A Correlation of Cytological and Genetical Crossing-over in *Zea mays*

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INTRODUCTION (REVISED)

When Alfred Sturtevant created the first genetic map,[1] he hypothesized that genetic recombination resulted from the actual exchange of chromatid fragments. However, at the time there was no hard evidence that proved recombination is accomplished via such a mechanism. The same genetic results could be explained if only alleles are exchanged during recombination, leaving the bulk of the chromatid arm unaffected. Since the two hypotheses make equivalent predictions regarding the distribution of alleles, they cannot be distinguished using purely genetic methods. (Figure I.1)

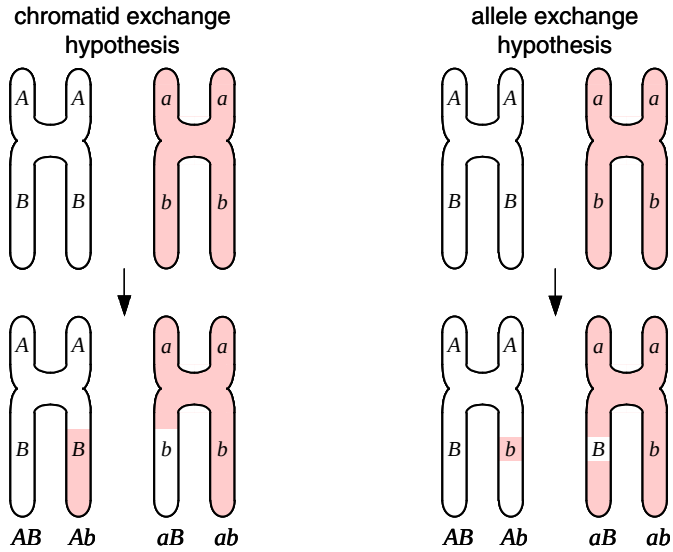

Figure I.1 An illustration of two different cytological explanations of genetic recombination for linked loci. In possibility 1, an actual exchange of entire chromatid fragments is postulated. In possibility 2, only the alleles at a given locus are exchanged. Notice that both hypothesized processes yield gametes that are genetically and cytologically equivalent. (To illustrate the differences between the two possibilities, the two different homologs have been shaded differently in this diagram. In actuality, however, the two different homologs would be physically indistinguishable when viewed under the microscope.)

[1] Sturtevant, Alfred H. 1913. The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. *Journal of Experimental Biology*, 14:43-59. Available from http://www.esp.org.

Attempting to demonstrate that genetic recombination is accomplished via the physical exchange of chromatid arms poses a problem similar to that encountered by Thomas H. Morgan when he first hypothesized that genes might be carried on the X chromosome.[2] Although Morgan's *genetic* hypothesis of X-linkage provided an explanation for the inheritance of the white-eye allele in *Drosophila*, the notion that genes are actually carried on the X chromosome was not proven until Calvin Bridges provided *cytological* evidence to confirm the genetic observations.[3] Bridges established a one-to-one correspondence between the abnormal distribution of eye-color alleles and the abnormal distribution of X chromosomes. That is, he established a relationship between *genetic markers* (the eye color alleles and their associated inheritance patterns) and *cytological markers* (the presence of abnormal sets of sex chromosomes).

Figure I.2 shows how similar logic could be applied to recombination, provided that homologous chromosomes with visually detectable physical differences (*i.e.*, heteromorphic homologous chromosomes) could be obtained for crossing. Notice that this demonstration would actually require three logically distinct steps.

1. Heteromorphic pairs of homologous chromosomes would have to be discovered so that cytological markers could be used for the direct observation of physical exchanges between homologs.
2. Initial crosses involving cytologically marked chromosomes would have to be made to demonstrate that observable exchanges of chromatid arms do in fact occur.
3. Once such physical exchanges were observed, crosses involving chromosomes carrying *both* cytological and genetical markers would have to be made to determine if there is a regular and reliable correlation between the two exchange processes.

