The Genetic Analysis of Distributive Segregation in *Drosophila melanogaster*. II. Further Genetic Analysis of the *nod* Locus

Ping Zhang and R. Scott Hawley

Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York 10461

Manuscript received April 27, 1989
Accepted for publication January 23, 1990

ABSTRACT

In *Drosophila melanogster* females the segregation of nonexchange chromosomes is ensured by the distributive segregation system. The mutation nod^a specifically impairs distributive disjunction and induces nonexhcange chromosomes to undergo nondisjunction, as well as both meiotic and mitotic chromosome loss. We report here the isolation of seven recessive *X*-linked mutations that are allelic to nod^a. As homozygotes, all of these mutations exhibit a phenotype that is similar to that exhibited by nod^a homozygotes. We have also used these mutations to demonstrate that *nod* mutations induce nonexchange chromosomes to nondisjoin at meiosis II. Our data demonstrate that the effects of nod^a on meiotic chromosome behavior are a general property of mutations at the *nod* locus. Several of these mutations exhibit identical phenotypes as homozygotes and as heterozygotes with a deficiency for the *nod* locus; these likely correspond to complete loss-of-function or null alleles. None of these mutations causes lethality, decreases the frequency of exchange, or impairs the disjunction of exchange chromosomes in females. Thus, either the *nod* locus defines a function that is specific to distributive segregation or exchange can fully compensate for the absence of the nod^+ function.

TWO parallel systems function to disjoin chromosomes in female meiosis of *D. melanogaster*. The primary means of ensuring homologous disjunction is an exchange-mediated system that uses chiasmata to segregate homologous chromosomes (for reviews see BAKER and HALL 1976; HAWLEY 1988). The other is the distributive system that disjoins those chromosomes that have failed to undergo exchange (reviewed by GRELL 1962, 1976). Although distributive disjunctions are as reliable as those governed by the exchange-mediated system, the distributive system is exchange-independent.

Systematic searches for meiotic mutants in *D. melanogaster* have provided a means to dissect the meiotic processes (BAKER and HALL 1976). Four mutations have been shown to affect the distributive system: *mei-S51*, (ROBBINS 1971); *ald* (O'TOUSA 1982); *Axs* (ZITRON and HAWLEY 1989) and *nod* (CARPENTER 1973). Studies of these mutations have led to a three-stage model of distributive segregation (CARPENTER 1973; O'TOUSA 1982): (1) identification of nonexchange chromosomes; (2) choice of disjunctional partners; and (3) orientation and segregation.

The first step in this model is defined by the *ald* mutation. In *ald* females *X* chromosomes that have undergone exchange enter the distributive system at a high frequency (O'TOUSA 1982). The second step in this process, namely partner choice, is defined by the *ald*, *Axs*, and *mei-S51* mutations (ROBBINS 1971; O'TOUSA 1982; ZITRON and HAWLEY 1989). All of these mutations induce or allow incorrect or nonhomologous segregation events, such as disjunctions of the *X* chromosomes from the small fourth chromosomes, to occur at high frequencies. The third step in this process, disjunction, is defined by the nod^a mutation (CARPENTER 1973).

nod^a is a female-specific recessive mutation that maps between *v* and *m* on the *X* chromosome and has been precisely localized to position 10C2-3 on the standard polytene map (BAKER and CARPENTER 1972; CARPENTER 1973; VOELKER *et al.* 1985). The most dramatic effect of nod^a on female meiosis is on the disjunction of the always nonexchange fourth chromosomes. Fourth chromosome nondisjunction frequencies approach 90% in nod^a/nod^a females and the vast majority of the exceptional gametes are nullo-*4* ova. CARPENTER (1973) also observed 3% *X* chromosome nondisjunction in nod^a/nod^a females. These exceptional ova result from random *X* chromosome disjunction in those meiocytes in which the two *X* chromosomes had failed to undergo exchange. Both the frequency of exchange and the disjunction of exchange bivalents was shown to be unaffected by the nod^a mutation. These data argue that the nod^a mutation defines a locus whose function is limited to the distributive system.

Based on an analysis of secondary nondisjunction in nod^a/nod^a females, CARPENTER (1973) concluded that *nod* does not impair the process of partner choice within the distributive system, but rather specifically affects the disjunctional process *per se*. The term secondary nondisjunction refers to a process whereby the

nonexchange *X* chromosomes borne by *XXY* females segregate from the *Y* chromosome rather than from each other (BRIDGES 1916). *XX*↔*Y* segregations presumably result from the formation of a trivalent in which each arm of the metacentric *Y* chromosome segregates from one of the acrocentric *X* chromosomes (COOPER 1948). As a consequence of trivalent formation two meiotic events occur, coorientation of the two *X* chromosomes (*i.e.*, a commitment to segregate to the same pole at meiosis I), and the segregation of the two cooriented *X* chromosomes from the *Y* chromosome. Using females with normal sequence *X* chromosomes, CARPENTER (1973) demonstrated that only the latter process is ablated in the presence of *nod*. *X* chromosomal coorientation still occurs normally. Carpenter interpreted this observation to mean that proper trivalent formation occurs even in the presence of the *nod* mutation (allowing normal coorientation), but that the actual *XX*↔*Y* segregation event is randomized.

CARPENTER (1973) also demonstrated that nonexchange chromosomes derived from nod^a/nod^a mothers were occasionally mitotically unstable, resulting in the production of mosaic offspring in which some lineages had lost the maternal *X* or fourth chromosome. *nod*-induced mitotic chromosome loss is restricted to nonexchange chromosomes and determined solely by the maternal genotype with respect to *nod* (*i.e.*, the frequency of *nod* induced mitotic loss is not influenced by the zygotic genotype at the *nod* locus).

In this report we describe the isolation and characterization of seven additional alleles of *nod*. We demonstrate that the aberrant chromosome behavior(s) observed in females carrying these *nod* alleles is limited to nonexchange chromosomes, and that the pleiotropic effects on meiotic and mitotic chromosome segregation (high frequencies of meiotic nondisjunction and loss of nonexchange chromosomes and subsequent mitotic loss) are general properties of all *nod* alleles. Finally we discuss several possible functions for the wild type product of the nod^+ locus.

MATERIALS AND METHODS

Genetic stocks: With the exception of the nod^a mutation, the *FM7a* balancer chromosome (referred to here as *FM7*) and *Df(1)nod*, all mutations referred to in this report are described in LINDSLEY and GRELL (1968). The phenotype of the nod^a mutation (CARPENTER 1973) is described fully in the text. The stock used in these studies was obtained from A. T. C. CARPENTER.

FM7 is a multiply inverted *X* chromosome balancer comprised of *In(1)1B2-3;20F* + *In(1)4D7-E1;11F2-4* + *In(1)15D-E;20A-E* (LINDSLEY and ZIMM 1987). This chromosome was derived as a result of crossing over in an *FM6/In(1)Sc*^{8} + *In(1)d1-49* female and is marked with y^{31d}, sc^8, w^a, v^{0f} and *B*. The structure of this chromosome is diagrammed in Figure 1. When heterozygous with a normal sequence *X* chromosome, this chromosome strongly suppresses the occurrence of exchange, as evidenced by frequencies of secondary nondisjunction which exceed 70% in *FM7/X/Y* females (ZITRON and HAWLEY 1989; R. S. HAWLEY, unpublished results).

Df(1)nod is a deletion from 10B10-12 to 10C3-4 induced on the *FM7* chromosome. This deletion encompasses several vital genes as well as nod^a. Both the lethality and the nod phenotype of this deficiency are fully covered by $B^S v^+ Yy^+$ (LEFEVRE 1971), a *Y* chromosome carrying an *X* chromosomal duplication that includes the nod^+ locus.

