GENETIC FINE STRUCTURE OF THE *Y* CHROMOSOME OF *DROSOPHILA HYDEI*

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ABSTRACT

A genetic map of the *Y* chromosome of *Drosophila hydei* has been constructed from deletion/complementation experiments, with the aid of male sterile mutants of the *Y* chromosome. A central conclusion of our experiments is that not more than a single complementation group can be detected in each of the lampbrush loop forming sites. Additional complementation groups, functionally independent of lampbrush loops, reside between these loci. Six complementation groups have been defined by several methods of mapping. An additional ten complementation groups are indicated, but their exact definition requires further investigation. The "synthetic sterility" of mutations in these ten loci contributes to the difficulty in unequivocally establishing their individual boundaries. Mapping problems also arise from the instability of certain mutants.

THE *Y* chromosome of *Drosophila melanogaster* has been subjected to many genetic and cytological studies (BRIDGES 1916; STERN 1927, 1929; SAFIR 1920; SHEN 1932). Compared with the other chromosomes of Drosophila, it is relatively devoid of genes. The few genetic loci identified include genes for ribosomal RNA (STERN 1927; RITOSSA and SPIEGELMAN 1965) and genes related to male germ cell development (STERN 1929). In spite of extensive studies of these loci with functions related to spermiogenesis (NEUHAUS 1939; BROSSEAU 1960; WILLIAMSON 1970, 1972; reviews: WILLIAMSON 1976; LINDSLEY and TOKUYASU 1980) our knowledge of the genetic structure of this chromosome is still incomplete.

A series of nine male fertility genes of the *Y* chromosome of *D. melanogaster* has been described by NEUHAUS (1939). BROSSEAU (1960) concluded that at least seven loci must exist in the *Y* chromosome, two in the short arm, five in the long arm. One additional locus, related to male fertility, was assumed to be present in both *X* and *Y* chromosomes. WILLIAMSON (1970, 1972) described a number of male sterile mutations in this chromosome. Mapping experiments with these mutations indicated the presence of at least 24 loci.

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MEYER, HESS and BEERMANN (1961) observed that *Y* chromosomal loci develop lampbrush structures in primary spermatocytes of *D. melanogaster*. Such structures were subsequently found in other Drosophila species studied (HESS and MEYER 1963). In particular *D. hydei* males develop distinct *Y* chromosome lampbrush loops favorable for cytological analysis (MEYER 1963). Five loop pairs, each with a specific morphology, were identified and their approximate positions in the *Y* chromosome were determined (HESS 1965). Two loops were localized at the end of the long arm ("threads," hereafter referred to as Th, and "pseudonucleolus," Ps). Two additional loops were found in a proximal region of the long arm close to the kinetochore ("tubular ribbons," Tr, and "clubs," Cl). One loop (although HESS, 1967, says two loops) is located in the short arm ("nooses," Ns) (Figure 1). The exact relationship between these loops and the

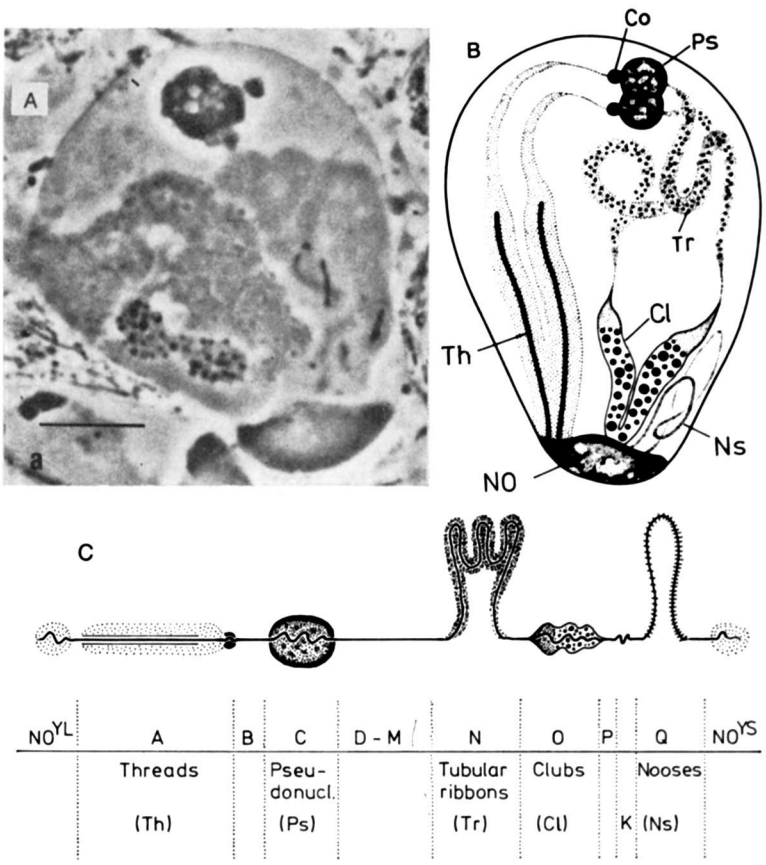

FIGURE 1.—Primary spermatocyte nuclei of *D. hydei* (A) In the phase contrast lampbrush loop-like structures can be seen. (B) Diagram of a wild-type spermatocyte nucleus displaying the different loops. (C) Diagram of one chromatid of the *Y* chromosome with the sequences of loops. From HENNIG 1967 and 1978a). Th: "threads"; Ps: "pseudonucleolus"; Tr: "tubular ribbons"; Cl: "clubs"; Ns: "nooses"; NO^{YL} and NO^{YS}: Nucleoli; NO: Nucleolus; K: kinetochore; A-Q: Complementation groups.

genetic loci involved in sperm differentiation remains unknown, although several observations indicate a relationship between loop-forming loci and fertility genes (HESS 1967). The amount of DNA involved in loop formation suffices to code for more than 100 polypeptides (HENNIG *et al.* 1974). Cytological evidence, on the other hand, favors the assumption that each loop behaves as a single functional unit at the transcriptional level (HENNIG 1978a).

Evidence of the effect of *Y*-chromosome loci on male fertility in *D. hydei* was obtained by LEONCINI (1977), who isolated a number of temperature-sensitive mutations. Temperature-conditional lesions in microorganisms are conventionally considered to result from the production of thermolabile proteins (WITTMANN and WITTMANN-LIEBOLD 1966; JOKUSCH 1966). Similarly, temperature-sensitive mutations in Drosophila have also been assumed to produce proteins that temperature can inactivate (SUZUKI *et al.* 1967; BAILLIE, SUZUKI and TARASOFF 1968; SUZUKI 1970; AYLES *et al.* 1973). Although temperature sensitivity might also stem from thermal instabilities in RNA molecules, modification at the protein level appears more likely. Additional indirect evidence for the function of *Y*-chromosome genes in spermiogenesis stems from studies of testis proteins (HENNIG *et al.* unpublished). The formation of a structural protein of the sperm flagellum depends on *Y*-chromosome gene expression in the Tr and Cl regions. In addition, a nucleolus organizer region exists at each end of the *Y* chromosome (MEYER and HENNIG 1974; HENNIG, LINK and LEONCINI 1975; SCHÄFER and KUNZ 1975).

