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Detection and isolation of induced chromosome aberrations in Lepidoptera

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Summary. An embryo chromosome technique combined with larval testes sampling permits isolation within 1 generation of lepidopteran strains carrying chromosome aberrations.

Identification and isolation of chromosomally aberrant strains of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), by conventional genetic techniques are complicated by 2 factors. Firstly, fertilities of single pair matings in the laboratory normally range from 40 to 95%, making detection of translocation carriers by reduction in egg hatch uncertain. Secondly, an obligatory 20-week 2nd instar larval diapause limits the number of generations reared per year to two. An embryo chromosome technique used in conjunction with larval testis sampling reduces the number of generations required to isolate strains carrying particular types of chromosome aberrations from 3 to 1 and minimizes the effort involved in rearing.

Materials and methods. Spruce budworm obtained from stocks maintained at this Institute were reared and mated as previously described[1]. Chromosome counts were obtained from embryos in the following manner. Samples of 24-h-old eggs were gently teased from egg masses and agitated in 1% NaOH at 35 °C for 10 min to separate the eggs[2]. After a brief rinse in modified Ringer-Castillo[3] without Ficoll, eggs were transferred individually to 0.3 ml of 0.02% colchicine in Ringer-Castillo in plastic chambers of a Shandon Cyto-Centrifuge (Shandon Southern Products Ltd, Runcorn, Great Britain). After 20 min at 22 °C, 0.9 ml of sterile distilled water was added to each chamber, eggs were carefully torn open, and the chamber contents gently mixed. After 5 min, cells were centrifuged onto glass slides at 900 rpm for 10 min. Slides were subsequently air-dried for 30 sec, post-fixed for 3 min in ethanol-acetone (1:1) at −20 °C, air-dried, stained with 2% acetocarmine and examined under phase contrast. Usable chromosome preparations were routinely obtained from 9 to 10 of the 12 embryos that could be processed at one time.

Testes were sampled from 5th instar larvae[4]. Survival of surgically-treated larvae to adulthood averaged 77%.

In establishing the utility and reliability of the method, 1-day-old adult males were exposed in groups of 10 to 0, 2 or 10 kR of X-irradiation (400 R/min, Faxotron 804, Field Emission Corp., Oregon) and individually mated to normal virgin females. Egg masses were collected daily and at least 9 embryos/mating were examined for inherited chromosome aberrations.

Results and discussion. Chromosome aberrations were detected in at least some embryos from all 6 successful 2 kR matings. This induced chromosome damage was not reflected by reduction in egg hatch compared to controls. Among embryos examined from the 5 successful 10 kR matings, 71% carried detectable aberrations. Average egg hatch in these families was 52% of the controls. No aberrant embryos were found in the 7 control families.

Aberrations detected were limited to numerical changes, as the small size and large number (2n = 60) of *C. fumiferana*

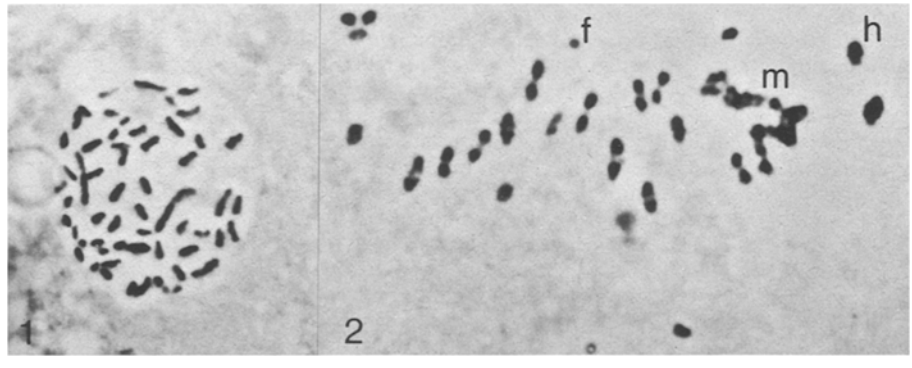

Fig. 1. Inherited chromosome aberrations in *C. fumiferana* embryo that reduced chromosome number from 2n = 60 to 2n = 58. × 1570.
Fig. 2. Meiotic metaphase from male offspring of an adult male irradiated with 10 kR. Note presence of fragment (f), heteromorphic bivalent (h) and multivalent (m). × 1570.

chromosomes prevent identification of other than gross morphological changes in mitotic chromosomes. The level of damage encountered differed between the 2 treatment levels. At 2 kR simple numerical changes predominated; most embryos examined fell in the chromosome number range 2n=57 to 2n=66 (figure 1). Much more extensive damage was found in the 10 kR series, the presence of numerous chromosome fragments making exact determination uncertain in many cases.