Throughout this report the fourth chromosome mutation spa^{pol} will be abbreviated as *pol*. Similarly, the $Y^S X \cdot Y^L$, *In(1)EN* chromosome will be denoted simply as $\hat{XY}$ and the *Muller-5* balancer chromosome as *Mu-5*.

Crosses were performed at 23.5° on standard medium. Bottles and vials were set up on day 0, transferred on day 5 and parents were discarded on day 10. All crosses were scored until the 18th day.

Gamma-ray mutagenesis: Males, 0–2 days old, were irradiated with 4000 R using a ^{60}Co source at a dose rate of 118 rad/min. Males were mated immediately after irradiation.

The measurement of primary nondisjunction: The basic experiment for measuring *X* and *4* nondisjunction is a cross between *FM7/y*; *pol/pol* females and $\hat{XY}$, *v f B*/O; *C(4)RM, ci ey*^{R}/O males. This cross allows one to recognize *X* chromosome nondisjunctional offspring produced by the mother as either yellow females (diplo-*X* exceptions) or as vermilion forked Bar males (nullo-*X* exceptions). Similarly, one can recognize fourth chromosome nondisjunctional offspring as either sparkling-poliert flies (diplo-*4* exceptions) or as cubitus interuptus eyeless-Russian flies (nullo-*4* exceptions). Haplo-*4 Minute* offspring show severely reduced viability and, although counted and recorded, are not reported in these data.

In all crosses the number of *X* chromosome exceptional progeny is doubled prior to calculation. This correction accouts for the inviability of diplo-*X* ova fertilized by $\hat{XY}$-bearing sperm and nullo-*X* ova fertilized by sperm which do not carry the $\hat{XY}$ chromosome. This correction is also reflected in the Adjusted Total. Sex-chromosome nondisjunction frequencies are then calculated as the sum of exceptional progeny classes divided by the adjusted total.

As shown in Table 3, matings of *FM7/FM7* or *FM7/FM7 Df(1)nod* females to $\hat{XY}$, *v f B*/O, *C(4)RM, ci ey*^{R}/O males resulted in the recovery of only a few *FM7*/O male progeny. The basis of this reduced viability, which is not observed when $FM7/nod^a$ mothers are used, is not understood. To correct for the erratic recovery of this class of regular progeny, the number of *FM7*/$\hat{XY}$ female progeny is doubled to estimate the number of progeny which resulted from ova bearing a single *X* chromosome (*i.e.*, regular ova). This correction is performed regardless of the fourth chromosome constitution of each progeny class and is reflected in the adjusted total.

Fourth chromosome nondisjunction frequency are calculated as (diplo-*4* exceptions) + (nullo-*4* exceptions) + 2(simultaneous *X*, *4* exceptions) divided by the adjusted total.

The measurement of nondisjunction at meiosis II: In several of our crosses, female progeny bearing two identical maternal *X* chromosomes were recovered. These offspring presumably arise as a consequence of nondisjunction at meiosis II. While *y* w^a *cv* nod^a *f*/*y* w^a *cv* nod^a *f* exceptional females are easily recognized, *FM7/FM7* diplo-*X* exceptions differ in appearance from *FM7/y* w^a *cv* nod^a *f* primary exceptions

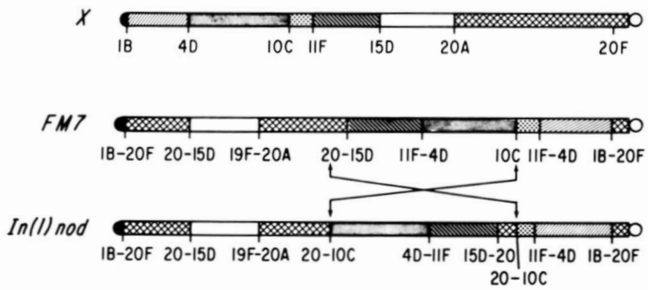

FIGURE 1.—Structure of the normal *X* chromosome, the *FM7* balancer chromosome, and *FM7,In(1)nod*. Cross-hatched blocks represent heterochromatin. Other blocks represent euchromatic intervals which are rearranged in the *FM7* and *FM7,In(1)nod* chromosomes relative to the normal *X*. Open circles represent centromeres and filled semicircles represent telomeres.

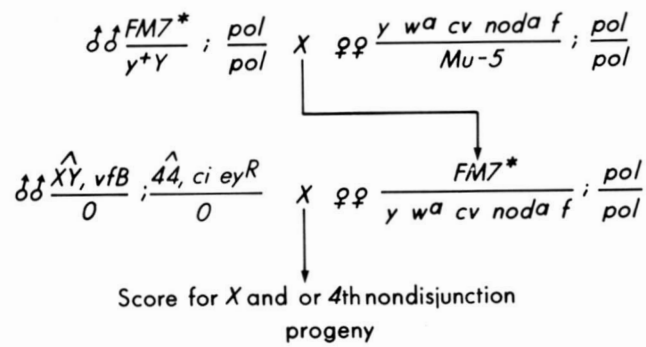

FIGURE 2.—Scheme used to isolate new *FM7,nod* alleles following γ-ray mutagenesis. For details see text. The symbol *FM7** indicates a mutagenized chromosome.

only by the severity of the Bar phenotype. This difference cannot be reliably scored in the presence of an eyeless-Russian phenotype. Accordingly, we calculated the frequency of meiosis II nondisjunction using only the number of $y\ w^a\ cv\ nod^a\ f/y\ w^a\ cv\ nod^a\ f$ daughters. The frequency of meiosis II nondisjunction is calculated as twice the number of such progeny divided by the total number of $y\ w^a\ cv\ nod^a\ f$/O males recovered.

Measurement of mitotic *X* chromosome loss: Mitotic *X* chromosome loss was indicated by the appearance of gynandromorphs. In the basic experiment *FM7*, $nod^x/y\ w^a\ cv\ nod^a\ f$ females are crossed to $\hat{X}Y$, *v f B/O* males. Gynandromorphs in progeny resulting from regular ova, which result from maternal chromosome loss, are recognized as nonyellow male-female mosaics while mitotic loss events in progeny derived from *XX* exceptional ova are recognized as yellow gynandromorphs (in those cases where the *FM7* chromosome was lost, the male tissue was marked with *f*).

Although no examples of paternal loss were observed in this study, they could have been easily detected as nonyellow gynandromorphs in which the male tissue was marked with *y* or y^{31d}.

The frequency of gynandromorphs is calculated as the number of regular gynandromorphs plus the number of exceptional gynandromorphs divided by the total number of *XX* or *XXY* zygotes. Because only those loss events that are visable structural mosaics are recognizable, the measured frequencies of mitotic loss may well underestimate the actual frequency of early chromosome loss.

Statistics: The standard errors on nondisjunction frequencies were assumed to be binomial, and thus calculated as $\text{SE} = (p(1-p)/N)^{1/2}$ where p is the frequency of exceptions and N is the adjusted total of progeny observed in the experiment.

RESULTS

Rationale: Our screen for new *nod* alleles was initiated to answer three specific questions, namely: (1) is the pleiotropic phenotype of nod^a a general characteristic of all mutations at the *nod* locus; (2) what is the phenotype of a null allele at this locus; and (3) is the *nod* locus essential for meiotic and mitotic processes other than distributive segregation? To accomplish these objectives, it was necessary to be able to detect *nod* mutations that might exhibit only a partial phenotype (*i.e.*, increased *X* nondisjunction but normal, or near normal, fourth chromosome segregation, etc.) or that might exhibit other phenotypes such as lethality or male-sterility.

