Selective screen to recover pairs of deletion-tandem duplications

L. OMELYANCHUK

Institute of Cytology and Genetics, Novosibirsk 630090, Russia

A method of selecting pairs of deletion-tandem duplications in an autosome arm of *Drosophila* has been developed. Males containing autosome 2 and the right and left free arms of this autosome produce gametes with the metacentric and the free arm through meiotic non-disjunction. Unequal exchanges induced in such gametes give deletions and tandem duplications within the left or right chromosomal arms. This approach removes the necessity of using markers to identify deletions in the chromosome region.

Keywords: deletions, *Drosophila*, duplications, free-arm, non-disjunction.

Introduction

Chromosomal deletions can be effectively used in genetic analysis and a number of methods have been developed for their recovery. The approach most commonly used is the so-called ‘uncovering’ of a recessive marker (Mohr, 1927). Lindsley *et al.* (1972) obtained a collection of autosome-Y translocations which allowed the synthesis of deletions and duplications of any autosome locus. The screening system, based upon Craymers autosynaptic elements (Craymer, 1981), allows the recovery of chromosomal deletions and duplications within a small genetic region (Gubb *et al.*, 1988).

A selective screen has been developed to obtain ‘complementary’ pairs of deletions — tandem duplications. This approach does not involve the use of a marker in the area under investigation because all deletions and duplications in the arm are being isolated. Deletions and duplications obtained are located in metacentrics or in free arms and do not contain additional material of other chromosomes as a rule.

The method is to induce unequal exchanges in differently marked homologous autosomes after meiosis. As the output of rearrangements is highest with irradiated mature sperm, we decided to use male meiosis. To enhance the frequency of non-disjunction appreciably, males heterozygous for a metacentric and for left and right free arms were used allowing non-disjunction of the metacentric and one of the free arms (Chadov *et al.*, 1986).

Materials and methods

The crosses used are diagrammed in Fig. 1. Heterozygous males containing autosome 2 marked with *net dp b* and free arms *F(2L)2, pr* and *F(2R)1, +* were irradiated with 3,000R γ -rays ^{60}Co and crossed to females containing compound *C(2L)RM, b* and two free arms *F(2R)1, +*. To collect eggs, females were transferred to fresh vials after a 2-day mating period.

The sons with wild-type phenotypes were picked out and crossed with homozygous *net dp b pr cn* females. The males arising from spermatozoa without exchanges between the metacentric and a left free arm gave progeny of two phenotypes — *pr* and *net dp b* — and were discarded. Males with other classes of phenotype were mated with females of constitution

Fig. 1 Crosses used when screening deletions and duplications.

C(2L)RM,b;F(2R)1, +. In subsequent generations stocks obtained in this way were maintained by mating wild-type males with *b* females. As the rearrangements are maintained only in males, crossing over between metacentric and the arm is depressed.

In each stock, temporary aceto-orcein squashes of larval salivary glands and giemsa-stained neural ganglion mitoses were examined. This allows independent determination of the number of 2L arms in the individuals with the rearranged 2L arm, analysis of rearrangements with heterochromatic breaks, and specification of a deletion or duplication to the metacentric or the free arm.

Results and discussion

Thirteen out of 354 tested males gave progeny different from *net dp b* and *pr*. These phenotypes and the results of cytological analysis of corresponding stocks are given in Table 1. Stocks NG4, NG6, NG7, NG12, NG14 contain cytologically visible chromosome rearrangements. The chromosomes in stocks NG4, NG6, NG7, NG12 appear to be two-break rearrangements and their structures, together with the marker constitution, are shown in Fig. 2. As may be seen, the structures in combination with chromosome *net dp b pr cn* generate phenotypes that agree with those in Table 1 (NG7 excluded). The correspondence includes only one class of progeny because the other one is inviable. All this may be applied to the NG14-complex

Fig. 2 Structures of the rearrangements obtained. Material from metacentrics is denoted by a faint line. A heavy line denotes the 2L free arm. The material deleted is denoted by a dashed line.