The developmental effects of the *Y*-chromosome fertility genes during sperm differentiation in *D. melanogaster* are still unknown. Several investigators conclude that there is no correlation between the inactivity (or absence) of distinct fertility genes and the pattern of distortion of spermiogenesis (BROSSEAU 1960; AYLES *et al.* 1973). More specific effects in terms of "early" or "late" disruption of differentiation processes have been described for *D. hydei* (MEYER 1968; HESS and MEYER 1968). Our observations indicate, however, that most of these "early" or "late" effects are more likely related to the number of *Y*-chromosome genes deleted than to specific gene function.

In this paper we provide a genetic map of the *Y* chromosome of *D. hydei*. Cytological observations and additional genetic aspects will be presented separately.

MATERIALS AND METHODS

Origin and breeding of stocks

Flies were kept on a medium containing maize, dried brewer's yeast, soya, agar, malt, syrup and fresh yeast at 25° or 18°. The stocks used were *C(1)RM, v f/Y* Tübingen; *C(1)RM, y m ch/Y* Tübingen; *C(1)RM, w lt/Y* Leiden; 340/1 (HENNIG 1972) (this strain has no *X*-chromosome nucleolus organizer because the entire heterochromatic arm is missing). *C(1)RM Tp(1)* w^{ml} y^{Lt} Geneva (BECK 1976); *C(1)RM, w lt/R(Y)* w^{m} (BECK, BREUGEL and SRDIC 1979). To achieve a consistent nomenclature corresponding to that used for *D. melanogaster*, we renamed some of the stocks used in prior investigations:

340/2 (HESS 1965): *T(X;Y)50* (cytology: Cl, Ns)
340/7 (HESS 1965): *T(X;Y)48* (cytology: Tr^{m}, Cl, Ns)
340/10 (HESS 1965): *T(X;Y)20* (cytology: Th)

290/1 (HESS 1965): *T(X;Y)58* (cytology: Ns)
697/16 (HESS 1967): *T(X;Y)37* (cytology: Th, Ps)
290/2 (HESS 1967): *T(X;Y)47* (cytology: Tr, Cl, Ns)
T(A;Y) from v1 (HESS 1967): *T(A;Y)2* (cytology: Th, Ps)
T(Y;A) from v1 (HESS 1967): *T(Y;A)1* (cytology: Tr, Cl, Ns)
TS54 (LEONCINI 1977): *ms(Y)K1^{ts}*
TS807 (LEONCINI 1977): *ms(Y)L1^{ts}*
TS829 (LEONCINI 1977): *ms(Y)A2^{ts}*

Induction of mutations

Induction of temperature-sensitive mutations

Temperature-sensitive mutations were induced by treating wild-type males with EMS (LEONCINI 1977), which were then crossed with *C(1)RM, w lt/Y* females. Single female offspring were mated to wild-type males at 26° (restrictive temperature). The male progeny of this cross were tested for fertility at 26°. The female parents of sterile progeny were crossed with wild-type males at 18° (permissive temperature). Male offspring were crossed with wild-type females to produce stable strains. The sterile males of the 26° cross were mated to wild-type females at 18°. In most cases both procedures also resulted in male fertility and stable strains could be maintained (see LEONCINI 1977).

Induction of sterile mutations

1) *Mutagenesis with EMS*: Mutagenesis with EMS was carried out according to LEWIS and BACHER (1968) or by feeding adults a modified medium containing 0.24 ml EMS (Serva, Heidelberg), 1.00 ml DMSP (Merck, Darmstadt) and 100 ml of 5% sucrose for 24 hr. In one experiment the EMS treatment was followed by an X-ray irradiation of approximately 9500R to test whether a different mutational pattern might be obtained (SANKARANARAYANAN and SOBELS 1976).

2) *Mutagenesis with X rays*: X-ray irradiation was carried out using an X-ray therapy instrument RT 100 (C. H. F. Müller) at 100 kV and 0.8 mA with a 1.03 mm aluminum filter. The dosage was determined withe the aid of a Simplex-Universal-Dosimeter (Pychlau) (doses between 2000R and 7000R were used). For irradiation up to 9500R, an OEG 60 X-ray tube (Machlett Labs, Inc.; focus S, Target w, no external filters, 50 kV 11 mA, at a rate of approximately 1900R/min) was used. *D. hydei* is less radiation sensitive than other, related species (for example *D. neohydei*).

3) *Isolation of* Y *chromosome mutations leading to male sterility*: Nonconditional male sterile *Y*-chromosome mutations can only be kept in a stock if complemented with appropriate *Y*-chromosome translocations or a free *Y* chromosome. Repeated attempts to construct a compound *X-Y* chromosome carrying the entire *Y* have failed. We used the two *X-Y* translocations *T(X;Y)37* and *T(X;Y)47* which together cover the entire *Y* chromosome. However, both translocation fragments may share a small region of the *Y* chromosome between the PS and the Tr.

EMS was fed to aged wild-type males for 24 hr, and they were immediately mated to virgin *C(1)RM, v f/Y* females in mass cultures at 26°. Single F_1 females were crossed with both *T(X;Y)37* and *T(X;Y)47* males (which carry different markers on their *X* chromosomes). Such double matings were usually successful, although in some instances only one type of male offspring was produced. These two classes of second-generation males, carrying the same maternally-inherited *Y* chromosome, were tested for fertility by mating to *C(1)RM, v f/Y* females.

If one of these test crosses did not produce progeny within 14 days at 26° the strain was preliminarily designated "*ms(Y)*." Progeny females of the complementary fertile substrain were backcrossed to their fathers to establish a stable *ms(Y)* stock.

Males of the established stocks were subsequently crossed with wild-type females and the male progeny tested for fertility by crossing with sibling families. Only stocks that produced no progeny in at least three independent experiments were kept as *ms(Y)* strains. In the rela-

tively rare cases where germ line mosaicism was suspected, stable stocks were constructed from single-pair matings.

For the induction of sterile mutations in *X-Y* translocation chromosomes [stocks *T(X;Y)37* and *T(X;Y)47*], males were fed EMS or irradiated with X rays as described above, then immediately mated to virgin females of the constitution *C(1)RM, v f/Y* or *C(1)RM, w lt/Y* in mass cultures. Single offspring males were mated to virgin attached-*X* females to establish a stable strain. After the appearance of larvae in these crosses, the same males were mated individually to females of either the *C(1)RM, y m ch/T(A;Y)2* or the *C(1)RM, y m ch/T(Y;A)1* constitution. Male progeny of these crosses did not possess a free *Y* chromosome [constitution: *T(X;Y)37/T(Y;A)1* or *T(X;Y)47/T(A;Y)2*]. The two translocation fragments of the *Y* chromosome promoted normal sperm development unless a *ms(Y)* mutation occurred in the *T(X;Y)* chromosome. Only stocks that, in repeated tests, produced sterile *T(X;Y)37(X;A)1* or *T(X;Y)47/T(X;Y)2* males were retained.

