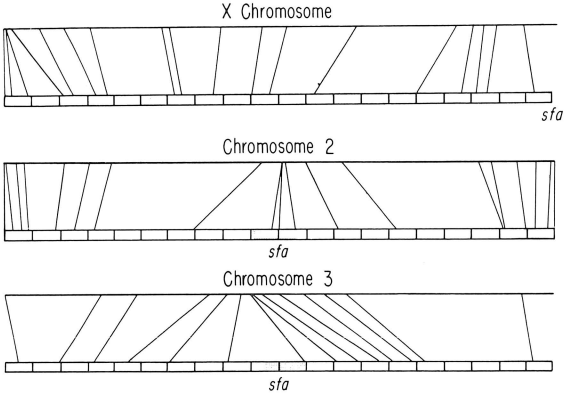

Figure 6. Comparisons between linkage maps (above) and salivary chromosome maps (below) in Drosophila melanogaster. Shaded portions represent heterochromatin. From LINDSLEY and GRELL (1968).

and GRELL (1968), regions near the centromere are disproportionately large in the autosomes, whilst they are nearly equivalent for the X chromosome.

DIFFERENTIAL RECOMBINATION. The most widely accepted interpretation of these non-linear relationships between linkage and cytological maps is differential rates of recombination, according to which a contracted condition and high gene density (as in certain proximal regions) would signify a low rate of recombination. BEADLE (1932) provided experimental evidence in Drosophila by demonstrating a greatly increased rate of recombination in a proximal segment after translocation to a more distal site.

The recombination hypothesis is also supported by limited observations on the position of chiasmata. Thus, although it may not be possible to identify all plant chromosomes in diplotene and diakinesis, the nucleolar chromosome, which is of particular interest for these comparisons, can nearly always be recognized. The tomato is not ideal for these purposes: diplotene is refactory and in diakinesis the euchromatin, to which all chiasmata are restricted, is too diffuse for accurate counting of chiasmata. In spite of these difficulties, the majority of figures shows interstitial chiasmata in chromosome 2 and terminal, single exchanges in most other arms of the complement (Figure 7b). In maize it is clear from the statistics and illustrations of DARLINGTON's (1934) study (Figure 7a) that interstitial chiasmata are usually seen in 6L, often in the vicinity of the centromere, whereas in other bivalents the proportion of terminal or subterminal chiasmata is higher.

Only in barley has it been possible to make a satisfactory cytological comparison. Although this species possesses two nucleolar chromosomes, they can be distinguished by the strength of their nucleolar organizers and size of satellites. Additionally, several plants in our material (var. Aravat) were heteromorphic for the size

