CYTOGENETIC ANALYSIS OF CHROMOSOME 3 IN *DROSOPHILA MELANOGASTER*: MAPPING OF THE PROXIMAL PORTION OF THE RIGHT ARM[1]

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ABSTRACT

In order to define more precisely the most proximal portion of chromosome *3R* in *Drosophila melanogaster*, several new chromosome aberrations involving this region have been recovered and analyzed. These new arrangements were recovered as induced reversions of two dominant mutations, $Antp^{Ns}$ and dsx^{D}, located in the region of interest. The results of the analysis have allowed the localization of several existing mutations, have further elucidated the complex homoeotic locus which resides in this region, and have confirmed the efficacy of this type of screen in the analysis of specific chromosome regions.

THE proximal portion of *3L* from *st* to the centromere has been rather well saturated with chromosome aberrations by LINDSLEY *et al.* (1972) and further information is not really needed. However, the proximal region of *3R* is not saturated to the same extent. Two small deficiencies in the most proximal portion of *3R* (81F-82A and 82B-C) can be synthesized using the *Y-3* translocation stocks of LINDSLEY *et al.* (1972). However, in the region from 82C to 85E, it is possible to make only one deficiency (83E,F-84D). Since this segment (proximal *3R*) is the most likely locale of a large group of ts lethals (TASAKA and SUZUKI 1973), as well as a complex homoeotic locus (DENELL 1973), we set out to create several new chromosome aberrations in this area of the third chromosome.

The results of LINDSLEY *et al.* (1972) demonstrate that, except for the Minutes, most dominant mutations are located in regions which result in a normal phenotype when haploid, since their dominant phenotypes are not expressed in Deficiency/+ heterozygotes. They point out that it should be possible to revert such dominants by deleting them. Therefore, the method chosen to screen for chromosome aberrations in proximal *3R* was to search for radiation-induced loss of the phenotypes expressed by two dominant mutations known to be located near the centromere of the third chromosome. They are Nasobemia ($Antp^{Ns}$) and doublesex-Dominant (dsx^{D}). This report concerns the recovery and characterization of several newly induced revertants of these two mutations.

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MATERIALS AND METHODS

All flies were raised on standard dried yeast, cornmeal and dextrose medium and all routine work was carried out at 22° ± 1°. Larvae destined for salivary gland chromosome preparations were raised at 17° ± 1°. All irradiations were done by packing 50–100 adult males of varying ages into gelatine capsules and administering approximately 4000r of gamma radiation from a cobalt-60 Gammacell. Detailed descriptions of the properties of the mutations and chromosome aberrations used can be found in LINDSLEY and GRELL (1968); those of special interest to this study are listed with a brief description in Table 1.

TABLE 1

Description and symbols of third chromosome mutations used

Mutant	Symbol	Map position	Description
scarlet	*st*	44.0	Eyes bright red
transformer	*tra*	45	Female transformed into sterile male
inturned	*in*	47	Hairs and bristles directed toward midline
radius incompletus	*ri*	47.0	L2 interrupted
Polycomb	*Pc*	47.7	Sex combs on 2nd and 3rd legs of male
eagle^2	eg^2	47.3	Wings spread
Deformed	*Dfd*	47.5	Eyes reduced; recessive lethal
Deformed recessive	Dfd^r	47.5	Recessive allele of *Dfd*
Kinked	*Ki*	47.6	Bristles and hairs short and twisted
roughened eye	*roe*	47.6	Eyes rough
dark red brown	*drb*	47.7	Eyes dark red brown
proboscipedia	*pb*	47.7	Oral lobes tarsus or arista-like
rotund	*rn*	47.7	Wings short, sex combs absent, associated with *T(2;3)rn = T(2;3)40–41;80–81* and *In(3R)81F;84D*
Antennapedia	$Antp^B$	48	Antenna leg-like, associated with $In(3R)Antp^B = In(3R)84A;85E$
Extra sex comb	*Scx*	47	Sex combs on all legs of male
Multiple sex comb	*Msc*	48.0	Sex combs on 2nd and 3rd leg of male, associated with *In(3R)Msc = In(3R)84B;84F*
Nasobemia	$Antp^{Ns}$	48.0	Antenna leg-like
double sex	*dsx*	48	Male and female intersexual
double sex dominant	dsx^D	48	Female intersexual
pink peach	p^p	48.0	Eyes dull ruby
bowed	*bod*	48.3	Wings curved down
Humeral	*Hu*	51	Extra humeral bristles, associated with *In(3R)Hu = In(3R)84B3;84F2-3;86B4-C1*
Stubble	*Sb*	58.2	Bristles short and thick
bithorax	*bx*	58.8	Halteres enlarged, metathorax mesothoracic
Ultrabithorax	*Ubx*	58.8	Halteres enlarged
stripe	*sr*	62.0	Dark median stripe on thorax
ebony	e^s	70.7	Black body
Serrate	*Ser*	92.5	Wing tips notched
$Df(3R)Antp^{Ns+R72}$	*R72*		Nasobemia revertant *Df(3R)84B3;84D*
T(3;Y)P92	*P92*		Insertion of segment of *3R(84D10–11;85A1–3)* into *Y*

I. Isolation of $Antp^{Ns}$ reversions.

Adult males homozygous for $Antp^{Ns}$ were irradiated and mated to virgin *TM3, Sb Ser/ T(2;3)ap*^{Xa} females in quarter-pint milk bottles containing standard Drosophila medium (approximately 20♂♂:30♀♀ per bottle). These parents were allowed two three-day broods on fresh medium, after which the males were discarded and the females were transferred to fresh bottles for a further three days, at which point they were discarded. The $Antp^{Ns*}/T(2;3)ap^{Xa}$ and $Antp^{Ns*}$*/TM3, Sb Ser* progeny (where $Antp^{Ns*}$ denotes an irradiated chromosome) were collected as virgins and examined for the presence of wild-type antennae. Because the $Antp^{Ns}$ mutation is not fully penetrant, progeny with wild-type antennae were tested for true reversion of $Antp^{Ns}$ by individually mating them in shell vials to several *TM3, Sb Ser/T(2;3)ap*^{Xa} males or virgin fmales. Any of these vials which failed to give progeny displaying the Nasobemia phenotype were saved and the putative revertant $Antp^{Ns*}$ chromosomes were balanced with *TM3, Sb Ser* or *T(2;3)*^{Xa}. Seven separate radiation experiments were performed for this screen.

II. Isolation of dsx^D reversions.

Heterozygous dsx^D *Sb e/TM2* males were irradiated and mated in quarter-pint milk bottles to *TM3/T(2;3)ap*^{Xa} virgin females (approximately 20♂♂:30♀♀ per bottle). Note that the *TM3* balancer chromosome used in this screen does not carry the recessive lethal marker *Sb* and, therefore, is viable in heterozygous combination with the dsx^D *Sb e* chromosome. Parents were brooded as in I and *X/X*; dsx^{D*} *Sb e/TM3* and *X/X*; dsx^{D*} *Sb e/T(2;3)ap*^{Xa} progeny were selected and tested for fertility by mating five of these "females" to five *TM3/T(2;3)ap*^{Xa} males per shell vial. Vials containing progeny were saved and the revertant chromosomes were balanced with *TM6* or *CxD*. Eight separate radiation experiments were performed.