Induction of X-Y *translocations*

Aged virgin attached-*X* females with a wild-type *Y* or an *ms(Y)* chromosome were irradiated with X rays (5700R 7600R or 9500R). These relatively high doses provided 1% to 3% of detachments. The irradiated females were mated to appropriately-marked males and the F_1 progeny were screened for matroclinous detachment male progeny. These males were crossed separately to *C(1)RM/Y* females to establish stable stocks. When larvae were found, the parental male was crossed with *C(1)RM Tp(1)*, w^{m1} Y^{Lt}*/0* females to produce *T(X;Y)/0* males for cytological inspection of the primary spermatocytes. *C(1)RM/0* females were selected as individuals with a "bobbed" phenotype from a stock carrying *C(1)RM Tp(1)*, w^{m1} y^{Lt} (BECK 1976). Testes of *T(X;Y)/0* males were dissected and squashed in isolation medium (HENNIG 1967) and the primary spermatocytes cytologically studied. Where *Y*-chromosome lampbrush structures were found, the strains were maintained as translocation strains.

Mapping of the ms(Y) *mutations*

For complementation mapping, males carrying different *X-Y* translocations or *T(X;Y)ms* chromosomes were crossed with *C(1)RM/ms(Y)* females. F_1 males were tested for fertility by mating them to their female siblings at 26° or 18° in mass cultures. Combinations that did not produce larvae within 14 days at 26° or within 22 days at 18°, corresponding to at least three times the period required to achieve fertility after eclosion, were considered sterile. These intervals were sufficient to guarantee fertility of wild-type males at the specified temperatures (LEONCINI 1977). All complementation tests were carried out at least twice. Samples of males of the various combinations were routinely inspected for spermatocyte cytology and for the presence of motile sperm. Except in very rare cases, males with motile sperm produced offspring.

For some of the test crosses *Df(1R)/ms(Y)*$_i$*/ms(Y)*$_j$ were constructed. *Df(1R)* was from strain 340/1. It was convenient to use males with *Y* chromosomes labeled with "w^mCo" (VAN BREUGEL 1970) and "*tu-p*" (HESS 1964) markers with an altered morphology of the loop pair "threads." Such males *(Df(1R)/Y,* w^mCo*, tu-p/Y, tu-p)* were obtained by a spontaneous event from stocks of the constitution *C(1)RM, w lt/Y, tu-p/Y, tu-p* × *T(X;Y)38* w^mCo/Y*, tu-p/Y, tu-p.*

These males (phenotype "w^mCo") were crossed to *C(1)RM/ms(Y)*$_i$ females. F_1 males of this cross were selected for "w^mCo" markers and the primary spermatocyte cytology of some of the males was checked for the double-*Y* constitution and the *tu-p* marker chromosome. In a second step, such males were crossed to *C(1)RM, w lt/ms(Y)*$_j$ females. The progeny males were selected for absence of the "w^mCo" marker and subjected to feritlity tests, a cytological analysis of the primary spermatocytes and sperm development.

Induction of Y *fragments*

In offspring of irradiated *X, w/Y,* w^mCo males crossed with *C(1)RM, w lt/Y* females, females were found that carried no w^mCo marker. Cytological analysis of primary spermatocytes of males derived from these females frequently revealed that the loss of the markers was accompanied by changes in the morphology of lampbrush loops or their deletion. Isolation of such

chromosomes by complementation with suitable *X-Y* translocation chromosomes permitted the recovery of free *Y* chromosome fragments carrying deletions of various lengths in the long arm.

Such chromosomes were screened by irradiating males of the genotype *X*, *w/Y* $w^{m}Co$ with 2000R (60 kV, approximately 130R/min) and crossing with virgin *C(1)RM*, *w lt/Y* females.

F_1 flies were scored for exceptional females (*i.e.*, females with a *w lt* genotype, showing no *w*–variegation). These were crossed in single pair matings with males carrying an *X-Y* translocation chromosome containing the entire long arm of the *Y* (*i.e.*, *T(X;Y)56/Y* males). Progeny males (constitution $T(X;Y)56/Df(YL)_i$; $i = 1, 2, \ldots n$) were crossed to *C(1)RM*, *w lt/Y* females. After eclosion of progeny the parental males were crossed with their daughters, establishing stable stocks of the constitution $C(1)RM, w\ lt/Df(YL)_i \times T(X;Y)56/Df(YL)_i$. The primary spermatocytes and neuroblast metaphases were analyzed cytologically to confirm the constitution of their partially deficient *Y* chromosomes.

Induction of translocations of interstitial Y *fragments*

Methods used in earlier studies of the *D. hydei* genome did not permit the study of interstitial fragments of the *Y* chromosome, particularly due to the positions of rDNA (MEYER and HENNIG 1974; HENNIG, LINK and LEONCINI 1975; SCHÄFER and KUNZ 1975). The *Y* chromosome carries nucleolus organizers in distal positions of both arms. The *X*-chromosomal nucleolus organizer is in the heterochromatic arm. Thus the nucleolus organizers are deleted by translocations of interstitial *Y* fragments to the *X* chromosome. Therefore, to recover such translocations, the genome must be supplied with ribosomal DNA. The *C(1)RM Tp(1)*, $w^{m1}\ y^{Lt}$ chromosome, which carries a small number of ribosomal cistrons in both euchromatic arms, aids in this process.

Irradiation of *C(1)RM Tp(1)*, $w^{m1}\ y^{Lt}/Df(YL)_i$ females with 2000R induced detachment. The irradiated females were crossed to wild-type males. Exceptional male offspring were crossed with *C(1)RM*, *w lt/Y* females to establish stocks, and with *C(1)RM Tp(1)*, $w^{m1}\ y^{Lt}/O$ females to obtain primary spermatocytes of offspring *T(X;Y)/O* males for analysis. *T(X;Y)O* males with a bb+ phenotype were discarded since they could not have contained interstitial fragments. Males displaying a strong bobbed phenotype in a *T(X;Y)/O* constitution were classified according to their cytology (*i.e.*, the lampbrush loops found in primary spermatocytes). In many translocations none of the loops were found in primary spermatocytes. In some of these cases segregation of the putative *T(X;Y)* chromosome appears to be normal while in others nondisjunction of sex chromosomes is observed. Only those stocks with normal meiosis were further studied. The isolation of the translocation chromosomes with interstitial *Y* fragments has been described in an abstract (BECK 1978).