Such studies were first carried out on corn by Harriet B. Creighton and Barbara McClintock (this paper) and on *Drosophila* by Curt Stern.[4] Both studies appeared in 1931 and both are now recognized as classic investigations.

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[2] Morgan, Thomas H. 1910. Sex-limited inheritance in Drosophila. *Science*, 32:120-122. Available from http://www.esp.org.

[3] Bridges, Calvin B. 1914. Direct proof through non-disjunction that the sex-linked genes of Drosophila are borne on the X-chromosome. *Science*, NS vol. XL:107-109. Available from http://www.esp.org.

[4] Stern, C. 1931. Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Faktorenaustausches. *Biologische Zentral-blatte*, 51:547-587.

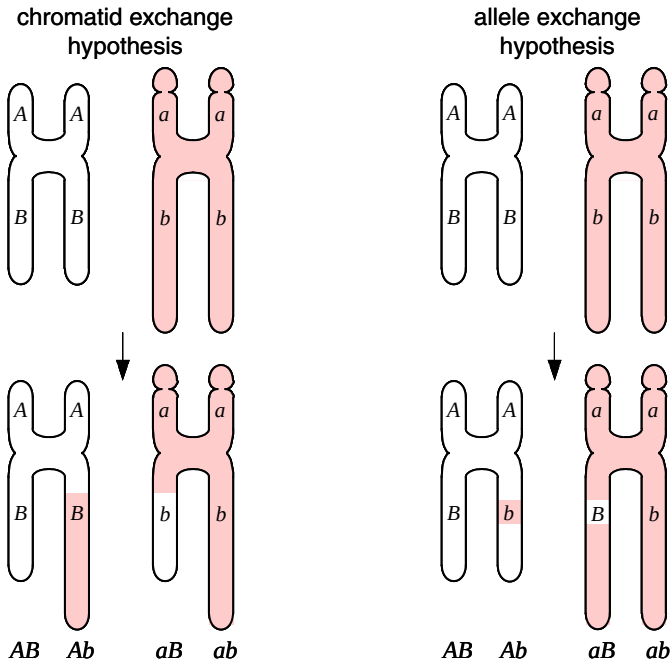


Figure I.2 An illustration of two different cytological explanations of genetic recombination for linked loci carried on heteromorphic chromosomes. Again, the two processes yield gametes that are genetically equivalent. However, because the homologs have observable, physical differences, in this cross the two different possibilities yield gametes that are morphologically distinct.

Creighton and McClintock's Analysis

Creighton and McClintock first obtained a strain of corn with an abnormal chromosome belonging to homologous pair number nine. This chromosome carried a distinct knob at one end and a detectable translocation at the other end. Figure I.3 illustrates the differences between the abnormal chromosome and the normal chromosome number nine.

By crossing corn carrying only the abnormal chromosomes with corn carrying only normal chromosomes they were able to obtain plants with heteromorphic pairs of chromosomes — that is, with one normal and one abnormal chromosome number nine. Then, they carried out crosses involving plants with heteromorphic chromosome pairs so that they could look for the occurrence of cytological recombination. Table

1 in their paper (page 3) presents results that show visible recombination occurring in 39.6% of the progeny in these crosses.

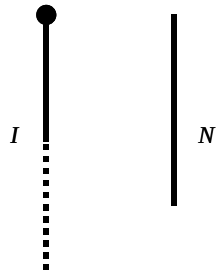
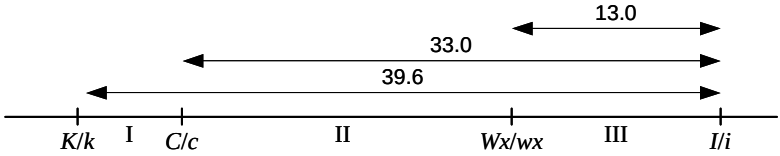


Figure I.3 A diagrammatic representation of the differences between an abnormal, interchange chromosome (*I*) and a normal chromosome (*N*). The interchange chromosome possesses visible abnormalities at both ends — a knob at the top and a translocated extension at the bottom.

