the white eye stock and cytological examination of the progeny (larvae) was carried out to assess which males were inversion heterozygotes. Only males were used during this step to rule out crossing over which would confuse the chromosome-marker relationship. In the following generation (G_3), two situations are possible depending on the location of the eye color. If it is located on chromosome 3, then all red eye flies will be inversion heterozygous and all white eye flies are normal. If the marker gene is on any other chromosome, half of both the red eye and white eye flies will be inversion heterozygous and half will be normal. Males being much easier to analyse, we checked in G_3, 18 w^+w (red) males which were all inversion heterozygotes, and 19 ww males which were all normal. This is a strong indication for the linkage of the eye color gene with chromosome 3. w^+w males were again test-crossed with normal ww females in order to repeat the analysis in the next generation G_4. Again all ww males (33) were normal, and all w^+w (red) males (7) inversion heterozygous.

To see whether recombination between the inversion and the marker gene occurs, we have analyzed the adult male G_4 progeny of w^+w females. It appeared that both w^+w and ww males can be inversion heterozygous or normal. A recombination of 40.2% was found (N=112). A ihgh incidence of double crossovers within the inversion being unlikely, this suggests that the eye colour locus is situated distally on the long arm of chromosome 3.

Linkage between the inversion and the red eye marker can be maintained by routinely repeating the G_3 and G_4 crossings without any further analysis of karyotypes or fertility.

This linkage enables sibcrosses to be made between inversion heterozygous males and females to obtain inversion homozygotes just by combining the red eye partners from red eye fathers. These homozygotes, if viable, can be used for genetic insect control. Another application for genetic insect control purposes, as suggested by PARKER[7], is the possibility of isolating compound chromosomes after irradiating inversion heterozygotes. This can be carried out with this inversion by irradiating w^+w, $In/+$ males and subsequently crossing them with the w^+w, $In/+$ females. After the induction by irradiation of crossingover in the male, duplication/deficiency gametes may occur which are complemented in a few combinations and give a compound karyotype. Compound strains have some intra-sterility, and complete sterility after crossing with the wild type in the field.

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Chloral Hydrate Induced Haploidization in *Aspergillus nidulans*

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Summary. This is the first report of induction of haploidization in *Aspergillus nidulans* by chloral hydrate, which is an efficient polyploidizing agent for higher plants and a psychotropic drug for man. A new procedure has been described to isolate haploids from diploids with a very high frequency, as compared to *p*-fluorophenylalanine, which is generally used for this purpose.

Chloral hydrate is widely used as a psychotropic drug and as an anaesthetic. It has been shown to interfere with the formation of mitotic spindle in plants[1], in grasshopper spermatocytes[2], in *Pleurodeles waltlii*[3] and in *Aspergillus nidulans*[4]. We wish to report here a new and interesting property of chloral hydrate in inducing haploidization in *Aspergillus nidulans* diploids.

Heterozygous diploids, which can be easily isolated in this fungus[5], give rise to new diploids and haploids spontaneously[6], though at a very low frequency. The haploid segregants can be visually detected in a diploid which is heterozygous for conidial colour markers.

Chloral hydrate was incorporated in the standard complete medium[7] to various final concentrations ranging from 0.001 *M* to 0.04 *M*, and conidia from diploid strains heterozygous for 2 colour markers as well as a number of nutritional markers and the corresponding haploids were plated on the surface. Viable counts were drastically affected by increasing concentrations of chloral hydrate, so much so that at 0.02 *M*, only 2% of conidia could form colonies. There was no differential effect on haploids and diploids. However, when a thick inoculum of conidia was streaked, rather than stabbed or plated, the growth was a little better. At a concentration of 0.02 *M* chloral hydrate, streaks of diploid strains produced well

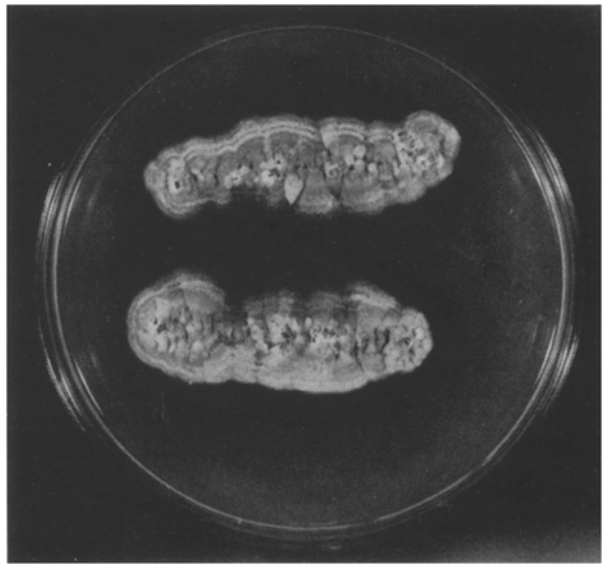

Fig. 1. A streak of a heterozygous diploid showing small sectors on complete medium + 0.02 *M* chloral hydrate after 12 days of incubation.