RESULTS

Analysis of new mutations

After an initial study with temperature-sensitive mutations in the *Y* chromosome of *D. hydei* (LEONCINI 1977), we isolated a large number of conditional and nonconditional male sterile mutations in free *Y* chromosomes or *X-Y* translocation chromosomes. Various mutation–induction techniques were used, which ensured that the results obtained were not dependent on the technique employed. The mutant chromosomes were tested for male fertility. Table 1 represents an extract of the essential features obtained from this study. The arrangement of the test cross results permits derivation of the number of identified complementation groups and their relative positions within the *Y* chromosome.

Validity of the complementation groups

Figure 2 shows that 16 different complementation groups can be established from our cross data. These results, however, have different confidence levels, which will be discussed in detail in this section.

TABLE 1

Complementation tests

	1 (23)	2 (8)	3 (5)	4a (21)	4b (3)	5 (14)	6 (10)	7 (8)	8 (1)	9a (2)	9b (1)	10 (1)	11a (5)	11b (1)	12a (25)	12b (1)	12c (1)	13 (1)	14 (4)	15 (1)
I(5)	S	S	S		S															
II(13)	F	S[1]	S[1]		S	S	F	S		S			S		S					
III(7)	F	F	F	S	S	S	F	S	S	S	S	S	S		S	S				
IVa(1)					F			F	S	S	S	S	S		S					
IVb(8)	F	F	F		F	F		F	F	S	S	S	S		S	S			S	
V(1)					F			F	F	S	S	S	S		S					
VI(2)	F	F	F	F	F	F		F	F	F	S	S	S		S			S	S	
VII(1)					F			F	F	F	F	S	S		S					
VIII(8)	S	S	S	S	S	S					F	F	F	F				F	F	
IX(2)	S		S									S	S	S				F	F	
X(1)												S	S					S	S	
XI(8)												S	S	S				F	F	
XII(4)		F		F	F	F		F	F	F	F				F			F		F
XIII(18)	S		S									S	S	S				S	F	
XIVa(1)	S	S	F	S	S	F						F	S							
XIVb(12)	S	S	F	S	S	F		S	S			S	S						S	
XV(1)	S	S	S	S	S	S	S	S	S	S	S	S	S		S				S	
XVIa(1)	S	S	S		S	F[2]						F	F					S	S	
XVIb(5)	S	S	S		S	F[2]		S							S					
XVIc(1)	S	S	F		S	S														
XVII(1)	F	S	S	S	S	S	F	S	S	S	S	S	S		S				S	

TABLE 1—Continued

	1 (23)	2 (8)	3 (5)	4a (21)	4b (3)	5 (14)	6 (10)	7 (8)	8 (1)	9a (2)	9b (1)	10 (1)	11a (5)	11b (1)	12a (25)	12b (1)	12c (1)	13 (1)	14 (4)	15 (1)
XVIII(5)	F	F	F	S	S	S	F	S	S	S		S	S		S				S	
XIX(1)	S		F		S	F						S	S							
XX(3)	S		F		S	F						F	S					S	S	
XXI(1)	S		F		S	F						F	F					F	F	
XXIIa(1)	S	S	F		S	F						F	F	S				S	S	
XXIIb(1)	S	S	S		S	F						F	F	S				S	S	
XXIIc(1)	F	F	F	F	F	F		F		F	F	F	F		F	S	F	S		
XXIII(3)												F	F	S				F	S	
XXIV(3)	S		F		S	F						F	F					S	S	
XXV(2)	S		F		S	F						F	S	S				S	S	
XXVI(1)	S				S							F	F							
XXVII(1)	S											F	S					S		
XXVIII(1)	S	S	S			F						F	F	F				F	F	

This table represents a summary of the data obtained. The complete list of results is available on request from the authors or from the editors.

S: Sterile combination; **F**: Fertile combination. Numbers 1–15 (first horizontal row) and I–XXVIII (first vertical row) refer to the stocks of the respective group numbers. Numbers in parentheses indicate the number of different chromosomes in the respective groups (as listed in the stocklist (see DISCUSSION).

S^1: Sterility of ts mutation only at restrictive temperature.

S^2: Some combinations are sterile because of inactivation of loci *A* and *B*.

Positions without these symbols indicate that the corresponding crosses have not been carried out. Either such crosses are not possible (for example combinations of two free *Y* fragments or of two *X–Y* translocations) or they are not required because the position of a particular mutation had been unequivocally established earlier.

We have subdivided the *Y* chromosome into four major regions (I to IV), which contain complementation groups as illustrated in Figure 2.

Region I (*loci* A *to* C): Region I is located at the distal end of the long arm of the *Y* chromosome (Figures 1 and 2). Cytologically it carries the two loop pairs, "threads" (Th) and "pseudonucleolus" (Ps) (HESS 1965). Genetically we distinguish three complementation groups, designated *A*, *B* and *C*. These loci are defined by crosses of the mutants in groups I to V, XIV to XXII and 1 to 5 (Figure 2 and Table 1). Two temperature-sensitive mutations were recovered in locus *A* and one in locus *B*. No temperature-sensitive mutation has been obtained for locus *C*. The loci *A* and *C* are apparently related to the lampbrush-loop-forming sites for Th and Ps (Figure 2). This follows from the cytological observation in spermatocyte nuclei, that when the Th or Ps are absent or modified, complementation does not occur at either the *A* or the *C* locus. An exception may be the *Y*, *tu-p* mutant, which HESS (1964) claimed was fully fertile. However we found this mutation to have reduced fertility.

In some mutants locus *B* is not affected; even if both adjacent loci *A* and *C* are mutated (for example, group XVIc or group 4b); or if one of the adjacent loci is deleted or nonfunctional (for example, mutants of groups II and XXIIa). This implies a functional autonomy of locus *B*.

Region II (D *to* F): Region II includes three loci (*D*, *E*, *F*), which are defined by complementation tests with free *Y* fragments and different *X-Y* translocations. None of our male sterile mutations in free *Y* chromosomes or in the *T(X;Y)37* chromosome maps in this region. Overlaps in this region, however, cannot yet be entirely excluded because the precise length of the *Y* fragments in the *X-Y* translocation chromosomes is unknown. Moreover, no cytological structure can be correlated with any of the three loci. Since the (genetically) longest translocation chromosome used for the definition of this region (group VII) completely covers region II and displays only the Th and Ps, this region cannot be correlated with the expression of the adjacent loop pair Tr. In addition, the longest free *Y* fragments used for the definition of this region carry region II entirely but do not display a Ps in primary spermatocytes. Therefore, we must conclude that region II is located somewhere between the loop pairs Ps and Tr. The position of this region relative to the subsequent region III (defined by a series of conditional male sterile mutations) is determined by the results of combinations of chromosomes from groups V, VI, VII and XIVa with chromosomes of group 10 and 11 (Table 1). Since additional difficulties in mapping this region arise from the abnormal segregation of the mutation bearing *Y* chromosomes and from the inactivation of *Y* fragments with breakpoints in this region, the definite validity of these three complementation groups remains to be proved.

