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STUDY OF JUXTAPOSITION EFFECT IN MAIZE USING THREE-GENE CHAINS

Mikhailov Mikhail
Institute of Genetics, Physiology and Plant Protection, MSU, Chisinau, Republic of Moldova
https://orcid.org/0000-0003-1204-410X, e-mail: mihail.mihailov@sti.usm.md

Summary. The recombination frequency in maize in marker chains *ws3-lg2-gl2* (chromosome 2) and *c1-sh1-wx1* (chromosome 9) was studied. In mosaic maize genotypes, in chromosomes of which homozygous and heterozygous regions alternate, the stimulating effect of external homozygous background on recombination frequency (the juxtaposition effect) depends on marked heterozygous interval length and increases as it decreases. The presence of homozygous fragments within the marked interval can lead to disappearance or weakening of the juxtaposition effect, as well as to redistribution of crossovers within the interval. In maize, unlike Arabidopsis, the juxtaposition effect is not accompanied by disappearance or decrease of interference.

Keywords: crossing over, maize, juxtaposition, heterozygote, homozygote

Introduction

Crossover, the reciprocal exchange of sections of homologous chromosomes during meiosis, is crucial for ensuring proper segregation of chromosomes and is the source of genetic variation within sexually reproducing populations [2,5]. In crop breeding, crossover is used for producing recombinant genotypes in which genes from different sources are combined. However, the relative scarcity of crossover events (usually 1-2 per chromosome) and their uneven chromosomal distribution can cause difficulties for breeders. Therefore, attention has long been paid to the study of factors regulating frequency and distribution of crossover events [1].

Particularly interesting phenomena were seen at the junction of heterozygous and homozygous regions. When studying mosaic genotypes composed of the genomes of two divergent parents, in the chromosomes of which homozygous and heterozygous regions alternate, it was found that the frequency of recombination in a heterozygous region increases if juxtaposed to a homozygous region [3,4,6]. Moreover, it has been shown that, crossover events are redistributed from homozygous to heterozygous regions, which the authors named a “juxtaposition effect” [6]. This effect was found in maize and Arabidopsis, and it is possible that this is a universal phenomenon for plants, independent of their reproductive strategies.

In maize, this effect was studied in two-gene chains [3]. Here, we continue the study in three-gene chains, which allows us to identify additional features of the manifestation of the juxtaposition effect associated with the length of the heterozygous region, disruption of its continuity and the internal distribution of crossover events.

Materials and methods

The initial material included inbred maize lines MK01, Ku123 and a multiple marked line 2-9m, carrying on chromosome 2 mutant markers *ws3* (white sheath), *lg1* (liguleless), *gl2* (glossy), and on chromosome 9 - *c1* (colorless aleurone), *sh1* (shrunken endosperm) and *wx1* (waxy endosperm).

In the course of backcrossing, markers from the 2-9m line were transferred to the genetic background of lines MK01 and Ku123 and the isogenic lines were obtained: M(*ws,lg,gl*)$_7$, M(*ws,lg*)$_7$, M(*c,sh,wx,R*)$_6$, M(*c,sh,wx,R*)cm$_7$, M(*c,wx,R*)$_7$, K(*c,sh,wx,R*)cm$_7$, M(*R*)$_7$, K(*R*)$_7$. The initial letters M and K mean MK01 and Ku123, the transferred mutations are given in brackets (here

and below without numbers for brevity), the subscript means the number of backcrossing. The *cm* symbol denotes a special way of line obtaining (combined), when the continuity of the three-gene chain was broken and a fragment of the original line could be introduced between the *sh* and *wx* loci. The dominant allele *R1* from chromosome 10 was transferred with the *c1* allele, so that the anthocyan kernel color appeared and segregation for *c1* could be observed.

In 2022-2023, the recombination rate between marker loci was estimated in hybrids: K(*R*)$_7$×M(*ws*,*lg*,*gl*)$_7$, MK01×M(*ws*,*lg*,*gl*)$_7$, K(*R*)$_7$×M(*ws*,*lg*)$_7$, MK01×M(*ws*,*lg*)$_7$, K(*R*)$_7$×M(*c*,*sh*,*wx*,*R*)$_6$, M(*R*)$_7$×M(*c*,*sh*,*wx*,*R*)$_6$, M(*R*)$_7$×M(*c*,*sh*,*wx*,*R*)cm$_7$, K(*R*)$_7$×K(*c*,*sh*,*wx*,*R*)cm$_7$, M(*R*)$_7$×M(*c*,*wx*,*R*)$_7$. For comparison purposes, data on a number of other hybrids studied earlier were also used [3].