III. Segregation analysis.

Because translocations and inversions with at least one breakpoint in heterochromatin cannot be differentiated by salivary gland chromosome analysis, all revertant chromosomes were tested genetically for translocations. Males from each revertant stock (with one exception) were mated to *y/y; SM1/Bl; TM2* or *TM3, Sb Ser/Tp(3)Vno;* spa^{pol}/spa^{pol} virgin females and F_1 males of the genotype *y/Y;+/SM1; revertant/TM2* or *TM3, Sb Ser*; $+/spa^{pol}$ were mated to spa^{pol}/spa^{pol} virgin females. One revertant ($Antp^{Ns+R16}$) did not produce males; therefore, females from the revertant stock were mated to males of the balancer stock and only F_1 females were crossed to spa^{pol}. The progeny of these crosses were scored to determine whether the markers *Cy* or spa^{pol} consistently segregated from the revertant chromosome as expected for translocations between the second and third or the third and fourth chromosomes, respectively. If the revertant chromosome appeared only in sons from this cross, then a *T(Y;3)* was indicated. An *X-3* translocation would have been indicated by the recovery of the revertant chromosome in the female progeny only.

IV. Salivary gland chromosome analysis.

Males from each of the stocks were mated to virgin Oregon-R females (10♂♂:10♀♀) in quarter-pint milk bottles. This mating was kept at 22° for a period of two to three days, at which point the parents were removed and the bottle shifted to 17° ± 1°. Late third instar larvae from these cultures were used for the preparation of salivary gland chromosomes by the usual methods. Those larvae carrying the balancer chromosomes (*TM3*, *TM6*, *T(2;3)ap*^{Xa} or *CxD*) were easily distinguishable from the newly-induced aberrations as well as revertants not associated with changes in the polytene chromosomes.

V. Removal of *Sb* and *In(3R)C* from the dsx^D revertant chromosomes.

A cytological examination of the dsx^D *Sb e* chromosome revealed that it carries an inversion with breakpoints that match the description of *In(3R)C*. To facilitate the genetic analysis of dsx^D revertants, this inversion and the recessive lethal marker *Sb* had to be removed by crossing over. Since dsx^D acts as a dominant female sterile, this cannot be accomplished in the dsx^D stock. Therefore, males from each dsx^D revertant stock were mated to homozygous *Ki* p^p *bx sr* e^s

females and heterozygous virgin females carrying the dsx^D revertant chromosome were selected from the progeny. These females were then testcrossed to *Ki* p^p *bx sh* e^s males and male progeny phenotypically *Ki*/+, *bx*, *sr* and e^s were selected as crossovers carrying the dsx^D reversion but lacking *In(3R)C* and *Sb*. The crossover chromosome was then balanced over *TM3, Sb Ser* or *CxD*.

VI. Complementation tests.

Each revertant chromosome was tested for viability when homozygous and for its ability to complement all other revertants as well as several mutations thought to be located in proximal *3R*. Crosses were made in shell vials (5 ♂♂ : 5 ♀♀ per vial) at 22° and the parents were discarded after ten days. Revertants involving *Y-3* translocations were tested from male parents only and the single revertant involving an *X-3* translocation was tested from female parents only. All other complementation crosses were performed reciprocally. In general, one vial was scored for each reciprocal cross and at least two vials were scored for each complementation test. At least 150 flies were scored in determining a lethal mutant combination. A mutant combination was said to be lethal if no heterozygotes survived and semi-lethal when fewer than 5% of the expected number of heterozygotes survived.

The homozygous lethality of revertant chromosomes not involving *X-3* or *Y-3* translocations was indicated simply by the stability of balanced stocks. Revertants involving *Y-3* translocations were tested for homozygous viability by mating exceptional *X/X; T(Y;3)/TM3, Sb Ser* females arising spontaneously in each stock to their *X;T (Y;3)/TM3, Sb Ser* brothers. The presence of non-*Sb* male progeny (*X; T(Y;3)/T(Y;3)*) from these crosses indicated homozygous viability of the revertant chromosomes.

In addition to the $Antp^{Ns}$ and dsx^D revertants recovered in this study, three other revertants were included in complementation tests. They are $Df(3R)Antp^{Ns+R72}$, a deficiency for the material between 84B3 and 84D isolated as an $Antp^{Ns}$ revertant by DENELL (1973), and $Antp^{+R1}$ and $Antp^{+R2}$, two spontaneous *Antp* revertants found by M. M. GREEN (personal communi-

TABLE 2

Cytogenetic characterization of Antp, $Antp^{Ns}$ *and* dsx^D *revertants*

Mutant	Cytology and segregation*
$Antp^{+R1}$	*In(3R)84B3;85C & Tp(3)?;87B*
$Antp^{+R2}$	*Df(3R)84B3;84D1–2*
$Antp^{Ns+R1}$	*In(3R)81F;90B-C*
$Antp^{Ns+R2}$	*T(3;4)84B1–3;86E1–4;102F*
$Antp^{Ns+R3}$	*T(Y;3)84A4-B2;Y*
$Antp^{Ns+R6}$	*In(3LR)79D1–2;84A4-B2*
$Antp^{Ns+R8}$	+
$Antp^{Ns+R13}$	*T(2;3)84A4-B2;40–41*
$Antp^{Ns+R16}$	*T(X;3)11F1–2;97D3–4 & In(3R)75A-B;82B-C & In(3R)80C;84A4-B2*
$Antp^{Ns+R17}$	*Df(3R)84B1–2;84D11–12*
$Antp^{Ns+R18}$	*T(Y;3)84A4-B2;Y*
$Antp^{Ns+R19}$	*T(Y;3)84B1–3;Y*
dsx^{D+R1}	*Df(3R)84D9–12;84F16*
dsx^{D+R2}	*Df(3R)84D9–12;84F16*
dsx^{D+R3}	*In(3R)84D11–12;85E1–2*
dsx^{D+R4}	+
dsx^{D+R5}	*Df(3R)84F2–3;84F16*

* Segregation analysis was used to characterize those aberrations which have at least one break in heterochromatin.

cation) (Table 2). All $Antp^{Ns}$ revertants recovered in this study and $Df(3R)Antp^{Ns+R72}$, $Antp^{+R1}$ and $Antp^{+R2}$ were tested for complementation *inter se* and with the known mutants *Dfd*, *Hu* and the dominant homoeotic mutations listed in Table 1. All revertants except $Antp^{+R1}$ were also mated to the mutations *Ki*, *roe*, *pb*, *rn*, *dsx* and *bod* (Table 1).

In(3R)C and *Sb* were separated from dsx^D revertants 2, 3, 4 and 5 by crossing over and all complementation tests with these revertants were done with the recombinant chromosome. Complementation tests with dsx^{D+R1} were made while this revertant chromosome carried *Sb* and *In(3R)C*. The five dsx^D revertants were tested for complementation *inter se* and with *tra*, *Pc*, *Dfd*, $Antp^B$, *Scx*, *Msc*, *Ns*, *dsx*, *Hu*, $Antp^{+R2}$, $Antp^{Ns+R17}$ and $Antp^{Ns+R72}$. All dsx^D revertants except dsx^{D+R1} were tested for complementation with dsx^D.

