Table III. NADP phosphatase[a] activity in detached wheat leaves treated with nicotinic acid, nicotinamide, NAD, benzimidazole or kinetin

	Protein (mg/ml)		Specific activity[b] (EU/mg protein)	
	Experiment No. I	II	Experiment No. I	II
Immediately detached	11.0	10.2	2.19	2.3
Water	9.8	9.5	2.33	2.40
Nicotinic acid	9.5	9.2	1.77	2.22
Nicotinamide	9.6	9.0	3.27	2.89
NAD	10.0	9.1	1.44	1.89
Benzimidazole	10.2	9.8	2.51	3.09
Kinetin	10.1	9.8	3.53	3.38

[a]Spectrophotometric assay for NAD formed in 33 m*M Tris* at pH 6.0.
[b]One enzyme unit is defined as the amount of enzyme which catalyses the formation of 1 µmole of NAD in 5 min.

NAD and nicotinic acid. The leaves floated on kinetin and benzimidazole showed an increase in NADP phosphatase activity.

The NADP-phosphatase in this study resembles the enzyme from pea leaves isolated by FORTI *et al*[11]. Both are acid phosphatases which hydrolyze NADP at high rates. The K_{NADP} value of the acid phosphatase from wheat leaves was 1.4×10^{-4} *M* in this study (c.f. 3×10^{-4} *M* for the pea leaf enzyme). Orthophospate was found to be a competitive inhibitor of both wheat leaf phosphatase and pea leaf phosphatase. The K_{iq} ($K_{i\ (Pi)}$) was 1.7×10^{-4} *M* in this study.

The specific activity of NADP-phosphatase in excised wheat leaves treated with solutions of kinetin or benzimidazole increased approximately 20% while the leaves treated with solutions of nicotinic acid or NAD lost about 20% of the specific activity. However, MISHRA and WAYGOOD[6] reported that wheat leaves treated with benzimidazole or kinetin had an increas ed NADP content and a fairly constant NAD-NADP ratio in fresh leaves. As the NADP phosphatase was an active enzyme even in the crude wheat leaf extract this indicates that a regulatory factor other than the enzyme level controls the NAD/NADP ratio in the leaves. If so, an increase in the specific activity of NADP-phosphatase in vitro will not necessarily cause the decrease of NADP content in vivo. This regulation is facilitated further by the compartmentation of the enzyme and substrate in separate loci. The main pool of NADP in leaves treated with the cytokinins is possibly in the chloroplasts while NADP-phosphatase was located outside the chloroplasts. Benzimidazole by maintaining the structural integrity of the chloroplasts prevents 'leaching' of the NADP from chloroplasts and hence the enzymic hydrolysis to NAD. Benzimidazole also favours the synthesis of NADP (presumably from NAD). Benzimidazole sustitution for the nicotinamide moiety of NAD has been reported by KAPOOR and WAYGOOD[13]. This substitution reaction together with enhanced transhydrogenase[14] and presumably also NAD kinase activity and the spatial separation of NADP-phosphatase from its substrate possibly explain the seemingly contradictory situation of an increase in the levels of both NADP and NADP-phosphatase in leaves treated with benzimidazole[15].

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[14] C. E. JOU, M. Sci. Thesis, University of Manitoba (1970).
[15] E. RACKER in *Methods of Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York, 1955), vol. 1, p. 500.
[16] E. R. WAYGOOD, Can. J. Res. *26*c, 461 (1948).

Location of an Eye Mutant in the Onion Fly *Hylemya antiqua* Meigen Using a Pericentric Chromosome Inversion

C. van HEEMERT and W. J. van den BRINK

Institute for Atomic Sciences in Agriculture, Association EURATOM-ITAL, P. O. Box 48, Wageningen (The Netherlands), 23 October 1975.

Summary. With the use of a pericentric inversion in chromosome 3, an eye color mutant in the onion fly was located in chromosome 3. No recombination occurs in males; 40.2% recombination was observed in females. This linkage through the male facilitates further cytogenetic research on structural aberrations involving chromosome 3.