These hybrids differ in the homozygous or heterozygous state of the rest of the chromosome (genetic background), the length of the marked region, the presence or absence of internal homozygosity in the marked interval (Fig. 1). The recombination rate was estimated by F_2 or BC data, in the latter case, the values for male and female meiosis were averaged.

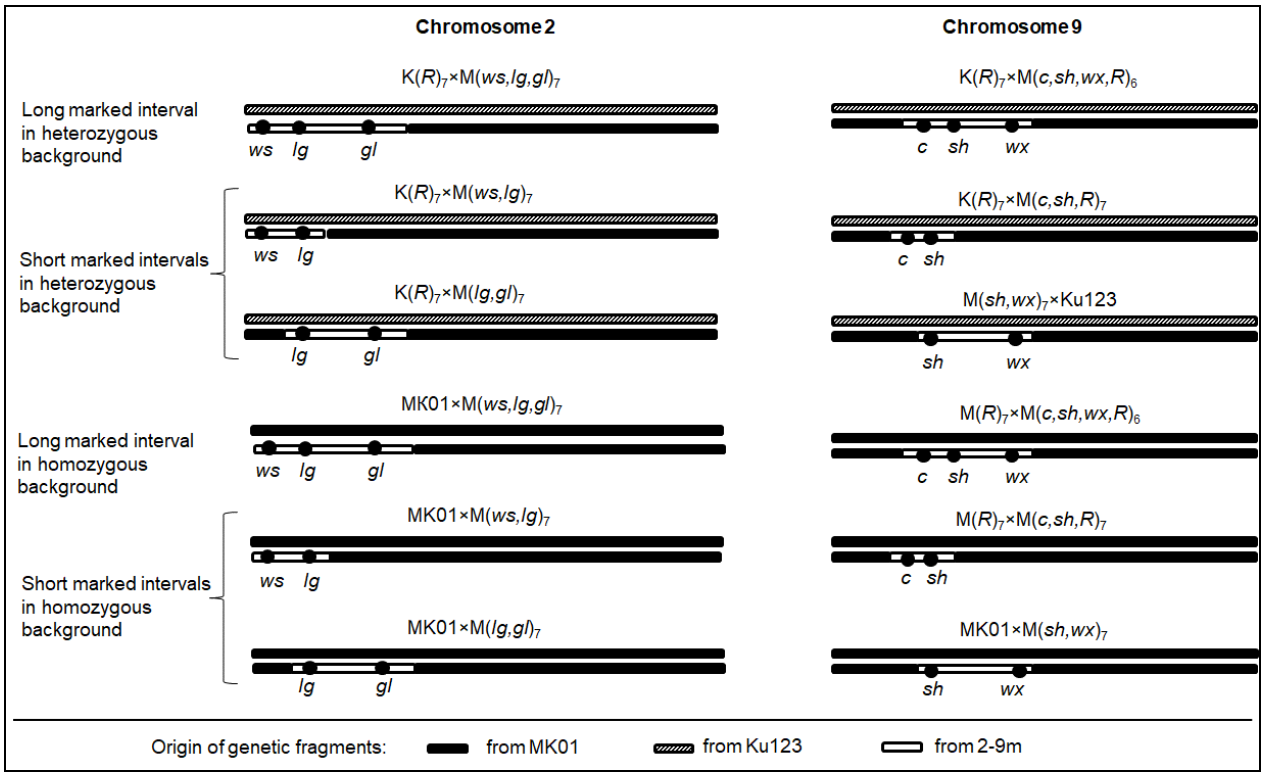


Figure 1. Marked intervals in the genotypes analyzed

Results and discussions

Comparison of the recombination frequency (RF) in hybrids differing in the marker interval length showed that in the heterozygous genetic background the marker interval length is of no importance, and for all four marker pairs the average recombination frequencies are approximately the same both in shortened (two-gene) intervals and in extended (three-gene) ones (Fig. 2). However, in the homozygous background the extension of the heterozygous interval containing markers is accompanied by a decrease in the recombination frequency. The juxtaposition effect does not disappear in this case, but weakens, and the RF takes intermediate values. This pattern was manifested in all four studied marker pairs: *ws-lg*, *lg-gl*, *c-sh*, *sh-wx* (Fig. 2, in some cases earlier data were used for comparison).