Having demonstrated that physical exchange between chromatids did in fact occur, Creighton and McClintock proceeded to establish a relationship between the process of physical exchange and the occurrence of genetic recombination. First, they mapped two loci that occurred on chromosome number nine between the knob and the translocation. One of these loci carried alleles that produce either colorless (*c*) or colored (*C*) aleurone. The other locus carried alleles that produce either waxy (*wx*) or starchy (*Wx*) endosperm. The overall map relationships (Table 2, p.5, and from a previous publication) were as follows.



Here *K* = presence of knob, *k* = normal; *I* = presence of interchange (translocation); *i* = normal.

At this point, note that two possibilities exist: (1) genetic and cytological recombination might be accomplished by the same process or (2) genetic and cytological recombination might be accomplished via different processes. Because these two hypotheses lead to different quantitative predictions regarding the relative frequency of different recombinant-type gametes, the two hypotheses may be distinguished by assaying the gametic output of an individual heterozygous for the two

loci and heteromorphic for the knob and the translocation. The different quantitative predictions may be derived as follows.

1. If both genetic and cytological recombination are accomplished by the same event, then a single recombination event in either region I or region III would yield gametes that are recombinant for the heteromorphologies but parental for the two loci. However, a single recombination event in region II would yield gametes that are recombinant for the loci and also for the heteromorphologies. Thus, given the map distances as established and assuming that few multiple-crossover progeny will be obtained, the following specific predictions may be made. (a) Nearly all of the progeny that are recombinant for the two loci should also be recombinant for the heteromorphologies. (b) Approximately half of the progeny that are recombinant for the heteromorphologies should also be recombinant for the two loci. (c) Few, if any, progeny should occur that are recombinant for the two loci but nonrecombinant for the heteromorphologies.
2. On the other hand, if genetic and cytological recombination are accomplished via separate (and thus presumably independent) processes, then the expected frequencies for the different recombinant classes can be calculated using the product rules of probability. Given the map distances, for the two loci the probability of obtaining recombinant progeny is .20 and the probability of nonrecombinant progeny is .80. For the heteromorphologies the probabilities are .39 recombinant and .61 nonrecombinant. If the two processes are independent, the joint probabilities for the loci and the heteromorphologies may be calculated by multiplying together the appropriate single probabilities, resulting in the following predictions. (a) Only 39% of the progeny that are recombinant for the two loci should also be recombinant for the heteromorphologies. (b) Approximately 20% of the progeny that are recombinant for the heteromorphologies should also be recombinant for the two loci. (c) Approximately 61% of the progeny should occur that are recombinant for the two loci but nonrecombinant for the heteromorphologies.

To perform the necessary gametic assay, Creighton and McClintock peformed the following cross

$$K\text{-}C\text{-}wx\text{-}I \;/\; k\text{-}c\text{-}Wx\text{-}i \;\times\; k\text{-}c\text{-}Wx\text{-}i \;/\; k\text{-}c\text{-}wx\text{-}i$$

and obtained 27 progeny. In keeping with the discussion above, we present their results by first scoring each progeny as being recombinant or parental (*i.e.*, nonrecombinant) for the loci and for the hetero

morphologies. Not all of the progeny could be scored unambiguously as parental or recombinant due to the complications associated with the presence of the dominant *Wx* allele in the second parent and to the difficulties of carrying out a full analysis for some of the progeny. However, enough of the progeny can be unambiguously scored for both the loci and the heteromorphologies to allow a determination between the two hypotheses. The results of this scoring are given in Table I.1.[5]

Table I.1 Results of Creighton and McClintock's cross involving an individual carrying doubly heteromorphic chromosomes with two intervening heterozygous loci. Each of the 27 progeny are scored as being recombinant (rec) or parental (par) for the loci and for the heteromophologies. Individual progeny that could not be unambiguously scored are indicated with "?".

