sacs were smaller but completed the three divisions. Seed-set was good although less than on a normal ear.

Essentially the same technique for locating genes has been used in Drosophila. X-rayed males homozygous for the dominant alleles were crossed on females carrying the recessives. F_1 flies that showed the recessive character were crossed again to the multiple recessive stock and the larvae examined cytologically using the salivary glands. Deficiencies could be recognized by the fact that one member of the synapsed pair was shorter and had a section with certain bands missing (Mackensen, 1935, Alexander, 1959).

Phenotypic effects

In Drosophila many of the dominant characters are associated with small deficiencies in heterozygous condition, e.g. the Minutes (short, fine bristles), Delta (wing veins broadened at junction with margin), Gull (spread wings), Notch (notched wing margins, 131 listed, all in X), Plexate (plexus-like thickening of veins at margin), and Vein (gap in one vein). Pale, a specific diluter of eosin eye color, is associated with a deficiency and a transposed segment. Deficiencies are usually lethal when homozygous, and include a complex of secondary effects. If in the X chromosome, they may be lethal to males. One good example is the class of mutants known as Minutes. Ninety are listed, some in each of the four chromosomes. Heterozygotes have short, fine bristles and in addition secondary effects "such as small body size, larger, somewhat rougher eyes, missing aristae, thin-textured wings with a tendency to plexus venation, missing bristles (usually postverticals), and sterility or low fertility, especially in females" (p. 121, Bridges and Brehme 1944). Certain Minutes may: increase the frequency of somatic segregation, enhance dominance of certain recessive wing venation or bristle characters; or produce a complementary dominant lethal effect with other dominant characters. For most of those given a careful salivary gland chromosome analysis there was a deficiency for one up to several bands. When the deficiency is in the chromocentric portion of any of the chromosomes, the analysis for the extent of the deficiency is not critical. An example of this is Schultz' Minute M (2) S 10 (M = minute; (2) = chromosome 2; S 10 designates this Minute). The heterozygote shows no crossing over in the region from light at 55.0 to cinnabar 57.5, but there is no detectable change in the salivary-gland chromosomes. Metaphase chromosomes show a deficiency of the heterochromatin of the right arm.

Behavior of deficiency homozygotes

In somatic segregation experiments certain X-chromosome loci for lethals were found to act as cell lethals, although surrounded by normal tissue (Demerec 1934, 1936). These were lethal in the hypodermal cells which normally develop the yellow body color used as the linked marker. They were not necessarily lethal in other tissues.

Of 15 X-chromosome loci for visible characters, 13 were cell-lethal while only 10 of 24 X-chromosome loci for lethals were cell lethal. He concluded from this that the visible characters tend to be of greater importance to the individual. The lethals that were studied cytologically were deficient for one or more salivary chromosome bands.

Unequivocal evidence that some recessive mutations are the consequence of homozygous small deficiencies has been obtained in Drosophila and in maize. Individuals with the phenotype of certain known recessives have appeared when they were homozygous for a small deficiency which included the normal alleles of those characters. For example, flies homozygous for a deficiency for the $+^{y}$ locus have a yellow body, as reported by Ephrussi (1934) and others; and flies homozygous deficient for $+^{w}$ have white eyes (Panshin 1938).

Similar evidence has been obtained in maize by Creighton (1937) and by McClintock (1938a, 1938b, 1941a, 1941b, 1944). In Creighton's study a terminal deficiency in the short arm of chromosome 9 was produced by X-irradiation.

The methods used by McClintock will be described in detail, because of the importance of the findings and of the techniques. In one series, McClintock (1944) used a stock having a normal #9 chromosome and one #9 with a duplication of the short arm, a reverse repeat marked with the dominant C factor for aleurone color and Sh Wx, as shown in Fig. 6. A more complex rearrangement involving three breaks in 9 was the source of similar deficiencies in the short arm, but is not shown here.