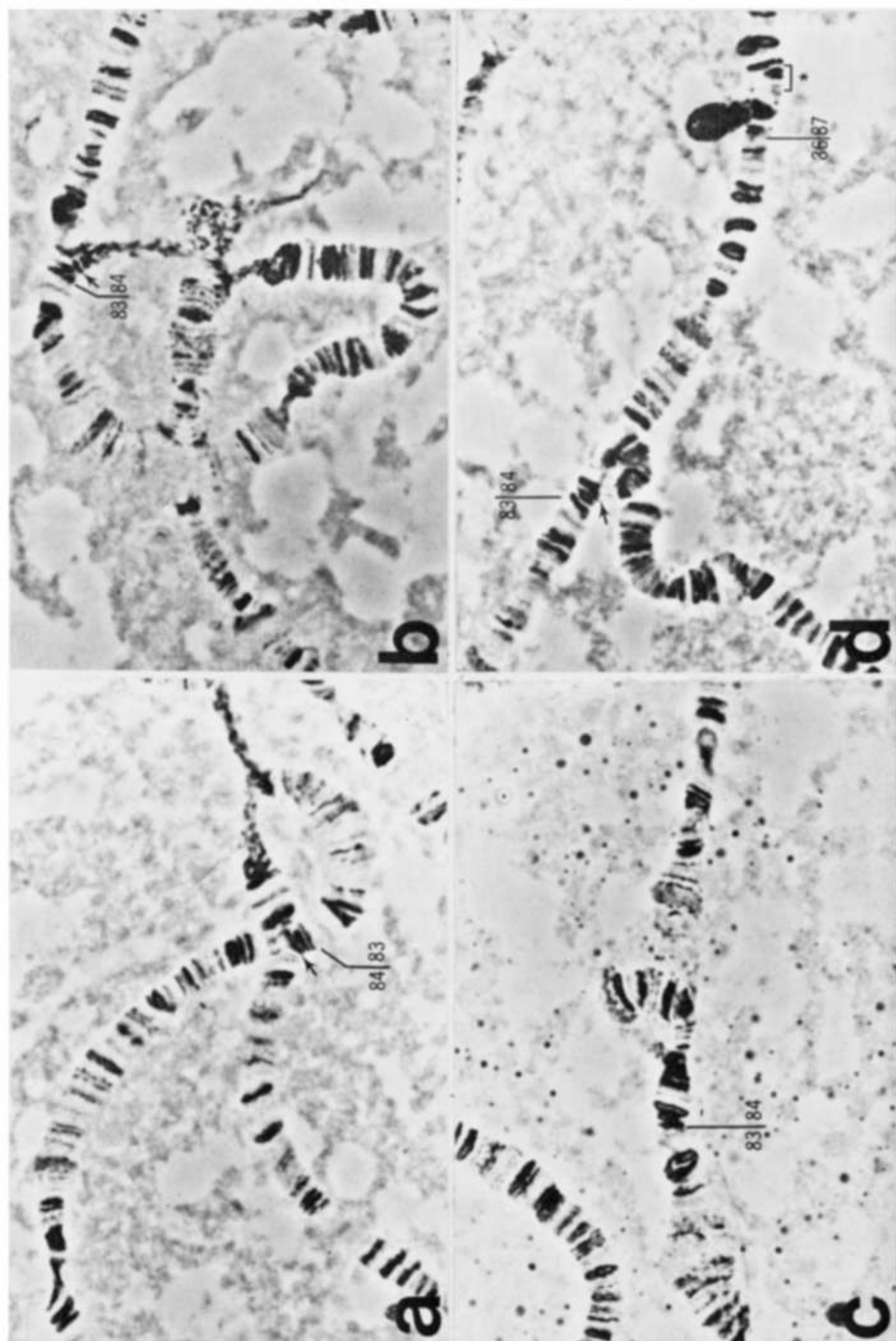
Those revertants associated with cytologically visible deficiencies were tested for complementation with all of the mutations listed in Table 1 mapping between 45 and 51. Also included in complementation tests was *Dp(3;Y)P92*, a duplication for the material between 84D and 85A synthesized by E. B. LEWIS (Table 1). Since this duplication is carried on the *Y* chromosome, complementation tests were carried out in males only. *Dp(3;Y)P92* was tested for its ability to complement the recessive lethality or mutant effects of the double sex-dominant revertants (except dsx^{D+R1}) and the mutations *Dfd*, *dsx* and p^p.

RESULTS

Among 58,669 $Antp^{Ns*}/T(2;3)ap^{Xa}$ and $Antp^{Ns*}/TM3, Sb\ Ser$ progeny examined, 2,556 were $Antp^{Ns+}$. In backcrosses to $T(2;3)ap^{Xa}/TM3, Sb\ Ser$, all but nineteen of these proved to still be heterozygous for $Antp^{Ns}$; these nineteen carried non-$Antp^{Ns}$ derivatives of the irradiated $Antp^{Ns}$-bearing chromosomes and were designated $Antp^{Ns+R}$, followed by a superscripted identifying number. Since our aim was to recover chromosomal aberrations, seven putative revertants that were viable when homozygous and cytologically normal were discarded, as were two putative revertants which were lethal when homozygous, but were cytologically normal and complemented the recessive lethality of $Antp^B$. Thus, ten revertants were retained for further study. Eight of these $Antp^{Ns}$ revertants 2, 3, 6, 8, 13, 16, 17 and 19) (Table 2) exhibit complete reversion of the Nasobemia phenotype but are lethal in combination with $Antp^B$. Two, $Antp^{Ns+R1}$ and $Antp^{Ns+R18}$, are partial revertants which express the Nasobemia phenotype with low penetrance and are viable with $Antp^B$.

In the screen for dsx^D revertants, 13,464 *X/X* progeny carrying irradiated $dsx^D\ Sb\ e$ chromosomes were tested for fertility. Five fertile females were recovered and the revertant chromosomes were designated dsx^{D+R1} through dsx^{D+R5} (Table 2).

The type of segregation observed for each revertant is shown in Table 2. Three ($Antp^{Ns}$ revertants 3, 18 and 19) show the presence of a *T(Y;3)*; two ($Antp^{Ns+R2}$ and dsx^{D+R1}) show a *T(3;4)*; one ($Antp^{Ns+R16}$) a *T(X;3)* and one ($Antp^{Ns+R13}$) a *T(2;3)*. The $Antp^{Ns+R6}$ stock has also been shown to carry a *T(Y;2)* which is not involved with the reversion of $Antp^{Ns}$, since the $Antp^{Ns+}$ phenotype is maintained when the translocation and the third chromosome are separated. The remaining ten revertants assort independently of all other chromosomes. Thus, out of seventeen revertants recovered and retained, seven carried a translocation involving chromosome 3 and another element.


b
83|84
d
83|84
a
84|83
c
83|84

Of the above 17 revertants, two ($Antp^{Ns+R8}$ and dsx^{D+R4}) had no obvious cytological anomaly. The remaining fifteen are all associated with some type of aberration. We have classified five as being primarily inversions and these are described in Table 2 and shown in Figure 1. Five revertants (all are $Antp^{Ns+R}$) are associated cytologically with translocations. These are described in Table 2 and shown in Figure 2. The remaining five revertants analyzed cytologically are deficiencies, the type of aberration we actually wanted to recover. Again, these are described in Table 2 and shown in Figure 3.

Dp(3;Y)P92, an additional chromosome aberration involving proximal *3R*, was supplied to us by DR. E. B. LEWIS, and while it is not a revertant chromosome, its importance to our analysis is sufficient to warrant our including its cytological properties. The cytological extent of the duplicated material from *3R* inserted into the *Y* is given in Table 1 and is shown in Figure 3F.

The $Antp^{+R1}$, $Antp^{+R2}$, $Antp^{Ns+R72}$ and all $Antp^{Ns}$ revertant chromosomes were tested for viability when homozygous and for complementation *inter se*. All revertant chromosomes are lethal when homozygous, except $Antp^{Ns+R18}$. The results of the *inter se* crosses are shown in Table 3. Crosses between $Antp^{Ns}$ revertants involved in *Y-3* translocations were not performed as these chromosomes normally only appear in the male. In addition, it was not possible to perform all crosses *inter se* with $Antp^{Ns+R16}$ because this stock was lost before the complementation tests were completed. It can be seen in Table 3 that $Antp^{Ns+R1}$ and $Antp^{Ns+R18}$ complement the recessive lethality of all of the retained revertant chromosomes. Every other *inter se* combination tested is lethal.

