Genet. Res., Camb. (1976), **26**, *pp.* 313–317
With 1 *plate and* 1 *text-figure*
Printed in Great Britain

New compound (1) chromosomes and the production of large quantities of *X/O* males in *Drosophila hydei*

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(*Received* 11 *September* 1975)

SUMMARY

In view of the exceptional usefulness of *Drosophila hydei* for the analysis of *Y*-chromosome activity, a technique has been developed which permits the production of large numbers of males lacking a *Y* chromosome. It is based on the synthesis of new compound(1) chromosomes which carry sufficient rDNA genes to secure survival in the absence of any *Y*-chromosomal rDNA. Through non-disjunction in males of suitable stocks, fertile Compound(1) females which lack the *Y* chromosome, phenotypically distinguishable from their normal sibs, are produced in sufficient number to allow the subsequent breeding of *X/O* males in gram quantities for biochemical studies.

1. INTRODUCTION

Drosophila hydei is one of the most suitable species to study the functional activity of the *Drosophila Y* chromosome owing to its large spermatocytes, which show a number of distinct structures developed by this entirely heterochromatic chromosome, and owing to the relatively advanced genetics of this species compared to others with suitable spermatocyte nuclei (review in Hess & Meyer, 1968). Biochemical and electron-microscopical studies on these *Y*-chromosome structures, likened to the loops of lampbrush chromosomes by Meyer (1963), necessitate a parallel study of males of the same species which do not possess them. However, no effective system to produce reasonable quantities of such males has so far been available in *D. hydei*. Furthermore, the existing C(1) chromosomes (Compound (1), usually called attached-*X* and symbolized $\overline{XX}$) are lethal in the absence of a *Y* chromosome (Hennig, 1968; Beck, 1973) because the location of the *X*-chromosomal nucleolus organizer region (NOR) distally in the heterochromatic arm (van Breugel, 1970) will preferentially exclude its presence in C(1) chromosomes (see also Fig. 1*B*). Therefore attempts to produce a system lacking free *Y* chromosomes, as for example $XY^{L}.Y^{S} \times \overline{XX}/O$ in *D. melanogaster* (Lindsley & Grell, 1968), imply the existence of C(1) chromosomes carrying sufficient rDNA at the NOR to secure viability in the absence of any *Y*-chromosomal rDNA. In this communication the synthesis of such Compounds, their phenotype and cytology as well as methods for producing large numbers of males lacking a free *Y* chromosome are described.

2. MATERIALS AND METHODS

Stocks used: *Tp(1)* w^{m1} y^{Lt}: Transposition *white-mottled-1*, homozygous viable, order: 20-17B2/h^{L}NOR/17B1.1h^{S}.h^{L}/NOR/h^{L} (van Breugel, 1970). bb^{l}: homozygous lethal *bobbed* allele from wild population. bb^{A1}, bb^{4}, bb^{8}: *bobbed* alleles with different bristle