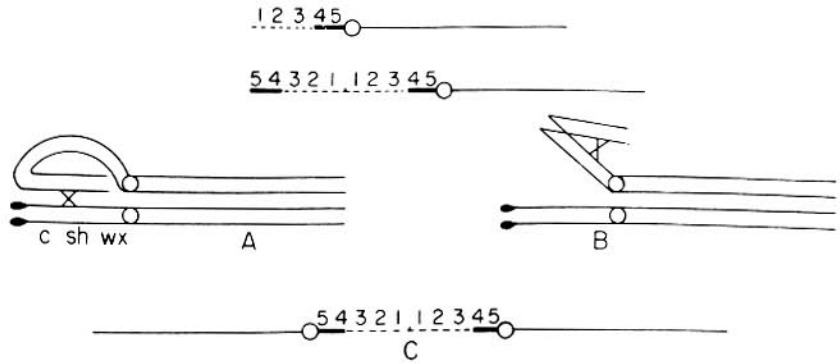


FIG. 6. Diagrams illustrating one of the duplications used by McClintock in a study of deficiencies. Top line: normal chromosome 9 (modified from McClintock 1941, Fig. 3, p. 238. Genetics 26). Second line: chromosome 9 with a duplication of the short arm in reverse order. A, B, diagrams of meiotic prophase association between a normal chromosome 9 (with c sh wx) and one with the short arm duplicated, but in reverse order and carrying the dominant alleles. The single crossover shown in A produces a bridge configuration at anaphase I, the crossover shown in B produces a bridge at anaphase II.

Either crossover gives rise to the dicentric chromatid shown in C. Its genetic makeup depends on the region in which crossing over occurred. (A, B, C; modified from the McClintock 1941, Fig. 11, p. 261. Genetics 26). This and a more complicated duplication stock for chromosome 9 were the sources of the deficient chromosomes in McClintock's 1941, and 1944 reports.

The crossover shown between the duplicated arms in Fig. 6A will produce a dicentric chromatid which will produce a bridge at anaphase I and may break at various positions. Since the dicentric chromatid produced by the crossover shown in Fig. 6B involves ~~sister~~ chromatids, the bridge does not appear until anaphase of the second meiotic division. attached to the same centromere,

In the subsequent gametophyte divisions, the broken chromosomes become dicentric, either through fusion of broken ends when the chromatid becomes double or by non-reproduction at the broken end. This initiates the bridge-break-fusion-bridge cycle (Fig. 7) which continues in corn through successive cell divisions in the gametophytes and in the endosperm but not in the tissues of the embryo and in the resulting plant (McClintock 1941b,

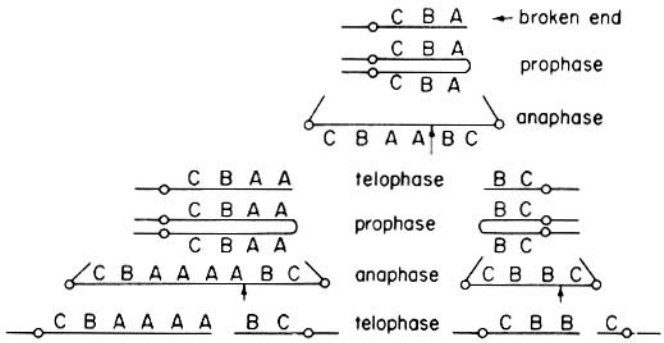


FIG. 7. Diagrams illustrating the break-fusion-bridge-break cycle. The broken chromosome is shown at the top. When it replicates; the broken ends are fused, thus forming a dicentric which at the next nuclear division forms a bridge. This may break at any point between the two centromeres, and the cycle is repeated. In corn this occurs in the meiotic and post meiotic divisions and in the endosperm. If the endosperm has two normal chromosomes carrying recessive markers, e.g. c sh and wx and the broken chromosome has the dominant alleles (C B A in the diagram), the break-bridge-fusion-break cycle will result in variegated kernels as a result of the irregular loss of the dominant alleles (McClintock 1941, Fig. 1, p. 235, Genetics 26).