The two *Antp* reverants, $Antp^{Ns+R72}$ and all of the $Antp^{Ns}$ revertant chromosomes, except the two partial revertants $Antp^{Ns+R18}$ and $Antp^{Ns+R1}$, fail to complement the recessive lethality of $Antp^{B}$ and *Scx* (Table 4). These results clearly demonstrate a functional interaction between *Antp*, *Scx* and $Antp^{Ns}$ and support DENELL's (1973) conclusion that they are allelic. $Antp^{Ns+R2}$ and $Antp^{Ns+R17}$ are lethal in heterozygous combination with *Msc*, while all other revertant chromosomes are viable with *Msc* (Table 4). All revertants are viable over *Pc* but interact with it to give a complex phenotypic complementation pattern (Table 4). Although the Nasobemia phenotype is never observed in stocks of $Antp^{Ns}$ revertants 2, 6 and 16 balanced with *TM3, Sb Ser* or *TM6*, when these revertants are heterozygous with *Pc*, the flies exhibit an antenna to leg transformation. DENELL (1973) reported that two of his $Antp^{Ns}$ revertants, including $Antp^{Ns+R72}$, interacted with *Pc* in a similar fashion and he attributed this interaction to an enhancement by the revertants of an antenna to leg trans-

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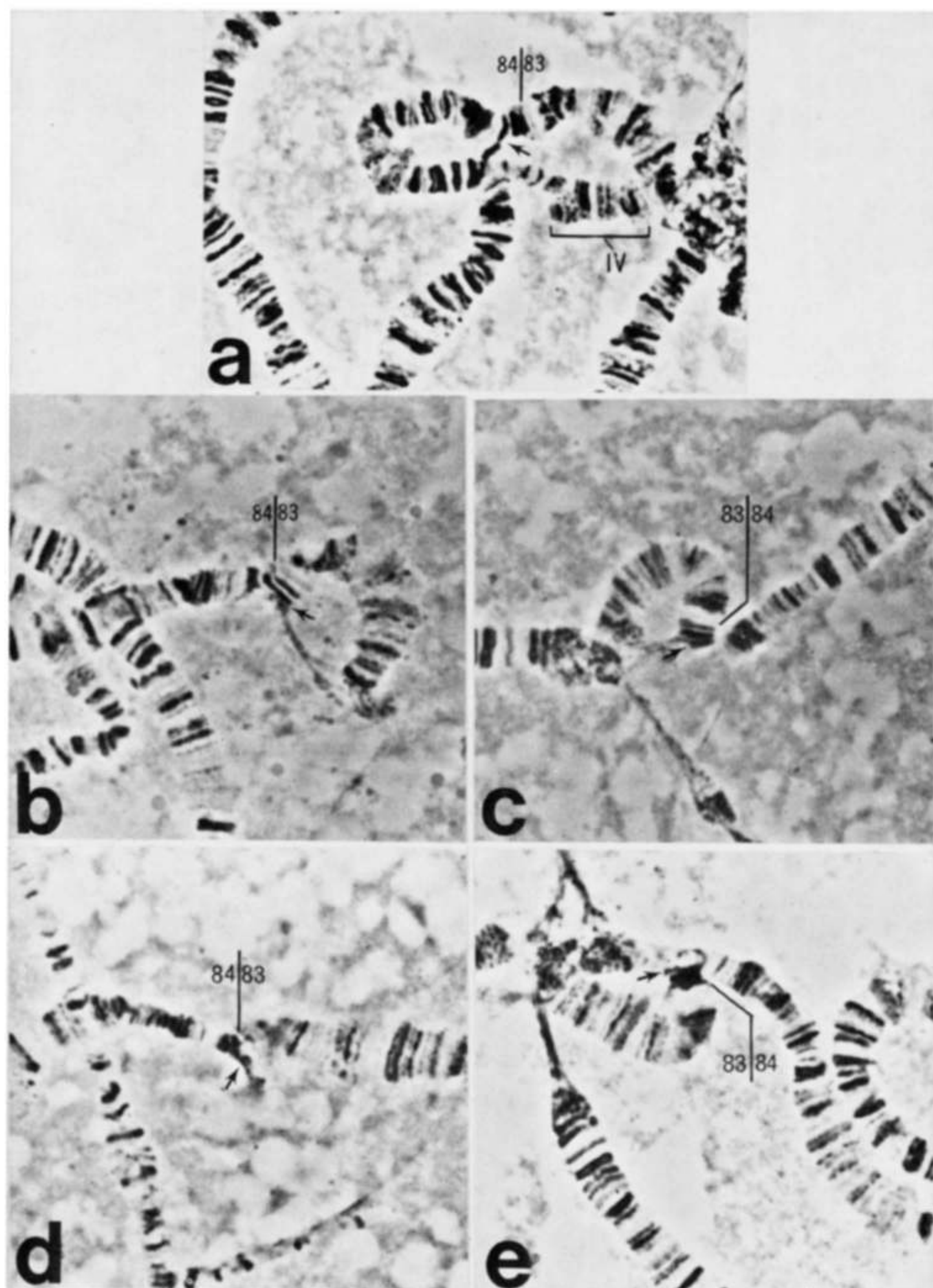
FIGURE 1.—Photomicrographs of inversion-bearing revertants:

a) $In(3LR)Antp^{Ns+R6}$ = *In(3LR)79D1-2;84A4–B2*. Arrow indicates *3R* breakpoint.

b) $In(3LR)Antp^{Ns+R16}$ = *In(3LR)75A-B;82B-C* & *In(3LR?)80–81;84A4–B2*. Arrow indicates *3R* breakpoint of the second inversion.

c) $In(3R)dsx^{+R3}$ = *In(3R)84D11–12;85F1–2*.

d) $In(3R)Antp^{+R1}$ = *In(3R)84B3;85C* & *Tp(3)?;87B*. Arrow indicates 84B breakpoint; asterisk and bracket show position of transposed material.


84|83
IV
a
84|83
b
83|84
c
84|83
d
83|84
e

formation associated with *Pc* (DENELL 1973). Also, heterozygotes with *Pc* of $Antp^{Ns}$ revertants 8, 13, 16 and 19 display humeral outgrowths as described by DENELL (1973). All $Antp^{Ns}$ revertant chromosomes are viable in heterozygous combination with $Antp^{Ns}$, *Dfd* and *Hu*.

By crossing over, *In(3R)C* and the recessive lethal marker *Sb* have been separated from dsx^{D} revertants 2, 3, 4 and 5 (but not from dsx^{D+R1}). Of five recombinant chromosomes recovered for dsx^{D+R3}, two are viable as homozygotes and the adults are sterile intersexes. Rare homozygotes are also found in three lines of the six recombinant chromosomes recovered for dsx^{D+R4}. *Like* dsx^{D+R3} *bx sr* e^{s} homozygotes, adult dsx^{D+R4} *bx sr* e^{s} homozygotes are intersexual. However, they also have roughened eyes and deformed legs. A single homozygous viable stock of each revertant was chosen and used in all complementation tests. Stocks of the other three revertants used in complementation are lethal when homozygous.

The dsx^{D} revertants were crossed in all combinations *inter se* and tested for complementation. The results are shown in Table 5. Flies heterozygous for dsx^{D+R3} and any of the other four dsx^{D} revertants are sterile intersexes. One dsx^{D+R4}/dsx^{D+R5} heterozygote was found out of 469 flies scored and five dsx^{D+R4}/dsx^{D+R1} heterozygotes were found out of 208 flies scored. All of these heterozygotes were intersexual and had roughened eyes and deformed legs. All other combinations of dsx^{D} revertants were lethal.

