THE ORDER OF THE GENES C, Sh AND Wx IN ZEA MAYS WITH REFERENCE TO A CYTOLOGICALLY KNOWN POINT IN THE CHROMOSOME

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The monoploid complement in *Zea mays* is composed of ten morphologically distinguishable chromosomes.[1] By means of trisomic inheritance it has been possible to associate certain linkage groups with particular members of the complement.[2] Thus the linkage group involving the genes *C* (colored aleurone), *sh* (shrunken endosperm) and *wx* (waxy starch) has been associated with the next to the smallest chromosome (chromosome 9) of the monoploid complement. It is the purpose of this paper to present evidence indicating the serial order of these genes with reference to a known point in the chromosome.

In a previous report[3] it was shown that in a certain strain of maize a segmental interchange had occurred between chromosomes 8 and 9. The diagram in figure 1 is similar to the one accompanying that report. By means of the close synaptic attraction of the homologous parts of chromosomes in early meiotic prophase it has been possible to determine the region in each chromosome at which the interchange occurred, and to recognize this region in the sporocytes in plants with the chromosome complex shown in *c*, figure 1. The conspicuous knob terminating the short arm of chromosome 9 (*n* in the diagram) is found only in certain maize cultures. However, in these cultures it is a constant feature of this chromosome. Evidence presented in this paper indicates that the loci of the genes *wx*, *sh* and *c* lie in the region between the interchange point and the knob, *wx* being nearest the interchange and *c* farthest from it.

Plants which are heterozygous for the interchange possess the chromosomes *nNIi* (see diagram) and show approximately 50% sterility in pollen and eggs.[4] At diakinesis the interchange complex appears as a ring of four chromosomes (*d*, Fig. 1). The four members of this ring distribute 2-by-2 at anaphase. Since in half of the sporocytes any two adjacent chromosomes in the ring go to the same pole, forming sterile combinations, and in the other half of the sporocytes and the adjacent members go to opposite poles, forming fertile combinations, six types of spores are formed, only two of which are viable: those containing *n* with *N* and those containing *I* with *i*. Occasionally the distribution at anaphase is such that 11 chromosomes, including 3 members of the ring, go to one pole and 9 to the other. In this manner viable 11-chromosome gametes are formed. Such gametes could possess the following chromosomes: (1) *iIN*, (2) *iIn*, (3) *nNI*, (4) *nNi*.

Through fusion of these 11-chromosome gametes with normal gametes four types of 21-chromosome plants can be obtained. The types of chromosome configurations seen at diakinesis in the resulting 21-chromosome individuals enable one to determine which of the four types of 11-chromosome gamete has functioned in any particular case. Experience has shown that fusion of a normal gamete with one of type (1) or type (2) will produce an individual which is approximately 25–30% sterile;[4] where-