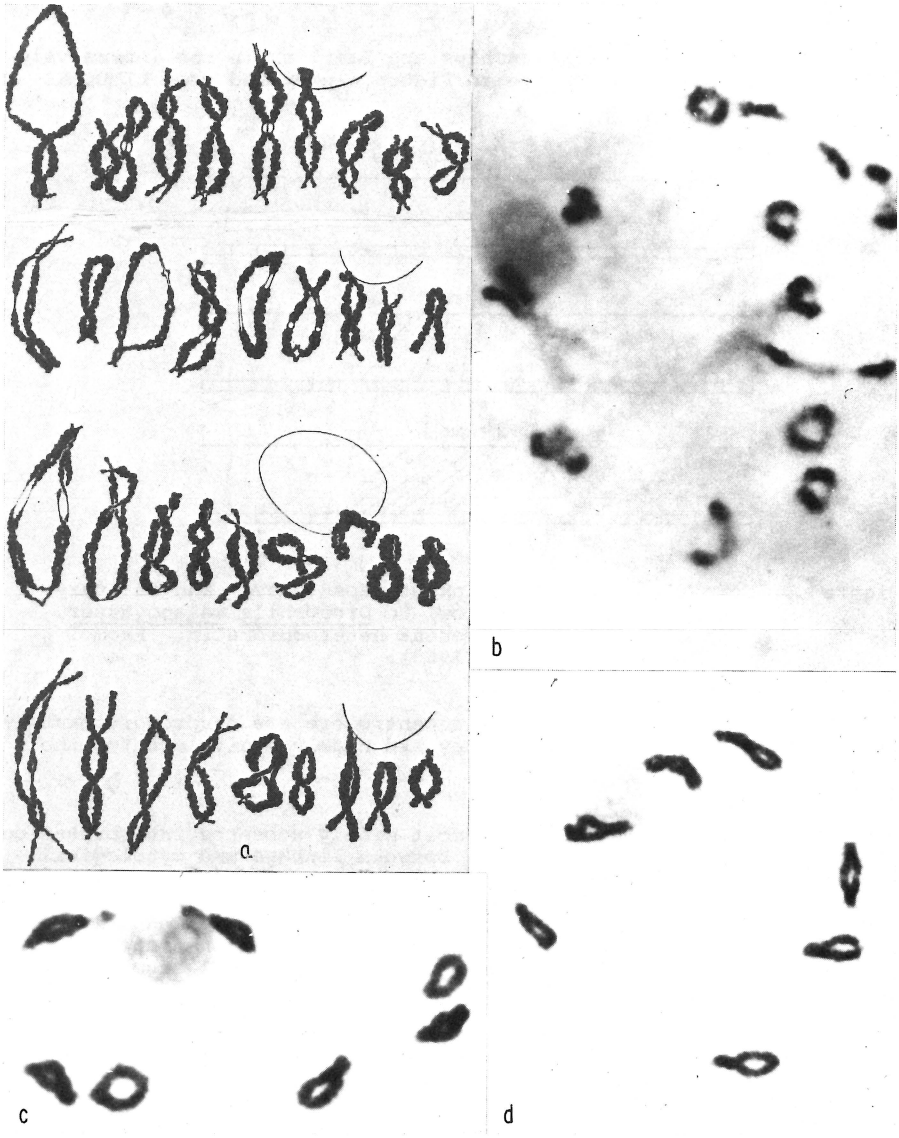

Figure 7. Meiotic chromosomes showing distribution of chiasmata. a. Successive stages in diplotene in maize (from DARLINGTON 1934, with the permission of the publisher); b. Diakinesis in tomato. Note the interstitial chiasma in the euchromatic (lighter) element of the nucleolar (second) chromosome; c. and d. Diakinesis in barley (x 1,000).

of satellite of the short arm of chromosome 6. Since only a few genes have been located on 6, we shall consider only the relationships between 7 and the remainder of the complement. At any rate, the distributions for chromosomes 6 and 7 are similar. The material was scored in diplotene and diakinesis, chiasmata being listed as either distal (terminal or subterminal) or proximal (median and nearcentric). In one sampling the proportion of proximal chiasmata

for chromosome 7 was 32% in diplotene and 30% in diakinesis, for the remainder, 8.7% in diplotene, and 4.5% in diakinesis; in another the comparable figures were for chromosome 7--53% in diplotene, 47% in diakinesis; remainder--8.3% in diplotene, 4.2% in diakinesis. All differences between 7 and the metacentrics are highly significant. Thus, within the limitations of such crude determinations, these cytological observations support the contention that recombination rates tend to be higher in proximal parts of nucleolar chromosomes. The fact that little change in either chiasmata numbers or position between diplotene and diakinesis suggests that terminalization might not be of great significance. Typical disposition of the bivalents is illustrated in Figure 7c; details will be published elsewhere.

That heterochromatin might conceivably play a role in suppressing recombination deserves consideration. The dearth of recombination in the proximal heterochromatin flanking the centromere in all arms of the Drosophila complement is well established. Similarly situated heterochromatin in the tomato is known to sustain little if any crossingover in chromosomes 4 (KHUSH and RICK 1967) and 9 (KHUSH and RICK 1968). The infiltration of small heterochromatic knobs into the adjacent euchromatin known in many arms might account for the reduced recombination there.

An influence of proximal heterochromatin itself on recombination in the adjacent euchromatic region might be considered. The varied pattern of heterochromatin distribution in maize chromosomes permits comparisons in that species. Arms lacking centric heterochromatin show a less marked accumulation of marker loci in the most proximal region than those possessing it (Figure 8). A 2:2 contingency

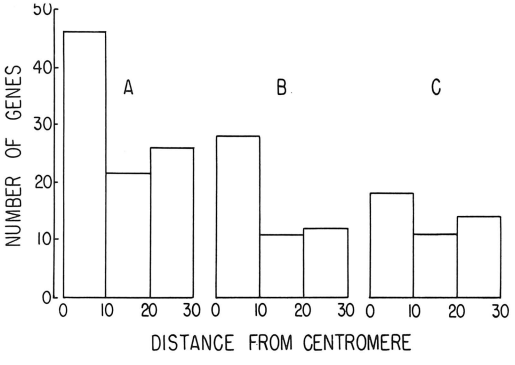

Figure 8. Density of genes in the proximal portion of maize chromosomes. Data pooled for all arms of at least 30 unit length excluding chromosome 6. A. all tested arms; B. arms with proximal heterochromatin; C. arms lacking proximal heterochromatin. Data from NEUFFER et al. (1968).