In order to identify new *nod* mutations based on either their effect on *X* or *4* disjunction, we chose to mutagenize a balancer *X* chromosome (*FM7*), and then to examine *X* and fourth chromosomal nondisjunction in $FM7^*/nod^a$ females (the symbol *FM7** denotes the mutagenized *FM7* chromosome). Because *X*-chromosomal exchange is strongly suppressed in such females, the *X* chromosomes are routinely segregated by the distributive system. Accordingly, *FM7,nod/nod* females might be expected to exhibit high levels of *X* nondisjunction as well as the high levels of fourth chromosome nondisjunction which are characteristic of nod^a.

Using this system, we were able to recover mutant-bearing *FM7* chromosomes exhibiting either increased *X* nondisjunction, increased fourth chromosome nondisjunction, or both. In fact, various chromosome aberrations exhibiting only *X* or fourth chromosome-specific effects on disjunction were commonly recovered in these screens. Nonetheless, as shown below, all of the mutations that failed to complement nod^a exhibited a phenotype similar, if not identical, to that observed for nod^a.

Mutant isolation: In the course of four mutageneses, more than 100,000 mutagenized chromosomes were screened by the method shown in Figure 2. *FM7/* $y^+\ Y$; *pol/pol* males were irradiated using 4000R of gamma rays and then mass-mated to $y\ w^a\ cv\ nod^a$ *f/Mu-5*; *pol/pol* females. The resulting $FM7^*/y\ w^a\ cv\ nod^a\ f$; *pol/pol* daughters were then tested in groups of 4–5 females per vial by crossing to $\hat{X}Y$,*v f B*/O; $C(4)RM,ci\ ey^R$/O tester males. Each vial was scored for the production of exceptional progeny (*i.e.*, those resulting from *X* and/or *4*th chromosome nondisjunction).

Vials were retested if they produced more than one simultaneous *X* and *4* nondisjunctional offspring, or more than three *X* chromosome exceptions, or more than three fourth chromosome exceptions. *FM7*/*

nod^a or $FM7*/\hat{XY}$; $C(4)RM,ci\ ey^R$ females from each of these vials were used to establish sublines (up to ten sublines per tested vial). $FM7*/nod^a$; pol/pol females constructed from these sublines were then retested by crossing to $XY,v\ f\ B/O$; $C(4)RM,ci\ ey^R/O$ males.

These four mutageneses resulted in the recovery of eight chromosomes which exhibited a strong *nod* phenotype when heterozygous with nod^a. One of these chromosomes was shown to carry a deficiency for the *nod* locus (*Df(1)nod,10B10-12;10C3-4*, see MATERIALS AND METHODS). We will present evidence in the following section that the remaining seven chromosomes carry new alleles of the *nod* locus.

In the first mutagenesis 15,000 treated *FM7* chromosomes were tested and two new *nod* alleles (nod^{b27} and nod^{b34}) were recovered. The *Df(1)nod* chromosome was also recovered during this mutagenesis. In the second mutagenesis three new alleles (nod^{b1}, nod^{b17} and nod^{b29}) were recovered from 45,000 treated chromosomes. Only one new allele, nod^{bd}, was recovered among the 18,000 chromosomes screened in mutagenesis III. The nod^{bd} chromosome was shown to carry both an *X* chromosomal inversion (*In(1)15D1E;10C*), as well as an unrelated *X*-autosome translocation (*T(1;3)17A;92A*).

The final mutagenesis, which screened 38,000 mutagenized chromosomes, resulted in the recovery of five *FM7* chromosomes that exhibit a strong *nod* phenotype when heterozygous with nod^a. One of these five chromosomes also carried an unrelated translocation between the distal region of the *X* chromosome and chromosome *3*. Molecular analysis of the *nod* locus (Zhang and Hawley, unpublished data) has shown that all five of these mutations are associated with the insertion of a 5.8-kb transposable element known as *3S18* (BELL *et al.* 1985) within the genomic region corresponding to the *nod* locus. This insertion is absent in both the parent *FM7* strain and in several nod^+ *FM7* chromosomes retained from this mutagenesis because they carry other mutations that exhibit other recessive visible phenotypes (such as two new alleles of *miniature*).

We propose the following sequence of events to explain this cluster of identical mutations. In the germline of one of the mutagenized *FM7* males, the *3S18* element transposed premeiotically into the *nod* locus, resulting in a cluster of mutant-bearing spermatocytes. Gamma-ray mutagenesis then superimposed an *X*-autosome translocation onto one of the *FM7,nod* chromosomes to produce the *X*-autosome translocation. Although we favor this explanation, it is also possible that this particular *nod* mutation preexists at a low level within our *FM7* stock, or that all five mutational events are the result of independent site-specific transposon-insertions. Regardless of which of these possibilities is correct, it seem prudent to assume that all five of the mutations represent a single mutational event at the *nod* locus. We have chosen a cytologically normal representative of this group (nod^{b9}) for further study.

Demonstration of allelism with nod^a: Four lines of evidence argue that the new mutations map to the *nod* locus and are in fact allelic to nod^a. First, as shown in Table 1, all seven new mutations fail to complement nod^a in $FM7,nod/nod^a$ females, and thus produce high levels of both *X* and fourth chromosome nondisjunction. Second, as shown in Table 2, with the exception of nod^{bd}, all of these mutations are recessive. (nod^{bd} exerts a dominant effect on *X* chromosomal nondisjunction as a consequence of an *X*-autosome translocation that is presumedly unrelated to the *nod* mutation.) Third, as shown in Table 3, at least nod^{b34}, nod^{b27}, nod^{b17}, and nod^{b29} exhibit a strong *nod* phenotype when heterozygous for a deficiency that includes the *nod* locus, namely *Df(1)nod10B10-12;10C3-4* (Table 3). Fourth, the two alleles so far tested (namely nod^{b34} and nod^{b17}) are fully complemented by the $B^{S-}v^+Yy^+$ chromosome, a *Y* chromosome that bears an *X* chromosomal duplication which includes the nod^+ locus and which fully rescues nod^a.

Ascertaining the null phenotype: The phenotype of nod^a is comprised of three components namely, random disjunction of nonexchange *X* chromosomes; high frequencies of fourth chromosome loss; and zygotic loss of maternal nonexchange chromosomes. As discussed below, all seven *nod* alleles induced on *FM7* exhibit each of these components of the *nod* phenotype.

Table 3 compares the effects of four *nod* mutations (nod^{b27}, nod^{b34}, nod^{b29}, and nod^{b17}) as homozygotes and when heterozygous with *Df(1)nod*. (The low levels of *X* chromosome nondisjunction observed in these females results from the fact that, as a consequence of recombination between the two *FM7* chromosomes, only a small fraction of *X* chromosomes are nonexchange. This effect will be considered below.) In no case is a significant difference observed between the *nod/nod* females and the corresponding *nod/Df(1)nod* females for either *X* or *4* chromosomal nondisjunction (*i.e.*, the 95% confidence limits for the means are overlapping). Similar data have been obtained for the nod^a allele using *Df(1)N71,10B5;10D4* by A. T. C. CARPENTER (personal communication). Thus, at least in terms of their effect on *X* and fourth chromosomal disjunction and loss, these five mutations likely define complete loss-of-function mutations or null alleles.