All dsx^{D} revertants were also tested for the ability to complement the known mutants *dsx*, *tra*, *Dfd*, *Hu* and the homoeotic mutants listed in Table 1. All dsx^{D+D}/dsx heterozygotes are sterile intersexes while all dsx^{D+R}/tra heterozygotes are fertile males and females. The inability of the dsx^{D} revertants to complement *dsx*, as well as their ability to complement *tra*, supports DENELL and JACKSON's (1972) conclusion that dsx^{D}, originally thought to be allelic to *tra* (GOWEN and FUNG 1957), is actually an allele of *dsx*. We have crossed dsx^{D} to *dsx* and all dsx^{D}/dsx heterozygotes were found to be morphologically males, possessing sex combs and the male number (5) of sternites. Thirty-one of these apparent "males" were individually mated to Ore-R virgin females as a test for fertility. Eighteen were fertile and 13 were sterile. The sterile "males" were noticeably larger than the fertile males and were not seen to mate with the Ore-R females, while their fertile brothers rapidly mated with these females. These observations are consistent with the conclusion (DENELL and JACKSON 1972) that

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FIGURE 2.—Photomicrographs of translocation-bearing revertants:

a) $T(3;4)Antp^{Ns+R2} = T(3;4)84B1\text{-}3;86E1\text{-}4;102F$. Arrow indicates *3R* translocation breakpoint; bracket indicates fourth (IV) chromosomes. The distal portion of chromosome *4* caps the *3R* base and is not visible in the photograph.

b) $T(Y;3)Antp^{Ns+R3} = T(Y;3)Y;84A3\text{-}B2$.

c) $T(2;3)Antp^{Ns+R13} = T(2;3)40\text{-}41;84A3\text{-}B2$.

d) $T(Y;3)Antp^{Ns+R18} = T(Y;3)Y;84A3\text{-}B2$.

e) $T(Y;3)Antp^{Ns+R19} = T(Y;3)Y;84B1\text{-}3$.

Arrows in b, c, d and e indicate *3R* breakpoint.

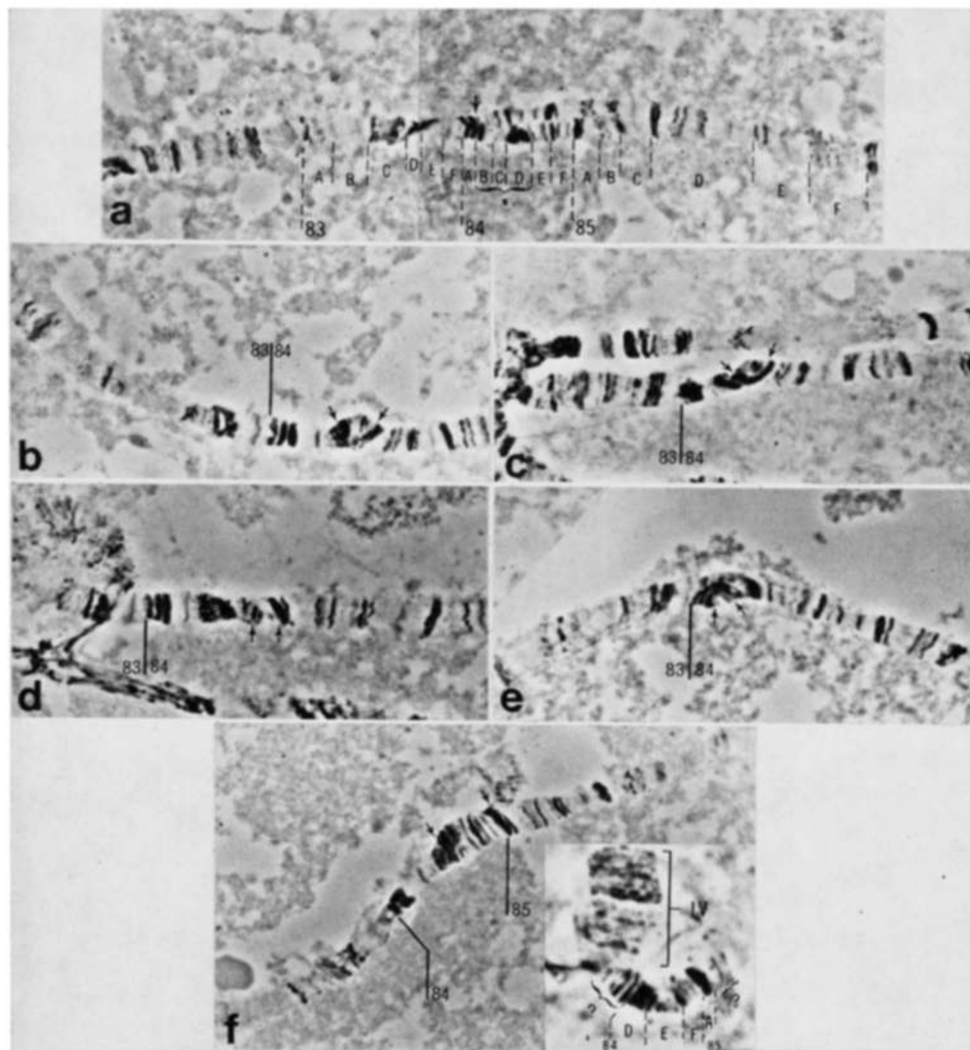

FIGURE 3.—Photomicrographs of deficiencies and duplication used in this analysis:

a) $Df(3R)Antp^{Ns+R17} = Df(3R)84B1\text{–}2;84D11\text{–}12$. The deficiency-bearing and normal homologs are unpaired. The lower segment shows the normal sequence; the amount of material deficient is indicated by bracket and asterisk. The arrow on the upper segment shows the point at which the deleted material has been removed.

b) $Df(3R)dsx^{D+R1} = Df(3R)84D9\text{–}12;84F16$.

c) $Df(3R)dsx^{D+R2} = Df(3R)84D9\text{–}12;84F16$.

d) $Df(3R)dsx^{D+R5} = Df(3R)84F2\text{–}3;84F16$.

Segment between arrows in b, c and e is the amount of material deleted in the deficient homolog.

e) $Dp(3;Y)P92 = Dp(3;Y)84D10\text{–}11;85A1\text{–}3;Y$. Large section shows duplication paired to normal homolog; arrows indicate length of duplication. Inset shows free duplication associated with fourth chromosome. Origin of banded material delineated by brackets is uncertain. The order of the *3R* material within and with respect to the *Y* chromosome is not known.

TABLE 3

Results of complementation among Antp *and* $Antp^{Ns}$ *revertants*

	$Antp^{+R1}$	$Antp^{+R2}$	$Antp^{Ns+R1}$	$Antp^{Ns+R2}$	$Antp^{Ns+R3}$	$Antp^{Ns+R6}$	$Antp^{Ns+R8}$	$Antp^{NsR+13}$	$Antp^{Ns+R16}$	$Antp^{Ns+R17}$	$Antp^{Ns+R18}$	$Antp^{Ns+R19}$	$Antp^{Ns+R72}$
$Antp^{+R1}$	L												
$Antp^{+R2}$	L	L											
$Antp^{Ns+R1}$	V	V	L										
$Antp^{Ns+R2}$	L	L	V	L									
$Antp^{Ns+R3}$	L	L	V	L	L								
$Antp^{Ns+R6}$	L	L	V	L	L	L							
$Antp^{Ns+R8}$	L	L	V	L	L	L	L						
$Antp^{Ns+R13}$	L	L	V	L	L	L	L	L					
$Antp^{Ns+R16}$	*	*	V	L	**	L	L	L	L				
$Antp^{Ns+R17}$	L	L	V	L	L	L	L	L	*	L			
$Antp^{Ns+R18}$	V	V	V	V	**	V	V	V	**	V	V		
$Antp^{Ns+R19}$	L	L	V	L	**	L	L	L	**	L	**	L	
$Antp^{Ns+R72}$	L	L	V	L	L	L	L	L	*	L	V	L	L

* Crosses not completed due to loss of stock $Antp^{Ns+R16}$.
** Crosses not performed due to translocation nature of revertant.
L = Lethal.
V = Viable.