Region III (G *to* N): Region III is identified by a series of temperature-sensitive mutations (groups 10 and 11a in Table 1 and Figure 2). Although Table 1 does not unambiguously indicate that the temperature-sensitive mutations in group 10 and 11a belong to different complementation groups, the temperature-sensitive mutations do differ in time and duration of their temperature-sensitive phase (LEONCINI 1977 and unpublished data). We therefore

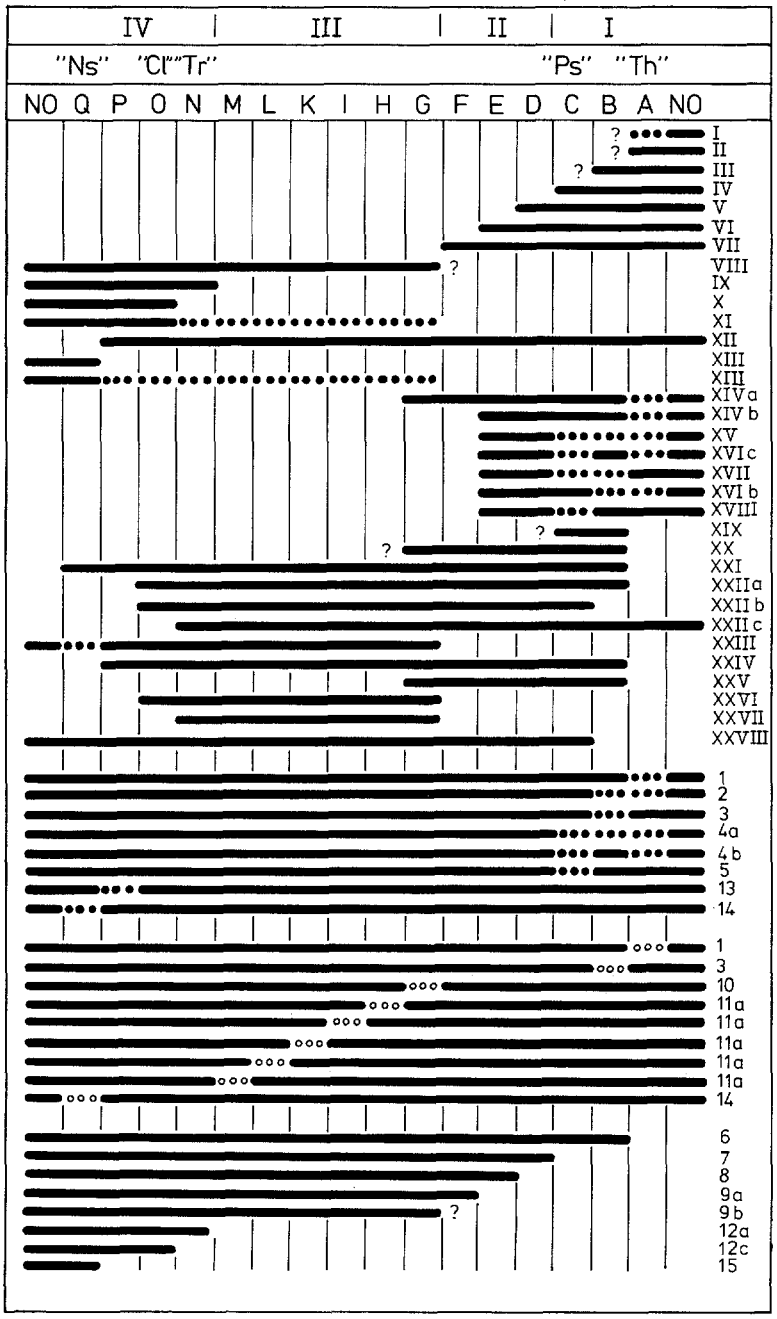

FIGURE 2.—Genetic map of the *Y* chromosome of *D. hydei*. The correlation between the results from Table 1 and the cytology of the *Y* chromosome is shown. Complementation groups are defined by capital letters A through Q, the positions of the nucleolus organizer regions by "NO". "Th," "Ps," "Tr," "Cl" and "Ns" refer to the positions of the lampbrush loops (Figure 1c).

The numbers on the right side of the figure correspond to those in Table 1, representing groups of mutant chromosomes. The Roman numbers are translocation chromosomes with (for

assume that the six temperature-sensitive mutations belong to separate complementation groups. The data in Table 1 indicate that at least two loci must be present. Moreover, LEONCINI (1977) has evidence showing that *ms(Y)K1^{ts}* and *ms(Y)L1^{ts}* are also in different locations.

A phenomenon that we call "synthetic sterility" causes problems in the analysis of the conditional mutations by *X/Y/Y* complementation tests (see below) (Tables 2 and 3). Complementation within the region carrying the loop pairs Tr, Cl and Ns is more difficult to establish since complete or partial duplication of this region frequently results in reduced fertility. On the other hand, the *ms(Y)G1^{ts}* chromosome is somewhat leaky, as is *ms(Y)H1^{ts}*, although with a lower frequency. Another problem is the low yield of mutations obtained in this region. The number of mutated *Y* chromosomes recovered from the proximal region is five to ten times lower than from the distal part of the long arm. This cannot be explained simply by a smaller target size because the proximal part of the long arm extends over approximately two thirds of the entire *Y* chromosome.

It is uncertain whether loci in region III are correlated with any lampbrush loops. We assume that no such correlation exists; a correlation with the Tr cannot be entirely excluded. At present, the loop-forming site of the Tr is defined by the two mutations in group IX (Table 1) and by a few other mutations. The two mutations in group IX are related to Tr with a morphology different from that of other strains having longer proximal *X-Y* translocation fragments. A complex structure for this locus (or these loci) has already been suggested (HENNIG 1967). Since all long translocations that include the proximal portion of the long arm and the short arm of the *Y* chromosome contain the *ms(Y)G1–ms(Y)M1* region, we do not know whether these loci can be separated from the region of the chromosome responsible for the formation of the Tr. It is possible that the expression of the Tr locus is required before loci *G* to *M* can be expressed, although this kind of dependence has not been found for other *Y* chromosome loci.

Region IV (O *to* Q): Three complementation groups can be distinguished in this region; two in the proximal part of the long arm and one in the short arm of the *Y* chromosome.

The definition of locus *O* is at present based mainly on the combination of partially deficient chromosomes since no sterile or conditionally-sterile mutations have been discovered in free *Y* chromosomes. That such mutations can be induced

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example, *XV*) or without (for example, *II*) mutations in the *Y* region of the translocation chromosome. The black lines represent the cytological length of the *Y* region in the *X-Y* translocation chromosome, as derived from metaphase chromosome structure and from cytology of primary spermatocytes. The cytology of spermatocyte nuclei from *T(X;Y)/O* males permits determination of the minimum length of the *Y* fragment in the translocation chromosome. Question marks indicate that the length of the respective *Y* fragment could not be more precisely determined and that the fragment might be longer than is indicated by the black line. Dotted regions indicate the presence of mutations. Open circles indicate a ts mutation.