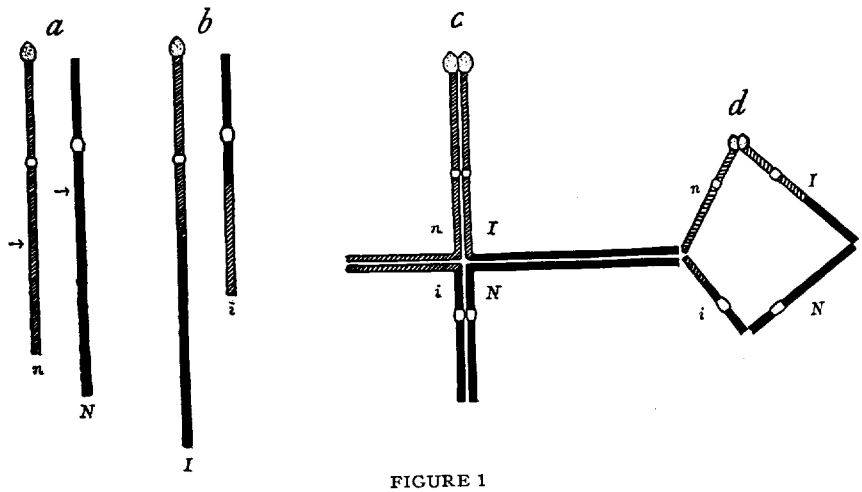

FIGURE 1

a.—Diagram of the two normal chromosomes which were involved in the segmental interchange. The clear portions in the chromosomes represent the spindle fibre attachment regions. The smaller chromosome terminated in an enlarged, deeply staining knob. The arrows indicate the places in the chromosome at which the interchange occurred to produce the situation shown in *b*. *b*.—The two chromosomes produced as the result of the segmental interchange. *c*.—The type of synaptic complex in midprophase of meiosis obtained by combining a normal chromosome complement with an interchange complement through crossing. *N*, larger normal chromosome; *n*, smaller normal chromosome; *I*, larger interchange chromosome; *i*, smaller interchange chromosome. *d*.—The ring formed at diakinesis by the opening out of chromosomes from the complex shown in *c*. (Chromosomes labeled as in C.)

as, fusion of a normal gamete with a gamete of type (3) or type (4) will produce an individual which is approximately 18% sterile. In the former cases the chromosome configuration at diakinesis is frequently a closed ring of four chromosomes with the extra chromosome attached. In the latter cases the configuration is not a ring, but consists either of a "chain" of five chromosomes, a bivalent plus a trivalent or, infrequently, 2 bivalents plus a univalent. The "chain" is composed of two bivalent chromosomes, bivalent 8 (*N*) and bivalent 9 (*n*), held together by the extra chromosome (*I* or *i*) which is synapsed at one end with 8 (*N*) and at the other end with 9

(*n*) (Fig. 2). Opening out of the members of the bivalents in late prophase produces a chain-like structure.

Plant 270_{11} belonged to the low sterile class. This plant was suspected of having the formula *nnNNI* since it was noted that in those sporocytes which showed a trivalent instead of a chain of five chromosomes, the trivalent always consisted of one long and two small chromosomes. Since no knob was present at the end of either the *n* or the *I* chromosome it was impossible to be certain from such observations. Pollen counts from several anthers indicated that the plant was approximately 18% sterile. If this plant had the formula *nnNNI*, four types of functional gametes would be produced: (1) *nN*, (2) *nNN*, (3) *nnN*, (4) *nNI*. Since 11-chromosome pollen grains seldom function in competition with grains containing only the monoploid complement, selfing such a plant would produce four types of individuals. All the 20-chromosome individuals would be ordinary normals. Of the 21-chromosome individuals there would be three kinds. The functioning of gametes of types (2) and (3) would produce individuals trisomic for chromosomes 8 (*N*) and 9 (*n*), respectively. The diakinetic configuration shown by such individuals would be nine bivalents plus a trivalent or ten bivalents plus a univalent. No sterility above that found in normal diploid individuals (approximately 2–5%) would be observed in pollen or eggs. The functioning of type (4) would produce an individual of the same chromosome constitution as the parent (*nnNNI*). It would show a chain of five chromosomes at diakinesis and exhibit 18% sterility in pollen and eggs.

Twenty-two individuals resulting from selfing plant 270_{11} were grown and examined cytologically. Eight of these were 20-chromosome individuals. They were ordinary diploids, showed no sterility and gave disomic ratios for *c*, *sh* and *wx*. Twelve of the thirteen 21-chromosome individuals must have come from gametes of type (4) since they showed 18% sterility in pollen and eggs. Cytological observations indicated that the remaining 21-chromosome individual was trisomic for chromosome 8 (*N*) and therefore must have involved a gamete of type (2). It was non-sterile and gave disomic inheritance for the genes *c*, *sh* and *wx*.

On the basis of the appearance of the chromosomes at diakinesis the assumption has been made that the formula for the parent plant and all its partially-sterile offspring was *nnNNI*. To test this assumption several of the partially sterile offspring were crossed to ordinary normal plants whose number 9 (*n*) chromosomes terminated in conspicuous knobs. Chromosome counts were obtained from nineteen individuals resulting from such a cross. There were nine 20-chromosome individuals, all normals. Among the ten 21-chromosome individuals seven were partially sterile like the female parent and three were non-sterile. Two of the three non-sterile 21-chromosome individuals were examined and were found to be

trisomic for chromosome 8 (N). Among the partially sterile plants two were examined for chromosome configurations at diakinesis. In each of them a chain of five chromosomes was observed. If the extra chromosome in these 21-chromosome plants were the long interchange chromosome (I), the knob should be found only in two positions.* These positions are illustrated in figure 2.