test on the two categories for loci in the first ten units vs. those in the 11-30 unit region yields a $\underline{X}^2$ of 5.23, which is significant, but only at the 5% level. On the other hand, the aforementioned consistent difference in pattern of recombination in nucleolar chromosomes argues against any universal effect of proximal heterochromatin.

Pertinent to these considerations are observations that proximal regions tend to be more sensitive to factors that modify

recombination rates. RHOADES and DEMPSEY (1966) thus report that the K10 chromosome in maize increases recombination in homomorphic chromosomes 3, but the enhancement is restricted to the proximal Gl-Lg region, which is known to be rich in heterochromatic knobs. CHANG (unpublished thesis research) has determined that the recombination in chromosome 9 of maize enhanced by the presence of K10 is limited to the proximal, heterochromatic region. These results are reminiscent of the well-known temperature-induced increase of recombination values in Drosophila and Neurospora, which are largely restricted to proximal regions.

DIFFERENTIAL MUTABILITY. The presence of some heterochromatin in the proximal euchromatic zones and their nearness to the main blocks of heterochromatin also suggest a different hypothesis--differential mutability--regions of higher marker density are subject to increased mutation rates. High genetic instability is exemplified in Drosophila by position effects wrought by centric heterochromatin after transposition into euchromatic regions. Pertinent also is M. M. GREEN's (unpublished) experiment in which w loses its high mutability rate when moved away from its normally neighboring interstitial heterochromatic region in 3C of the Drosophila X chromosome.

The mutability hypothesis is supported by the distribution of multiple alleles in the tomato map. According to our latest survey, 80 such alleles have been reported, of both spontaneous and X-ray-induced origin. By definition the distribution of such alleles must reflect the distribution of loci of previously located genes; consequently, the distribution of multiple alleles is meaningful only if compared with that of all known loci. Our data permit a comparison in the distribution in testable arms for the first thirty units from the centromere. The pooled data yield 20 loci for the first ten units, 4 for the next, and 7 for the third interval, in contrast to 32, 17, and 18 for all known loci. Thus, for this entire region, 65% of the multiple alleles are found in the most proximal section, while only 48% of the known loci are found there. Tested by 2:2 contingency, the χ^2 is 1.87, corresponding to a probability of 0.1-0.2. Thus, although appreciable, the difference is not statistically significant. If real, it could be explained by differential mutability, not by recombination.

The large differences in density of markers between tomato chromosomes 11 and 12 (and between maize chromosomes 8 and 10) bear no apparent relation to recombination rates, yet could be explained in terms of differential mutability. The fact that the tomato metacentric chromosomes usually pair as ring bivalents (i.e., with at least two chiasmata apiece) at diakinesis implies that even the smallest chromosomes must have a map length of roughly 100 units or more. It is therefore highly unlikely that a very high level of recombination causes the reduced gene density on chromosome 12 and vice versa for chromosome 11. JAIN and RAUT (1965) reported a differential susceptibility of tomato chromosomes to breakage by hydroxylamine and (1966) differences in the chromosomal distribution of mutations induced by different mutagens. They construe these results to indicate an inhomogeneity of base pair distribution and suggest that it might have significance in relation to the observed asymmetrical gene distribution. Otherwise we have no clue as to the basis for mutability differences.

In summary, although none of the evidence is compelling, when considered in the aggregate it leads to the conclusion that non-random gene distribution within chromosomes of higher plants reflects some factor in addition to differential recombination--most likely differential mutability.

THE SILENT REGIONS

Large sections of the linkage maps of tomato and maize are "silent"--i.e., they lack loci of mutant genes. Such unmarked regions are scattered throughout the chromosomes of both genomes, but, as already observed, are commonest in distal regions. That these sections actually have comparable chromosomal counterparts, not negligible physical units with extremely high recombination rates, has been proven by PHILLIPS (1969) in his breakage-point analysis of maize chromosomes. In distal 7L, for example, he relates the silent bn-bd region of 38 crossover units with approximately 24% of the cytological length of that arm.

