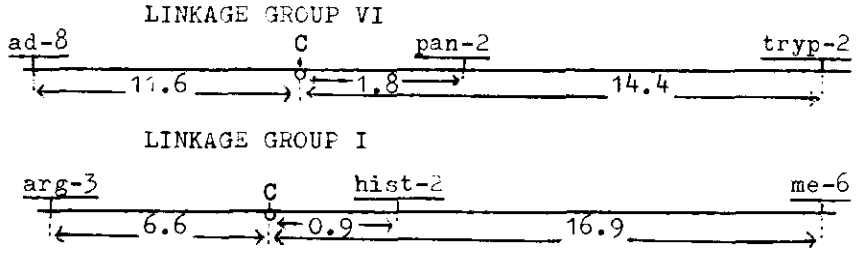
Prakash, V. Changes in cross-over frequency in Neurospora crassa mediated by chelating agents.

During recent years, a number of workers have recorded modifying effects on the frequency of genetic exchanges by employing primarily one chelating agent, ethelenediamine tetraacetic acid (EDTA). It is presumed that this agent facilitates in breaking the linear continuity of the chromatids incidental to crossing-over as it chelates metallic ions, especially calcium and magnesium, essential to the maintenance of structural integrity of chromosomes.

In the present study, EDTA and another chelating agent, 8-hydroxy quinoline were used. To screen the effects of different concentrations of EDTA and 8-HQ on the frequency of crossing-over, ascospores, representing the population of meiotic products of the crosses made in Neurospora crassa, were plated on a selective medium in which only particular recombinants could grow. After heat shock, the plates were incubated at 25° C. Depending on the approximate size of colonies from the germinated ascospores, the initial score was done after 16 to 18 hours of incubation, and to score ascospores with delayed germination

a final count was undertaken after 25 to 27 hours of incubation. The frequency of crossing-over was determined by observing prototrophic recombinants (i.e., recombinants which had the same minimum requirements that characterize the wild-type or prototype) and certain auxotrophic recombinants (i.e., recombinants which had nutritional requirements). Ascospores from crosses treated with the highest concentration which was not completely inhibitory were plated. A total of 29394 ascospores from various crosses were analysed out of the total 35868 ascospores plated. Average percentage viability for a cross was about 82%.

All crosses were made on the cottoned media (See "Perithecial production - use of non-absorbent cotton" in this Newsletter) at 25°C. After inoculating the protoperithecial parent, the cross was made after 48 hours by adding a suspension of conidia from the other parental strain. Six hours later, chelating agents were added and solutions adjusted to the specific molar concentrations. The strains employed were ad-8 (74Y 152-M7); arg-3 (30300); hist-2 (C94); me-6 (75001); pan-2 (74A-YU370-1A); tryp-2 (75001). These strains, before use, were back-crossed as to provide them with fairly closely related genetic background. A recurrent perithecial parent in the back-cross series was the Lindegren wild-type strain while the fertilizing parent was always an isolate from the preceding generation. Three sets of crosses were made. The first set consisted of ad-8 + tryp-2 x + pan-2 +, involving markers on linkage group VI. The second and third sets involved markers on linkage group I. The second set was made up of arg-3 hist-2 + x + + me-6, and the third set consisted of arg-3 + me-6 x + hist-2 + . Centromere distances, derived from independent tetrad analysis data, for the loci under study, are given in a diagram as follows:



Tryp-2 in the first set of crosses was a leaky type and showed a limited growth on the minimal medium but was readily distinguishable from the type of growth shown by true prototrophs. As it is scorable even without supplying its biochemical requirement, anthranilic acid or tryotophan, it is a useful marker for screening purposes. Its score represents the frequency of cross-over in region I of linkage group VI. Whereas the prototrophs showed 2-strand and a part of 3-strand double cross-overs, the adenine auxotrophs showed the cross-overs in region II, of linkage group VI. In the second set of crosses, the frequency of cross-overs in region II was shown by the prototrophs and in region I was indicated by the histidine auxotrophs on linkage group I. In the third set of crosses, the percentage of prototrophs represented the percentage of 2-strand and a part of 3-strand double cross-overs in region I and II of linkage group I. Whereas arginine auxotrophs showed the frequency of cross-overs in region II, methionine auxotrophs showed the frequency of cross-overs in region I. As the prototrophs would also germinate on the supplemented medium, appropriate correction was made in each set of crosses to arrive at the respective auxotroph number.

