November 8, 1948

Dr. Joshua Lederberg
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Dear Lederberg:

I hope that you will forgive this delay in answering your letter of October 4th. When your letter came, I was recuperating from an illness that began in September. Almost no mental activity went on for some weeks but I am back to normal now. It was a disappointment to have missed your talk in New York. My father met wth an accident and on that day was taken to the hospital for an operation. I remained with my family much of the day.

The enclosed sheets represent the analysis I worked out Saturday night and yesterday to interprete your results as I understand them from your letter. Although it may not be the correct or even desired solution, still it does work according to the understanding I have and the assumptions given in the analysis. It calls for nothing very special in the manner of chromosomal or genetic behavior. There is nothing in the maize work like it that I could use as a model so I can not refer to any work there.

Thank you for writing me about this work. I am thoroughly interested in your investigations and find them stimulating. All good luck with this work.

Sincerely yours,

Barbara McClintock

General Considerations

I. Origin of aberrant prototroph:

1. Cross of parents with different combinations of factors of B_1 M B Mal_1 Lac V_1 T L.

2. Single aberrant prototroph from this cross.

II. Behavior of aberrant prototroph:

1. This prototroph is some form of a heterozygote as it appears to be heterozygous for some of the factors in the cross.

2. This is shown by segregants arising from prototroph which are crossover types.

3. The type of crossover chromatids recovered in the segregants are not the expected ones in that many unusual "multiple crossover" chromatids are represented.

4. These segregants are stable and do not throw other segregants.

III. Behavior of segregants in crosses:

1. Crossed to standard stocks, the segregants may yield prototrophs, some of which are heterozygous for some of the factors involved. Thus, these segregants may be heterozygous producers.

2. The heterozygous prototrophs are hemizygous for a specific region of the chromosome including the Mal locus and loci adjacent to it.

3. These heterozygous prototrophs segregate stable forms that ~~show~~ are crossover recombinants.

4. The types of recombinants differ from the usual recombinants coming from crosses of standard stocks in that there is a predominant class of segregants and few of other types. The predominant class obtained depends upon:

   (1) The factors introduced into the cross.

   (2) The contrasting combinations of these factors in the parents.

   (3) The particular heterozygote chosen for a study of its segregants following one kind of cross.

IV. Questions that require an answer:

1. Was the chromosome constitution of one of the parents giving rise to the original aberrant prototroph altered in some way? Can this alteration start the chain of events leading to the cycle outlined above?

2. Why is the prototroph heterozygous for some factors introduced into the cross (and homozygous for others)?

3. Why are the heterozygotes hemizygous for one specific region of one chromosome?

4. Why do the heterozygotes give segregants that are stable?

5. Why are the proportions of the various recombinants so different from those given by normal zygotes?

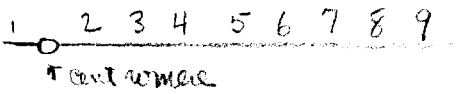
   Why are these biased in favor of certain recombinations depending upon the heterozygote itself?

   Why do the "elusive multiple mutants" appear in these segregants?

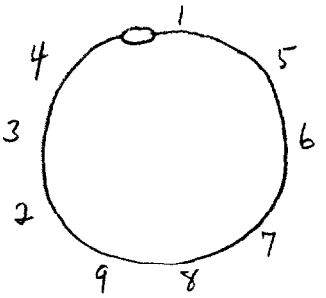
6. Why are these stable segregants "heterozygous" producers?

Working Hypothesis.

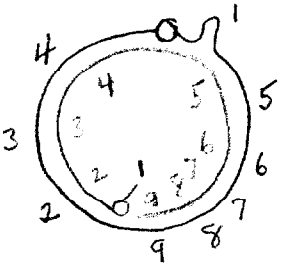
1. Assumption taken for organization of chromosome in K-12 stock:



2, In the original cross, one of the parents had this chromosome in the form of a ring following an aberration that also rearranged segments of the chromosome. For the purposes of this description, I have selected one kind of rearrangement. The type required would depend on an analysis of the data, not available to me. The nature of the normal chromosome would also need clarification, depending on the data. For the purposes of illustrating the general thesis of reasoning in the working hypothesis, the selection is quite arbitrary. Thus, the ring chromosome and the organization of its parts are:



3. Synapsis of this ring chromosome with a normal chromosome would give:

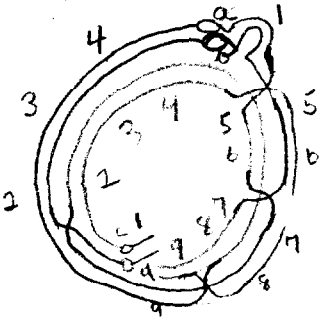


The loop region is too short to synapse with its homologous part in the rod chromosome. This is consistent with what we know of synapsis in maize.

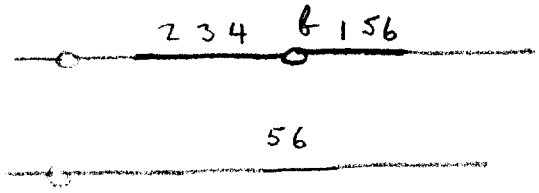
The combination of ring and rod, above, is the chromosome composition of the zygote arising from the original cross that gave the first aberrant prototroph.

4. From this zygote, a heterozygote must arise. It must be heterozygous for some of the factors involved in the cross.

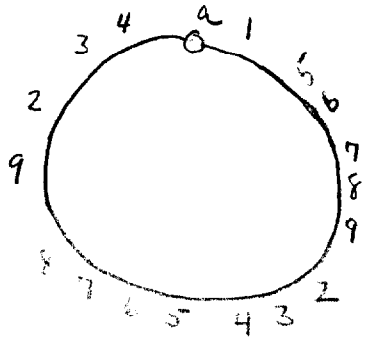
5. Such a heterozygote will arise following specific types of crossing-over in the synapsed ring and rod. One such quadruple crossover is diagramed:



This will give the following chromatids:



This is a dicentric chromosome Individual receiving it will be inviable.



This is a normal chromosome and a double crossover chromatid type.

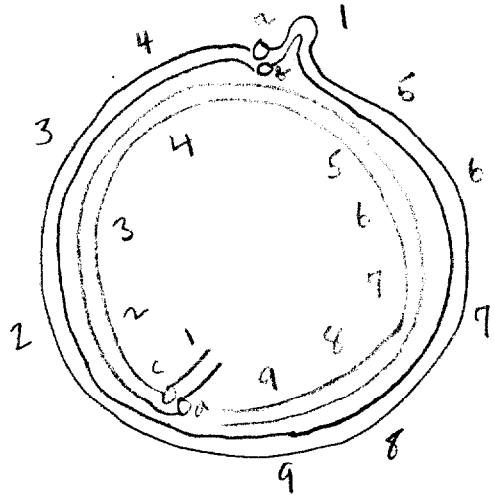
Individuals receiving this chromosome will be diploid for regions 2 to 9 but haploid for region 1. Since only one centromere is present this constitution could maintain itself. Through proper selection methods, the aberrant types following any irregular behavior of the ring chromosome would be eliminated.

This particular "heterozygote" is homozygous for regions 5 and 6, and 9, heterozygous for regions 2,3,4,7 and 8 and hemizygous for region 1.

It may be seen from the crossover diagram that the regions that will be heterozygous or homozygous in any heterozygote arising from such a zygote will depend on just where the crossovers occurred.

At this point, the various types of crossovers from the combinatin of such a ring with a normal rod may be indicated. It will show why the unusual types of crossovers will survive and the usual types be lost through inviable chromosomal constitutions or organizations. It is not necessary to go over this until after the behavior of the "heterozygote" has been considered. I will not try to diagram all types but merely indicate some of them and the consequences:

Side A



Side B

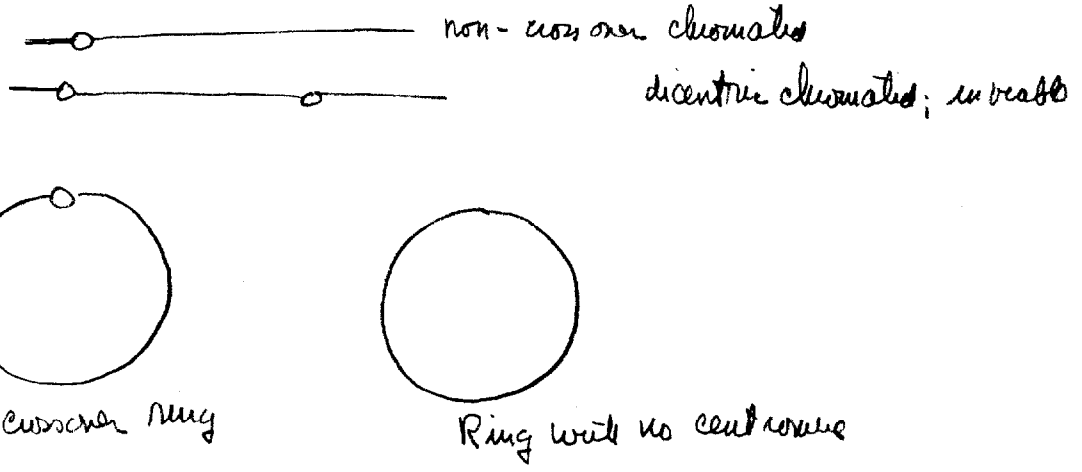
Single crossovers on sides A and B will differ but lead to the same result with respect to inviable individuals. The viable chromatids will be the non-crossovers only, a ring and a rod. The crossover chromatids will give dicentric chromosomes, the organization depending upon whether the crossover occurred on side A or side B. Since these crossover chromatids lead to inviable individuals, the single crossovers may be eliminated from consideration.

Double crossovers. Here the two sides must be considered.

1. 2-strand doubles

   a) Same side: Viable ring and rod, each a double crossover chromatid.

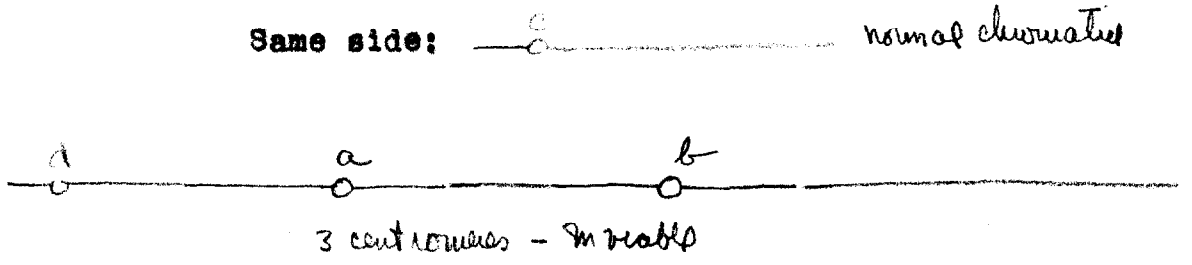
   b) Opposite sides. ~~Considering the b and c strands~~, will give:



Of the 2-strand doubles, only those on one side will produce crossover chromatids with viable comstitutions.

2. 3-strand doubles: Consider b and d and a and d strands. Same side or opposite sides will give inviable crossover chromatids and one non-crossover normal rod.

Same side:



Opposite side: Quite similar type - a three centromere chromatid; inviable constitution.

3. 4-strand double.

   a) Same side: Will give viable crossover chromatids like same-side single crossovers.

b) Opposite sides: Will give 2 dicentric chromatid; inviable constitutions.

Triple and quadruple crossovers: Some viable constitutions can come out of these crossovers that are unusual in their recombinants. The heterozygous ring is produced following a multiple crossover.

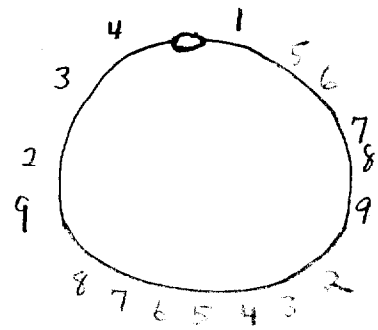
Many of the crossovers give inviable chromosome constitutions. Therefore, selection of viable types means selection of normally infrequent "recombinants", i.e., normally relatively infrequent recombinants. The selection of viable types will include:

(1) Non crossover rings and rods

(2) Double crossovers or multiple crossover rings and rods

(3) The "heterozygote" or diploid - a double sized ring, 1 centromere, hemizygous for a specific region.