1942). By crossing this stock heterozygous for the duplication and for c sh and wx on a colorless aleurone (cc) stock used as the ♀ parent, many of the kernels that had received a broken chromosome 9 could be identified by the variegation for colored aleurone, produced whenever the breakage of the bridge produced a chromosome deficient for the C locus. The embryos of these seeds also should have received a broken chromosome from the same nuclear division. Over 500 plants from such kernels were examined at pachytene, and many self-pollinations were made.

Part of the plants were classified as having received a chromosome 9 that was approximately normal, others as having received a deficient 9. These deficiencies ranged from extensive to very short. Those with deficiencies for approximately one to six terminal chromomeres had ratios for aleurone color that deviated greatly from the normal ratio of 3 colored : 1 colorless. Further tests indicated that the deficiency was not transmitted through the pollen. Those that had deficiencies shorter than these produced when selfed either normal or nearly normal ratios of colored : colorless (C:c) since this locus is not included in these short terminal deficiencies and since transmission was normal. A test of 30 plants that had no cytologically obvious deficiency produced only normal seedlings, although about 25% of them were expected to be homozygous for any deficiency that might have been present. In contrast, segregation for seedling characters linked with C occurred in the progeny of plants heterozygous for certain of the deficiencies that were obvious cytologically. One type was pale yellow (py)*, another white (w)*, both lethal. Seven of the former and six of the latter were selected for further tests. These tests included (1) a check of transmission in relation to the presence or absence of a cytologically demonstrable deficiency, (2) intercrosses between mutants of similar phenotype, (3) tests of mutants against longer deficiencies, and (4) tests of allelism between the pale yellow and the white mutants and between them and the previously known yellow green-2 (yg_2) mutant.

(1) Transmission in relation to deficiency

Of the surviving green plants in progenies segregating for the mutant only those heterozygous for the deficiency segregated for the mutant character. For example, in tests involving the white mutants, there were 36 green plants with two normal 9 chromosomes and 93 with one normal and one deficient. The former did not segregate w, while all the latter did and all but two in normal 3:1 ratios. These two showed lower transmission through the ♂. The 7 py cultures showed normal transmission. In the py heterozygotes, one chromosome 9 was deficient for the large terminal knob plus the short thin strand between it and the first chromomere. In the w heterozygotes, those portions were missing and in addition a short piece of the first chromomere (Fig. 8).

(2) Intercrosses between mutants

Intercrosses between plants heterozygous for the pale yellows produced only 3 green : 1 pale yellow. Similar results were obtained for the white mutants. As she stated it, this establishes the isoallelic if not the identical nature of the mutants within each group.

(3) Tests of the mutants against longer deficiencies

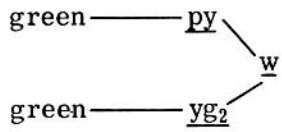
Pollen from heterozygous deficient plants from the different cultures segregating py placed on plants heterozygous for longer deficiencies that were only ♀ transmissible produced progenies segregating pale yellow (py) seedlings. Similar tests of the cultures segregating w produced progenies segregating white seedlings. These are precisely the results expected if the py character for example were associated with a very short terminal deficiency; w a somewhat longer one, but still shorter than the deficiencies they were tested against. That is, the shorter terminal deficiency contributed by the ♂ cannot be

* pyd and wd in McClintock's reports.

covered by the longer terminal deficiency contributed by the ♀. Plants homozygous for the short deficiency have the recessive phenotype.

(4) Tests for allelism

Crosses of py heterozygotes on w heterozygotes segregated in the immediate progeny in every case (42 crosses) for py only; showing that py and w are allelic with py dominant over w. Tests of py with yg_2, a naturally occurring mutant known to be in the same general region, produced only green progeny, but tests of w with yg_2 segregated yg_2. Hence py and yg_2 are non-allelic, but w and yg_2 are allelic. Thus they form two series as to dominance with w common to both:



These fit into one scheme if the yg_2 locus is between py and w: i.e. py is associated with a short deficiency that does not include the yg_2 locus, whereas the w is associated with a deficiency that includes both py and yg_2. These relations are shown in Figure 8.

