Effect of Temperature on Crossingover in Heterozygous T_{5-6c} With Homozygous Inversion in Chromosome 5 in Maize

Shams-ul-Islam Khan¹

Received October 18, 1954

Introduction

Effects of high and low temperature have been studied in many countries with variedly different ends in view. In recent years much interest has been shown on the effects that temperature changes produce in meiosis and related phenomena of chromosomes. A great mass of literature has accumulated on this subject now but a few may be cited which have a direct bearing on the topic of this paper. Dobzhansky (1931) and Sturtevant and Beadle (1936) have observed a decrease in the amount of crossingover in *Drosophila* near the inversion and translocation points. Steinberg (1937) and others have also reported similar results. In regard to the effects of temperature on crossingover, the evidence available is rather conflicting. Plough (1917, 1921), Graubard (1934) and others have reported an increase in crossingover percentage while Earnst Hans (1938) and Whittaker (1939) have observed a reduction and Bryden (1935) and Clark (1943) have detected no effect of temperature changes on crossingover. Kirssanow (1933) studied the combined effects of temperature and X-rays on the frequency of crossingover in chromosome 3 of *Drosophila*, while in this paper working on similar lines, observations are presented on the effect of temperature and inversion on crossingover frequency in a translocated stock of maize.

Material and methods

The material for investigations described here consisted of seeds and the plants raised therefrom, of a heterozygous T_{5-6c} with a homozygous inversion in chromosome 5.

The normal chromosome 5 has an almost median centromere with the short arm being 201 and the long arm 234 cytological units long. In the long arm there is a darkly staining knob of heterochromatic material at about 163 c. units from the centromere. From the knob toward the end is a short region of about 45 c. units of lightly staining chromomeres (Burnham, unpub.). The normal chromosome 6 is always seen attached to the nucleolus in the meiotic prophase because of the presence of a nucleolus organizer in its short

¹ Pakistan research scholar for advanced studies at the University of Minnesota, St. Paul, Minn., U.S.A.

arm. It has a submedian centromere with the short arm being 52 and the long arm 189 c. units long. At the end of the short arm is the nucleolus organizer, then 2-3 darkly staining chromomeres and the terminal heterochromatic knob. The region between the nucleolus organizer and the centromere consists of darkly staining chromomeres. In T_{5-6c} the break in chromosome 6 occurs close to the centromere in the darkly staining region of the short arm carrying the organizer, and in chromosome 5 in the lightly staining region at the end of the long arm. Thus in the pachytene configuration of heterozygous T_{5-6c} the centromere of chromosome 6 is close to the intersection while that of chromosome 5 is about 63 crossover units away. This unequal distribution of the centromeres in the horizontal arms of the cross shaped configuration directly affects the frequency of rings and chains at the metaphase. This observation has further been substantiated by bringing the centromere of chromosome 5 closer to the intersection. An inversion in which the breaks occur on the left side and just close to the centromere and the heterochromatic knob, brings the centromere in close proximity of the knob and automatically reduces the crossover distance to about 14 crossover units, between the centromere and the intersection. The observed frequencies of rings and chains have been given in Table 1. It

Table 1. Frequency of rings:chains and 2:2::3:1 disjunction in heterozygous T_{5-6c} with and without a homozygous inversion in chromosome 5

Stock	Rings		Chains		Field		Greenhouse	
	Alter-nate	Adja-cent	Alter-nate	Adja-cent	2:2	3:1	2:2	3:1
10327-36 × 10413-3	202	204	137	129	380	0	334	0
Frequency	60.4%		39.6%		100%	0%	100%	0%
10327-29 × 10414-9	281	275	194	180	329	0	263	0
Frequency	59.8%		40.2%		100%	0%	100%	0%
10328-46 × 10413-1	215	212	150	134	279	0	285	0
Frequency	60.0%		40.0%		100%	0%	100%	0%
10415-6 × 10484-1*	229	225	350	330	404	4	334	5
Frequency	40.0%		60.0%		99%	1%	98.5%	1.5%

* Stock With a homozygous inversion in chromosome 5; the rest with normal chromosome 5.

may be noted that in heterozygous T_{5-6c} stocks with normal chromosome 5, the ratio of rings to chains is 3:2 (60%:40%) but it is almost reversed when a homozygous inversion is introduced in chromosome 5. A detailed account of the theoretical aspects of crossingover in heterozygous T_{5-6c} has already been published (Shams-ul-Islam, 1954) and will therefore be used here as a reference.