The silence of these regions might owe to their genetic inertness or to failure to detect mutations of genes residing there. The former alternative can be dismissed because their genetic activity has been repeatedly demonstrated. Thus, RHOADES (1968) revealed that deficiency for part of a silent region distal to the knob on the maize 3L arm is lethal to the development of gametophytes. It has been our experience (KHUSH and RICK 1968) in tomato that only very short deficiencies are tolerated in all tested longer arms, even in the hemizygous state. Certain regions, particularly distal ones, may lack markers simply because they have not been studied intensively enough. Some may not have received much emphasis, others may not have been within range of linkage testers or have only recently been adequately screened. While the silence of certain sections might thus be satisfactorily explained, others have been so intensively screened that markers within them could hardly have escaped detection. Thus, in the tomato 4L, the 26-unit silent region between ven and e has been intensively screened for over a decade with the testers clau and ful to the left and e, itself, on the right. The 31-unit gap distal to tf on 5S is another example, and so, in fact, as previously observed, is the whole of chromosome 12.

Duplication within the complement provides another hypothesis. The evidence for duplication in maize was assessed in 1951 by RHOADES. Whereas the occurrence of duplicate gene interaction and the grouping of certain duplicate genes in specific chromosomal regions suggested intragenomic duplication, such regions are not all silent, and their essentiality to gametophyte development has been demonstrated in translocation heterozygotes. The mutability hypothesis has already been discussed.

The silence of terminal regions presents a special problem. PHILLIPS' (1969) analysis in maize shows that sizeable regions at the tips of 5S, 5L, and 6L--three of the five scrutinized distal regions--lack mutant loci. For 6L at least the terminal third is silent. As for the tomato, relatively few loci have been positioned in distal zones. Induced deficiencies have detected markers for far portions of 2L, 3L, 6L, and 8L, but the situation is uncertain for the other arms. Cytogenetic analysis is impeded by several factors: (1) except for the tomato telomeres, these zones have no landmarks, (2) interstitial deficiencies there are difficult to induce, (3) once induced, the position of such deficiencies is obscured by a tendency toward non-homologous pairing, and (4) lack of genic loci. Until a special effort is made for its solution, this problem will continue to be vexing.

DEGREES OF DIPLOIDY

Polyploid species are defined as aggregations of the genomes of one or more diploid species. The diploid species are identified as those having the lowest or basic chromosome numbers of their

respective genera. Such diploids may not be absolute in the sense that their genomes are free of genetic duplication. Three plant species--maize, petunia, and tomato--are compared here in respect to their degrees of diploidy.

THE MAIZE SITUATION. RHOADES' (1951) aforementioned treatment of duplicate genes in maize led to the conclusion that the maize genome undoubtedly does contain duplicated regions. Pertinent information is also contributed by the comportment of aneuploids. A remarkable tolerance of chromosomal unbalance was revealed by McCLINTOCK (1929) and PUNYASINGH (1947) in the composition of progeny of a triploid (3n x 2n): all except 2% of the plants were aneuploids, which had from one to seven extra chromosomes (Figure 9). Not only does maize withstand much unbalance, but also the morphogenetic effect of single extra chromosomes is so limited that only six of the ten primary trisomics can be recognized by their phenotype and only certain ones like triplo-5 can be accurately classified in populations segregating for disomics/trisomics (RHOADES 1955). Vigor and fertility are severely impaired in the most unbalanced maize aneuploids.

Additional evidence of genetic duplication in maize is given by tolerance of chromosomal deficiencies. Small but detectable deficiencies can be transmitted through gametes (BURNHAM 1932, CREIGHTON 1934, STADLER 1929) and some can even be withstood by zygotes in homozygous condition (McCLINTOCK 1941, 1944). In the face of this evidence the presence of a considerable amount of duplication must be admitted in the maize genome.