The effect of mutations at the *nod* locus on meiosis I: The strong suppression of the *X* chromosomal exchange exhibited by *FM7/X* females results in all, or nearly all, of the *X* chromosomes disjoining via the distributive system. Depending on the allele tested, $FM7,nod/nod^a$ mothers produce between 50 and 57% *X* nondisjunctional progeny at meiosis I (Table 1). These values of *X* chromosomal nondisjunction are

TABLE 1

Results of crossing $\widehat{XY}$, *v f B/O*; *C(4)RM, ci ey^R/O* males to *X/X*; *pol/pol* females

Gamete types		Maternal genotype								
Mother	Father	*nod^a/nod^a*	*FM7a,Df(1)nod/ nod^a*	*FM7a,nod^{b27}/ nod^a*	*FM7a,nod^{b34}/ nod^a*	*FM7a,nod^{b9}/ nod^a*	*FM7a,nod^{b1}/ nod^a*	*FM7a,nod^{b17}/ nod^a*	*FM7a,nod^{b29}/ nod^a*	*FM7a,nod^{bd}/ nod^a*
Regular										
X 4	$\widehat{XY}$ *44*	664	286	117	63	78	72	321	67	180
X 4	O $\widehat{44}$	638	136	70	52	71	78	231	42	122
X nondisjunctional										
O *4*	$\widehat{XY}$ *44*	5	190	59	31	43	59	131	33	63
XX 4	O $\widehat{44}$	6	127	68	30	43	45	153	34	55
4 nondisjunctional										
X 44	$\widehat{XY}$ O	211	65	19	19	15	17	51	13	10
X 44	O O	185	34	18	8	24	13	49	17	10
X O	$\widehat{XY}$ $\widehat{44}$	1517	1359	534	282	375	394	919	241	169
X O	O $\widehat{44}$	1337	687	409	215	281	323	995	218	148
X, 4 nondisjunctional										
XX 44	O O	4	24	5	2	8	9	20	4	8
O O	$\widehat{XY}$ $\widehat{44}$	22	1113	337	157	300	294	597	221	152
XX O	OO $\widehat{44}$	8	538	187	100	130	161	396	104	98
O *44*	$\widehat{XY}$ OO	4	30	5	3	3	5	22	4	2
Total		4601	4589	1828	962	1371	1470	3885	998	1017
Adj. total		4650	7464[1]	2489	1285	1898	2043	5204	1398	1395
Nondisjunctional frequency										
X ± SE		0.02 ± 0.01	0.54 ± 0.01	0.53 ± 0.01	0.50 ± 0.01	0.55 ± 0.01	0.56 ± 0.01	0.51 ± 0.01	0.57 ± 0.01	0.54 ± 0.01
4 ± SE		0.71 ± 0.01	0.84 ± 0.01	0.82 ± 0.01	0.81 ± 0.01	0.83 ± 0.01	0.82 ± 0.01	0.78 ± 0.01	0.83 ± 0.01	0.61 ± 0.01
Gynandromorphs										
Regular		0	49	10	2	1	3	20	3	1
Exception		0	58	15	6	6	5	19	5	5
Frequency		0.0	0.034	0.023	0.014	0.009	0.009	0.016	0.009	0.009
Meiosis II nondisjunction										
nod^a/nod^a females		0	14	1	1	4	0	4	2	2
Frequency		0	0.032	0.005	0.010	0.041	0	0.010	0.027	0.016

Due to the inviability of the *FM7*, *Df(1)nod* chromosome, the number of *X*O male offspring is estimated using the number of *XXY* female offspring when calculating the adjusted total.

TABLE 2

Results of crossing $\widehat{XY}$, *v f B*/O; *C(4)RM, ci ey*^{R} males to *FM7a/y cv v f car*; *pol/pol* females heterozygous for the indicated *nod* allele

Gamete types		Maternal genotype									
Mother	Father	*nod*^{a}	*Df(1)nod*	*nod*^{b27}	*nod*^{b34}	*nod*^{b17}	*nod*^{b9}	*nod*^{b1}	*nod*^{b29}	*nod*^{bd}	*nod*$^{+}$
Regular											
X 4	$\widehat{XY}$ *44*	1547	3823	677	367	145	398	446	593	526	1459
X 4	O *44*	1645	4762	469	204	128	359	293	434	334	1336
X nondisjunctional											
O *4*	$\widehat{XY}$ *44*	15	9	1	0	0	3	3	0	142	5
XX 4	O *44*	9	10	3	1	1	1	0	1	43	5
4 nondisjunctional											
X 44	$\widehat{XY}$ O	1	0	0	0	0	0	0	3	0	1
X 44	O O	5	4	0	2	0	0	0	1	0	0
X O	$\widehat{XY}$ *44*	5	8	1	0	0	0	0	0	0	5
X O	O *44*	2	18	0	0	0	0	0	1	0	2
X,4 nondisjunctional											
XX 44	O O	1	0	0	0	0	0	0	0	0	0
O O	$\widehat{XY}$ *44*	1	4	0	0	0	0	0	0	0	1
XX O	O *44*	0	0	0	0	0	0	0	0	0	0
O *44*	$\widehat{XY}$ O	1	0	0	0	0	0	0	0	0	0
Total		5067	8638	1151	574	274	761	742	1033	1045	2814
Adj. total		5089	8661	1155	575	275	765	747	1034	1230	2825
Nondisjunctional frequency											
X ± SE		0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.30 ± 0.01	0.01 ± 0.0
4 ± SE		0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0	0	0	0	0.00 ± 0.01	0.00 ± 0.0
Gynandromorphs		0	0	0	0	0	0	0	0	0	0

TABLE 3

Results of crossing $\widehat{XY}$, *v f B*; *C*(4) *RM, ci ey*^{R} males to *FM7a/FM7a*; *pol/pol* females bearing the indicated *nod* alleles

Gamete types		Maternal genotype								
Mother	Father	+/+	nod^{b27}/ nod^{b27}	nod^{b27}/ *Df(1)nod*	nod^{b34}/ nod^{b34}	nod^{b34}/ *Df(1)nod*	nod^{b29}/ nod^{b29}	nod^{b29}/ *Df(1)nod*	nod^{b17}/ nod^{b17}	nod^{b17}/ *Df(1)nod*
Regular										
X 4	$\widehat{XY}$ $\widehat{44}$	1058	67	100	71	57	33	33	44	71
X 4	O $\widehat{44}$	514	70	58	10	44	23	11	10	36
X nondisjunctional										
O *4*	$\widehat{XY}$ $\widehat{44}$	6	0	4	2	0	0	1	1	1
XX 4	*O* $\widehat{44}$	2	0	1	1	0	2	2	4	2
4 nondisjunctional										
X 44	$\widehat{XY}$ O	0	1	22	0	12	5	0	6	1
X 44	O O	0	3	6	0	4	2	0	4	0
X O	O $\widehat{44}$	0	181	360	71	122	117	49	129	99
X O	$\widehat{XY}$ $\widehat{44}$	1	188	448	299	303	165	135	293	336
X,4 nondisjunctional										
XX 44	O O	0	0	0	0	0	0	0	0	0
O O	$\widehat{XY}$ $\widehat{44}$	0	10	23	11	20	8	6	22	19
XX O	O $\widehat{44}$	0	7	2	1	5	4	3	5	5
O *44*	$\widehat{XY}$ O	0	0	0	0	0	0	0	1	1
Total		1581	527	1024	466	567	359	240	519	571
Adj. total		2133	544	1200	770	794	435	360	752	872
Nondisjunctional frequency										
X ± SE		0.01 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
4 ± SE		0.01 ± 0.01	0.75 ± 0.02	0.83 ± 0.01	0.81 ± 0.01	0.86 ± 0.01	0.84 ± 0.02	0.80 ± 0.02	0.87 ± 0.01	0.83 ± 0.01
Gynandromorphs										
Regular		0	0	0	0	0	0	0	1	0
Exceptional		0	0	1	0	0	0	0	0	0
Frequency		0	0	0.004	0	0	0	0	0.005	0

more than 20-fold greater than the frequency of *X* nondisjunction observed in nod^a/nod^a females. Nonetheless, given that the frequencies of nonexchange tetrads in nod^a/nod^a and *FM7*, *nod/nod* females are approximately 5% (CARPENTER 1973) and 100%, respectively, it may be concluded that in both cases the disjunction of nonexchange *X* chromosomes is apparently randomized.