Till recently the most commonly used cytological method for the calculation of crossover frequency has been by ascertaining the chiasma frequency. In this investigation however, a new method recently used by Burnham

(unpub.) has been found to give very satisfactory results. It consists of the use of translocation between any chromosome and the sixth chromosome associated with the nucleolus by virtue of its organizer. It has already been shown (McClintock, 1934) that in the absence of the organizer the nucleolar

Fig. 1. a, quartet type 1, 2. b, quartet type 3. c, quartet type 4. d, e, quartet type 8. f, quartet type 5. g, quartet type 10. h, anaphase-I, showing 3:1 disjunction.

material remains dispersed and does not organize itself into a nucleolus. If therefore each cell of the quartet receives an organizer a nucleolus will be organized in each, otherwise not. The non-crossover and the crossover quartet types as worked out show a difference in the nucleolar organization,

the former has either one nucleolus in each cell of the quartet or two in each of the two adjacent ones and two with dispersed nucleolar material, while in the latter there is a nucleolus in each of the three cells and none in the fourth. The nine different quartet types possible on theoretical considerations have been shown along with those actually observed in Figs. 1 and 2. The crossover quartet types are 4, 5, 6, 7, 8 and 9, and the non-crossover quartet types are 1, 2 and 3. One of very rare occurrence is quartet type 10.

Fig. 2. Quartet types.

Plants were grown in 12" pots in the greenhouse maintained at a temperature range of 70–78°F. The cold rooms where the plants were exposed to low temperature treatment were run at $36 \pm 2^\circ\text{F}$ and each lighted by two fluorescent tubes at a height of 5'.6" from the ground for a period of 18 hours daily.

The microsporocyte samples were fixed in acetic-alcohol (1:3) and immediately transferred to the refrigerator for storage. Belling's acetocarmine method as modified by McClintock was used for staining the microsporocytes. The pollen grains were stained in 1% Iodine solution for pollen counts. As far as possible microsporocyte and pollen samples were taken from each plant.

Experiments and results

In a preliminary experiment designed to select a more cold resistant stock, several similar ones were exposed to $36 \pm 2^\circ\text{F}$ for a week at different stages of growth. Stock No. 10415-6 $\times$ 10484-1 was selected from this trial experiment for further more extensive work.

In order to study the influence of temperature on crossover frequency through the entire active growth period, from seedling stage to the meiotic prophase stage in the tassel the following experiment was planned. About sixty seeds of 10415-6 $\times$ 10484-1 were germinated directly in pots in the greenhouse. After five days the coleoptile pierced through the soil and by the end of the first week it had attained a height of 1''-2''. Then 45 seed-

Table 2. Average percentage of crossover quartet and pollen abortion in heterozygous T_{5-6c} with a homozygous inversion in chromosome 5, after exposure to $36\pm 2^\circ\text{F}$

	C. o. quartet	Pollen abortion	
	Observed	Predicted*	Observed
Normal	11.63	46.73	50.70
I	11.15	46.38	50.66
II	10.61	47.57	50.74
III	11.44	46.77	51.52
IV	11.77	47.37	51.85
V	12.87	46.99	53.48
VI	10.45	47.04	50.26
VII	10.55	46.32	50.75
VIII	10.65	46.43	50.58

* Predicted from percentage of c.o. quartet.

lings of almost equal height and vigour were selected and the rest discarded. On the 7th day five plants were transferred to the cold room for an exposure of one week. At the end of the week, the five plants were transferred back

Fig. 3. Comparison of the effect of different temperatures on crossingover in Heterozygous T_{5-6c} with a homozygous inversion in chromosome 5. ----- at 50°F . — at 36°F .

to the greenhouse and another lot of five shifted into the cold room. This procedure was repeated for eight weeks and by then meiosis in the tassel had almost finished. The results of quartet and pollen counts have been summarised in Table 2, and plotted in thin line in Fig. 3. The high crossover value of 12.87 as the average of the fifth week shows a significant trend of the effect of low temper-

ature. In the case under consideration the increase is not much, not because there is little or no effect of low temperature but because the crossover

distance between the centromere and the intersection has been reduced to only 13.2 crossover units, and therefore the crossover value is not expected to rise above this level.

Discussion

Although the crossover values in heterozygous T_{5-6c} with homozygous inversion in chromosome 5 vary from 10.6% to 12.9% but the range of variation of pollen sterility remains limited to the 50% level. This suggests an internal controlling mechanism which maintains the pollen sterility at 50% level regardless of the values of crossingover, higher or lower than the normal. The observations presented above in this paper indicate, as has already been hinted at earlier, the possibility of an internal mechanism which controls the frequency of plane I and plane II disjunction. It has been reported that equal axes of the pachytene configuration of a heterozygous translocation produce equal frequencies of the aneuploid types while unequal axes give rise to unequal frequencies (Dobzhansky, 1933), and since in the stock under discussion there is a monomodial intersection, it seems plausible to suppose that the absence or low frequency of plane II disjunction helps to reduce the percentage of abortive pollen grains, or indirectly helps to maintain the percentage of pollen sterility at 50%.