The phenotypes of plants carrying various combinations of chromosomes 1, 2 and 3 shown in Fig. 8 were as follows:

1 + 1 = yellow green seedling
2 + 2 = pale yellow "
3 + 3 = white "
1 + 2 = green "
1 + 3 = yellow green "
2 + 3 = pale yellow "

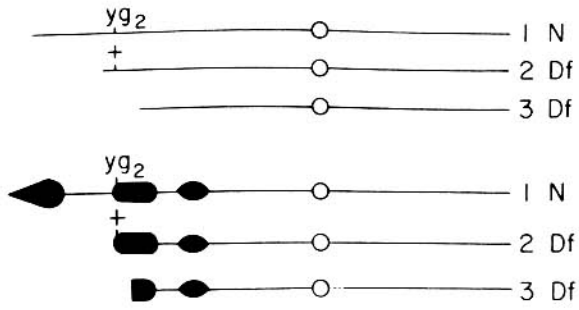


FIG. 8. Diagrams illustrating the terminal deficiencies in chromosome 9 which when homozygous produced the pale yellow (py) and the white (w) seedlings. The locus of the previously known yellow-green (yg_2) mutant is shown also. The relative lengths of the normal and deficient chromosomes are shown in the upper three chromosomes, the chromomeres involved are shown in the lower three. The chromosomes numbered 1, 2 and 3 represent respectively the position of yg_2 in the normal chromosome, the deficient chromosome which accounts for py but has the normal allele of yg_2, and the chromosome which has a somewhat longer deficiency that accounts for w. The stocks in which py and w appeared had the normal allele of yg_2 and also had the large terminal knob. The yg_2 stock did not carry the knob. (Modified from McClintock (1944) Fig. 2, p. 494, Genetics 29).

Thus the reasons for the allelic relationships are clear from the combined evidence from genetics and cytology. Had only the genetic behavior been available the results would have been difficult to explain.

In another series of experiments, McClintock (1941a, 1938b) obtained male- and female-transmissible mutants associated with homozygous small deficiencies generated by a ring chromosome. These recessive mutants appeared among the progeny of plants carrying a chromosome 5 that was deficient for a short segment adjacent to the centromere and also carrying a ring chromosome that had the deficient loci and a centromere. Together the two constituted the genic material present in a normal chromosome 5. Two stocks were available, one with a rod chromosome 5 deficient for chromomeres 1-4 in the short arm adjacent to the centromere and the other with a rod chromosome deficient for chromomeres 1 to 9 but with those loci present in each case in a compensating ring chromosome.

In most somatic divisions such a ring chromosome was lost. Occasionally the ring became double in size, supposedly as a result of somatic crossing over. In occasional divisions the ring broke, and one portion passed to each pole. The broken ends became

fused and thus smaller rings were produced. One or the other might contain a small deficiency. In a plant with two deficient number 5 chromosomes and two ring chromosomes, this change might occur first in one ring and then in the second ring in cells descending from the first change. If the two deficiencies overlapped, the resulting cell and its descendants would be homozygous for a short deficiency. When the changes occurred during development, longitudinal sectors in the leaves had the deficiency. If the sector were included in the reproductive tissue, offspring carrying the deficiency would be produced.

Within the short segment of four chromomeres adjacent to the centromere, four distinct non-allelic mutants, all male and female transmissible, were distinguished in this manner. In one the deficiency produced a brown midrib character similar in appearance to bm_1, the gene for which was known previously to be in that general region. Three more non-allelic mutants were discovered in chromomeres 5 to 9 adjacent to the segment ~~deficient~~ present in the smaller ring. These were obtained from the line with the larger ring.