Fig. 1 illustrates the comparative effects of four EDTA concentrations on the frequency of crossing-over. In Fig. 2 are shown the comparative effects of five 8-HQ concentrations on the frequency of crossing-over. An increase in molar concentration of EDTA and 8-HQ among the treated crosses appears to have prompted a gradual decrease in the frequency of observable double exchanges (2-strand and a part of 3-strand doubles) over the control cross. The modifications in the frequency of exchanges brought about either by EDTA or by 8-HQ, when compared, appear to differ between genetic regions and between linkage groups. With respect to region I of linkage group VI, a significant increase of exchanges over the control crosses is observed among the crosses treated with 10×10^{-5} M and 20×10^{-5} M EDTA but there appears to be no significant increase among the crosses treated with 8-HQ. A significant increase in the cross-over frequency in region II of linkage group VI is indicated in the crosses treated with 10×10^{-5}M

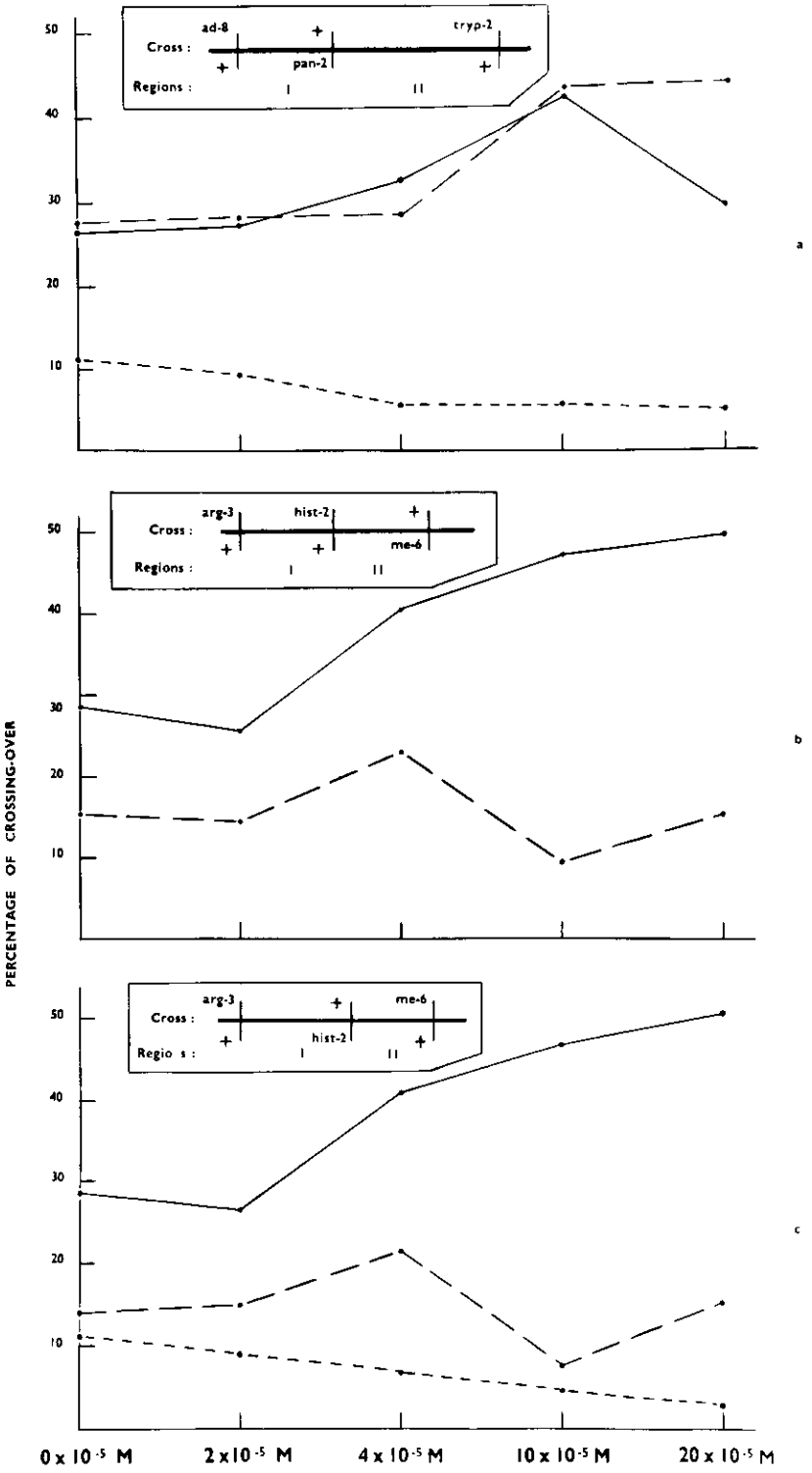


Fig. 1

Fig. 1: Comparative effects of four EDTA concentrations on frequency of crossing-over. Percentage cross over values are based on ascospore plating data (screening crosses). Large broken lines represent cross-overs in region I, full lines represent cross-overs in region II and small broken lines represent 2-strand double and part of 3-strand double cross-overs.