Genetic research on the onion fly *Hylemya antiqua* Meigen has been undertaken in the context of genetic insect control. Several X-ray induced chromosomal rearrangements (translocations and inversions) associated with reduced fertility have been studied[1–3]. Unfortunately, no morphological markers have yet been isolated. Such markers are useful for the isolation of homozygous translocations or inversions; cytogenetic analysis alone being more time-consuming. Recently we have received a white eye (*ww*) marker stock from Canada[4]; the wild type flies are red eyed (w^+w^+). This mutant is not sex-linked[5], thus it will be located on 1 of the 5 autosomes ($2n=12$). With the aid of a pericentric inversion, it was possible to assess on which chromosome this white eye marker is located.

Inversion *In(3)2* was used in which chromosome 3 is involved[1], see Figure. In mitotic metaphases, the inversion heterozygous (*In*/+) karyotypes could be discriminated from the +/+ types on the basis of the different arm-ratio of the inverted chromosome in combination with the transposition of a secondary constriction from the short arm of the normal chromosome to the long arm of the inverted chromosome (Figures c and e). In meiotic stages, the inversion heterozygous types could be identified by the presence of a clear ring or bump at one end of the chromosome 3 bivalent (Figures d and f). The fertility is approximately 30% reduced (70% egg hatch), as measured from test-crossed inversion heterozygous (*In*/+) females (chiasmate), while *In*/+ males have a normal fertility due to the absence of chiasmata.

An analogous situation has been described for a few pericentric inversions in *Drosophila*[6].

Crossing scheme:

G_1. ♀ red: w^+w^+, *In*?/+ × ♂ white: *ww*, +/+. Of females which proved to be heterozygous for the inversion, sons were retained for the next generation:

G_2. ♀ white: *ww*, +/+ × ♂ red: w^+w, *In*?/+. Progenies from males heterozygous for the inversion were retained for the next generation:

G_3. a) ♀ white: *ww*, +/+ × ♂ red: w^+w, *In*/+. Progenies were 50% *ww*, +/+; 50% w^+w, *In*/+. b) ♀ red: w^+w, *In*/+ × ♂ white: *ww*, +/+. Progenies contained both red and white with and without the inversion due to recombination in the female.

The crossing scheme shows how in the first generation (G_1), females of the inversion stock were testcrossed individually with *ww* males. About half of the females showed reduced fertility, due to crossing-over in the inversion loop, and half had a normal fertility. Males could be used as well, but in that case cytological analysis of the males or their progeny is essential in order to establish the karyotype. In G_2 all the flies had red eyes (w^+w). Males were testcrossed individually with normal *ww* females of