		heteromorphologies			
		par	rec	?	
genetic markers	par	8	2	0	10
	rec	0	3	1	4
	?	6	6	1	13
		14	11	2	27

As can be seen, the results of the experiment match almost exactly with the predictions of the hypothesis that both genetic and physical recombination are accomplished via the same event. Specifically, (a) all (three out of three) of the progeny that were recombinant for the loci were also recombinant for the heteromorphologies; (b) approximately half (three out of five) of the progeny that were recombinant for the heteromorphologies were also be recombinant for the two loci; (c) no progeny occurred that were recombinant for the two loci but nonrecombinant for the heteromorphologies. Thus, Creighton and McClintock concluded their paper by noting,

> The foregoing evidence points to the fact that cytological crossing-over occurs and is accompanied by the expected type of genetic crossing-over.

[5] Given the difficulties associated with the occurrence of the dominant *Wx* allele in the tester individual, one might wonder why the authors would choose to make such a cross. The answer is simply this: no plant with the *k-c-wx-i*/*k-c-wx-i* genotype was available and it would have taken at least a year to generate such a plant. As is often the case with breakthrough papers, part of the critical skill of the authors lies in their ability to conduct crucial science with less than perfect material.

Although the results did strongly suggest that genetic and cytological recombination were produced by the same underlying physical process, the limited nature of the data set (of the 27 progeny studied, only 13 could be unambiguously assigned parental or recombinant status for both genetic and cytological markers) caused some contemporaneous writers[6] to express reservations about the results, which in turn led Creighton and McClintock to publish another short note containing additional, corroborating findings.[7]

If this paper is read in isolation, the authors' discussion of their results can, at times, be difficult to follow. When this paper was originally published, however, it was accompanied by another paper[8] that immediately preceded it in the journal and that provided a more detailed discussion of the genes and chromosomal abnormalities used in the study. The two papers should be read together, with the first, descriptive paper serving as a critical and necessary introduction to the second, experimental work.

For another discussion of this important work, see Edward Coe and Lee B. Kass (2005) Proof of physical exchange of genes on the chromosomes. *Proceedings of the National Academy of Sciences, USA*. 102:6641-6646.

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Seattle, Washington 2018

6 Brink, R. A. and Cooper, D. C. 1935. A Proof That Crossing Over Involves an Exchange of Segments Between Homologous Chromosomes. *Genetics*, 20: 22-35.

7 Creighton, Harriet B., and McClintock, Barbara. 1935. A correlation of cytological and genetical crossing-over in Zea mays. A Corroboration. *PNAS*, 21:148-150. Available from http://www.esp.org.

8 McClintock, Barbara. 1931. The Order of the Genes *C*, *Sh* and *Wx* in *Zea Mays* with Reference to a Cytologically Known Point in the Chromosome. *PNAS* August 1, 1931. 17:485-491. Available from http://www.esp.org.

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A CORRELATION OF CYTOLOGICAL AND GENETICAL CROSSING-OVER IN *ZEA MAYS*[1]

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A REQUIREMENT FOR THE GENETICAL STUDY OF CROSSING-OVER is the heterozygous condition of two allelomorphic factors in the same linkage group. The analysis of the behavior of homologous or partially homologous chromosomes, which are morphologically distinguishable at two points, should show evidence of cytological crossing-over. It is the aim of the present paper to show that cytological crossing-over occurs and that it is accompanied by genetical crossing-over.

In a certain strain of maize the second smallest chromosome (chromosome 9) possesses a conspicuous knob at the end of the short arm. Its distribution through successive generations is similar to that of a gene. If a plant possessing knobs at the ends of both of its 2nd-smallest chromosomes is crossed to a plant with no knobs, cytological observations show that in the resulting F_1 individuals only one member of the homologous pair possesses a knob. When such an individual is

1 The authors wish to express appreciation to Dr. L. W. Sharp for aid in the revision of the manuscripts of this and the preceding paper. They are indebted to Dr. C. R. Burnham for furnishing unpublished data and for some of the material studied.