Table 1 Phenotype classes in the progeny of the males analysed and cytological characteristics of the corresponding stocks

Stock's index	Phenotypes in the progeny of males tested	Cytology
NG1	<i>net dp b pr</i>	No exchange
NG3	<i>net dp b pr</i> and <i>net</i>	No exchange
NG4	+	34B2-7; 36B
NG5	<i>b</i> and <i>pr</i>	No exchange
NG6	<i>net dp pr</i>	23E1-2; 30C1-5
NG7	<i>net pr</i> and <i>net dp b</i>	36C1-2; 39B-C4
NG8	<i>b</i> and <i>net dp b</i>	No exchange
NG9	<i>net pr</i> and <i>pr</i> and <i>net dp b</i>	No exchange
NG10	<i>dp</i>	No exchange
NG11	<i>dp</i> and <i>net dp b</i> and <i>pr</i> and <i>net pr</i>	No exchange
NG12	<i>net dp</i>	30B-C; 38E-F
NG13	<i>net</i>	Not analysed
NG14	<i>net dp</i>	30B2-5; 32F2-4; 40; 80; 90C

chromosome rearrangement, whose structure is not presented herein.

The marker *net* was present in two phenotypic classes in NG3, NG7, NG9, NG11. This may mean that exchanges in these cases occur at the 4-chromatid stage. This may occur after paternal chromosome replication and before caryogamy or as a result of mitotic crossingover after caryogamy. Phenotype classes in NG7, NG5, NG8 and NG3 agree with single and double mitotic exchanges correspondingly. Another reason for *net* phenotypes in NG3, NG7, NG9, NG11 might be that irradiated chromosomes carry dominant enhancers of *net* but this seems less probable.

Males of cultures NG9 and NG11 gave progeny phenotypes similar to those expected as the result of gonadal mitotic crossing over. Stocks NG1 and NG10 probably contain induced but cytologically non-visible exchanges. Unexpectedly, stock NG7 contains both deletion-duplication and mitotic exchange.

Fourteen individuals with phenotype *pr*, eight with *net dp* and one with *net* found in the first mating progeny and evidently being mitotic crossovers confirm the mitotic nature of exchanges in a part of the stocks.

These phenotypes could appear as the result of exchanges at the 4-chromatid stage in the regions pre-centromere, *dp-b*, or *net-dp* respectively. The large number of exchanges in the *pr*-centromere region may imply the presence of heterochromatin as early in development as the male pronucleus.

As shown in this study, it is possible to recover pairs of deletion — tandem duplications in any part of an autosome of *Drosophila*. If it is necessary to concentrate on a particular chromosome region, the screen could be made with markers on both sides of the region. Mitotic exchanges could then be almost completely excluded based upon progeny phenotypes. The main difficulties in the experiment were the low level of meiotic non-disjunction in irradiated males, and the insufficient number of males. We now have free 2L and 2R arms giving high non-disjunction of the metacentric and the arm.

The collection of deletions and duplications could be extended by complementation between a mutated chromosome with the deletions or duplications already available.

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References

- CHADOV, B. F., CHADOVA, E. V. AND KHOCKINA, E. A. 1986. Chromosome conjugation in *Drosophila* III. Similarity and differences between chromosome pairing in females and males. *Genetica*, **22**, 71–79.
- CRAYMER, L. 1981. Techniques for manipulating chromosomal rearrangements and their application to *Drosophila melanogaster* 1. Pericentric inversions. *Genetics*, **99**, 75–97.
- GUBB, D., MCGILL, S. AND ASHBURNER, M. 1988. A selective screen to recover chromosomal deletions and duplications in *Drosophila melanogaster*. *Genetics*, **119**, 377–390.
- LINDSLEY, D. L., SANDLER, L., BAKER, B. S., CARPENTER, A. T. C., DENELL, K. E., HALL, J. C., JACOBS, P. A., MICLOS, G. L. G., DAVIS, B. K., GETHMANN, R. C., HARDY, R. W., HESSLER, A., MILLER, S. M., NOZAVA, H., PARRY, D. M. AND GOULD-SOMERO, M. 1972. Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. *Genetics*, **71**, 157–184.
- MOHR, O. L. 1927. Exaggeration and inhibition phenomena. *Avh. Norske Vidensk. Akad.*, **6**, 1–19.