Arabic numbers define groups of mutations in either free *Y* chromosomes (*1–5*, *10*, *11a*, *13*, *14*) or in the free *Y* fragments (numbers *6–9*, *12*, *15*). The mutant chromosomes used for these are described elsewhere. (Drosophila Information Service, in press).

TABLE 2

Fertility test of X/Y/Y males (first experiment)

Constitution of males	Number of fertile vials	Number of sterile vials
Df(1R) / ms(Y)L1^{ts} / ms(Y)B1^{ts}	8	2
Df(1R) / ms(Y)L1^{ts} / ms(Y)Q4^{ts}	0	10
Df(1R) / ms(Y)L1^{ts} / ms(Y)L1^{ts}	0	2
Df(1R) / ms(Y)L1^{ts} / ms(Y)H1^{ts}	0	2
Df(1R) / ms(Y)L1^{ts} / ms(Y)G1^{ts}	0	2
Df(1R) / ms(Y)H1^{ts} / ms(Y)B1^{ts}	7	2
Df(1R) / ms(Y)H1^{ts} / ms(Y)Q4^{ts}	17	1
Df(1R) / ms(Y)H1^{ts} / ms(Y)H1^{ts}	2	17
Df(1R) / ms(Y)H1^{ts} / ms(Y)I1^{ts}	2	4
Df(1R) / ms(Y)H1^{ts} / ms(Y)G1^{ts}	1	4
Df(1R) / ms(Y)I1^{ts} / ms(Y)B1^{ts}	0	3
Df(1R) / ms(Y)I1^{ts} / ms(Y)Q4^{ts}	0	9
Df(1R) / ms(Y)I1^{ts} / ms(Y)L1^{ts}	0	4
Df(1R) / ms(Y)I1^{ts} / ms(Y)H1^{ts}	0	2
Df(1R) / ms(Y)I1^{ts} / ms(Y)I1^{ts}	0	2
Df(1R) / ms(Y)I1^{ts} / ms(Y)G1^{ts}	0	6
Df(1R) / ms(Y)G1^{ts} / ms(Y)B1^{ts}	0	1
Df(1R) / ms(Y)G1^{ts} / ms(Y)Q4^{ts}	0	4
Df(1R) / ms(Y)G1^{ts} / ms(Y)L1^{ts}	0	2
Df(1R) / ms(Y)G1^{ts} / ms(Y)H1^{ts}	0	1
Df(1R) / ms(Y)G1^{ts} / ms(Y)G1^{ts}	1	1
Df(1R) / ms(Y)L1^{ts} / ms(Y)I1^{ts}	0	2

Construction of males with constitutions as indicated in the first column is described in MATERIALS AND METHODS. Fertility was assumed whenever any offspring larvae were found. Mutations *ms(Y)G1^{ts}* and *ms(Y)H1^{ts}* are not fully sterile at restrictive temperature.

The fertility of the males was tested by crossing 5 to 10 males of each constitution to 20 females.

is shown, however, by sterile mutations of this locus in *X-Y* translocation chromosomes (group X) and *Y* fragments (group 12b). Some of these sterile mutations display modified morphology of the loop pair Cl. This is consistent with the deletion mapping: the primary spermatocyte nuclei of all fertile combinations carrying a functional locus *O* display this loop pair in an unchanged morphology. Without this loop pair no combination has been fertile; therefore, the locus O must be correlated with the loop-forming site of the Cl.

Locus *P* has been detected on the basis of a single sterile mutation (group 13). Because it is sterile in combination with *X-Y* translocations of group XXIIa,b (both carrying cytologically visible Cl), group XXIIc and group XVIa (cytologically without Cl) its position must be proximal to locus *O*. No lampbrush loop pair can thus be attributed to this locus.

Locus *Q* is the only site found in the short arm of the *Y* chromosome. Its identification relies on three nonconditional sterile mutations and one temperature-

TABLE 3

Fertility test of X/Y/Y *males (second experiment): Synthetic sterility*

Constitution of male	Fertile (f) / Sterile (s)
Df(1R) / ms(Y)AC1 / Yw^{m}Co, tu-p	f
Df(1R) / ms(Y)AC1 / ms(Y)B2	f
Df(1R) / ms(Y)AC1 / ms(Y)B3	f
Df(1R) / ms(Y)AC1 / ms(Y)Q4^{ts}	s*
Df(1R) / ms(Y)P1 / ms(Y)Q2	s*
Df(1R) / ms(Y)P1 / ms(Y)Q1	s*
Df(1R) / ms(Y)P1 / ms(Y)Q3	s*
Df(1R) / ms(Y)P1 / ms(Y)Q4^{ts}	s*
Df(1R) / ms(Y)Q2 / ms(Y)P1	s*
Df(1R) / ms(Y)Q2 / ms(Y)Q2	s
Df(1R) / ms(Y)Q2 / ms(Y)Q1	s
Df(1R) / ms(Y)Q2 / ms(Y)Q3	s
Df(1R) / ms(Y)Q2 / ms(Y)Q4^{ts}	s
Df(1R) / ms(Y)Q1 / ms(Y)Q1	s
Df(1R) / ms(Y)Q1 / ms(Y)Q3	s
Df(1R) / ms(Y)Q1 / ms(Y)Q4^{ts}	s
Df(1R) / ms(Y)Q3 / ms(Y)P1	s*
Df(1R) / ms(Y)Q3 / ms(Y)Q2	s
Df(1R) / ms(Y)Q3 / ms(Y)Q3	s
Df(1R) / ms(Y)Q3 / ms(Y)Q4^{ts}	s
Df(1R) / ms(Y)Q4^{ts} / ms(Y)Q1	s
Df(1R) / ms(Y)A2^{ts} / msYtu-p	s*

Constitution of males as indicated in the first column was obtained as indicated in MATERIALS AND METHODS. The fertility tests were carried out in mass matings with 30 to 100 males and 50 females per vial.

* Constitution displaying synthetic sterility. They all achieve advanced stages of spermiogenesis.

sensitive mutation. No further distinction among these lesions has been achieved. They are sterile with each other in *Df(1R)/ms(Y)$_i$/ms(Y)$_j$*-constitutions (Table 3). In combination with translocation chromosomes carrying the short arm of the *Y*, they are fertile (Table 1). The function of this locus correlates strictly with the cytological expression of the loop pair Ns. We cannot entirely exclude the presence of another complementation group, since the *Df(1R)/ms(Y)$_i$/ms(Y)$_j$*-constitution sometimes leads to "synthetic sterility" (see below).