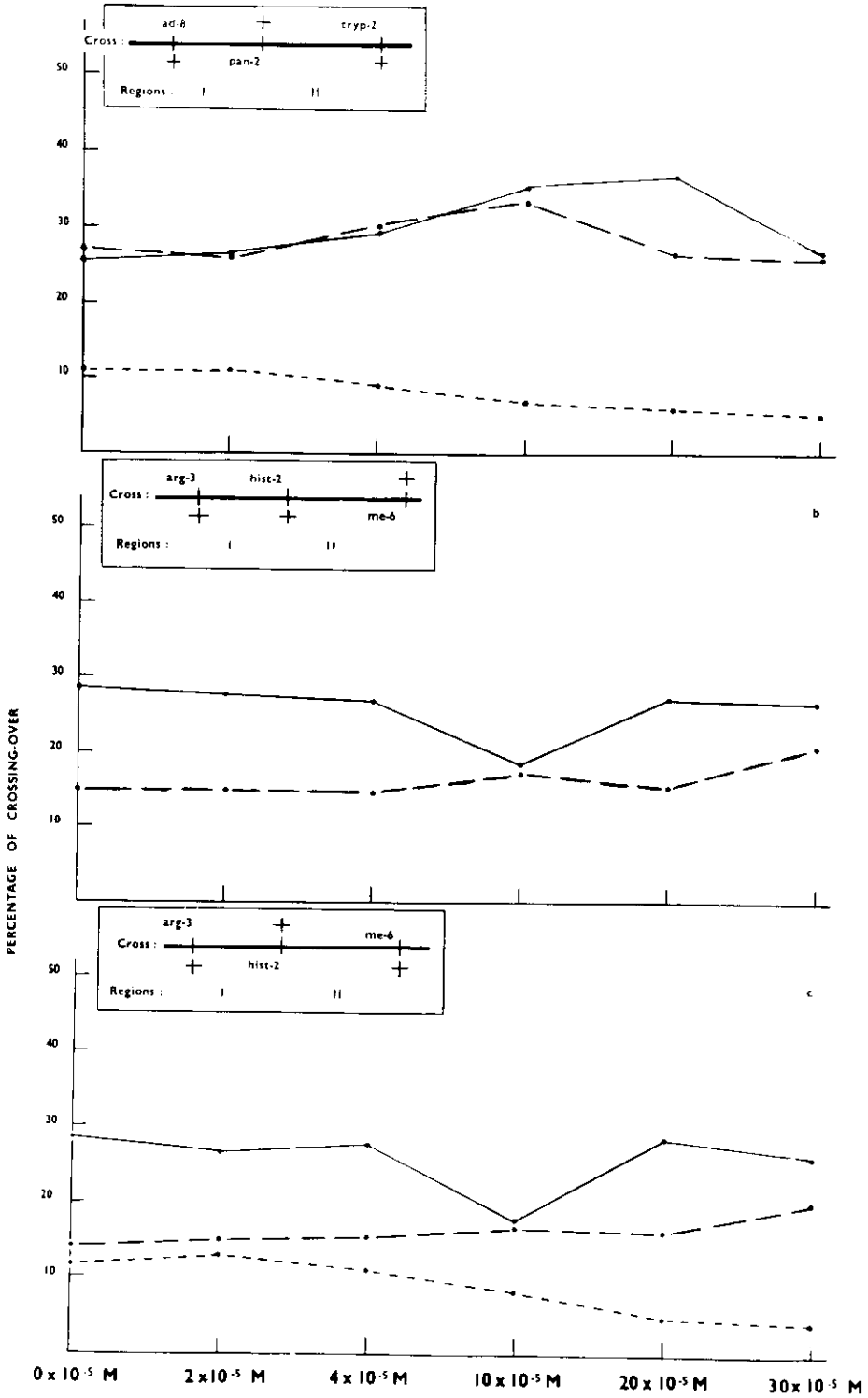


Fig. 2

Fig. 2: Comparative effects of five concentrations of 8-Hydroxyquinoline on frequency of crossing-over. Percentage cross-over values are based on ascospore plating data (screening crosses). Large broken lines represent cross-overs in region I, full lines represent cross-overs in region II and small broken lines represent 2-strand double and part of 3-strand double cross-overs.

EDTA and with 20 x 10^{-5}M 8-HQ over the untreated crosses. With respect to region I of linkage group I, a significant increase in the cross-over frequency is shown in the crosses treated with 4 x 10^{-5}M EDTA and with 30 x 10^{-5}M 8-HQ. Whereas a significant increase in the cross-over frequency is observed in region II of linkage group I among crosses treated with 4 x 10^{-5}M, 10 x 10^{-5}M and 20 x 10^{-5}M EDTA, a significant decrease is indicated in a cross treated with 10 x 10^{-5}M 8-HQ.

Different concentrations of the chelating agents appears to have a variable pattern of modifications in the cross-over values among chromosomal segments. This may be a reflection of their differential effects on the general inter-cellular ionic environment bringing about an interaction at the macromolecular level, in such a way that it causes modifications in genetic exchanges. Further investigations, however, may reveal whether by the treatment with the specific molar concentrations of chelating agents, the hypothetical links of the macromolecular complexes of component nucleic acids and proteins are weakened or a chain of physiological processes directed towards the ultimate modifications in the activity of genetic material are initiated.--Department of Botany, University of Malaya, Kuala Lumpur, Malaya.