Sampling of larvae from F1 families descended from irradiated males confirmed the presence of inherited numerical modifications, as well as revealing morphological changes undetectable in mitotic cells. Meiotic analysis disclosed altered chromosome configurations ranging from simple trivalents and quadrivalents in the 2 kR series to fragments, complex multivalents and heteromorphic bivalents in the 10 kR series (figure 2). In general, the extent of inherited chromosome aberrations predicted for each family on the basis of embryonic sampling was closely confirmed by meiotic analysis, indicating that embryo sampling is useful in detecting both presence and extent of inherited aberrations.

From a 2 kR family known by embryonic and meiotic analysis to be carrying low frequencies of aberrations, 6 males were identified by testis sampling as heterozygous for 1 or 2 chromosome fusions. Of the 4 that became adults, 3 mated successfully with normal females. Egg hatch was reduced in all 3 families, and embryonic sampling gave the following chromosome number classes: family 1, ♂ parent n=28 II+1 III, 12 embryos with 2n=60, 14 with 2n=59; family 2, ♂ n=28 II+1 III, 16 embryos with 2n=60, 9 with m=59; family 3, ♂ n=26 II+2 III, 5 embryos with 2n=58, 7 with 2n=59 and 4 with 2n=60. Establishment of segregation ratios in F2 embryos demonstrates 2 points; the techniques can be used to establish translocation-bearing strains in 1 generation, and embryo sampling can be used to investigate meiotic behavior of translocations in the holokinetic chromosome system of Lepidoptera, hitherto not studied in detail.

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Effect of B-chromosomes on the duration of mitotic cycle in pearl millet

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Summary. Effect of B-chromosomes on the duration of the mitotic cycle was studied by a colchicine shock method in the root-tip cells of pearl millet *(Pennisetum typhoides)*. 3 B-chromosomes were found to have very little effect, whereas 5 B-chromosomes resulted in an increase of the duration by about 39%.

Several aspects of cytogenetics of B-chromosomes of *Pennisetum typhoides* (S. & H.) have been reported[2-4]. The B-chromosomes at pachytene are largely heterochromatic; the short arm appears to be wholly heterochromatic, while the long arm consists of proximal heterochromatin followed by an euchromatic region with 8 chromomeres. This B-chromosome varies in number both in pollen mother cells (PMCs) and in somatic cells, between and within the plants. The B-chromosomes in small numbers (1-4) do not seem to influence the mean chiasma frequency of the normal (A) chromosomes, though 5 or more B's have a negative effect. Further, they increase the variance of the mean; this effect increases with the increase in the number of B-chromosomes per PMC. 5 or more B's per PMC also produce deleterious effects on the behaviour of A-chromosomes at meiosis and on exophenotypic characters[4].

B-chromosomes in general are known to have an influence on many different gene-controlled processes. They produce an extension of mitotic cycle time and reduction in cell number; these effects together with changes in cell size in the presence of B's provide a physiological basis for many effects of B-chromosomes like reduction in vigour, delay in germination and delay in flowering[5]. The present study was undertaken to investigate whether B-chromosomes affect the mitotic cycle in pearl millet.

Materials and methods. Seeds from stock without (0 B), with 3 (3 B), and with 5 (5 B) B's were germinated on moist filter papers at 30±2 °C. After 24 h germinating seedlings with 5-6 mm long roots were chosen. The roots were pretreated with 0.25% colchicine for 1 h and, after thorough washing in tap water, seedlings were replaced on the moist filter

Accumulation of metaphases expressed as percentage, in root tips of diploids without (0B) and with 3 or 5 (3B, 5B) B-chromosomes in pearl millet

Sampling time (h)	0B		3B		5B	
	2×	4×	2×	4×	2×	4×
1	10.9		9.0		5.5	
2	9.3		8.6		6.6	
3	10.0		16.3		8.0	
4	13.0		9.5		7.3	
5			13.7		1.6	
6			16.0			
7					0.08	
8						
9			5.0		2.5	
11					3.0	
12			0.03			
15			3.7		2.4	
16	0.3	0.8		2.0		4.0
17						
18		2.0				
19				0.03		5.3
20		9.0		0.0		
21						0.04
22		6.0		2.0		1.5
24		7.0		3.0		
25						5.0
26		8.0		14.6		
27						
28		0.0		0.0		3.0
29						2.7
30				3.4		2.3
31						3.3
32				4.4		8.8