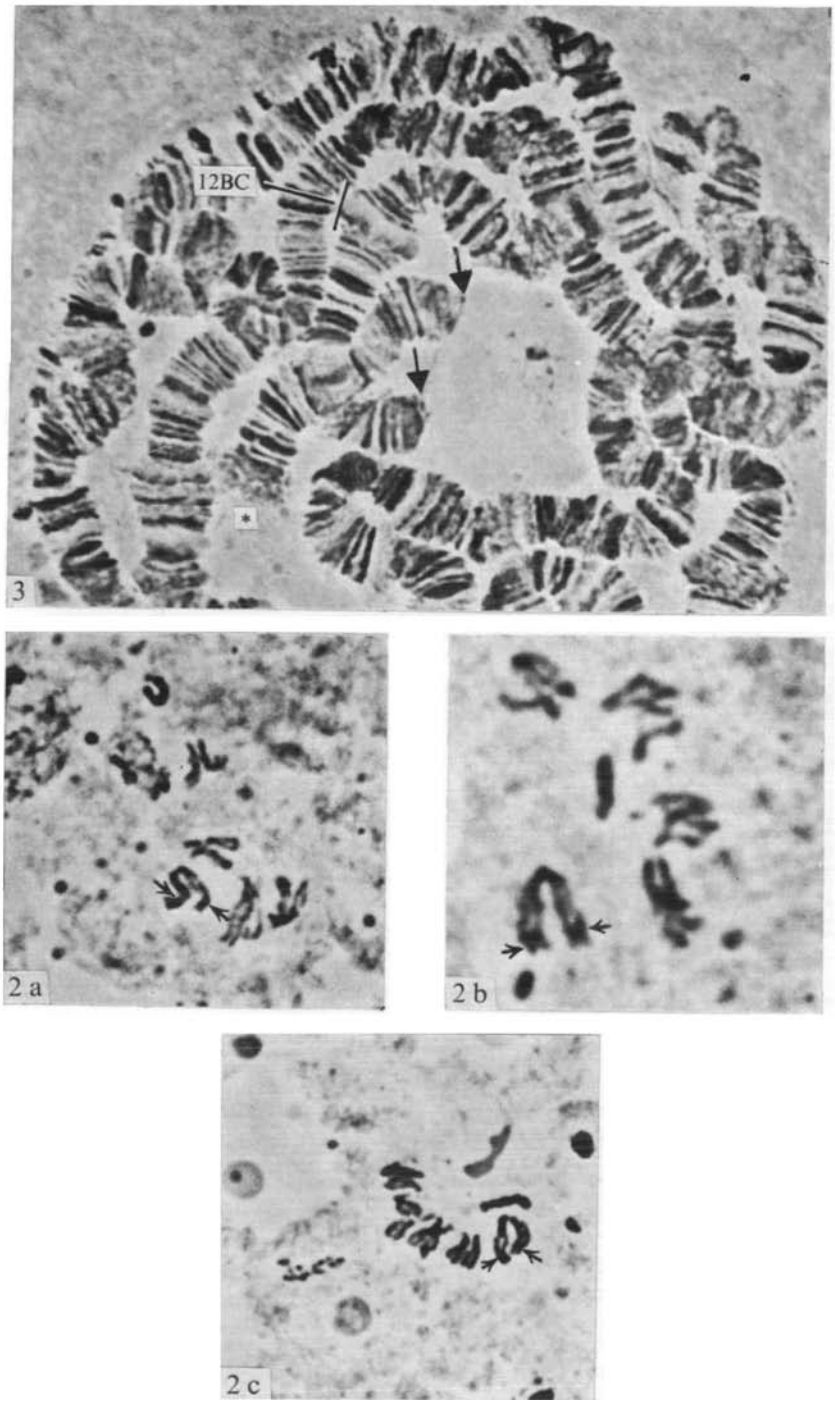

Fig. 2. Chromosomes of three new Compound (1) stocks derived from $Tp(1)w^{m1}y^{L1}$: *a*, $C(1)^1$; *b*, $C(1)^2$; *c*, $C(1)^3$. Note asymmetry of centric heterochromatin and presence of heterochromatin distally in each chromosome arm (arrows). Photographs by F. M. A. van Breugel. × 3600 approx.

Fig. 3. Salivary gland chromosomes of $C(1)^2Tp(1)$ showing nucleolus inserted in region 17B (arrows). The tip of the *X* chromosome is marked by an asterisk; 12BC indicates a 'landmark' proximal of the break and roughly half way through the length of the *X* chromosome. × 1800 approx.

a part of the NOR inserted into euchromatin in salivary chromosome region 17B and probably a fragment of the NOR at the normal position distally in the heterochromatic arm of the *X* chromosome (van Breugel, 1970). Formation of a Compound(1) may occur after the induction of a break near the centromere to the left in one chromosome and a break in the heterochromatic arm of the partner chromosome. In this case the NOR portion transposed into euchromatin is preserved in both arms of the Compound.

Fig. 1(*B*) for comparison shows the type of C(1) chromosome obtained from normal *X* chromosomes. Though the inclusion of a NOR into C(1) chromosomes derived from *X* chromosomes with normal sequence does not seem impossible, no such case has been found (Beck, 1973).

As can be seen from Plate 1, Fig. 2(*a–c*), the three new C(1) chromosomes obtained after irradiation of homozygous *Tp(1)* w^{m1} y^{Lt} females, and referred to as *C(1) Tp(1)* in the text, show only little centric heterochromatin. This excludes the presence of a NOR in the normal position. In all three cases the heterochromatin in the distal part of each chromosome arm is clearly visible. That this region develops a nucleolus is seen from Plate 1, fig. 3, where the nucleolus is attached to salivary chromosome region 17B (see legend to Fig. 3). In many nuclei the nucleolus is detached and a break is seen where it was located. Thus the same pictures are obtained as by van Breugel (1970), with the exception that the chromosomes are attached and have lost a large portion of the heterochromatic arms including the NOR fragment which probably had been present in the original chromosome. The position of the transposed heterochromatin indicates that all three C(1) chromosomes are reversed metacentrics and their full description may be given as follows: *C(1)* $RM^{1,2,3}$*Tp(1)* w^{m1} y^{Lt}, where the superscripts designate each of the independently obtained Compounds.