THE PETUNIA SITUATION. The garden petunia (Petunia hybrida) is included in this survey because it is an example of extreme tolerance of aneuploidy. Although this plant is useful cytogenetically, it has not received much attention. In unpublished studies of a 3n x 2n cross, I counted chromosomes in 88 progeny summarized as follows: 2n (7_{II}) - 8, (2n + 1) - 28, (2n + 2) - 31, (2n + 3) - 12, (2n + 4) - 3, (2n + 5) - 4, (2n + 6) - 2 (Figure 9). In similar material LEVAN (1937) found the same distribution of chromosome numbers but lower frequencies of the higher aneuploids. Thus in a relatively small sample every possible aneuploid between diploid and triploid was found; furthermore, the viability of the most unbalanced plants was not noticeably reduced. So little morphological effect was wrought, moreover, that it was impossible to distinguish phenotypically between the aneuploids--even the most unbalanced types--or between them and diploids. Such tolerance of aneuploidy contrasts markedly with the results observed in the tomato and many other diploid plants (see below). On the other hand, a parallel indifference in respect to viability and phenotype has been observed in Clarkia unguiculata (n = 9) (VASEK 1956) and Collinsia heterophylla (n = 7) (DHILLON and GARBER 1960), revealing that this situation is not exceptional. Other inklings of internal duplication in the petunia genome are found in the lack of well-defined Mendelian segregation; blending inheritance is typical of the characters that I have studied and observed in trial grounds of commercial seed firms.

As I reflected previously (RICK 1943), it is astonishing that the petunia should emerge as an example of extreme genic redundancy, for it has the lowest chromosome number (n = 7) in the entire nightshade family. In contrast, tomato, datura, and Nicotiana sylvestris (GOODSPEED and AVERY 1941) behave more like strict diploids in respect to the criteria considered here.

Whether petunia or maize is more tolerant of chromosome unbalance is debatable. They do not differ markedly in respect to

the survival of aneuploid categories (Figure 9), although the complete range of numbers between 2n and 3n has been observed in the progeny of 3n x 2n crosses only in petunia and extra chromosomes modify morphology and vigor less in this species.

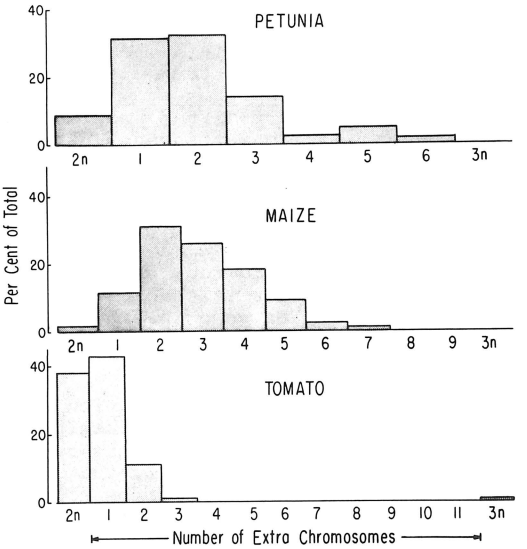

Figure 9. Frequency distributions of chromosome numbers in the progeny of 3n x 2n crosses. Petunia data from RICK (unpublished); maize data from McCLINTOCK (1929) and PUNYASINGH (1947); tomato data from RICK and BARTON (1954).

THE TOMATO SITUATION. The tomato exemplifies a plant species with minimal duplication. It is remarkably free of the duplicate type genic interaction. Examples have been proposed from time to time, as in the determination of crimson and high-pigment fruit color mutants, but thorough investigation usually reveals that only one gene of major effect is responsible for each. Even in the well-documented case of the "citrine" character (REEVES 1968, 1970), which is unquestionably a digenic recessive, each recessive homozygote *per se* produces a phenotype distinguishable from normal.

The tomato is highly intolerant of aneuploidy: the pooled progeny (799) of many triploids included 38% diploid, 43% (2n + 1), 16% (2n + 2), and 1% (2n + 3) (Figure 9), the degree of phenotypic modification and sterility increasing strikingly with the number of extra chromosomes (RICK and BARTON 1954). Each primary trisomic can be distinguished so clearly by its phenotype that trisomics and diploids can be identified, even in relatively complex genetic segregations. According to KHUSH's (1970) review, similar limits to tolerance of aneuploidy are known in 12 other diploid plant species (*Antirrhinum majus*, *Beta vulgaris*, *Datura stramonium*, *Fragaria* sp., *Hordeum vulgare*, *Lotus pedunculatus*, *Nicotiana sylvestris*, *Oenothera biennis*, *Oryza sativa*, *Pennisetum typhoides*, *Secale cereale*, and *Sorghum vulgare*). In these and nine other species (*Arabidopsis thaliana*, *Avena strigosa*, *Capsicum annuum*, *Crepis capillaris*, *C. tectorum*, *Matthiola incana*, *Oenothera blandina*, *Oe. lamarckiana*, and *Spinacea oleracea*), single extra chromosomes drastically alter

the phenotype. A high degree of sensitivity thus appears to be widespread amongst diploid plants. Somewhat reduced effects in the above specified characteristics were noted in a primitive tomato cultivar (RICK and NOTANI 1961), but, even so, the most extreme aneuploid encountered (2n + 4) was found only once among 94 progeny. A similar relationship is known between wild and cultivated barley (TSUCHIYA 1958, 1960).