TABLE 4

Results of complementation between Antp *and* $Antp^{Ns}$ *revertants and selected dominants on chromosome* 3

	$Antp^B$	*Scx*	*Msc*	*Pc*	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{+R1}$	L	L	*Msc*	*Pc*	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{+R2}$	L	L	*Msc*	*Pc*	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R1}$	V	V	*Msc*	*Pc*	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R2}$	L	L	L	*Pc Ns**	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R3}$	L	L	*Msc*	*Pc*	$Antp^{Ns\text{-}ho}$	*Dfd*	*Hu*
$Antp^{Ns+R6}$	L	L	*Msc*	*Pc Ns**	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R8}$	L	L	*Msc*	Pc^{ho}	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R13}$	L	L	*Msc*	Pc^{ho}	$Antp^{Ns\text{-}ho}$	*Dfd*	*Hu*
$Antp^{Ns+R16}$	L	L	*Msc*	Pc^{ho} *Ns**	$Antp^{Ns\text{-}ho}$	*Dfd*	*Hu*
$Antp^{Ns+R17}$	L	L	L	*Pc*	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R18}$	V	V	*Msc*	*Pc*	$Antp^{Ns}$	*Dfd*	*Hu*
$Antp^{Ns+R19}$	L	L	*Msc*	Pc^{ho}	$Antp^{Ns\text{-}ho}$	*Dfd*	*Hu*
$Antp^{Ns+R72}$	L	L	*Msc*	*Pc Ns**	$Antp^{Ns}$	*Dfd*	*Hu*

ho = flies show humeral outgrowths.
* = flies show both *Pc* and Nasobemia-like phenotypes.
L = lethal.
V = viable.

the dsx^D/dsx combination transforms genetic females into sterile males, but has no effect on genetic males.

All dsx^D revertants, except dsx^{D+R1}, were mated to dsx^D. In every mating, all of the heterozygous, i.e., dsx^D/dsx^{D+R}, offspring were found to be morphologically "males", many of which had female body size. Thus, the dsx^D revertants behave very much like *dsx* when heterozygous with dsx^D. The dsx^D/dsx^{D+R4} and dsx^D/dsx^{D+R5} heterozygotes also showed roughened eyes and deformed legs.

None of the dsx^D revertants interacted with any of the dominant homoeotic mutants or *Hu*. However, the dsx^{D+R} chromosomes, which carry deficiencies

TABLE 5

Complementation results of inter se *crosses involving* dsx^D *revertants*

	dsx^{D+R1}	dsx^{D+R2}	dsx^{D+R3}	dsx^{D+R4}	dsx^{D+R5}
dsx^{D+R1} *Sb e*	Lethal				
dsx^{D+R2} *bx sr* e^s	Lethal	Lethal			
dsx^{D+R3} *bx sr* e^s	Sterile intersex	Sterile intersex	Sterile intersex		
dsx^{D+R4} *bx sr* e^s	Sterile intersex rough eye deformed legs	Lethal	Sterile intersex	Sterile intersex rough eye deformed legs	
dsx^{D+R5} *bx sr* e^s	Lethal	Lethal	Sterile intersex	Sterile intersex rough eye deformed legs	Lethal

(Table 2), are lethal when heterozygous with two different chromosomes carrying *Dfd*.

All revertant chromosomes associated with cytologically visible deficiencies (Table 1 and 2; Figure 3) were crossed *inter se* and were mated to the known mutations listed in Table 7. Figure 4 shows the relationship of these deficiencies to the chromosome as well as several genes localized to the proximal portion of *3R*.

In *inter se* crosses, the $Antp^{+R2}$ deficiency is viable when heterozygous with dsx^{D+R1}, dsx^{D+R2} and dsx^{D+R}. The dsx^{D+R5} deficiency is viable with $Antp^{Ns+R72}$ and $Antp^{Ns+R17}$ (Table 6). All other combinations of deficiencies are lethal. These complementation results are consistent with the cytological map in Figure 4 in that deficiencies which cytologically overlap are lethal when in heterozygous combination, while non-overlapping deficiencies are viable.

The recessive mutations p^p, *bod* and *pb* are complemented by all deficiencies. In addition, the dominant mutations *Ki* and *Hu* show no striking interaction with any of the deficiency chromosomes, and all $Antp^{Ns}/Df$ heterozygotes are viable and show a Nasobemia phenotype (Table 7). $Antp^{Ns+R72}/drb$ heterozygotes show a slightly darker eye color than their *TM6/drb* siblings; nevertheless, we do not feel that *drb* is located within the limits of $Df(3R)Antp^{Ns+R72}$ since *drb* is complemented by all other deficiencies. Although *Pc* interacts with $Df(3R)Antp^{Ns+R72}$ to cause an antenna to leg transformation (DENELL 1973), we do not believe that *Pc* is located in the 84B to 84F interval, since all deficiencies complement the recessive lethality of *Pc*. As stated previously, two different *Dfd*-bearing chromosomes tested were lethal with the three dsx^{D+R} deficiency chromosomes. Because of their interaction with *Dfd*, the three dsx^{D+R} deficiencies were mated to Dfd^r, thought to be a recessive allele of *Dfd*. However, Dfd^r does not appear to be exposed by any of these deficiencies.

As mentioned previously, the $Antp^{+R2}$, $Antp^{Ns+R72}$ and $Antp^{Ns+R17}$ deficiencies, as well as seven other $Antp^{Ns}$ revertants associated with breaks in or very near to the 84B1,2 doublet, fail to complement the recessive lethality of $Antp^B$ and *Scx*. However, of all revertant chromosomes, only $Antp^{Ns+R2}$ and $Antp^{Ns+R17}$ are lethal when heterozygous with *Msc*. The breakpoints of the *Msc* inversion have been reported by DENELL (1973) to be at 84B1,2 and 85C. It therefore appears that a recessive lethal is associated with the 84B1,2 breakpoint of

TABLE 6

Results of inter se *complementation of deficiency-bearing chromosomes*

	$Antp^{+R2}$	$Antp^{Ns+R72}$	$Antp^{Ns+R17}$	dsx^{D+R1}	dsx^{D+R2}	dsx^{D+R5}
$Antp^{+R2}$	Lethal					
$Antp^{Ns+R72}$	Lethal	Lethal				
$Antp^{Ns+R17}$	Lethal	Lethal	Lethal			
dsx^{D+R1}	Viable	Lethal	Lethal	Lethal		
dsx^{D+R2}	Viable	Lethal	Lethal	Lethal	Lethal	
dsx^{D+R5}	Viable	Viable	Viable	Lethal	Lethal	Lethal

In(3R)Msc since $Antp^{Ns+R17}$ is deficient for 84B1,2, but not 85C, and $Antp^{Ns+R2}$ carries a break in the 84B1,2 doublet.