Table 1. *Emergence of* C(1) Tp(1)/Y *and* C(1) Tp(1)/0 (bb) *females on consecutive days, from the cross* C(1) Tp(1)/Y♀ × T((In); X 3)s♂ *(data were collected from 10 uncrowded culture bottles*

Day of emergence	Females emerged		
	$\overline{XX}/Y$	$\overline{XX}/O$	% $\overline{XX}/O$
1	143	4	2·7
2	125	16	11·3
3	156	32	16·4
4	60	10	14·2
5	46	8	14·8
Total	530	70	11·6

Since the homozygotes of the stock used show a moderate *bb* phenotype and *Tp(1)* w^{m1} $y^{Lt}/0$ males are viable and *bb* in phenotype, it could be predicted that the *C(1) Tp(1)* females derived from this stock, when lacking a *Y* chromosome, would show a *bb* phenotype. That this is the case could be demonstrated by crossing *C(1) Tp(1)* females to males carrying the translocation *T((In) X; 3)s* or to males of R-stocks. The presence of such translocations results in frequent non-disjunction of sex chromosomes in the male (H. Frei, personal communication, and my own unpublished results) and consequently females lacking a *Y* chromosome are expected in F_1. Indeed, somewhat late-hatching females (Table 1) showing a marked *bb* phenotype were found at a frequency of 11·6% among the daughters from the cross to *T((In) X; 3)s* males. Similar frequencies were obtained in crosses with males of the R-stocks mentioned above.

The exceptions are readily recognized by their short scutellar bristles, which reach only 60% of the wild-type bristle length, and they could also be diagnosed by a very faint large-spotted mottling phenotype. Since a cytological proof of *Y*-chromosome

absence in these exceptions is difficult, these females were further characterized through a series of crosses which allowed the demonstration of the absence of Y chromosomes in the male progeny (Table 2). Males lacking a Y chromosome are clearly recognized by their *bb* phenotype when their X chromosome carries a *bb* allele (Beck, 1972) and can also be diagnosed by the absence of the characteristic loops formed by the Y chromosome in spermatocytes (Meyer, 1963), and further by their complete sterility. All the results in Table 2 make it clear that no Y chromosomes were transmitted from the mothers to the sons, hence these females must be of the $\overline{XX}/O$ constitution.

Table 2. *Phenotype of male progeny from crosses of* C(1) Tp(1)/0 *females to males carrying various* bb *alleles* (*explanation in the text*)

Cross ♀ $C(1)^2Tp(1)$ × male:	Male progeny		
	Phenotype	Spermatocytes	Fertility
X^{bb+}/Y^{bb+}	Wild type	No loops	Sterile
X^{bbl}/Y^{bb+}	Lethal	—	—
X^{bbA_1}/Y^{bb+}	Extreme *bb*	No loops	Sterile
X^{bb^4}/Y^{bb+}	Strong *bb*	No loops	Sterile
X^{bb^8}/Y^{bb+}	Medium *bb*	No loops	Sterile

The *bb* phenotype of such females may be explained by the loss of a portion of the NOR at the moment when the transposition occurred, as already van Breugel (1970) suggested, and a further loss at the formation of the Compound chromosomes. It cannot be excluded, however, that the two X chromosomes of the Compound together contain a normal (wild-type) amount of rRNA genes, but that the two polycistronic regions are partially hampered in their function through a position effect (Baker, 1971). The question, whether the whole NOR or only part of it has been transposed into euchromatien can therefore not be answered at present.

Since the $\overline{XX}/O$ females can easily be scored, this system now allows the production of large numbers of X/O males. The procedure consists in collecting *bb* daughters from a cross of $C(1)$ $Tp(1)$ females to X-autosome translocation males, followed by crossing these *bb* females to any males desired.

The method formerly used in our laboratory for obtaining X/O males consisted in the scoring of large progenies from *bb* females of stock T7. These females produce, by primary non-disjunction of their sex chromosomes, a certain amount of X/O sons (Frei, 1974). Compared to this method, the system described here permits an approximately 15- to 20-fold increase in X/O recovery. This makes biochemical work with X/O males much more attractive and saves time and culture vessels.

In addition, the new system facilitates a systematic search for a fertile XY compound chromosome, since large numbers of gametes can be tested by crossing mutagenized males to $C(1)$ $Tp(1)/0$ females, with automatic selection of fertile male progeny in mass culture. Such experiments are currently in progress.

I am indebted to Prof. H. Gloor, Drs H. Frei, H. R. Kobel and F. M. A. van Breugel (Leiden, The Netherlands) for their help in various aspects of this work. The technical assistance of Mrs Annie Legast, Mrs Claraberta Michelini and of my wife, Mrs Edith Beck, is gratefully acknowledged.

This work received support from the Fonds national suisse de la recherche scientifique (crédit no. 3.224.69).

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