In *FM7*, *Df(1)nod/nod*^{a} females the frequency of nullo-*X* ova exceeds that of diplo-*X* ova by almost twofold. This excess of nullo-*X* ova is presumably the consequence of meiotic loss events. Curiously, in *FM7,nod/nod*^{a} females, an excess of nullo-*X* exceptions is observed only when the ova are simultaneously nullo-*4*. Near equal frequencies of nullo-*X* and diplo-*X* exceptions are observed in ova which possess one or two fourth chromosomes. The significance of this observation will be considered in the Discussion.

The levels of fourth chromosome nondisjunction in *FM7,nod/nod*^{a} females are similar to those observed in nod^a/nod^a females. As was the case for nod^a/nod^a females, more than 90% of the fourth nondisjunctional gametes produced by *FM7,nod/nod*^{a} females were nullo-*4*. This excess of nullo-*4* ova is most likely due to meiotic chromosome loss.

The *nod*$^+$ gene product is only required for nonexchange chromosomes: If, as is true for *nod*^{a}, these new mutations only affect the disjunction of nonexchange (*i.e.*, distributively segregating chromosomes), then *X* chromosome disjunction should be regularized in *FM7,nod/FM7,nod* females. Since such females carry isosequential *X* chromosomes, they should show normal levels of recombination, only a low level of *nod*-induced *X* nondisjunction should be observed.

As shown in Table 3, in *FM7,nod/FM7,nod* females the frequency of *X* chromosome nondisjunction was only 4–9% (see Table 3), a sharp decline from 50% *X* nondisjunction seen with *FM7,nod/nod*^{a} females. If only nonexchange *X* chromosomes undergo nondisjunction in such females, then, assuming nonexchange *X* chromosomes will disjoin at random, the fraction of bivalents that failed to undergo exchange E_0 can be estimated to be 8–18%. These values are substantially higher than those obtained by tetrad analysis for normal sequence *X* chromosomes (5–7%), suggesting that exchange in *FM7/FM7* females may be somewhat reduced (either as a consequence of structural effects or due to weak recessive exchange-defective mutations borne by the *FM7* chromosome).

We have estimated the frequency of nonexchange tetrads in *nod*$^+$ *FM7* homozygotes by assaying the frequency of secondary nondisjunction. This method has recently been reviewed by RUTHERFORD and CARPENTER (1988); however, the exact relationship of the frequency of secondary nondisjunction to the E_0 tetrad frequency requires further mention here. Although secondary nondisjunction (*i.e.*, $XX \leftrightarrow Y$ segregation) represents the predominant class of segregation events for nonexchange *X* chromosomes in *XXY* females that are heterozygous for a structural aberration, $X \leftrightarrow XY$ segregational events involving nonexchange *X* chromosomes are commonly observed in females bearing isosequential *X* chromosomes (GRELL 1962; ZIMMERING, 1976). Indeed, studies by GRELL (1962) demonstrate that isosequential nonexchange *X* chromosomes segregate from the *Y* in only 40–70% of the cases. In *FM7,nod*$^+$*/FM7,nod*$^+$*/y*$^+$*Y* females the frequency of secondary nondisjunction is 6.42% (N = 612). Thus, the frequency of E_0 tetrads in *FM7/FM7* females may be estimated to be 9.1–16%. These values correlate remarkably well with the estimate of 8–18% observed in *FM7,nod/FM7,nod* females.

Therefore, the *X* nondisjunction observed in *FM7,nod/FM7,nod* females most likely reflects only the expected effect of *nod* on nonexchange chromosomes, and like *nod*^{a}, these new *nod* alleles do not appear to reduce exchange *per se* or to impair the disjunction of exchange bivalents.

The effect of *nod* on meiosis II: In crosses of *FM7,nod/nod*^{a}; *pol/pol* (females) to $\hat{XY}$*,v f B*/O, *C(4)RM,ci ey*^{R}/O (males) (Table 1), gametes bearing two identical maternal *X* chromosomes (nod^a/nod^a) were produced at frequencies of 1 to 4%. These progeny result from maternal nondisjunction at meiosis II. Nullo-*X* ova resulting from meiosis II nondisjunction or loss are not distinguishable from those resulting from nondisjunction or loss at meiosis I. Thus, if the frequency of loss at meiosis II exceeds the frequency of nondisjunction, the observed frequency of *nod*-induced *X* chromosomal nondisjunction at meiosis II may underestimate the true frequency of abnormal chromosome behavior at meiosis II.

The effect of *nod* on early mitosis: *X* chromosomal mitotic loss, as assayed by gynandromorph production, was also elevated in daughters of *FM7,nod/nod*^{a} mothers and the chromosomes lost in these gynandromorphs were always of maternal origin (see Table 1). This is true even when *FM7,nod/nod*^{a} females are crossed to males bearing a normal sequence *X* (data not shown). The observation that *X* chromosome somatic loss occurred in both exceptional (*FM7,nod/nod*^{a}) progeny and in regular (*FM7,nod/*$\hat{XY}$ and *nod*^{a}*/*$\hat{XY}$) progeny demonstrates that maternal chromosome loss is independent of zygotic genotype and that the probability a given maternal *X* chromosome will undergo mitotic loss is independent of its disjunctional behavior at meiosis.

Although no effort was made to quantitate the relative amounts of male and female tissue, the vast majority of gynandromorphs contained very large patches of male tissue, and half male-half female mosaics were common. This observation suggests that most *nod*-induced loss events occur during early cleav-

TABLE 4

Secondary nondisjunction in *FM7a, nod/nod^a* females

Female genotype	Exceptional ova				Frequency of X chromosome nondisjunction (%)	Total progeny
	XX	*Y*	*XXY*	O		
FM7a/nod^a/y$^+$Y	357	202	10	17	61	1338
FM7a,nod^{b27}/nod^a/y$^+$Y	222	172	211	309	58	2221
FM7a,nod^{b34}/nod^a/y$^+$Y	214	197	192	324	58	2264

FM7a/X/y$^+$Y; spa^{pol}/spa^{pol} females were crossed to *XY, y v f B*/O; *C(4)RM, ci ey^R*/O males.
nod^a = y w^a cv nod^a f.

age, perhaps at the first mitotic division. The significance of this observation will be considered in the discussion.

In all but two cases the two maternal *X* chromosomes (*FM7,nod* and *nod^a*) underwent mitotic loss at equal frequency. The two exceptions to this generalization are *FM7,Df(1)nod* and *FM7,nod^{bd}*. The *FM7,Df(1)nod* mutation carries a recessive lethal mutation that must also be a cell lethal because no exceptional gynandromorphs which had lost the *y w^a cv nod^a f* homolog were observed. Similarly, loss of the *FM7,nod^{bd}* translocation would result in significant autosomal aneuploidy and thus death.

X chromosomal mitotic loss is not observed in *FM,nod/nod$^+$* females (Table 2) and its frequency is greatly diminished in the progeny of *FM7,nod/FM7,nod* females (Table 3). This latter result confirms the suggestion of CARPENTER (1973) that *nod*-induced mitotic chromosome loss is likely restricted to those chromosomes that have passed through the distributive system.