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back-crossed to one having no knob on either chromosome, half of the offspring are heterozygous for the knob and half possess no knob at all. The knob, therefore, is a constant feature of the chromosome possessing it. When present on one chromosome and not on its homologue, the knob renders the chromosome pair visibly heteromorphic.

In a previous report[3] it was shown that in a certain strain of maize an interchange had taken place between chromosomes 8 and 9. The interchanged pieces were unequal in size; the long arm of chromosome 9 was increased in relative length, whereas the long arm of chromosome 8 was correspondingly shortened. When a gamete possessing these two interchanged chromosomes meets a gamete containing a normal chromosome set, meiosis in the resulting individual is characterized by a side-by-side synapsis of homologous parts. Therefore, it should be possible to have crossing-over between the knob and the interchange point.

In the previous report it was also shown that in such an individual the only functioning gametes are those which possess either the two normal chromosomes *(N,n)* or the two interchanged chromosome *(I,i)*, i.e., the full genom in one or the other arrangement. The functional gametes therefore possess either the shorter, normal, knobbed chromosome *(n)* or the longer, interchanged, knobbed chromosome *(I)*. Hence, when such a plant is crossed to a plant possessing the normal chromosome complement, the presence of the normal chromosome in functioning gametes of the former will be indicated by the appearance of ten bivalents in the prophase of meiosis of the resulting individuals. The presence of the interchanged chromosome in other gametes will be indicated in other F_1 individuals by the appearance of eight bivalents plus a ring of four chromosomes in the late prophase of meiosis.

If a gamete possessing a normal chromosome number 9 with no knob, meets a gamete possessing an interchanged chromosome with a knob, it is clear that these two chromosomes which synapse along their homologous parts during prophase of meiosis in the resulting individual are visibly different at each of their two ends. If no crossing-over occurs, the gametes formed by such an individual will contain either the knobbed, interchanged chromosome (*a*, Fig. 1) or the normal chromosome without a knob (*d*, Fig. 1). Gametes containing either a knobbed normal chromosome (*c*, Fig. 1) or a knobless, interchanged chromosome (*b*, Fig. 1) will be formed as a result of crossing-over. If such an individual is crossed to a plant possessing two normal knobless chromosomes, the resulting individuals will be of four kinds. The non-

[3] McClintock, B., *Proc. Nat. Acad. Sci.* 16:791-796 (1930).

crossover gametes would give rise to individuals which show either (1) ten bivalents at prophase of meiosis and no knob on chromosome 9, indicating that a gamete with a chromosome of type *d* has functioned or (2) a ring of four chromosomes with a single conspicuous knob, indicating that a gamete of type *a* has functioned. The crossover types will be recognizable as individuals which possess either (1) ten bivalents and a single knob associated with bivalent chromosome 9 or (2) a ring of four chromosomes with no knob, indicating that crossover gametes of types *c* and *b*, respectively, have functioned. The results of such a cross are given in culture 337, table 1. Similarly, if such a plant is crossed to a normal plant possessing knobs at the ends of both number 9 chromosomes and if crossing-over occurs, the resulting individuals should be of four kinds. The non-crossover types would be represented by (1) plants homozygous for the knob and possessing the interchanged chromosome and (2) plants heterozygous for the knob and possessing two normal chromosomes. The functioning of gametes which had been produced as the result of crossing-over between the knob and the interchange would give rise to (1) individuals heterozygous for the knob and possessing the interchanged chromosome and (2) those homozygous for the knob and possessing two normal chromosomes. The results of such crosses are given in cultures A125 and 340, table 1. Although the data are few, they are consistent. The amount of crossing-over between the knob and the interchange, as measured from these data, is approximately 39%.