Since the presence of an inversion suppresses crossingover and increases the frequency of non-disjunction, it is believed that the presence of an inversion in the 5th chromosome involved in the T_{5-6c} increased the frequency of 3:1 disjunction and that this is further increased by exposures to low temperature (tables 1 & 2). A 1.0% 3:1 disjunction will give rise to 1.0% of the quartet type 10 and this in turn will mean an increase of 4% in pollen sterility. It is probably due to this that the mode of the range of pollen sterility in heterozygous T_{5-6c} with homozygous inversion in chromosome 5 is above the 50% level, unlike that of the heterozygous T_{5-6c} with normal chromosome 5 in which it is below this level.

The observations show that as a result of the effect of temperature, the maximum limit of crossingover (13.2) has almost been obtained (12.9%). Further it appears that the low temperature has an effect on interference. As a result of this the percentage of singles is increased and that of the multiples is decreased and this ultimately increase the crossover value. Hence it seems plausible to infer that low temperature increases the crossover frequency by increasing the interference. As to how this effect of temperature on interference is manifested it seems that the viscosity hypothesis offers a good explanation.

Summary

1. Heterozygous T_{5-6c} with homozygous inversion in chromosome 5 has

been used to study the effect of exposure to $36 \pm 2^\circ\text{F}$ on crossingover.

2. Crossingover frequency has been calculated by ascertaining directly the frequency of the crossover quartet.

3. Data on the effect of exposure of heterozygous T_{5-6c} with homozygous inversion in chromosome 5 to $36 \pm 2^\circ\text{F}$ have been presented.

4. The presence of an inversion increased the frequency of chains from 40% to 60%.

5. The period of maximum response to the influence of low temperature exposures is 4-6 day period in the 5th week. The increase in crossover frequency is from 10.6% to 12.9% almost approaching the maximum limit of 13.2%.

6. It has been suggested that the effect of low temperature on crossingover is through its influence on interference.

The writer wishes to express his deep appreciation of Dr. C. R. Burnham for his continued interest, kind criticism and many helpful suggestions during the earlier course of this investigation. He is thankful to him for the stocks he made available for research. To Dr. A. O. Dahl of the Department of Botany, University of Minnesota the writer is indebted for constructive criticism during the later course of this investigation.

Literature cited

- Bryden, Wm. 1935. Some observations upon the mitotic and meiotic divisions in the Wistar rat. I. The effects of changes in temperature. *Cytologia* 6 (2/3): 300-307.
- Clark, A.M. 1943. Linkage relations in *Habrobracon* and the effects of temperature, X-radiations and age on crossingover, *Proc. Penn. Acad. Sci.* 17: 47-64.
- Dobzhansky, Th. 1931. The decrease in crossingover observed in translocations and its probable explanation. *Amer. Natl.* 65: 214-232.
- 1933. Studies on chromosome conjugation II. The relation between crossingover and disjunction of chromosomes. *Zeitschr. Indukt. Abstamm. u. Vererbungsl.* 64: 269-309.
- Ernst, Hans. 1938. Meiosis und Crossingover, *Cytologische und Genetische Untersuchungen an Antirrhinum majus* L. *Zeitschr. Bot.* 33 (6/7): 241-299.
- Graubard, Marc A. 1934. Temperature effects on interference and crossingover. *Genetics* 19: 83-94.
- Kirssanow, B.A. 1933. Über die Nachwirkung verschiedener Temperaturen und der X-strahlen auf des Crossingover in dritten Chromosome bei *Drosophila melanogaster*. *Biol. Zentralbl.* 51 (10): 529-533.
- McClintock, B. 1934. The relation of a particular chromosomal elements to the development of the nucleoli in *Zea mays*. *Zeitschr. Zellforsch. u. mikr. Anat.* 21: 294-328.
- Plough, H.H. 1917. The effect of temperature on crossingover. *Jour. Exp. Zool.* 24: 147-209.
- 1921. Further studies on the effect of temperature on crossingover. *Jour. Exp. Zool.* 32: 187-202.
- Shams-ul-Islam, K. 1954. Theoretical aspects of crossingover in heterozygous T_{5-6c} in corn, *Zea mays* L. *La Cellule*, 56, 3: 309-327.
- Steinberg, A.G. 1937. Relations between chromosome size and effects of inversions on crossingover in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. U.S.A.* 23 (2): 54-56.
- Sturtevant, A.H. and Beadle, G.W. 1936. The relations of inversions in the X-chromosome of *Drosophila melanogaster* to crossingover and disjunction. *Genetics* 21: 537-554.
- Whittaker, T.W. 1939. The chromosome complement of *Cyphomandra betacea*. *Jour. Arnold Arb.* 14: 113-117.