Mitotic segregation induced by chloral hydrate in different diploids

Diploid used	No. of streaks analyzed	No. of segregants analyzed				No. of haploid segregants	No. of diploid segregants	No. of classes of haploid segregants
		Yellow	White	Green	Total			
On chloral hydrate alone								
riboA1, biA1; fpaX71 MSE [a]	1	8	9	0	17	17	0	13
On chloral hydrate and then transferred to complete medium								
riboA1, biA1; fpaX70 MSE	2	13	7	1	21	21	0	15
riboA1, biA1; fpaX72 MSE	2	10	4	2	16	15	1	12

[a] MSE, Master Strain 'E'[8].

conidiating and fast growing sectors after about 10–12 days of incubation. Each streak gave rise to about 15 distinct sectors, which when isolated and tested separately for conidial diameter and biochemical requirements, were found to be haploids and did not segregate further on a medium containing *p*-fluorophenylalanine (FPA). About 85% of them were different from one another, indicating that the clonal factor was greatly diminished. The growth of the diploid in the streak is not detectable visually, and all the segregants produced are haploids indicating a selective effect of chloral hydrate against diploids and disomics. Even the haploids do not grow very well and appear after 8–10 days as small sectors (Figure 1). If the streak is transferred to complete medium after 5 days on chloral hydrate, the sectors grow out vigorously and sporulate normally within 2 days of the transfer (Figure 2), thereby facilitating isolation. Although all growth appears brownish grey on chloral hydrate, on complete medium sectors with different colours are extremely prominent because of profuse conidiation. Out of 36 isolates picked up from streaks first grown on chloral hydrate and then transferred to complete medium, 35 turned out to be haploids and only one was a diploid (Table), indicating thereby that, during 5 days on chloral hydrate, there is almost complete haploidization, following which the haploid sectors grow out of the streak.

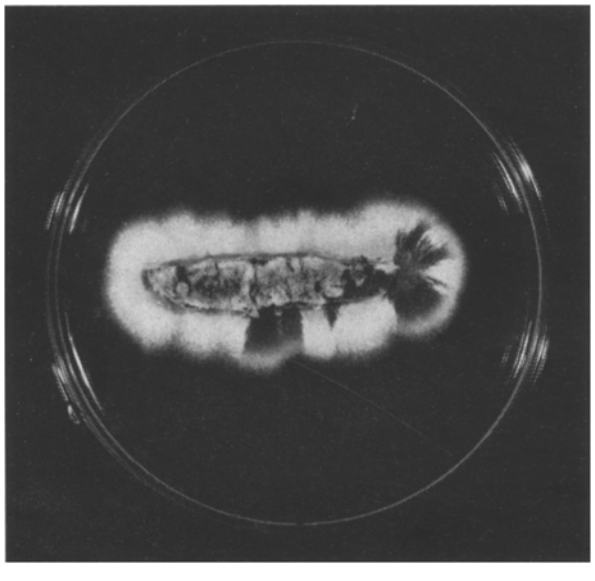

Fig. 2. The diploid was first streaked on complete medium + 0.02 *M* chloral hydrate, incubated for 5 days and then transferred to complete medium. After 2–3 days of the transfer, fast growing and profusely conidiating sectors are visible.

Using chloral hydrate as the haploidizing agent, 2 newly isolated FPA-resistant mutants (*riboA1, biA1; fpaX70* and *riboA1, biA1; fpaX71*) were assigned to the linkage group VI, and the third new isolate (*riboA1, biA1; fpaX72*) was assigned to the linkage group I. These assignments were confirmed by using FPA as the haploidizing agent[9]. Segregation of all other markers was as expected on the basis of their linkage group locations. The diploid between *fpaX70* and MSE gave out only green sectors when FPA was used as the haploidizing agent, but on choral hydrate well growing and profusely conidiating yellow, white and green sectors could easily be obtained.

The above-mentioned chemical and the procedure make the induction and isolation of haploids from a diploid less time consuming and very easy, thus facilitating the entire procedure of genetic mapping based on mitotic segregation. Also, chloral hydrate is much cheaper than *p*-fluorophenylalanine. It is unique because of its dual function – induction of c-mitosis and polyploidy in higher plants and haploidization in *Aspergillus*. To our knowledge, there is no report of efficient induction of haploidization by any of the polyploidizing agents in any organism.

Thus, chloral hydrate deserves more attention as a potential haploidizing agent for plant and animal cells. Since it is a widely used drug, such investigations would add to our knowledge regarding its effects on human beings.

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