The $Antp^{Ns+R17}$ deficiency also fails to complement the mutations *roe* and *rn*, whereas all other deficiencies complement these mutations. As stated above, our examination of $Antp^{Ns+R17}$ has shown that the 84B1,2 doublet is deleted in this deficiency (see Figure 3a). DENELL (1973) has reported that $Antp^{Ns+R72}$ is not deficient for 84B1,2, but extends from 84B3 to 84D. In addition, we have found that 84B1,2 is not deleted in the $Antp^{+R2}$ deficiency (see Figure 3e). The failure of $Antp^{Ns+R17}$ to complement *roe* and *rn* and the ability of $Antp^{+R2}$ to complement these mutations indicate that *roe* and *rn* are located either in or near 84B1,2 or in 84D (Figure 4). However, the failure of $Antp^{Ns+R72}$ to complement dsx^{D+R1} and dsx^{D+R2} would indicate that these deficiencies overlap in section 84D much as $Antp^{Ns+R17}$ and dsx^{D+R1} do (Figure 4). Because these three deficiencies all complement *roe* and *rn* (Table 7), it would seem that *roe* and *rn* are located in or just proximal to the 84B1,2 doublet and not in 84D. However, the possibility does exist that $Df(3R)Antp^{Ns+R72}$ does not actually physically overlap the two dsx^{D+R} deficiencies but instead carries a recessive lethal exposed by them. If this were the case, then a small region in 84D could be deleted in $Antp^{Ns+R17}$ but not deleted in the other three deficiencies and *roe* and *rn* could be located in that region. In support of this alternative are the observations that, in addition to the heterochromatic translocation breakpoints, the *T(2;3)rn* chromosome carries an inversion with a break in 84D (Table 1); and that *roe* maps genetically to the right of $Antp^{Ns}$ (.02 map units) (M. M. GREEN, personal communication).

The three deficiencies recovered as revertants of dsx^D all fail to complement *dsx*. However, it is interesting to note that neither of the breakpoints of *In(3R)*-dsx^{D+R3} falls within 84F, the limits of the smallest revertant deficiency (Table 2, Figure 1c). *Dp(3;Y)P92* was tested for the ability to complement the recessive lethality or mutant effects of *Dfd*, *dsx*, p^p and dsx^D revertants 2 through 5. The duplication does not cover the recessive lethality of dsx^{D+R2} and dsx^{D+R5} or the semi-lethality of dsx^{D+R4}. However, cytologically it does extend beyond the limits of the material missing in the two deficiency chromosomes (Figure 4). It would seem, therefore, that these chromosomes carry lethal and/or semi-lethal mutations which are not located in the 84D-84F interval but which were likely present on the original irradiated dsx^D *Sb e* chromosome. Such semi-lethals probably account for the roughened eye and deformed leg phenotype observed in several of the complementation crosses involving these chromosomes. *Dp(3;Y)P92* complements the recessive lethality of *Dfd*, indicating that this lethality is located

FIGURE 4.—Diagrammatic representation of proximal *3L* and *3R* from 79A through 85C (after BRIDGES 1941a, b), showing relative sizes of deficiencies (dotted lines) and duplication (solid line) used in this study. The location of several genes is also indicated above the chromosome. *Spl* = Splayed; *M* = Minute; *Tpl* = Triplolethal; *Msc* = Multiple sex comb; *Scx* = Extra sex comb; *Antp* = Antennapedia; *Ns* = Nasobemia; dsx^D = double sex dominant; *roe* = roughened eye; *rn* = rotund; *dsx* = double sex; *p* = pink. Description of deficiencies are given in Table 2.

TABLE 7

Results of complementation between deficiency-bearing chromosomes and selected third chromosome mutations

	Pc	*Dfd*	Dfd^r	*Ki*	*roe*	*drb*	*pb*	*rn*	$Antp^B$	*Scx*	*Msc*	$Antp^{Ns}$	*dsx*	dsx^D	p^p	*bod*	*Hu*
$Antp^{+R2}$	*Pc*	*Dfd*	+	*Ki*	+	+	+	+	L	L	*Msc*	$Antp^{Ns}$	+	female inter-sexual	+	+	*Hu*
$Antp^{Ns+R72}$	*Pc*	*Dfd*	+	*Ki*	+	"*drb*"	+	+	L	L	*Msc*	$Antp^{Ns}$	+	female inter-sexual	+	+	*Hu*
$Antp^{Ns+R17}$	*Pc*	*Dfd*	+	*Ki*	*roe*	+	+	*rn*	L	L	L	$Antp^{Ns}$	+	female inter-sexual	+	+	*Hu*
dsx^{D+R1}	*Pc*	L	+	*Ki*	+	+	+	+	*Antp*	*Scx*	*Msc*	$Antp^{Ns}$	*dsx*	L*	+	+	*Hu*
dsx^{D+R2}	*Pc*	L	+	*Ki*	+	+	+	+	*Antp*	*Scx*	*Msc*	$Antp^{Ns}$	*dsx*	female sterile male	+	+	*Hu*
dsx^{D+R3}	*Pc*	L	+	*Ki*	+	+	+	+	*Antp*	*Scx*	*Msc*	$Antp^{Ns}$	*dsx*	female sterile male	+	+	*Hu*

L = Lethal
+ = Wild type
L* = Lethal due to *Sb* on both chromosomes.

in the 84D-85A region. The ability of *Dp(3;Y)P92* to complement the recessive lethality of *Dfd* was tested in flies heterozygous for *Dfd* chromosomes from different stocks to reduce the probability of homozygosis of recessive lethals other than *Dfd*. The duplication, however, does not cover the *Dfd* eye phenotype, and *Dfd/Dfd/Dp* flies have an extreme *Dfd* phenotype.

Flies homozygous for *dsx* or dsx^{D+R8} and carrying *Dp(3;Y)P92* are not intersexes, indicating that *dsx* is located within the limits of this duplication. Also, homozygotes for p^p which carry *Dp(3;Y)P92* have wild-type eye color. HILDRETH (1965) has reported that *dsx* is to the right of p^p. Our cytological observations (see DISCUSSION), however, do not agree with this conclusion. To test the veracity of this report, we have mated *st in ri* eg^2/p^p *dsx* females to p^p *dsx/TM3*, *Sb* males and selected recombinants between p^p and *dsx*. Among 1,023 phenotypically p^p non-*Sb* progeny scored, three males and one female were not intersexual. The three males were individually mated to females homozygous for *st in ri* eg^2 and from each cross phenotypically *st*, *in*, *ri* and eg^2, progeny were produced. Since the markers *st*, *in*, *ri* and eg^2 are known to be located in proximal *3L* (HOLM *et al.* 1969; LINDSLEY and GRELL 1968), these results clearly demonstrate that *dsx* is to the left of p^p.

DISCUSSION

As has been shown by this study and others (SUTTON 1943; LIFSCHITZ and FALK 1969; MANGE and SANDLER 1973; DENELL 1973), the induction of reversions of dominant mutations is an efficient screening device for recovering chromosome aberrations in specific regions of the Drosophila genome. Using this technique, we have recovered several translocations, inversions and deficiencies in the proximal portion of *3R*. Unfortunately, the deficiencies, the class of aberration most useful to us, appear to be restricted entirely to section 84 of the polytene chromosome map. The reason for this restriction at the proximal end is clear. LINDSLEY *et al.* (1972) found a region of the genome (*Tpl*) in 83D-E which was lethal in either the hypo- or hyperploid state. Therefore, any deficiency extending from section 84 to the left would probably encounter this area and not be recovered. The reason for the dsx^D revertant deficiencies stopping at section 85A, however, is not as clear, but it is interesting to note that of three deficiencies, all are broken just to the left of 85A1. Whether or not a haplo-inviable locus exists in section 85 remains to be seen.

It should also be noted that the above method of screening is not foolproof. There exists in the proximal portion of *3R*, a dominant mutation Kinked (*Ki*), which we have attempted to revert using gamma rays. Thus far, we have scored 49,275 chromosomes and have not found one revertant. The reason for our lack of success could be one of many, not the least of which is that a deletion of the *Ki* locus does not result in the loss of the dominant phenotype. The point is, however, that there do exist mutations which seemingly should be revertible by their deletion but for one reason or another are not.