In studies of elements in the nucleus that control mutation, McClintock (1951, p. 30-31, 1956) found that certain mutants in maize produced by the activator-dissociator (Ac-Ds) system were homozygous for a chromosomal deficiency. She found also that sectors homozygous for a deficiency for the C, Bz or Wx loci were phenotypically like the corresponding homozygous recessives, colorless aleurone, bronze aleurone, and waxy endosperm respectively. This does not mean that these characters in normal stocks necessarily are deficiencies. Nor does it mean that all artificially produced mutations are deficiencies. Some may be, others not.

In these experiments the deficiencies could be recognized easily because of their position. Internal deficiencies of a similar magnitude in corn chromosomes that were otherwise normal would be recognizable only rarely. The known cytological and genetic techniques cannot distinguish between a change in the gene and a deficiency. Mutants that are deficiencies might not be expected to back mutate. Back mutations from mutants produced by mutagens have been reported by Giles (1956). This indicates that some induced mutants, at least in Neurospora, may not be deficiencies. However the explanation of back mutation may not be a simple one. In certain cases they represent mutations in a suppressor, in others not.

So-called pollen abortion 'genes' have been reported in Datura and in corn. Plants heterozygous for them show about 50% of aborted pollen, but normal seed-set. Transmission is only through the ovules. Ratios for markers linked with them are abnormally low in selfs or tests through the pollen. They were used extensively in Datura as a means of locating genes in the chromosome. They may be short deficiencies, although cytological examination and other tests in corn did not detect a deficiency (Burnham 1941).

Effects of duplications and deficiencies on crossing over

In Drosophila females heterozygous for a deficiency, crossing over does not occur in the deficient region, as shown earlier. In tests for the effect of the first deficiency in Drosophila on recombination between r and fu on either side, there was a decrease of 0.7 unit which is only slightly greater than the distance (0.5) between f and B which were included in the deficiency. In subsequent papers, a decrease in crossing over was noted also in regions adjacent to the deficiency; but there was a tendency for a slight increase in regions farther away.

A deficiency for the dachs locus in chromosome 2(Df (2) d) in Drosophila gives decreased crossing over in the d-b region in the heterozygote (Bridges 1919). These genes are located at 31.0 and 48.5.

Genetic studies of a deficiency-duplication in corn identified by Frances Clark Beard as resulting from the insertion of a segment of the long arm of chromosome 3 in the short arm of 9 have been reported by Rhoades (1958a). An F_1 of the following composition in chromosomes 9 and 3: $\frac{Dp9\ Wx}{N9\ wx}\ \frac{Df3\ A_1}{N3\ a_1}$ was used in backcrosses to $\underline{a_1}\ \underline{wx}$. If no crossing over occurs between Dp9 and Wx, or between Df3 and A_1, four kinds of spores are expected in equal numbers: Dp9 Wx Df3 A, Dp9 Wx N3a, N9 wx Df3A, and N9 wx N3a. Of these, only the N9 Df3 mega- and micro-spores are regularly aborted. Through the female, the other three showed normal transmission. When the heterozygote was used as the pollen parent, the Dp9 N3 class of pollen functioned only occasionally. The following data were obtained by backcrossing this F_1 as female with $\underline{wx}\ \underline{wx}\ \underline{a_1}\ \underline{a_1}$.

Wx A	Wx a	wx A	wx a	total
2618	2409	431	2262	7720

Wx : wx in % = 65.1 : 34.9

A : a in % = 39.5 : 60.5

Had there been no crossing over between Wx and Dp9, the ratio of Wx : wx through the ♀ should have been 2:1, very close to the observed. If A and Df3 had been completely linked the ratio should have been 1A:2a. The observed value of 39.5% A indicates that some crossing over occurred. Since the N9wx Df3A class of spores aborts, if Wx and Dp are completely linked, the wx A offspring should represent recombinations between A and the deficiency, and the wx a class the corresponding parental frequency. On this basis, the recombination value was 16%. Of 142 plants from Wx A kernels, 127 had a Df3 and 15 had a normal 3. The latter 15 represented crossovers between A and Df3. A similar test of 60 plants from a Wx kernels showed only six that were Df3. Based on the total of the three tests, the locus of the deficiency in 3 was about 16 recombination units from A. Also, heterozygotes with the constitution: $\frac{Dp9\ +\ +}{N9\ gl_6lg_2}\ \frac{Df3\ +}{N3\ a_1}$ were backcrossed to $gl_6\ lg_2\ a_1$. For comparison plants without the deficiency in 3 but with the same genetic markers; one group heterozygous for Dp9, the other without Dp9 were also backcrossed to $gl_6\ lg_2\ a_1$. The data are in Table 3.