The effects of the new *nod* alleles on secondary nondisjunction: In *XXY* females, the presence of a free *Y* chromosome greatly increases the frequency of the nondisjunction of nonexchange *X* chromosomes, a phenomenon known as secondary nondisjunction (BRIDGES 1916). This is presumably a result of the formation of a *XXY* trivalent and subsequent *XX↔Y* disjunction (COOPER 1948; GRELL 1962). CARPENTER (1973) observed that the frequency of *X* chromosomal nondisjunction in *nod^a/nod^a/Y* females significantly exceeded that observed in *nod^a/nod^a* females and was, in fact, identical to that observed in *nod$^+$/nod$^+$/Y* controls. However, in the controls, the nondisjunctional progeny were of only two gamete types, namely, *XX* and *Y*, while *nod^a/nod^a/Y* females produced ova with *XX*, *XXY*, *Y* and O at equal frequencies. These results from *nod^a/nod^a/Y* females were interpreted by CARPENTER (1973) to be the consequence of correct orientation of the *XXY* trivalent followed by defective disjunction, such that the two *X* chromosomes still cosegregate while the *Y* disjoins at random.

We have analyzed *FM7,nod^{b27}/nod^a/y$^+$Y* and *FM7,nod^{b34}/nod^a/y$^+$Y* females, in which secondary nondisjunction would be expected to occur at high frequency as a result of the exchange-suppression. As shown in Table 4, *FM7,nod$^+$/nod^a/y$^+$Y* and *FM7,nod/nod^a/y$^+$Y* females produced approximately equal numbers of *X*-chromosome nondisjunctional ova. However, in *FM7,nod/nod^a/y$^+$Y* females *XX*, *Y*, *XXY*, and O bearing ova are recovered at approximately equal frequencies, while the nondisjunctional ova produced by *FM7,nod$^+$/nod^a/y$^+$Y* females are almost exclusively *XX* or *Y*. The consistent excess of nullo-*X* gametes observed in each of the two *FM7,nod/nod^a/y$^+$Y* crosses may be another example of the *nod*-induced chromosome loss events.

Clearly, *XX↔Y* segregation appears to be inoperative in *FM7,nod/nod* females. However, because the frequencies of secondary nondisjunction observed in *FM7,nod/nod^a/Y* females do not greatly exceed those *X* chromosome nondisjunction frequencies observed in *FM7,nod/nod^a* females, we cannot distinguish between a model in which orientation occurs normally and segregation is defective and one in which the entire process is disrupted.

Position effect variegation and *nod^{bd}*: *FM7,nod^{bd}* is male sterile even in the presence of *B^{S}v$^+$Yy$^+$* and also exhibits approximately 30% *X* nondisjunction in *FM7,nod^{bd}/+* females (see Table 2). Cytological analysis of the *FM7,nod^{bd}* chromosome shows that it has an inversion with one breakpoint at or near the *nod* locus in 10C and the other within the heterochromatin located at the 15DE breakpoint in the middle of the *FM7* chromosome (Figure 1). In addition, the *nod^{bd}* chromosome carries an unrelated translocation between 17A of the *X* chromosome and 92A on the right arm of the third chromosome, explaining both the male sterility and dominant effect on *X* chromosome nondisjunction (CHANDLEY 1965; LIFSCHYTZ and LINDSLEY 1972).

FM7,nod^{bd}/nod^a females exhibit lower frequencies of the fourth chromosome nondisjunction than do other *FM7,nod/nod^a* females (see Table 1). Since this mutation is associated with an inversion which places the *nod$^+$* locus near heterochromatin, it was possible that the lower level of fourth chromosome nondisjunction in *FM7,nod^{bd}/nod^a* females is a consequence of a variegating position effect (for review see SPOFFORD 1976). This hypothesis is supported by the ob-

TABLE 5

Position effect variegation at the *nod* locus

Female genotype	Nondisjunction % X nd	% 4 nd	% X, 4 nd	Total progeny
FM7, nod^{b27}/nod^{+}	0.7	0.1	0	1151
FM7, nod^{b27}/nod^{a}	53.1	86.7	48.2	2489
FM7, nod^{bd}/nod^{+}	30.1	0	0	1230
FM7, nod^{bd}/nod^{a}	54.5	61.3	37.2	1395
FM7, nod^{b27}/$\overline{XY}$, nod^{a}	30.0	81.3	34.5	4638
FM7, nod^{bd}/$\overline{XY}$, nod^{a}	44.2	0.9	0.2	921
FM7, nod^{bd}/nod^{a}/y^{+}Y		*2.8*		*809*
FM7, nod^{bd}/nod^{a} (29°)	44.1	62.3	23.3	2148
FM7, nod^{bd}/nod^{a} (23.5°)	52.9	57.4	23.1	2139
FM7, nod^{bd}/nod^{a} (19.0°)	38.1	34.3	9.9	1256

Females of the indicated genotype were crossed to $\overline{XY}$, *v f B*/O; *C(4)RM, ci ey^{R}*/O males.

servation that the *nod* phenotype exhibited by *nod^{bd}* is suppressed both by the addition of heterochromatin and by lower temperatures.

As shown in Table 5, the frequency of fourth chromosome nondisjunction in *FM7,nod^{bd}/nod^{a}/y^{+}Y* females is reduced to 2.8%. A similarly low frequency of fourth chromosome nondisjunction, 0.9%, is also observed in *FM7,nod^{bd}/XY,nod^{a}* females (as compared with 81.3% observed in *FM7,nod^{b27}/XY,nod^{a}* females). These results demonstrate that the presence of a *Y* chromosome suppresses the *nod^{bd}* mutation, a characteristic feature of variegating position effects. Further support for the suggestion that the *nod* phenotype observed in the presence of *nod^{bd}* results from position-effect variegation is derived from the observation that fourth chromosome disjunction becomes more regular at lower temperatures.

Thus, the *nod^{bd}* mutation most likely results from an inversion whose heterochromatic breakpoint lies near to, but not within, the *nod* locus. The *nod* phenotype then results from inactivation of the *nod* locus as a consequence of a heterochromatic position effect. To the best of our knowledge, the *nod^{bd}* mutation represents the first documented example of position effect variegation in the germline.

DISCUSSION

We have extended and verified Carpenter's analysis of the original *nod^{a}* allele by isolating and characterizing seven new alleles of *nod*. The *nod^{a}* allele is pleiotropic with respect to its effect on chromosome behavior (high frequencies of nondisjunction, loss at meiosis I and meiosis II, and the loss of maternal nonexchange chromosomes at early cleavage). Our study has shown that the pleiotropic effects of *nod^{a}* are a general property of mutations at the *nod* locus and thus are likely to be the result of a single biochemical defect. Moreover, our screen was designed to allow the recovery of *nod* alleles which might, as homozygotes or hemizygotes, result in sterility or even lethality. No such mutations were recovered, very strongly suggesting that the *nod* locus is not essential either for oogenesis or for mitotic chromosome behavior after early cleavage. Thus, the *nod^{+}* locus encodes a function which, while dispensable for fertility and viability, is necessary for the proper disjunction of nonexchange chromosomes at meiosis I and during early cleavage.

The effect of *nod* on disjunction at meiosis I: By inducing these mutations on a balancer chromosome, we have been able to compare the effect of *nod* mutations in the presence and absence of *X* chromosomal exchange. Our data corroborate those of CARPENTER (1973) in demonstrating that the *nod^{+}* product is required primarily, if not exclusively, by nonexchange chromosomes. This is to say that either there exist meiotic functions that are specific to the distributive system, or that exchange can compensate for the *nod* defect.

Our studies extend those of CARPENTER (1973) by demonstrating that in *FM7, nod/nod* females an excess of nullo-*X* gametes is observed only among nullo-*4* ova. No excess of nullo-*X* ova is observed among haplo-*4* or diplo-*4* progeny. The failure to observe an excess of nullo-*X* progeny among the diplo-4 exceptions argues that *X* chromosome loss is not simply correlated with fourth chromosomal nondisjunction. Rather, *X* chromosome loss is apparently restricted to those ova in which fourth chromosome loss has also occurred. It is not possible to ascertain whether these loss events occur at meiosis I or at meiosis II. It is possible that this effect reflects a heterogeneous population of oocytes in *nod* mothers, such that some meiocytes are more likely to allow chromosome loss than are others.