Table 1

$\frac{\text{Knob-interchanged}}{\text{Knobless-normal}}$		×	Knobless-normal, culture 337 and knobbed-normal cultures A125 and 340	
	Plants possessing 2 normal chromosomes		Plants possessing an interchanged chromosome	
Culture	Non-crossovers	Crossovers	Non-crossovers	Crossovers
337	8	3	6	2
A125	39	31	36	23
340	5	3	5	3
Totals	52	37	47	28

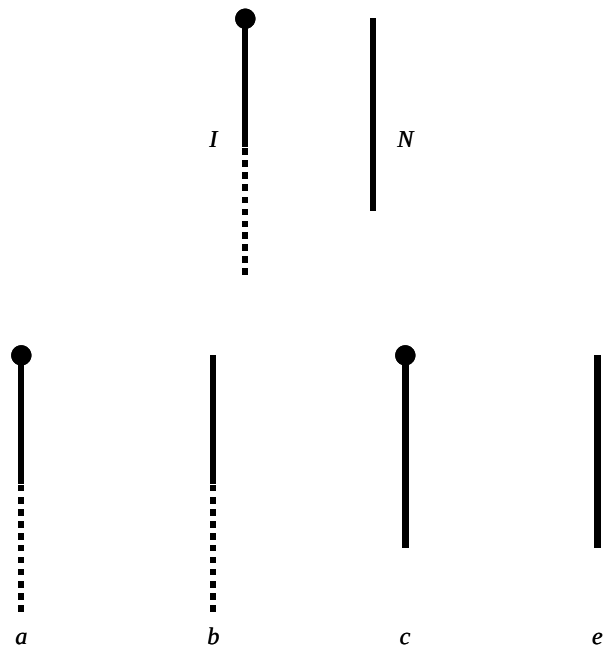

Figure 1. *Above*, Diagram of the chromosomes in which crossing-over was studied. *I*, Interchange chromosome; *N*, normal chromospome. *Below*, Diagram of chromosome types found in gametes of a plant with the constitution shown above. *a* – Knobbed, interchanged chromosome; *b* – Knobless, interchanged chromosome, *c* – Knobbed, normal chromosome; *d* – Knobless, normal chromosome. *a* and *d* are non-crossover types. *b* and *c* are crossover types.

In the preceding paper it was shown that the knobbed chromosome carries the genes for colored aleurone (*C*), shrunken endosperm (*sh*) and waxy endosperm (*wx*). Furthermore, it was shown that the order of these genes, beginning at the interchange point is *wx*-*sh*-*c*. It is possible, also, that these genes all lie in the short arm of the knobbed chromosome. Therefore, a linkage between the knob and these genes is to be expected.

One chromosome number 9 in a plant possessing the normal complement had a knob and carried the genes *C* and *wx*. Its homologue was knobless and carried the genes *c* and *Wx*. The non-crossover gametes should contain a knobbed-*C*-*wx* or a knobless-*c*-*Wx* chromosome. Crossing-over in region I (between the knob and *C*) would give rise to knobless-*C*-*wx* and knobbed-*c*-*Wx* chromosomes. Crossing-over in region 2 (between *C* and *wx*) would give rise to knobbed-*C*-*Wx* and knobless-*c*-*wx* chromosomes. The results of

crossing such a plant to a knobless-*c*-*wx* type are given in table 2. It would be expected on the basis of interference that the knob and *C* would remain together when a crossover occurred between *C* and *wx*; hence, the individuals arising from colored starchy (*C*-*Wx*) kernels should possess a knob, whereas those coming from colorless, waxy (*c*-*wx*) kernels should be knobless. Although the data are few they are convincing. It is obvious that there is a fairly close association between the knob and *C*.