Table 3. Backcross data from plants carrying a duplication in chromosome 9, a deficiency in 3 and heterozygous for the $gl_6\ lg_2\ a_1$ markers in chromosome 3 (cross #1), and from plants with the normal chromosome 3 with and without the duplication in 9 (crosses 2 and 3 respectively). The plants are tabulated only for the genetic markers. (Rhoades, 1958a, by permission).

		Regions				Total	Recombination	
		0	1	2	1-2	plants	Gl-Lg	Lg-A
1.	$\frac{Dp9\ +}{N\ 9}\ \frac{+\ Df3\ +}{gl\ lg\ N\ a}$	273-504	292-147	44-83	32-19	1396	35.1	12.8
2.	$\frac{Dp9\ +}{N\ 9}\ \frac{+\ N\ +}{gl\ lg\ N\ a}$	347-338	154-168	155-156	32-53	1403	29.0	28.2
3.	$\frac{N9\ +}{N9}\ \frac{+\ N\ +}{gl\ lg\ N\ a}$	381-370	168-180	188-200	41-55	1583	28.0	30.6

A comparison of the recombination values for cross #1 with those for the control in #3 in Table 3, shows reduced crossing over in the Df3 heterozygote only in the relatively long Lg-A region. Recombination was increased in the adjacent Gl-Lg region. The results suggest that the deficiency may be included in the Lg-A region. Data are needed for

marked regions distal to the deficiency.

Comparisons of the frequencies of complementary crossovers in this same experiment show about a 2:1 ratio for the singles in region 2 and for the doubles in 1 and 2. Since N9Df spores abort, this would be expected if the deficiency (Df) were closer to Lg than to A. The duplication in 9 had no influence on recombination in the normal pair of number 3 chromosomes as shown in cross 2 of Table 3, although the region inserted in 9 was present in quadruplicate. This result is contrary to data from Drosophila in duplications that originated from heterozygous translocations (Rhoades 1931, Dobzhansky 1934). The presence of the duplication in one chromosome greatly reduced the crossing over in the homologous region of the normal pair. The high frequency of pairing between the duplication and its homologue in a different chromosome as seen in salivary chromosomes, may account for the different behavior in Drosophila.

In Rhoades' experiment in corn, the segment of chromosome 3 was inserted in the Bz-Wx region close to Bz, the order being Yg Sh Bz Dp Wx. Plants heterozygous for this duplication showed very little crossing over throughout the short arm of chromosome 9. The values were 1.96 for Yg-Sh and 1.32 for Sh-Wx based on 2280 backcross seeds (normal values 23 and 20 respectively). It is possible that pairing may be more variable in a duplication-bearing arm that is relatively short.

Possible uses

1. Duplications experimentally produced serve, as stated by Muller, to "determine the effects of genic disproportions of all portions of the germ plasm and to compare these with the effects of gene mutations in the same region." Tests should be made for possible physiological effects not associated with gross phenotypic changes. Position effects which might be desirable changes in chemical composition or in physiology might conceivably accompany certain transpositions which result in duplications, or other changes in chromosome structure. Extensive tests made on a great range of varieties and stocks would probably be needed, since results are unpredictable.

2. In species with detailed genetic and cytogenetic information crosses may be planned to produce duplications for known chromosomal segments or for known genes; as waxy, high amylose, and sugary in corn. This may have an advantage over polyploidy in which the entire genome is duplicated.