Returning to the "heterozygote":



This ring is diploid for all ~~factors~~ regions except region 1. It is haploid for region 1.

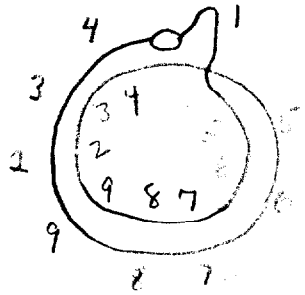
Heterozygous and homozygous regions:

1	2	3	4	5	6	7	8	9
-	2	3	4	5	6	7	8	9
hemi	heterozygous			homo		hetero		homo.

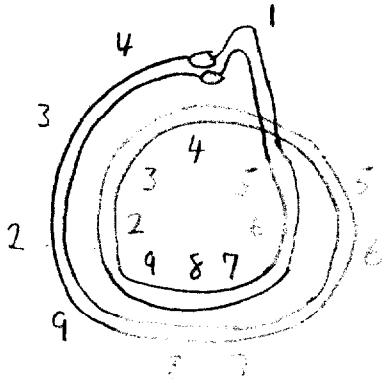
It is necessary now to make an assumption that is unusual but still probable, if this type of analysis is any where near correct. This assumes that the individual receiving this ring, the "heterozygote", continued to divide and produce new individuals with the same chromosome constitution until the physiological conditions produced a meiotic condition in some of the individuals. This means meiotic prophase stages and synapsis of homologous parts of chromosomes followed by crossing over. Because the ring chromosome is diploid, homologous parts of the ring can synapse, crossing-over can occur and segregants obtained.

These segregants will be of very specific types as the diagrams will show.

The synapsis of the single ring chromosome:



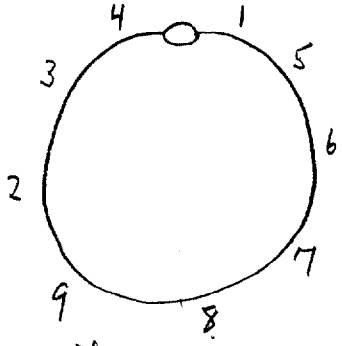
Strands in the synapsed ring following reduplication of the chromatids:



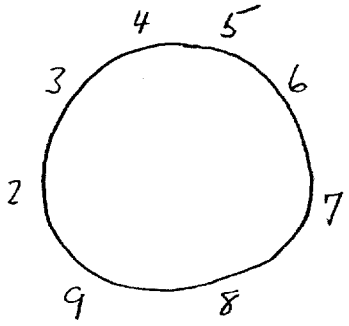
The crossovers:

Single crossovers:

a) involving same chromatid of ring chromosome. Will give a haploid ring and a ring with no centromere and deficient for region 1. The haploid ring with the centromere is viable.



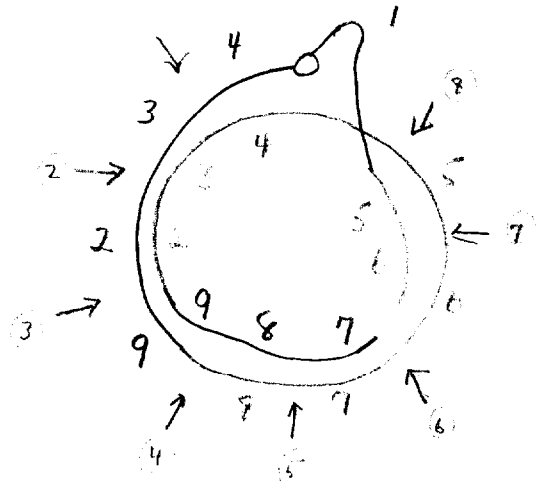
and



Haploid ring. Like original ring chromosome that initiates the heterozygous condition of the original aberrant heterozygous prototroph

no centromere. Deficient for region 1. Inviable constitution.