The RF decreases especially significantly between closely located markers (pairs *ws-lg* and *c-sh*). This is obviously due to the fact that the homo/hetero boundaries are moved far away, going

beyond the very distant *gl* and *wx* loci. A gradual weakening of the juxtaposition effect with the increasing distance from the homo/hetero transition point was also observed in Arabidopsis [6]. It must be assumed that the redistribution of crossovers between homozygous and heterozygous regions is a local phenomenon and has a limited range of action.

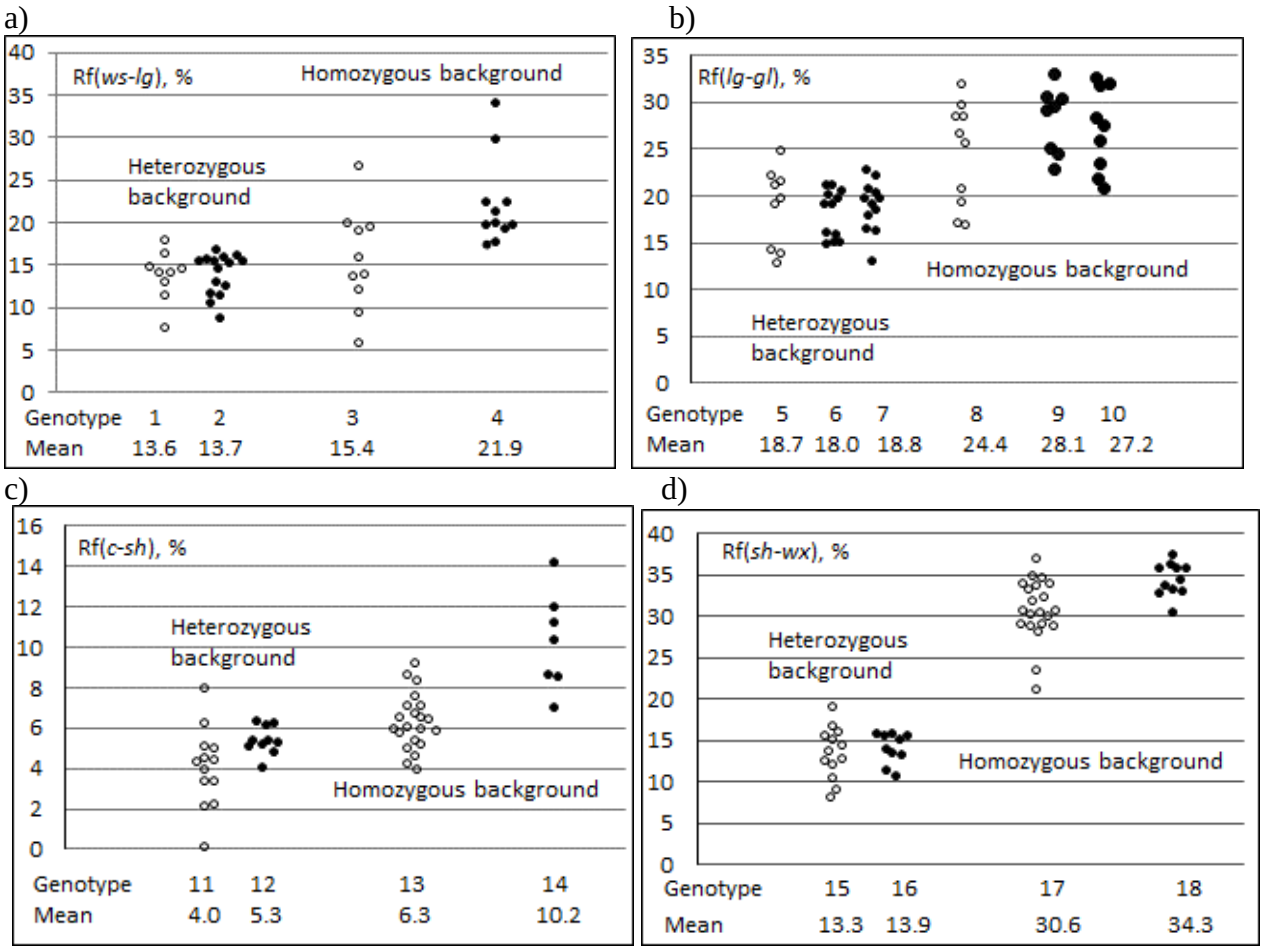


Figure 2. Recombination frequency (Rf) in shortened and extended heterozygous marked intervals at different genetic backgrounds.

Black points - short (digenic) interval, empty points – long (trigenic) interval.