The effect of *nod* on meiosis II nondisjunction and on mitotic chromosome loss: Our data demonstrate a dramatic effect of *nod* on nondisjunction at meiosis II. Although high levels of *X* chromosomal nondisjunction at meiosis II were not observed when CARPENTER (1973) examined *nod^{a}/nod^{a}* females bearing normal sequence *X* chromosomes, they were observed in *nod^{a}/FM7,Df(1)nod* females. This result suggests that the induction of nondisjunction at meiosis II is a general property of *nod* mutations. The failure to observe meiosis II nondisjunction in *nod^{a}/nod^{a}* females is most reasonably explained by suggesting that *nod*-induced meiosis II nondisjunction, like meiosis I misbehavior and mitotic loss, is restricted to nonexchange chromosomes.

As shown in Table 1, homozygous *nod* mothers also produce gynandromorphic offspring at high frequency. *nod*-induced mitotic loss is independent of zygotic genotype and is strictly limited to maternal nonexchange chromosomes. We consider three hy-

potheses to explain these data.

First, as suggested below, *nod* mutations may disrupt the proper modification of a chromosomal organelle (such as the centromere) that is required for both segregation at meiosis I and/or several ensuing mitotic divisions. One might imagine that as a consequence of improper or incomplete modification during meiosis, defective chromosomes are produced which fail to segregate properly at meiosis II and in early cleavage, resulting in both nondisjunction (which would only be detectable at meiosis II) and in chromosome loss.

Second, since the first mitotic division in Drosophila is gonomeric (*i.e.*, male and female pronuclei undergo separate and parallel mitoses before pronuclear fusion), it is possible that the *nod* defect results from the persistence of a maternal spindle abnormality through meiosis II and into the first cleavage divisions. This hypothesis is consistent with the observations that most, if not all, *nod*-induced loss occurs in early cleavage. However, it is difficult to understand why only nonexchange chromosomes nondisjoin at meiosis II or are lost in early divisions, unless nonexchange chromosomes are defective, or marked in such a way as to make them more sensitive to *nod*-defect.

The third hypothesis argues that the observed meiosis II nondisjunction and somatic chromosome loss are not the direct result of the *nod* defect but rather a general property of chromosomes that are univalents at meiosis I. This hypothesis has been invoked by others to explain both the somatic chromosome loss observed in the progeny of *nod* mothers (CARPENTER 1973), and the observation that mosaic progeny are commonly recovered at low frequency in crosses involving one of a number of other mutations that impair various aspects of the meiotic process (BAKER and HALL 1976; HAWLEY 1988).

The genetic analysis of disjunction: The meiotic effects of mutations at the *nod* locus may be compared to that of mutations at two other loci, namely the ca^{nd} locus (DAVIS 1969; HINTON and MCEARCHERN 1963; SEQUEIRA, NELSON and SZAUTER 1989) and the $l(1)TW\text{-}6^{cs}$ mutations (WRIGHT 1974).

ca^{nd} alleles also induce chromosomal nondisjunction and loss at meiosis I and mitotic chromosome loss during early cleavage divisions. Like alleles of nod^{a}, mutations at the ca^{nd} locus also produce high frequencies of fourth chromosomal nondisjunction the vast majority of which are observed as nullo-*4* ova. Although ca^{nd} mutations induce both exchange and nonexchange *X* chromosomes to undergo nondisjunction at high frequency (DAVIS 1969; SEQUIERA, NELSON and SZAUTER 1989), nonexchange chromosomes nondisjoin and are lost much more frequently than are exchange chromosomes (CARPENTER 1973).

As pointed out by BAKER and HALL (1976) "the wild-type allele of ca^{nd} specifies a function required for the regular disjunction of nonexchange, as well as exchange chromosomes, but an exchange may serve as the partial equivalent of the function—it can facilitate regular disjunction of chromosomes even in the absence of the ca^{nd+} function." In this sense, *nod* may be considered to be highly analogous to ca^{nd}, with the exception that in the absence of nod^{+} product exchange virtually always regularizes disjunction. One can then imagine that both the *nod* and ca^{nd} loci encode components of the meiotic system that are required for all chromosomes, independent of their exchange status, and that defects at either locus can be partially or fully compensated for by proper chiasma function. Further evidence that the *nod* and ca^{nd} loci encode similar and perhaps overlapping functions is provided by our recent finding that $nod/+$; $ca^{nd}/+$ double heterozygotes display levels of *X* and fourth chromosomal nondisjunction that are some ten-to-fifty fold higher than are those observed for either single heterozygote (B. BRODEUR and R. S. HAWLEY, unpublished observations).

This hypothesis is supported by the observation that *nod*-induced nondisjunction of major autosomes is not restricted to nonexchange chromosomes (CARPENTER 1973). Indeed, although second chromosomes which have undergone exchange in *nod* mothers are much less likely to nondisjoin than are nonexchange chromosomes, more than 60% of the second chromosomal nondisjunction observed in *nod* females involves bivalents that had undergone one or more exchanges. Thus, as is true for ca^{nd}, the ability of an exchange to compensate for a lack of *nod* function may depend on such factors as chromosome size or structure and also on the position or number of exchanges.

The $l(1)TW\text{-}6^{cs}$ mutation exerts a dominant meiotic effect that is similar to that observed for alleles of *nod* and for ca^{nd}. In $l(1)TW\text{-}6^{cs}/+$ or $l(1)TW\text{-}6^{cs}/l(1)TW\text{-}6^{cs}$ females both *X* and fourth chromosomes undergo nondisjunction at high frequency, and nonexchange chromosomes appear to be more strongly affected than are exchange chromosomes (WRIGHT 1974; B. S. BAKER, personal communication). $l(1)TW\text{-}6^{cs}$ is pleiotropic and exhibits a recessive cold-sensitive maternal and zygotic effect on viability as well as on mitotic chromosome behavior (M. GATTI and B. S. BAKER, personal communication). $l(1)TW\text{-}6^{cs}$ maps to position 37.1 on the *X* chromosome (WRIGHT 1974). Based on their very close proximity and similar phenotypes, WRIGHT (1974) has raised the intriguing possibility that at least one component of the $l(1)TW$ mutation (which may well be complex) is a dominant antimorphic change at the *nod* locus. Direct tests of this proposal are now underway in this laboratory.

If $l(1)TW\text{-}6^{cs}$ is in fact allelic to *nod*, then given the effect of this mutation on the disjunction of exchange chromosomes, the *nod* and ca^{nd} loci may be more analogous in function than previously thought.

The cellular basis of the *nod* defect: Several studies have demonstrated that the physical basis of the ca^{nd} defect is the abnormal segregation of chromosomes by disorganized spindles with abnormal numbers of poles. This defect is manifested both at meiosis and during early cleavage divisions (WALD 1936; KIMBLE and CHURCH 1983). DAVIS (1969) has argued that mutations at the ca^{nd} locus result in aberrant spindle fibers, while others have suggested that the defect is in the centromeres (BAKER and HALL 1976) or in the microtubule organizing centers (KIMBLE and CHURCH 1983). It is entirely possible that a similar defect underlies the *nod* phenotype. However, at least in its simplest form, such a model cannot explain the specific effects of mutations at the *nod* locus on maternal nonexchange chromosomes.

PURO and NOKKALA (1977) have demonstrated at metaphase I in *Drosophila melanogaster* females, achiasmate bivalents co-orient on the same arc of the spindle and then move precociously towards opposite poles (often reaching their respective poles before chiasmate bivalents have even begun their poleward migration). Similar phenomenon have also been observed cytologically in a variety of insect meioses (HUGHES-SCHRADER 1969; NOKKALA 1986a, b). Perhaps these early disjunctions are more sensitive to defects in centromere or spindle formation or function than are the later chiasmate disjunctions.