The evidence for transmission of tomato deficiencies has been summarized by KHUSH and RICK (1967). Briefly, exclusively heterochromatic regions like 2S can be transmitted in duplicate without apparent deleterious effect on gametophytes and to a limited extent as deficiencies; a deficiency for part of the proximal heterochromatic zone of 9L is transmitted by female gametophytes, but only at a reduced rate; large euchromatic deficiencies are practically never viable in gametophytes: and, according to ratios obtained for linked markers, the same holds even for euchromatic deficiencies that are too small to be detected cytologically.

Thus, of the three species considered, the tomato behaves as the least tolerant of the tested kinds of chromosomal abnormalities. Consistent with the lack of duplicate gene interactions, these data detect no redundancy in the genome.

THE TOMATO MIMICS. Possible evidence of duplication and some curious spatial relationships are found in the tomato mimetic mutants. It has been our experience that, when a new mutant is found with phenotype identical or highly similar in syndrome to that of another previously known gene, they generally prove to be allelic. Such is not the case, of course, in such simple presence-vs.-absence characters as anthocyanin deficiencies or in the complete or partial deficiencies of chlorophyll, whose synthesis and disposition can be disturbed by many genes acting on various synthetic pathways. But, particularly in those mutants with a complex syndrome of associated pleiotropic characters, phenotypes of alleles are strikingly similar. The exceptions to this situation are of special interest here.

Such mimics prove to be relatively rare. Comparable statistics are difficult to derive, but I believe I am reasonably accurate in stating that for every 8-10 mutants with close phenotypic resemblance that prove to be allelic to previously known genes, one turns out to be situated at a different locus. Now the point of primary significance here is that, with only one exception, members of such mimic series have their loci on the same chromosome. The following series have been thus far reported:

1. The dwarf series. Plants compact; internodes, leaves, petioles, flower parts foreshortened; leaves bullate, with thick, stiff laminae: d (also d^{cr}, d^{x}) at 100, dpy at 78, and dd at 141 on chromosome 2 (HERNANDEZ-BRAVO 1969) (Figure 10).

2. The wiry series. Leaf margins irregularly eroded, becoming progressively more extreme from cotyledons to the uppermost leaves, of which only the rachis may remain; perianth parts filiform; ovary apocarpous; female sterile; w at 20, w-4 at 28 on chromosome 4 (Figure 10). Other known wiry genes are either extinct or have not yet been located.

3. The wilting series. Abnormal stomatal control leading to excessive transpiration, wilting, and even necrosis from uncontrolled dessication; not at 40, flc at 59 on chromosome 7; sit at 32 on chromosome 1 (Figure 11); mutants discovered by STUBBE (1957, 1958, 1959) and investigated physiologically by TAL (1966).

Figure 10. Tomato dwarf (above) and wiry (below) mimic series mutants. Metric scales at right.