3. It is also possible to incorporate different alleles in a true-breeding genotype. Irradiation of the 2-row/6-row heterozygote in barley is being used in an attempt to produce a duplication stock that will be VVvv (Beard 1960). Since the Vv genotype in normal diploids yields more than either VV or vv, plants homozygous for the duplication might incorporate this advantage.

4. Duplications and deficiencies may furnish tools for the study of special problems bearing on chromosome behavior, including pairing and crossing over. They may be used either directly or indirectly for the production of special stocks, for example the production of ring chromosomes.

   An example of their direct use might be in a study of the behavior of heteromorphic pairs. An example using a deficient chromosome for this purpose is an experiment set up by Novitski (1951) in Drosophila by using stocks with chromosomes synthesized by Raffel and Muller in which the right and left ends of certain scute inversions in the X chromosome were combined. One of these combinations lacked the long heterochromatic region and was about a third shorter than normal. The resulting X chromosome pair was heteromorphic.

The inclusion of genetic markers enabled Novitski to recognize crossovers and non-crossovers, and to compare the frequencies of complementary classes. For each class of non-crossovers and crossovers, those which received the shorter rod chromosome were in excess, varying from 67 to 75% of the total in that class. For one of the regions, the offspring with the shorter crossover chromosome had three recessive marker genes and were probably less viable than those with the longer crossover chromosome which had only one recessive marker (of the order of 78 to 86% as viable). This viability difference would decrease the frequency of recovery of the shorter crossover chromosome, and thus would be opposite in direction to the observed result. A check experiment involving inversions but without the length difference gave equality of the complementary classes. The results suggest that in oogenesis the two longer chromatids were oriented toward the inner two nuclei (non-functional).

Non-random segregation in maize in the ♀, dependent on the presence of an abnormally long chromosome 10 has been reported by Longley (1945) and Rhoades (1942, 1952). Genes linked and on the knobbed chromosome were recovered in excess, as if the longer chromatids were oriented toward the ends more frequently than expected by chance. The effect seemed to be explainable by the observed formation of additional loci with spindle fiber activity (neo centromeres) in the presence of the abnormal 10 chromosome (Rhoades 1942, 1952). However, Emmerling (1959) has reported that the effect is not always associated with neo-centromere activity. Non-random segregation for other chromosome pairs heterozygous for knobs also occurs when abnormal 10 is present.

5. Duplications appear to be an important factor in evolution (Metz 1947). They are one means of adding gene material.

6. Deficiencies may be used to locate recessive genes: a. By cytological examination of recessives in F_1 from crosses of recessive stocks with irradiated male gametes, or from developing heterozygotes X-rayed immediately after fertilization. b. In higher plants, deficiencies that are transmitted through the ♀, not through the male, can be established as stocks for locating genes also, e.g. the pollen-abortion "genes" in Datura. Their failure to be transmitted through the pollen makes it possible to determine the amount of recombination between a locus and the deficiency. For example, the plant that was deficient for part of the long arm of 6 (Fig. 5B) was used to pollinate yy (white endosperm) plants (yy x $\frac{\text{y deficient}}{\text{Y pl.}}$). There were 534 Y (yellow) : 101 y (white) kernels produced. The white grains (15.9%) must represent crossovers between y and the deficiency (McClintock 1931). The usual value for Y-Pl is 28.

CHAPTER 2. DEFICIENCIES AND DUPLICATIONS

In non-tandem duplications in Neurospora restoration of the normal condition occurs frequently at the interchange point by spontaneous breakage (Perkins et al., 1972).

The recombination value between C and Wx in plants homozygous for the chromosome 3 segment inserted between C and Wx in chromosome 9 was the same, 17%, as that for plants without the insertion. When B chromosomes were present, the value was more than doubled, 37% with 1 B, 40% with 2 B's, and 42% with 3 B's (Rhoades, 1960, 1968). The recombination in Lg-A in 3 in plants homozygous for Df in 3 was not less than in normals (Rhoades, 1966).