Mutation of locus *Q* always results in an early interruption of spermiogenesis: Differentiation does not proceed beyond the early spermatid stages. Locus *Q* is the only locus where a consistent correlation of gene function and a particular developmental block could be detected.

Synthetic sterility

Complementation tests with mutations in free *Y* chromosomes should be carried out most easily by constructing males with two *Y* chromosomes (MATERIALS AND METHODS). In *D. hydei*, however, this is not without problems since the only marked *Y* chromosome carries the unstable w^mCo marker (VAN BREUGEL 1970), which behaves like a transposon (HACKSTEIN, unpublished data). In all our experiments the *X/Y/Y* constitution was assumed by the cytological inspection of the primary spermatocytes. The use of a cytologically marked *Y* chromosome, such as the Y^{tu-p} chromosome, provides unequivocal proof of its genetic origin. Tables 2 and 3 summarize the results of such experiments. Any vial containing even a few larvae was identified as "fertile." It will be noted that some crosses deliver offspring although they should be sterile. Among these are the mutants *ms(Y)H1*^{ts} and *ms(Y)G1*^{ts}. However, these mutants are leaky at the restrictive temperature. Other consitutions are sterile even though complementation should take place, as determined by prior mapping of the mutations. This is obvious for the combination *ms(Y)Q4/ms(Y)AC1*. In this instance, two mutations are clearly in different regions of the chromosomes. The sterility of these combinations must be due to cooperative effects among the various gene products. Since this kind of interaction is principally comparable to "synthetic lethality" as originally established by DOBZHANSKY (1946) and defined in a more general way by LUCCHESI (1968), we adopted the term "synthetic sterility" for the phenomenon observed in our experiments.

Functional instabilities

In some crosses with mutants in group 5, which had previously mapped to locus *C*, we obtained a large number of sterile combinations where we expected complementation by translocations carrying *C*. Since cytological inspection of primary spermatocytes of such males gave indications of an inactivation of locus *A*, we tested for such instabilities by crossing females of the constitution *C(1)RM, v f/ms(Y)*$_i$ with *T(X;Y)56* males to assure complementation for this region of the *Y* chromosome. (The *T(X;Y)56* males carry the long arm of the *Y*.) Stocks of the constitution *C(1)RM, v f/ms(Y)*$_i$ × *T(X;Y)56/ms(Y)*$_i$ were established and cultured for ten generations, avoiding backcrossing to parental flies. Since any duplication of active *Y*-chromosome loci results in slower sperm development, a strong selection advantage is expected for males with reduced numbers of active-duplicated *Y* chromosome loci, *i.e.*, for males with progressive inactivity of loci within the *Y* chromosome. After ten generations males were crossed to *X/X* females and the spermatocyte cytology of the male progeny were studied. It became evident that the Th were no longer visible. Recently we found that such inactivation can occur within the first generation after induction of the mutation. Additional complementation tests confirmed that loci *A* and *B* had become inactive.

DISCUSSION

The number of complementation groups and their relationship to lampbrush loops: Earlier cytogenetic studies of the *Y* chromosome of *D. hydei* suggested

that the genetic activity in this chromosome is restricted to the sites forming lampbrush loops in primary spermatocytes (HESS 1967). This view must be modified. We have found several loci affecting male fertility that do not coincide with lampbrush loops. The best established example is locus *B*. All the loci in this region of the *Y* chromosome—loci *A*, *B* and *C*—are expressed autonomously [see *T(X;Y)* chromosomes in groups II, III, XIX, XXI b, 4b and the respective translocations (group XVI)]. Two types of loci in the *Y* chromosome sites can be distinguished from spermatocyte cytology according to whether or not they develop large structural modifications comparable to the lampbrush loops in amphibian oocytes (MEYER 1963).

Another fundamental conclusion from our experiments is that most of the loop-forming loci represent only one complementation group each. Our data demonstrate this unequivocally for the loops Th and Ps as determined by a variety of mapping approaches (deficiencies, *ms(Y)*^{ts} and *ms(Y)* mutants).

In the Ns forming the chromosome region, the evidence also points to the existence of just one complementation group. While HESS (1967) postulates the presence of two loop-forming sites within the short arm of the *Y* (Ns), we discern only one pair of Ns. The *X/Y/Y* experiments in particular indicate the presence of only one complementation unit. All of the possible combinations at this locus cause early blocks in sperm development. In combination with the mutant chromosome *ms(Y)P1*, however, development proceeds to advanced stages of spermiogenesis, which may be taken as an indication that the same genetic function is affected in mutants of complementation group *Q*.

We also conclude the presence of only one genetic locus for the loop pair Cl. The number of mutations induced in this region, however, is unusually small and complementation mapping with the aid of translocation chromosomes may not suffice to exclude the possibility of the existence of added complementation groups.

The correlation of the cytological and genetic data in the loop pair Tr is complicated. None of the complementation groups can unequivocally be assigned to this loop. This could indicate a considerable genetic complexity at this locus, which prevents genetic dissection with the methods used. Evidence in favor of such a situation is provided by the temperature-sensitive mutations mapping between Ps and Tr in region III. The mutant *ms(Y)G1*^{ts} complements *X-Y* translocations that do not carry the locus for Tr. Possibly some of the conditional mutations belong to loci that are functionally related to the expression of Tr. We have so far failed to produce a genetic constitution where the entire region between Ps and Tr is present while the loop-forming locus of the Tr is absent.

The apparent difficulty of inducing mutations within the middle region of the long arm of the *Y* could be interpreted as a consequence of the repetitive nature of certain genes. This does not necessarily imply that these multiple copies are tandemly arranged. The presence of repeated genes has been illustrated by hybridization experiments (HENNIG 1968; for discussion see also WILLIAMSON 1972, 1976) and by recent DNA studies (VOGT *et al.* unpublished). The assumption of an additional amplification of *Y* chromosomal genes in primary sper-

matocytes (WILLIAMSON 1972) is not necessarily convincing, since the hybridization studies giving evidence for the presence of multiple DNA copies were done with DNA extracted from flies rather than with testis DNA. Moreover, recent experiments demonstrate the presence of transcribed repeated DNA sequences in some *Y*-chromosome genes (VOGT, HENNIG and SIEGMUND, 1982).