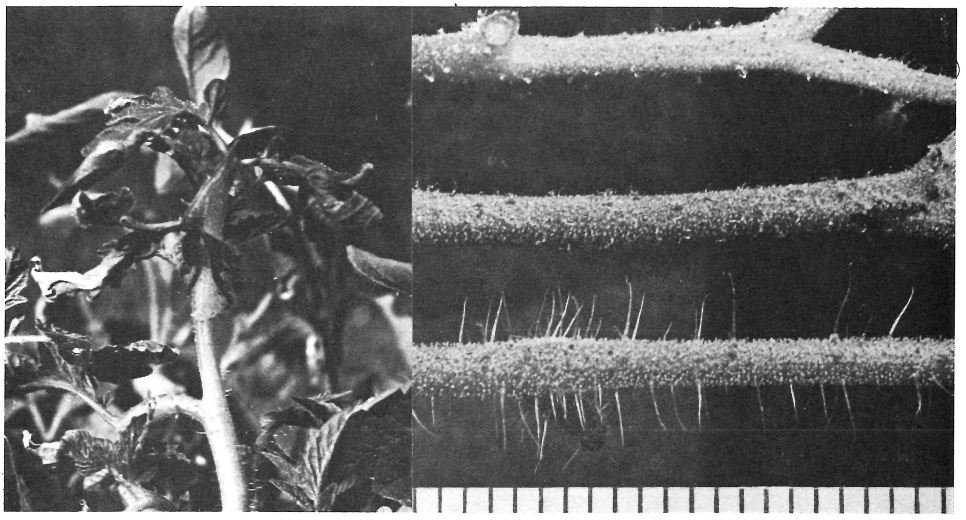

Figure 11. Tomato mutant *not*, representing the wilting mimic series (left); hairless mimic series on right; *ini* above, *hl* in middle, normal below (mm scale at bottom).

4. The *hairless* series. Stem cells absent or abortive in the larger trichomes, the multicellular pads intact; stems brittle; foliage color grayish; *hl* (*hl*^{2}) at 37, *ini* at 57 on chromosome 11 (Figure 11). Other hair-modifying genes are known, but they distort or eliminate certain classes of hairs or are otherwise phenotypically distinct from this series.

5. The *jointless* series. Pedicel articulation absent; inforescence indeterminate: *j* and *j-2* on chromosome 11.

The chances that these 11 genes would appear by random distribution on the same chromosome as their respective mimics are exceedingly remote. They might have originated by some form of intrachromosomal duplication, but since they do not interact in duplicate fashion, the doubling process must have been sufficiently ancient to permit each gene to assume independent function (diploidization?). The duplicated segments, if they exist, are probably small because no evidence has yet been encountered of sequences of repeated genes.

According to an alternative hypothesis, these mimics might have originated by the dispersion of structural genes in an operon, albeit *bona fide* operons are not known in eukaryotes. If they arose thusly, the mimics would not reflect duplication in the tomato genome. Thus, at the present time, we have no strong evidence of duplication in the tomato genome.

However inconclusive these findings may be, they do reveal that, even within the same botanical family, chromosome number alone is not a reliable index of the degrees of diploidy. Only extensive cytogenetic study can provide the critical data.

PROSPECTS

These comparisons reveal certain asymmetries in the distribution of genic loci between chromosomes and non-random situations within chromosomes of higher plants. In respect to the latter aspect, remarkable similarities exist between plants and such other intensively investigated eukaryotes as Drosophila and Neurospora. They are similar not only in the proximal aggregation of loci on the linkage maps of most chromosomes but also in a reverse tendency on the long arm of the nucleolar organizing chromosome. The terminal accumulation that is so pronounced on the X chromosome and some of the autosomal arms of Drosophila still lacks a parallel in other organisms, although admittedly mapping in the latter lags far behind. If this review serves no other purpose, I hope it underscores the urgent need for more intensive efforts to gain a better understanding of the linkage maps of higher plants and their relationships with cytological maps.

The proximal heterochromatin found in all arms of the tomato complement and probably in the majority of angiosperms is still poorly understood. Does it serve to stabilize the centromere? Is it essential to normal functions of the chromosome and cell? The unique map characteristics of the nucleolar chromosome invite further research. Do its anomalies owe to the presence of a nucleolus organizer or to some other factor? A study of recombination in translocated nucleolus organizers should be illuminating, but much effort would be required in synthesizing the appropriate marker stocks for testing the interchange homozygotes. A series of such translocations in maize that would be highly useful for this purpose was reported by BURNHAM (1950). Several were also reported in tomato by STRINGAM (1968), but they suffered the disadvantage of having their breakpoints in various positions in the euchromatin of 2L. Presumably the desired translocations should be attainable in tomato because its centromeres and adjacent heterochromatic zones are highly susceptible to radiation-induced breakage.

The silent regions pose some intriguing problems. Intensive efforts should be made to populate them. Pollen lethals might serve as the most useful genes for such purposes since they are common and their linkages can be detected simply by scanning segregating generations for distorted ratios of linked genes. Although their usefulness as markers would be greatly limited by problems of identification and stock maintenance, they might assist in such distribution tests in the same fashion as zygotic lethals do in Drosophila.

The enigma of the poorly marked terminal regions has already been mentioned. Prerequisite to progress in this area is the siting of markers as close as possible to the chromosome ends. Until this goal has been reached, we have no assurance that the selected linkage testers screen the entire genome. Mapping these regions could be expedited by synthesizing translocations that bring such useful cytological markers as knobs or heterochromatic blocks into association with the terminal regions.