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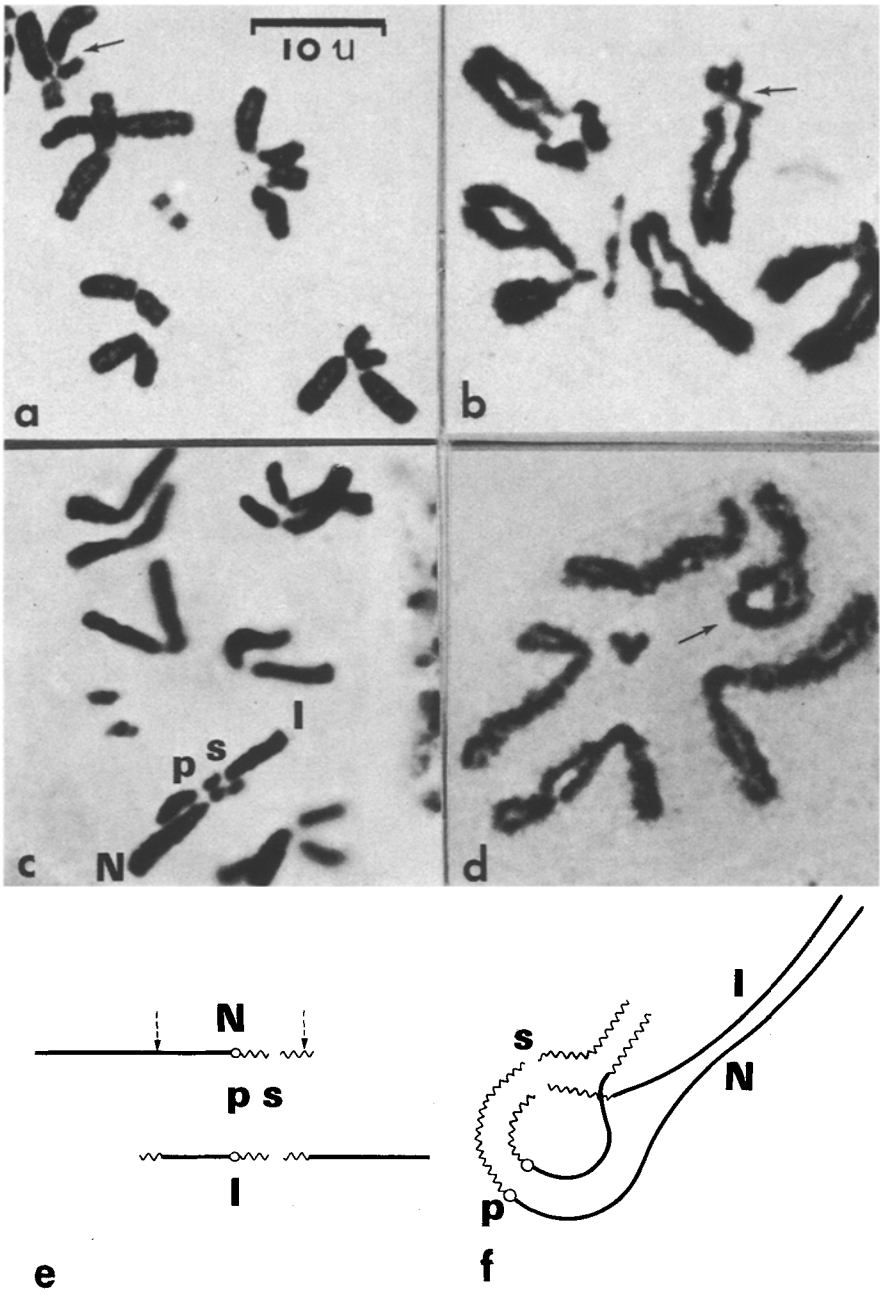

a) Normal karyotype, mitotic metaphase, of the onion fly in a larval brain cell. In larval stage, males ($2n = 10 + XY$) are not distinguishable from females ($10 + 2X$). b) Normal karyotype, diakinesis/prometaphase of a male. c) Inversion heterozygote, mitotic metaphase, as seen in an embryonic cell. d) Inversion heterozygote in meiotic prometaphase (♂). e) Diagrammatic illustration of the normal and inverted chromosome in mitotic metaphase. f) Diagrammatic illustration in meiotic prometaphase. N, normal chromosome (arm ratio = 2.6); I, inverted chromosome (arm ratio = 2.0); p, primary constriction; s, secondary constriction, ···➤ = breakpoint (appr.). Note the centric somatic pairing in a) and c); → = chromosome pair 3.

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*

M. SINGH and U. SINHA

Department of Botany, University of Delhi, Delhi–110007 (India), 26 January 1976.

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

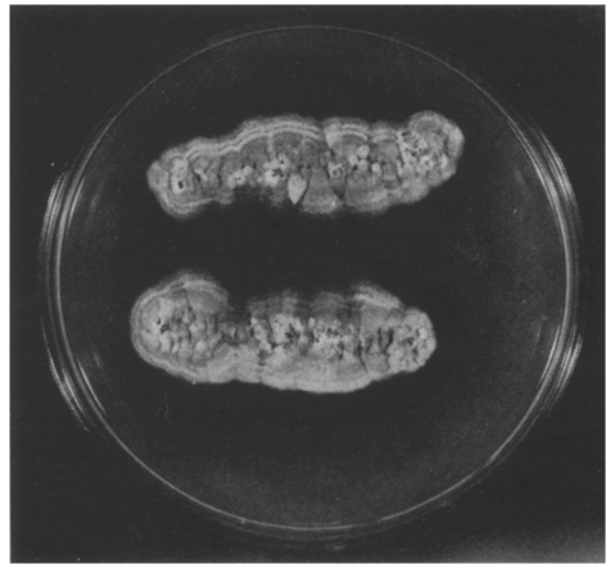

Fig. 1. A streak of a heterozygous diploid showing small sectors on complete medium + 0.02 *M* chloral hydrate after 12 days of incubation.