The conspicuous knob allows the configuration to be easily interpreted. In every sporocyte in which a chain of five chromosomes appeared the knob was found in one or the other of the positions illustrated. In some sporocytes a trivalent plus a bivalent appeared instead of a chain of five. The presence of the knob on one of the n chromosomes makes it possible to state that such a trivalent is composed either of nnI or NNI. This evi-

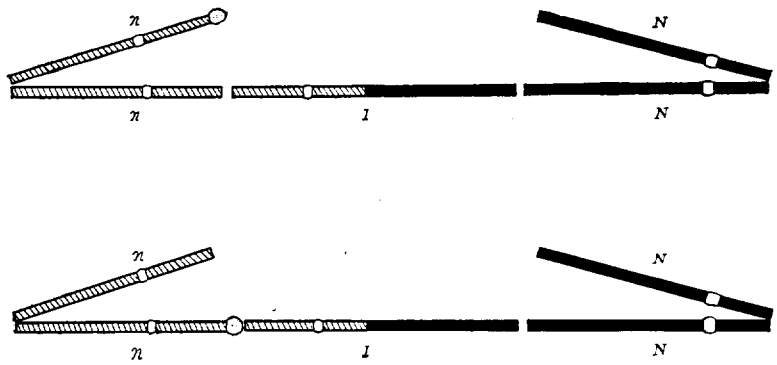

FIGURE 2

dence makes it certain that the extra chromosome in the complement of these sterile plants is the long interchange chromosome (I).

The configuration which would be seen if the short interchanged chromosome (i) were present would be a chain of five chromosomes, also. However, the long arm of chromosome 9 would be associated with the interchanged chromosome and so the knob would be where the two n chromosomes join. No such case was seen.

Chromosome I possesses nearly two-thirds of that chromosome (number 9) which carries the genes of the *c-sh-wx* linkage group. It is highly probable, therefore, that the loci of some of the known genes of this linkage group lie in this portion of the chromosome. Crossover data have shown that the locus of the gene *sh* lies between that of *c* and *wx*. The map distance between *c* and *sh* is 3.3, that between *sh* and *wx*, 20.3.[5] Unpublished data which Dr. C. R. Burnham has generously allowed me to use have shown that the percentage of crossing-over between *wx* and the interchange point is approximately 13 per cent, between *sh* and the interchange

approximately 32 per cent, between *c* and the interchange, approximately 33 per cent. If it could be shown that the long interchange chromosome (*I*) carries the genes *wx*, *sh* and *c* or their allelomorphs, their order with reference to the interchange point could be established. The following evidence is presented in support of this claim.

TABLE 1

RATIOS OBTAINED FROM SELFING PLANT 270_{11}

COLORED				COLORLESS			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
11	8	1	0	25	5	9	2

Plant 270_{11} was heterozygous for the genes *c*, *sh* and *wx* (table 1). Among the 21-chromosome, partially sterile individuals which came from the colored, non-shrunken, non-waxy kernels of 270_{11}, three were utilized for genetic investigations. These plants were numbered $388A_1$, $388D_1$ and $388D_2$. All had the formula *nnNNI*.

The pollen of plant $388A_1$ was examined for the waxy ratio and sterility (table 2). The waxy ratio in the pollen of this plant was approximately 15 *Wx*:1 *wx*. This is in contrast to the 1 *Wx*:1 *wx* ratio found among diploid individuals or individuals which are trisomic for some chromosome other than that which carries the genes of the *c-sh-wx* linkage group. In most anthers the sterility approximates 18%. For reasons as yet unknown, certain anthers show a considerably lower or higher percentage of sterility. When this pollen was used on a plant homozygous for *c*, *sh* and *wx* the following ratio was obtained:

296_9 ♀ × $388A_1$ ♂

C				*c*			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
27	22	3	0	0	0	172	5**

When crossed to a plant heterozygous for *c* but homozygous for *sh* and *wx* the following resulted:

$389C_1$ ♀ × $388A_1$ ♂

C				*c*			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
41	26	124	6	0	0	119	5**

In examining these data it should be remembered (1) that the functional gametes formed by plant $388A_1$ contain *nN*, *NNn*, *nnN* or *nNI* in varying

proportions according to the mode of distribution at meiosis (page 487), (2) that the spores with *nI* or *NI* abort and are the primary cause of the observed sterility and (3) that the 11-chromosome pollen grains do not function in competition with those containing the monoploid set. The ratio for waxy in the pollen and the ratios and linkage relations of the genes *c*, *sh* and *wx* in the backcross are distinctly abnormal. The ratios are explainable on the assumption that the interchange chromosome (*I*) has that portion of chromosome 9 which involves the loci of the genes *c*, *sh* and *wx*. Accordingly, it can be stated that plant $388A_1$ possessed two *n* chromosomes each with the genes *c-sh-Wx* and an interchange chromosome (*I*) with the genes *C-Sh-wx*.

The genic constitution of the two *n* chromosomes in plant $388D_1$ were *c-sh-wx* and *C-sh-Wx*, respectively; chromosome *I* contained the genes *C-Sh-wx*. This is clear from the following cross to the triple recessive:

296_{13} ♀ × $388D_1$ ♂

C				*c*			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
11	21	72	11	0	1	8	79

That one *n* chromosome of plant $388D_2$ contained the genes *c-sh-Wx*, the other *n* chromosome *C-Sh-Wx* and the long interchange chromosome (*I*) the genes *C-Sh-wx* can be seen from the following crosses:

$388D_2$ ♀ × 390_3 (homozygous *c-sh-wx*) ♂

C				*c*			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
108	3	3	0	1	0	38	0

296_{22}(homozygous *c-sh-wx*) ♀ × $388D_2$ ♂

C				*c*			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
194	34	2	0	5	0	130	4**

296_{21}(homozygous *c-sh-wx*) ♀ × $388D_2$ ♂

C				*c*			
Sh		*sh*		*Sh*		*sh*	
Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*
197	18	3	2	3	0	150	5**

The above results point to the fact that the loci for the genes *c*, *sh* and *wx* lie in that part of the long interchange chromosome (*I*) contributed by chromosome 9. That the genes lie in the order *wx-sh-c* beginning at the interchange point is indicated by the fact that *wx* shows 13%, *sh* 32% and *c* 33% crossing over from the interchange point. It is not known on which side of the spindle-fibre insertion region these genes lie. It is possible, however, that they may like in the short arm, between the insertion region and the knob, since evidence presented by Stadler[6] suggests that the gene *wx* lies closer to the spindle-fibre insertion region than the genes *sh* or *c*.

TABLE 2

POLLEN COUNTS FROM INDIVIDUAL ANTHERS OF PLANT 388 A_1

Wx	*wx*	ABORTIVE	PER CENT ABORTIVE	PER CENT *wx*
991	59	129	10.9	5.6
2029	142	937	30.1	6.5
632	48	145	17.5	7.0
701	55	164	18.0	7.2
1035	80	240	17.7	7.1
1415	69	347	18.9	4.7

The author wishes to state that no attempt has been made to discuss in detail the data herein presented. It was desired to present briefly the evidence at this time, since it lends valuable support to the argument in the paper which follows.

* The position of the knob resulting from crossing-over will be described in a later paper.

** Among the plants of the *c-sh-wx* cultures four or five silks were exserted before the ear shoot appeared above the leaf sheath and was bagged. It is possible that the high number of *c-sh-wx* kernels mostly represent self-pollinations rather than double crossovers or crossovers involving all three chromosomes. Similar crosses are being repeated.

[1] McClintock, B., *Sci.*, **69**, 629 (1929).

[2] McClintock, K. B., and Henry E. Hill, *Genetics*, **16**, 175–190 (1931).

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

[4] Burnham, C. R., *Ibid.*, **16**, 269–277 (1930).

[5] Mimeographed pamphlet on linkage in maize. Cornell University.

[6] Stadler, L. J., *Sci. Agr.*, **11**, 557–572 (1931).