These are a few directions in which future research might be usefully directed in order to tackle some of the mysteries of the genome in higher plants. Undoubtedly many other avenues exist, and I am also confident that the critical problems will attract talented, vigorous investigators, as they are at this campus.

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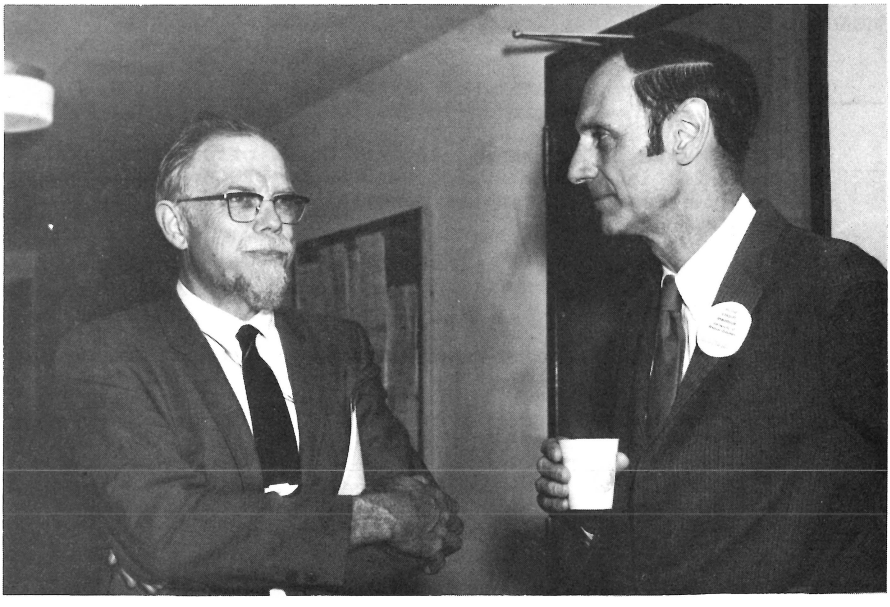
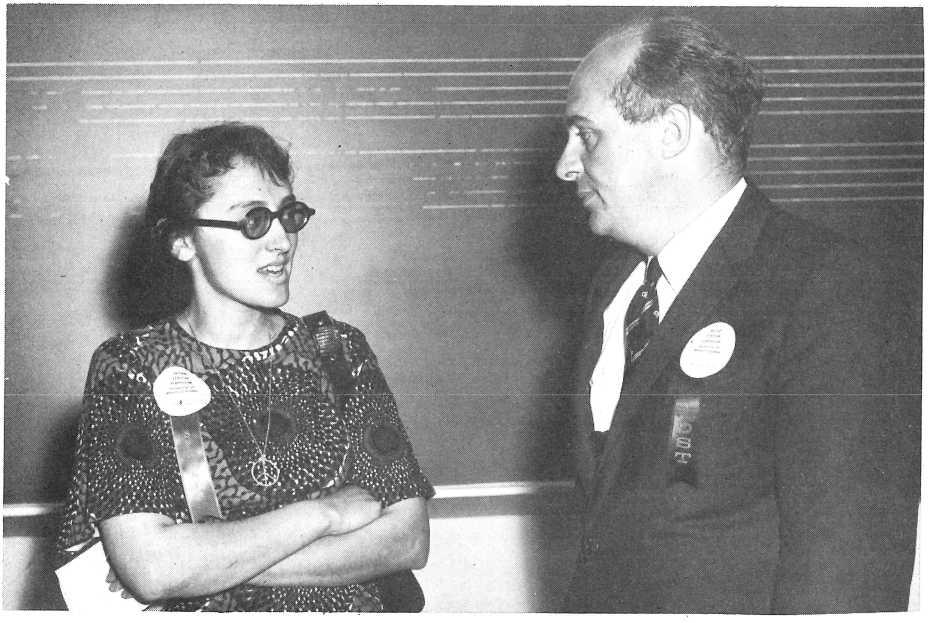


Dr. C. M. Rick (left) and Dr. H. L. Carson (right).



Dr. Lynn Margulis and Dr. Gordon Kimber

The speakers at the 2nd Stadler Symposium: Beadle, Sager, Peacock, Fogel, Rick, and Margulis.