Regions of single crossovers between homologous parts of same chromatid.



Constitutions of viable haploid rings:

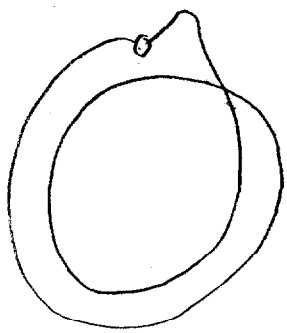
C.O. Region 1	4 3 2 9 8 7 6 5 1	}	simulate multiple crossover chromatids
" " 2	4 3 2 9 8 7 6 5 1		
" " 3	4 3 2 9 8 7 6 5 1	}	alike
" " 4	4 3 2 9 8 7 6 5 1		
" " 5	4 3 2 9 8 7 6 5 1	→	simulates a "multiple crossover chromatid"
" " 6	4 3 2 9 8 7 6 5 1	}	alike
" " 7	4 3 2 9 8 7 6 5 1		
" " 8	4 3 2 9 8 7 6 5 1		

This is the basis for the bias towards certain types of haploid stable segregants from heterozygotes. Some of the multiple cross-overs will give these same constitutions.

b) Single crossovers involving sister chromatids: These will give a very large dicentric ring, tetrapoid for all regions except region 1 which will be diploid. This is an inviable chromosome constitution.

Double crossovers:

To differentiate strands following reduplication, one strand will be drawn in black, the sister strand in green:



a) Same strand double crossovers (black with black and black with black) will give a "diploid" ring chromatid having the same organization as the diploid ring before synapsis. It will have varying genic constitutions depending upon where the crossovers occurred. Those new diploid rings that are heterozygous will again throw haploid segregants of certain types besides genically ~~new~~ different diploids, diploids with different genotypes.

b) Two sister strand exchanges (black with green and black with green).

Several types of products may be formed:

(1) Tetraploid dicentric rings, diploid for region 1. Inviable chromosome constitution.

(2) One dicentric large ring and one acentric large ring. inviable

(3) Two rings like those produce by a) above, i.e., viable heterozygotes (diploids).

c) Same strand and sister strand (black with black and black with green).

Several types produced:

(1) Tetraploid dicentric rings, diploid for region 1. Inviable constitution

(2) One viable <u>haploid</u> ring
One viable <u>triploid</u> ring, haploid for region 1

(3) Two viable haploid rings (each with centromere)
Two inviable haploid rings (deficient for region 1; no centromere)
These viable rings are like the single crossover types and multiple recombinants can come out.

In tabulating the above products of crossing over, I may have left out some expected classes from b) and c) but they will fall into the types already given above.

Many of the products of crossing over in the heterozygote (diploid) will give inviable chromosomal constitutions or heteroploid types that are unstable and will again segregate new types. The stable segregants are the haploid ring chromatids. These segregants will be "heterozygote producers" because they have the same chromosome constitution as the original individual with a ring chromosome. Following crosses of these segregants to standard stocks, the cycle begins all over again:

1) Selection of <u>viable</u> types produced through crossing over.

2) Selection will favor infrequent crossover types including the "diploid" individuals that are hemizygous for region 1 and homo-or heterozygous for various factors entering the cross, depending upon the regions where crossing over occurred.

3) These "heterozygotes" will split off new, stable haploids with special bias towards certain recombinants. Many multiple recombinants should appear.

4) Because the heterozygotes may vary in genic constitutions following any one cross, each heterozygote will have its own particular bias in the types of ~~xxxxx~~ stable segregants it gives.

5) The heterozygotes (diploids) will all be hemizygous for one specific region in the chromosome.

As far as I can determine from the information in your letter (as outlined according to my understanding under "General Considerations) the questions stated at the end of the general considerations are answered by this type of analysis. Your data may show that it is not the type of analysis you require. It may be possible to construct a similar system that will fit the accumulated data and allow for an experimental attack. In this letter, I am not including the required tests for this hypothesis. They would be obvious to you.