Genotypes: 1 - K(*R*)$_7$×M(*ws,lg,gl*)$_7$, 2 - K(*R*)7×M(*ws,lg*)$_7$, 3 - MK01×M(*ws,lg,gl*)$_7$, 4 - MK01×M(*ws,lg*)$_7$, 5 - K(*R*)7× M(*ws,lg,gl*)$_7$, 6 - K(*R*)$_7$×M(*lg,gl*)$_7$ (2021), 7 - Ku123×M(*lg,gl*)$_6$ (2017), 8 - MK01×M(*ws,lg,gl*)$_7$, 9 - MK01×M(*lg,gl*)$_7$, 10 - MK01×M(*lg,gl*)$_6$ (2017), 11 - K(*R*)$_7$×M(*c,sh,wx,R*)$^{in}_6$, 12 - K(*R*)$_7$×M(*c,sh,R*)$_7$ (2017), 13 - M(*R*)$_7$×M(*c,sh,wx,R*)$^{in}_6$, 14 - M(*R*)$_7$×M(*c,sh,R*)$_7$ (2017), 15 - K(*R*)$_7$×M(*c,sh,wx,R*)$^{in}_6$, 16 - M(*sh,wx*)$_7$×Ku123 (2017), 17 - M(*R*)$_7$×M(*c,sh,wx,R*)$^{in}_6$, 18 - MK01×M(*sh,wx*)$_7$ (2017).

Another class of phenomena under study is associated with the presence of internal homozygosity in the marked interval. Internal homozygosity occurs when an isogenic line containing a chromosome fragment of the original form in the marked interval participates in crossing. It was previously shown that the presence of a homozygous fragment in the *ws-lg-gl* chain of chromosome 2 leads to the disappearance of the juxtaposition effect [3]. However, in the present study, the presence of an internal homozygous fragment around the *sh* locus in the M(*R*)$_7$×M(*c,wx,R*)$_7$ hybrid weakened the juxtaposition effect, but did not eliminate it: the RF(c-wx) value was 29.0% versus 34.4% for the case of complete internal homozygosity in the M(*R*)$_7$×M(*c,sh,wx,R*)$_6$ hybrid. This is probably due to the close position of the *sh* locus to the marginal *c* locus.

In the M(*R*)$_7$×M(*c,sh,wx,R*)cm$_7$ and K(*R*)7×K(*c,sh,wx,R*)cm$_7$ hybrids, for which internal

homozygosity between the *sh* and *wx* loci is assumed, there was no general decrease in recombination frequency within the marked interval, but a significant redistribution of crossover events between the *c-sh* and *sh-wx* subintervals occurred. For these two hybrids, RF(*c-sh*) was 8.3% and 12.0%, and RF(*sh-wx*) was 25.2% and 26.4%, correspondingly. The *sh-wx/c-sh* ratio was thus 3.0 and 2.2 versus 4.9 in the M(*R*)$_7$×M(*c,sh,wx,R*)$_6$ hybrid, in which the entire marked interval is completely heterozygous. This result is consistent with the hypothesis of redistribution of crossover events in mosaic genotypes from homozygous to heterozygous regions of chromosomes.

The use of three-gene chains made it possible to evaluate interference, which was previously impossible with two-gene chains. In the chromosome 2, the interference coefficient was 0.57 in the heterozygous background and 0.79 in the homozygous background (K(*R*)$_7$×M(*ws,lg,gl*)$_7$ and MK01×M(*ws,lg,gl*)$_7$ hybrids). In the chromosome 9, these values were 0.35 in the heterozygous background and 0.62 in the homozygous background (K(*R*)$_7$×M(*c,sh,wx,R*)$_6$ and M(*R*)$_7$×M(*c,sh,wx,R*)$_6$ hybrids). Thus, in maize, the homozygous genetic background does not lead to the disappearance of interference, as in Arabidopsis [6], and it is possible that the mechanisms for implementing the juxtaposition effect in them are somewhat different.

Conclusions

In mosaic maize genotypes, in the genome of which homozygous and heterozygous regions alternate, the effect of the external homozygous background on the recombination frequency depends on the length of the marked heterozygous interval and increases as it decreases.

The presence of homozygous internal fragments under the conditions of the external homozygous background can lead to the disappearance or weakening of the juxtaposition effect, as well as to the redistribution of crossover events within the marked interval.

In maize, the juxtaposition effect is not accompanied by the disappearance or decrease of interference, which suggests differences between maize and Arabidopsis in the implementation mechanism of this effect.

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