Synthetic sterility: Certain combinations of sterile *Y* chromosomes lead to sterility (Tables 2 and 3). In some instances our prior mapping studies showed that the mutations in different complementation groups, particularly in males carrying the *ms(Y)Q4*^{ts} chromosome (mutated in the Ns to conditional sterility) in combination with male sterile *Y* chromosomes mutated in the middle of the long arm. We call this phenomenon "synthetic sterility," equivalent to the term "synthetic lethality" (DOBZHANSKY 1946; LUCCHESI 1968); it also has been used comparably by KRIMBAS (1960). Synthetic sterility results from the combination of certain mutations belonging to different complementation groups that in other combinations do not lead to sterility. The molecular basis for such synthetic sterility could be negative interaction between modified gene products of the different loci. The fact that in our experiments conditional mutations are preferentially involved in producing synthetic sterility supports such an interpretation. Similar (but formally different) phenomena occur within individual complementation groups. The combination of the *Y*, *tu-p* chromosome with *ms(Y)A2*^{ts} leads to sterility even at the permissive temperature, where both combined chromosomes should be fully functional because both mutations map to locus *A* (unpublished data). The *Y*, *tu-p* chromosome itself has been claimed to support normal fertility (HESS 1964), although we found that the number of offspring is generally reduced, especially in *X/Y*, *tu-p/Y*, *tu-p* constitutions.

The function of the Y *chromosome loci*: The role of *Y*-chromosome genes in the process of sperm differentiation is still unknown. The existence of temperature-sensitive mutants (LEONCINI 1977) indicates that the genes involved may code for proteins. Since *Y*-chromosome genes appear to interact with the expression of autosomal genes coding for structural proteins of sperm, some *Y* chromosome genes may code for products involved in activation, processing or assembly of structural proteins required for sperm morphogenesis (HULSEBOS, HACKSTEIN and HENNING, 1982).

Other evidence on the nature of the contribution from *Y*-chromosome genes can be derived from our observation that no obvious *trans* effects at the level of lampbrush loop expression have been observed. On the other hand, some mutations (for example *ms(Y)AC1*) could be considered as *cis*-dominant affecting several adjacent loci.

It seems that *trans* active loci interacting with *Y* chromosome genes are located outside the *Y* chromosome. LIFSCHYTZ (1974, 1975) described *X*-chromosome mutants affecting *Y*-loop morphology in primary spermatocytes. However, some of these mutants are also female sterile and they may affect the *Y*-chromosome loops in an unspecific way (see also LINDSLEY and TOKUYASU 1980).

Earlier we found that chromosome *2* of *D. hydei* might be involved in regulating the morphology and/or expression of *Y*-chromosome loci (HENNIG 1977).

Recently we isolated several recessive male sterile mutations in chromosome *2* that affect the expression of either the Th alone or the Th and the Ps. A concomitant parallel reaction of these two sites has also been observed in some of the mutants described in this paper (*i.e.*, mutants in group 4b and in group XV). It seems that the loci for Th, Ps and, possibly, locus *B* are, at least partially, under a common control mechanism.

Many of our EMS-induced mutations may be point mutations (JENKINS 1967, and others). This would imply that single-base substitutions are sufficient to inactivate several adjacent complementation groups (for example, group 4a). The successful induction of revertants of such mutations by EMS treatment strongly favors this interpretation (unpublished). Several authors (OSTER 1964; BISHOP and LEE 1969, 1973; WILLIAMSON 1970; LOVELESS 1966) and our own experiments (unpublished) show that complex chromosome rearrangements can be induced by EMS. Rearrangements would hardly be expected to be reversed by chemical mutagens.

While no *trans* effects could be established on the chromosomal level, a different situation exists at the gene product level. Our experiments indicate that cooperative effects can occasionally be found, particularly with conditional mutants. The most obvious example is the mutant *ms(Y)Q4*^{ts}, which causes sterility in any *X/Y/Y*-males bearing temperature-sensitive mutations (Tables 2 and 3). This implies that one reason for synthetic sterility is these cases may be incompatibility of gene products. Comparable effects have been described for *X/Y/Y*-constitutions in *D. melanogaster* (GRELL 1969). Synthetic sterility between *Y*-chromosome mutations in different locations may be one of the major reasons for the difficulties in definitely mapping the *D. melanogaster Y* chromosome.

Genetic structure of Y *chromosome loci*: Our genetic map of *Y*-chromosome genes has been obtained by conventional genetic mapping. This prevents us from reaching conclusions on the molecular structure of these genes. The problems that arise in transferring knowledge obtained on the genetic or cytogenetic level to the molecular level have been discussed at length (BEERMANN 1972; HENNIG 1978b).

Our data clearly demonstrate that at least some, and most likely all, of the lampbrush loop regions accommodate only a single complementation group, in spite of their exceedingly high DNA content (HENNIG *et al.* 1974). However, our genetic approach may be too crude to dissect more complex functional arrangements.

A striking observation in our experiments is the widely differing frequency of induced mutations in different regions of the *Y* chromosome. Mutations are most frequent in region I; but after EMS or X-ray treatment, regions II, III and IV show five to ten times fewer mutations than region I. One possible explanation is that mutation-resistant regions contain repeated gene copies. Point mutations might thus remain undetected. This is consistent with our observation that no conditional mutations could be recovered from the entire proximal region of the long arm of the *Y*. Alternatively, regions II to IV might give rise to a large proportion of "dominant" sterility mutations that could not be maintained in our

experiments. However, no evidence in favor of such an interpretation has been obtained. In addition, biochemical evidence from recombinant DNA work indicates an occurrence of transcribed repeated sequences in the *Y* chromosome (VOGT, HENNIG and SIEGMUND, 1982). This would imply that the distal portion of the long arm (carrying the loci *A*, *B* and *C*) might be preferentially composed of "nonrepeated" genes since conditional and nonconditional mutations are easily recovered. Nevertheless, these loop regions also contain extremely large amounts of DNA.

Inactivation of active regions: Some of the crosses with strains of group 5 give results contradictory to those of crosses with other strains. Certain combinations produce complementation while others, which are also expected to yield fertile offspring, are sterile. Cytological investigation of this phenomenon led to the conclusion that, where the Ps are missing, some newly-induced mutations display a strong tendency to inactivate adjacent loci as well. In a test experiment (communicated elsewhere) some of the new mutations were kept in various combinations with long overlapping *Y* regions. A large proportion of these chromosomes become inactive within several months (occasionally within a few generations). The inactivation might extend over the entire overlapping region or even over other parts of the chromosome. Similar observations in *X-Y* translocation chromosomes were mentioned by HESS (1967). A more extended study was done by HENNIG (1980) who studied hybrid *X-Y* translocation chromosomes (*i.e.*, chromosomes with an *X* of *D. hydei* and a *Y* fragment of *D. neohydei*). In "hybrid" translocations, such inactivation events apparently occur regularly while in *D. hydei* only specific chromosomes display such a phenomenon. In *X-Y* translocations induced in *D. neohydei*, inactivation of the translocated *Y* fragment is frequent (unpublished). Several features of this inactivation phenomenon are similar to the phenomenon of position effect variegation. Although different in some parameters, both phenomena may have a common molecular basis.

Inactivation of *Y* chromosome genes may represent a more common phenomenon and may thus be a major reason for problems in mapping the *D. melanogaster Y* chromosome. In the *D. melanogaster Y* chromosome such inactivation events are not easily detected because of a lack of suitable cytological markers.

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