Alternatively, one might imagine that the disjunction of nonexchange chromosomes requires chromosome modification to compensate for the lack of chiasma function. An example of nonexchange chromosomes that are specifically modified during meiosis has been documented in another organism. Ultrastructural studies in the mole cricket, *Neocurtilla hexadactyla*, revealed that nonexchange chromosome segregation involves a unique modification at the kinetochores in these chromosomes (KUBAI and WISE 1981). Interactions between the centromeres of the nonexchange chromosomes and microtubule may mediate the unique meiotic segregation of these chromosomes. Perhaps the *nod* mutation disrupts a kinetochore structure similar to the one in *N. hexadactyla*. Current efforts in several laboratories to study the *nod* and ca^{nd} loci at the molecular level, and thus to identify their protein products, should shed considerable light on this hypothesis.

We thank CHRISTINE NEW for technical assistance, and M. FEANY, B. BRODEUR, D. WRIGHT, A. ZITRON, and W. WHYTE for numerous discussions. We are also grateful to A. CARPENTER for critical comments on the manuscript. Finally, the authors are grateful to DONNA LOMBARDI for preparing this manuscript. This work was supported by a grant to R.S.H. from the Eukaryotic Genetics Program of the National Science Foundation (DCB-8815749). R.S.H. is also the recipient of a Faculty Research Award from the American Cancer Society (FRA-324) and of an Irma T. Hirschl Monique-Weill Caulier Career Scientist Award. This report was taken from an ongoing thesis of P.Z. in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Sue Golding Graduate Division of Medical Scinece, Albert Einstein College of Medicine, Yeshiva University.

LITERATURE CITED

BAKER, B. S., and A. T. C. CARPENTER, 1972 Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. Genetics **71:** 255–286.

BAKER, B. S., and J. C. HALL, 1976 Meiotic mutants: genetic control of meiotic recombination and chromosome segregation, pp. 351–434 in *The Genetics and Biology of Drosophila*, edited by M. A. ASHBURNER and E. NOVITSKI, Vol. 1A. Academic Press, New York.

BRIDGES, C. B., 1916 Nondisjunction as the proof of the chromosome theory of heredity. Genetics **1:** 1–51, 107–163.

BELL, J. R., A. M. BOGARDUS, T. SCHMIDT and M. PELLEGRINI, 1985 A new *copia*-like transposable element found in a *Drosophila* rDNA gene unit. Nucleic Acids Res. **13:** 3861–3871.

CARPENTER, A. T. C., 1973 A mutant defective in distributive disjunction in *Drosophila melanogaster*. Genetics **73:** 393–428.

CHANDLEY, A. C., 1965 Application of the "distributive pairing" hypothesis to problems of segregation in translocation heterozygotes of *Drosophila melanogaster*. Genetics **52:** 247–258.

COOPER, K. W., 1948 A new theory of secondary nondisjunction in female *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **34:** 179–187.

DAVIS, D. G., 1969 Chromosome behavior under the influence of claret-nondisjunctional in *Drosophila melanogaster*. Genetics **61:** 577–594.

GRELL, R. F., 1962 A new model for secondary nondisjunction: the role of distributive pairing. Genetics **47:** 1737–1754.

GRELL, R. F., 1976 Distributive pairing, pp. 435–486 in *The Genetics and Biology of Drosophila*, edited by M. A. ASHBURNER and E. NOVITSKI, Vol. 1A. Academic Press, New York.

HAWLEY, R. S., 1988 Exchange and chromosome segregation in eucaryotes, pp. 497–527 in *Genetic Recombination*, edited by R. KUCHERLAPATI and G. R. SMITH. American Society for Microbiology, Washington, D.C.

HINTON, C. W., and W. MCEARCHERN, 1963 Additional observations on the behavior of ca^{nd}. Drosophila Inform. Serv. **37:** 90.

HUGHES-SCHRADER, S., 1969 Distance segregation and compound sex chromosomes in mantispidae (*Neuroptera*: *Mantispidae*). Chromosoma **34:** 367–382.

KIMBLE, M., and K. CHURCH, 1983 Meiosis and early cleavage in *Drosophila melanogaster* eggs: effect of the claret-nondisjunctional mutation. J. Cell Sci. **62:** 301–318.

KUBAI, D., and D. WISE, 1981 Nonrandom chromosome segregation in *Neocurtilla* (*Gryllotalpa*) *hexadactyla*: an ultrastructural study. J. Cell Biol. **88:** 281–293.

LEFEVRE, G., JR., 1971 Salivary chromosome bands and the frequency of crossing over in *Drosophila melanogaster*. Genetics **67:** 497–513.

LIFSCHYTZ, E., and D. L. LINDSLEY, 1972 The role of *X* chromosome inactivation during spermatogenesis. Proc. Natl. Acad. Sci. USA **69:** 182–186.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*, Part 3, edited by W. HEDRICK, Drosophila Inform. Serv. **65:** 89.

NOKKALA, S., 1986a The mechanisms behind the regular segregation of autosomal univalents in *Calocoris quadripunctatus*. Hereditas **105:** 199–204.

NOKKALA, S., 1986b The meiotic behavior of B-chromosomes and their effect on the segregation of sex chromosomes in males of *Hemerobius marjinctus*. Hereditas **105:** 221–227.

O'TOUSA, J., 1982 Meiotic chromosome behavior influenced by

the mutation *altered disjunction* in *Drosophila melanogaster* females. Genetics **102:** 503–524.

PURO, J., and S. NOKKALA, 1977 Meiotic segregation of chromosomes in *Drosophila melanogaster* oocytes. Chromosoma **63:** 273–284.

ROBBINS, L. G., 1971 Nonexchange alignment: a meiotic process revealed by a synthetic meiotic mutant of *Drosophila melanogaster*. Mol. Gen. Genet. **110:** 144–166.

RUTHERFORD, S. L., and A. T. C. CARPENTER, 1988 The effect of sequence homozygosity on the frequency of *X*-chromosomal exchange in *Drosophila melanogaster* females. Genetics **120:** 725–732.

SEQUEIRA, W., C. R. NELSON and P. SZAUTER, 1989 Genetic analysis of the *claret* locus of *Drosophila melanogaster*. Genetics **123:** 511–524.

SPOFFORD, J. B., 1976 Position-effect variegation in *Drosophila*, pp. 955–1018 in *The Genetics and Biology of Drosophila*, edited by M. A. ASHBURNER and E. NOVITSKI, Vol. 1C, Academic Press, New York.

VOELKER, R. A., G. B. WISELEY, S. M. HUANG and H. GYURKOVICS, 1985 Genetic and molecular variation in the *RpII 215* region of *Drosophila melanogaster*. Mol. Gen. Genet. **201:** 437–445.

WALD, H., 1936 Cytological studies on the claret mutant type of *Drosophila simulans*. Genetics **21:** 264–279.

WRIGHT, T. R. F., 1974 A cold-sensitive zygotic lethal causing high frequencies of nondisjunction during meiosis I in *Drosophila melanogaster* females. Genetics **76:** 511–536.

ZIMMERING, S., 1976 Genetic and cytogenetic aspects of altered segregational phenomena in Drosophila, pp. 569–573 in *Genetics and Biology of Drosophila*, Vol. 1b, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

ZITRON, A. E., and R. S. HAWLEY, 1989 The genetic analysis of distributive segregation in *Drosophila melanogaster*. I. Isolation and characterization of *Aberrant X segregation, Axs*, a mutation defective in chromosome partner choice. Genetics **122:** 801–821.

Communicating editor: W. M. GELBART