Table 2

Knob-*C*-*wx* / Knobless-*c*-*Wx* × Knobless-*c*-*wx*							
C-*wx*		*c*-*Wx*		*C*-*Wx*		*c*-*wx*	
Knob	Knobless	Knob	Knobless	Knob	Knobless	Knob	Knobless
12	5	5	34	4	0	0	3

To obtain a correlation between cytological and genetic crossing-over it is necessary to have a plant heteromorphic for the knob, the genes *c* and *wx* and the interchange. Plant 338 (17) possessed in one chromosome the knob, the genes *C* and *wx* and the interchanged piece of chromosome 8. The other chromosome was normal, knobless and contained the genes *c* and *Wx*. This plant was crossed to an individual possessing two normal, knobless chromosomes with the genes *c*-*Wx* and *c*-*wx*, respectively. This cross is diagrammed as follows:

The results of the cross are given in table 3. In this case all the colored kernels gave rise to individuals possessing a knob, whereas all the colorless kernels gave rise to individuals showing no knob.

The amount of crossing-over between the knob and the interchange point is approximately 39% (table 1), between *c* and the interchange approximately 33%, between *wx* and the interchange, 13% (preceding paper). With this information in mind it is possible to analyze the data given in table 3. The data are necessarily few since the ear contained but few kernels. The three individuals in class I are clearly non-crossover types. In class II the individuals have resulted from a

crossover in region 2, i.e., between *c* and *wx*. In this case a crossover in region 2 has not been accompanied by a crossover in region I (between the knob and *C*) or region 3 (between *wx* and the interchange). All the individuals in class III had normal chromosomes. Unfortunately, pollen was obtained from only I of the 6 individuals examined for the presence of the knob. This one individual was clearly of the type expected to come from a gamete produced through crossing-over in region 2. Class IV is more difficult to analyze. Plants 6, 9, 10, 13, and 14 are normal and *WxWx*; they therefore represent non-crossover types. An equal number of non-crossover types are expected among the normal *Wxwx* class. Plants 1, 2, 4, 11 and 12 may be of this type. It is possible but improbable that they have arisen through the union of a *c-Wx* gamete with a gamete resulting from a double crossover in region 2 and 3. Plants 5 and 8 are single crossovers in region 3, whereas plants 3 and 7 probably represent single crossovers in region 2 or 3.

The foregoing evidence points to the fact that cytological crossing-over occurs and is accompanied by the expected types of genetic crossing-over.

CONCLUSIONS

Pairing chromosomes, heteromorphic in two regions, have been shown to exchange parts at the same time they exchange genes assigned to these regions.

Table 3

Knob-*C*-*wx*-interchanged / Knobless-*c*-*Wx*-normal	×	Knobless-*c*-*Wx*-normal / Knobless-*c*-*wx*-normal	
Plant number	Knobbed or knobless	Interchanged or normal	
Class I, *C*-*wx* kernels			
1	Knob	Interchanged	
2	Knob	Interchanged	
3	Knob	Interchanged	
Class II, *c*-*wx* kernels			
1	Knobless	Interchanged	
2	Knobless	Interchanged	
Class III, *C*-*Wx* kernels			*Pollen*
1	Knob	Normal	*WxWx*
2	Knob	Normal	. . . . .
3	. . . . .	Normal	*WxWx*
5	Knob	Normal	. . . . .
6	Knob	. . . . .	. . . . .
7	Knob	Normal	. . . . .
8	Knob	Normal	. . . . .
Class IV, *c*-*Wx* kernels			
1	Knobless	Normal	*Wxwx*
2	Knobless	Normal	*Wxwx*
3	Knobless	Interchanged	*Wxwx*
4	Knobless	Normal	*Wxwx*
5	Knobless	Interchanged	*WxWx*
6	Knobless	Normal	*WxWx*
7	Knobless	Interchanged	*Wxwx*
8	Knobless	Interchanged	*WxWx*
9	Knobless	Normal	*WxWx*
10	Knobless	Normal	*WxWx*
11	Knobless	Normal	*Wxwx*
12	Knobless	Normal	*Wxwx*
13	Knobless	Normal	*WxWx*
14	Knobless	Normal	*WxWx*
15	Knobless	Normal	*Wx*–