The results of all complementation tests allow us to draw several conclusions about gene-band associations in proximal *3R*. These conclusions are summarized

in Figure 4. No specific localization of *Ki*, *pb*, *bod* and *Hu* (Table 1) is possible except to note that they are not located in the 84B1,2-84F16 interval. Although *drb* does show an interaction with $Antp^{Ns+R72}$, we do not feel that this deficiency exposes the locus, as none of the other deficiencies show this interaction. Therefore, the most likely explanation is that the $Antp^{Ns+R72}$ chromosome carries a lesion association with *drb* but separate from the deficiency.

Our results entirely confirm those of DENELL (1973) with respect to the localization of $Antp^{Ns}$, *Antp* and *Scx*. All cytologically aberrant $Antp^{Ns}$ revertant chromosomes except one ($Antp^{Ns+R1}$) (Table 2) have at least one breakpoint in or very close to 84B1,2 and these revertants (Table 4) fail to complement the recessive lethality of both $Antp^{B}$ and *Scx*. To lend further support to the 84B1,2 localization of these homoeotic mutations, the two *Antp* revertant chromosomes also have breaks just to the right of the 84B1,2 doublet. Therefore, DENELL's (1973) suggestion to rename *Ns* as $Antp^{Ns}$ and *Scx* as $Antp^{Scx}$ should be adopted.

The gene *Pc* has now been shown to be in the left arm of chromosome 3 (PURO, NYGREN and NUUTILA 1973). The fact that all of our $Antp^{Ns}$ revertants complement the recessive lethality of *Pc* is consistent with this finding. However, there are certain interactions (possibly enhancement of *Pc*) between *Pc* and our $Antp^{Ns}$ revertants that warrant further study.

In complementation tests between *Msc* and our revertants, we found that the recessive lethality of this chromosome was complemented by all *Antp* and $Antp^{Ns}$ revertants except two, $Antp^{Ns+R2}$ and $Antp^{Ns+R17}$. Since $Antp^{Ns+R2}$ has a break within the 84B1,2 doublet and $Antp^{Ns+R17}$ is physically deficient for these bands, we have concluded that the recessive lethality of *In(3R)Msc* lies in the 84B1,2 doublet (this, however, does not preclude the presence of another lethal at the distal break of the inversion). DENELL's (1973) deficiency, $Antp^{Ns+R72}$, as well as $Antp^{+R2}$, are viable with *Msc*, are both broken just distal to 84B1,2 and are, therefore, not deficient for this doublet. DENELL (1973) has examined the *Msc* inversion cytologically and has concluded that the proximal inversion breakpoint is within these bands. He has also described a radiation-induced *Msc* revertant which carries, in addition to the original inversion, a new inversion with one breakpoint just proximal to 84B1,2 and another at 100A. This revertant suggests that the dominant sex comb phenotype of *Msc* is also associated with the 84B1,2 breakpoint of *In(3R)Msc*. Thus, it would appear that the same breakpoint (84B1,2) is associated with the extra sex comb phenotype and the recessive lethality of *Msc*. However, the relationship between the recessive lethal and the homoeotic effect is still not clear. Therefore, the placement of *Msc* in 84B1,2, along with *Antp*, $Antp^{Ns}$ and $Antp^{Scx}$, does not necessarily mean that *Msc* is allelic to these mutations. Indeed, *Msc* complements the recessive lethality of most of the *Antp* and $Antp^{Ns}$ revertants as well as the recessive lethality of $Antp^{Scx}$ (DENELL 1973) and $Antp^{B}$ (unpublished results). The simplest explanation is that there are at least two vital functions in or very near to the 84B1,2 doublet. However, it is also possible that there is only one vital function and that, in order to expose the recessive lethality of *Msc*, it is necessary to negate totally the function associated with this band.

As has already been pointed out (see RESULTS), our results confirm the conclusion of DENELL and JACKSON (1972) that the Hermaphrodite (*Hr*) gene, which was in turn deemed a dominant allele of transformer (*tra*) (GOWEN and FUNG 1957), is actually a dominant allele of double sex. None of our dsx^D revertants show any interaction with *tra*, yet they all fail to complement *dsx*. Furthermore, two of the revertants give a *dsx* phenotype when homozygous; and all chromosome aberrations associated with reversion of dsx^D are in proximal *3R*, the location of *dsx*; not in *3L*, the location of *tra*. Since the dsx^D/dsx combination results in the transformation of genetic females into sterile males, and flies heterozygous for *dsx* and $Df(3R)dsx^{D+R5}$ show this same transformation, it would, therefore, seem that the double sex recessive mutation is amorphic.

The three deficiencies induced as dsx^D revertants also allow the localization of the *dsx* gene to section 84F, the limits of the smallest deficiency, dsx^{D+R5} (Table 2; Figures 3 and 4). One discrepancy in this conclusion is that the breakpoints of $In(3R)dsx^{D+R3}$ do not fall within section 84F. The right breakpoint, 85E1,2, is clearly not involved with the reversion as none of the deficiencies expose this break, nor does *Dp(3;Y)P92* cover it (*P92* covers *dsx*). However, the proximal break of the inversion (84D11,12) is exposed by the two larger dsx^{D+R} deficiencies and is covered by the duplication (Figure 4). Fortunately, $Df(3R)Antp^{Ns+R17}$ overlaps this breakpoint (Table 2, Figure 4) and we know that this deficiency complements *dsx*. Therefore, we feel that *dsx* and dsx^D can be placed in 84F, and that $In(3R)dsx^{D+R3}$ may be involved with some type of position effect reversion of dsx^D.

The p^p mutation is not exposed by any of the deficiencies and is, therefore, not found in the 84B1,2–84F16 interval. However, p^p is covered by *Dp(3;Y)P92* and must, therefore, be located in the first few dark bands of 85A (i.e., 85A1,2,3) (Figure 4). This localization is a few bands to the right of the original localization of *p* by WARD and ALEXANDER (1952).

The recessive lethality of two different *Dfd*-bearing chromosomes is exposed by the deficiencies recovered as dsx^D revertants, putatively placing it in 84F (Figure 4). Furthermore, this recessive lethality is covered by *Dp(3;Y)P92* in *Dfd/Dfd/Dp(3;Y)P92* males, while their female non-duplication-bearing *Dfd/Dfd* siblings are lethal, lending further support to the 84F localization. However, Dfd^r is not exposed by any of the deficiencies in the 84B-F interval. We are, therefore, left with two plausible explanations: (1) *Dfd* is truly located in section 84F but Dfd^r is not, i.e., *Dfd* and Dfd^r are not allelic; (2) *Dfd* and Dfd^r are not in the 84F interval. However, there is a common recessive lethal on both *Dfd* chromosomes which does fall in section 84F (this would mean *Dfd* is homozygous viable). Unfortunately, our results do not allow us to choose between the two. However, genetic studies currently in progress should allow a more definitive answer.

The last two mutations we have analyzed are *roe* and *rn*. As has been stated (see RESULTS), both *roe* and *rn* are exposed by $Df(3R)Antp^{Ns+R17}$ but not by any of the other deficiencies. This fact, coupled with the 84D9,10 break found in the *rn* stock (Table 1) and the recent localization of *roe* to the right of $Antp^{Ns}$, makes

us believe that both *roe* and *rn* lie in the proximal portion of section 84D between the right breakpoint of $Df(3R)Antp^{+R2}$ and the left breakpoint of $Df(3R)dsx^{D+R1}$ (Figure 4).

As can be seen, there are still several unknowns in our attempt to analyze the proximal portion of *3R*. Nevertheless, further analyses of these existing aberrations which, when coupled with existing point mutations and the induction of new revertants, should allow us to determine a more definitive and, we believe, valuable picture of the structure and functional organization of this very interesting segment of the Drosophila genome.

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