The *lethal(1)TW-6^{cs}* Mutation of *Drosophila melanogaster* Is a Dominant Antimorphic Allele of *nod* and Is Associated With a Single Base Change in the Putative ATP-Binding Domain

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ABSTRACT

The *l(1)TW-6^{cs}* mutation is a cold-sensitive recessive lethal mutation in *Drosophila melanogaster*, that affects both meiotic and mitotic chromosome segregation. We report the isolation of three revertants of this mutation. All three revert both the meiotic and mitotic effects as well as the cold sensitivity, demonstrating that all three phenotypes are due to a single lesion. We further show that these revertants fail to complement an amorphic allele of the *nod* (*no distributive disjunction*) locus, which encodes a kinesin-like protein. These experiments demonstrate that *l(1)TW-6^{cs}* is an antimorphic allele of *nod*, and we rename it *nod^{DTW}*. Sequencing of the *nod* locus on a *nod^{DTW}*-bearing chromosome reveals a single base change in the putative ATP-binding region of the motor domain of *nod*. Recessive, loss-of-function mutations at the *nod* locus specifically disrupt the segregation of nonexchange chromosomes in female meiosis. We demonstrate that, at 23.5°, the meiotic defects in *nod^{DTW}*/+ females are similar to those observed in *nod/nod* females; that is, the segregation of nonexchange chromosomes is abnormal. However, in *nod^{DTW}/nod^{DTW}* females, or in *nod^{DTW}*/+ females at 18°, we observe a more severe meiotic defect that apparently affects the segregation of both exchange and nonexchange chromosomes. In addition, *nod^{DTW}* homozygotes and hemizygous males have previously been shown to exhibit mitotic defects including somatic chromosome breakage and loss. We propose that the defective protein encoded by the *nod^{DTW}* allele interferes with proper chromosome movement during both meiosis and mitosis, perhaps by binding irreversibly to microtubules.

IN most organisms exchange is the primary means for ensuring the segregation of homologous chromosomes during meiosis I (for review, see HAWLEY 1988). Although exchange is sufficient to ensure disjunction in *Drosophila melanogaster* females, those chromosomes that fail to undergo exchange are segregated faithfully by the exchange-independent mechanism known as the distributive system (reviewed by GRELL 1962, 1976; CARPENTER 1991). The *nod* (*no distributive disjunction*) locus, which has recently been shown to encode a kinesin-like protein (ZHANG *et al.* 1990), has been defined by loss-of-function mutations that specifically impair or abolish the distributive system (CARPENTER 1973; ZHANG and HAWLEY 1990).

In this paper, we show that a dominant mutation, *l(1)TW-6^{cs}*, which primarily disrupts distributive chromosome segregation in heterozygous females, is a dominant allele of the *nod* locus. This confirms a prediction of WRIGHT (1974), and we have renamed the allele *nod^{DTW}*. Sequence comparison of the *nod^{DTW}* with that of *nod^{+}* from Oregon R wild-type reveals only a single base change. This mutation predicts a substitution of asparagine for serine in the putative ATP-binding domain of the *nod* kinesin-like protein.

The *nod^{DTW}* mutation was induced by ethyl methanesulfonate (EMS) and initially isolated as a recessive cold-sensitive zygotic lethal (WRIGHT 1973, 1974). Studies of mitotic chromosome behavior using somatic cell markers suggested that this lethality was the consequence of a severe mitotic defect (BAKER, CARPENTER and RIPOLL 1978). Furthermore, at intermediate temperatures neuroblasts homozygous or hemizygous for *nod^{DTW}* show a high frequency of mitotic chromosome breakage that appears to result from the formation of anaphase bridges between homologous chromosomes (M. GATTI and B. S. BAKER, manuscript in preparation).

WRIGHT (1974) showed that *nod^{DTW}*/+ females also exhibit a high frequency of meiotic nondisjunction without an apparent decrease in the exchange frequency, and that nondisjunction in *nod^{DTW}*/+ females increases dramatically in the presence of balancer chromosomes. In this paper we show that *nod^{DTW}*/+ females at 23.5° are primarily defective in segregation of nonexchange chromosomes, confirming WRIGHT's (1974) suggestion that heterozygosity for the *nod^{DTW}*

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mutation primarily affects distributive segregation.

At low temperature or when homozygous, the nod^{DTW} mutation induces a tenfold higher level of nondisjunction, involving both exchange and nonexchange chromosomes. Thus, depending on both dosage and temperature, the nod^{DTW} mutation can exhibit a phenotype ranging from a specific effect on distributive chromosome segregation, to more generalized effects on exchange chromosome segregation and even to a profound defect in mitotic chromosome segregation.

The effect on mitotic chromosome behavior is surprising given that recessive loss-of-function alleles of *nod* only affect distributive chromosome segregation (ZHANG and HAWLEY 1990). However, the *nod* transcript, while ovary-specific in adults, is present throughout development (ZHANG *et al.* 1990). The effect of nod^{DTW} on mitotic segregation can be explained if the defective nod^{DTW} protein is present throughout development and impairs both meiotic and mitotic segregation, perhaps by interfering with the function of related kinesin-like proteins.

MATERIALS AND METHODS

Genetic stocks: All mutations and chromosomes referred to in this report are described in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1987, 1990), with the exception of the *FM7a,nod* alleles, which are described in ZHANG and HAWLEY (1990). Throughout this report the fourth chromosome marker spa^{pol} will be abbreviated as *pol*. The $Y^S X \cdot Y^L$,*In(1)En,y v f B*$\cdot y^+$ chromosome will be denoted simply as $\widehat{XY}$,*v f B*, and $Y^S X \cdot Y^L$, *In(1)En,y B* as $\widehat{XY}$,*y B*. The *X* chromosome balancer, $FM7a,y^{31d}\ sc^8\ w^a\ v^{0f}\ B$ is referred to as *FM7a*.

Crosses were performed on standard medium at 23.5° unless otherwise indicated. Bottles with 15 pairs of parents or vials with a single female and 3 males were set up on day 0 and transferred on day 6. Parents were discarded on day 13. The original vial was scored until day 19, and the transfers were scored until day 25.

EMS: Males 0–24 hr old were mutagenized with 3.25 mM EMS as described by LEWIS and BACHER (1968).

Measurement of primary nondisjunction: The frequency of *X* and fourth chromosome nondisjunction was measured by mating *f/FM7a*; *pol/pol* females to $\widehat{XY}$,*v f B*/O; $C(4)RM,ci\ ey^R$/O males. *X* nondisjunctional progeny are recognized as females heterozygous for the semidominant Bar mutation (diplo-*X* exceptions) or as vermilion forked Bar males (nullo-*X* exceptions). Similarly, fourth chromosome nondisjunctional offspring are recognized as either sparkling-poliert (diplo-*4* exceptions) or as cubitus interruptus eyeless-Russian flies (nullo-*4* exceptions). Haplo-*4* offspring are phenotypically Minute, and have severely reduced viability, and although recorded, are not reported in these data. Variations of the basic experiment using different markers are noted in the tables.

In all crosses the number of *X* chromosome exceptional progeny is doubled prior to calculation. This correction accounts for the inviability of diplo-*X* ova fertilized by $\widehat{XY}$-bearing sperm and nullo-*X* ova fertilized by sperm not carrying a sex chromosome. This correction is recorded as adjusted total in the tables. Sex chromosome nondisjunction is calculated as the sum of two times the exceptional progeny classes divided by the adjusted total.

Fourth chromosome nondisjunction was calculated by doubling simultaneous *X,4* exceptional progeny, adding fourth chromosome exceptions and dividing the sum by the adjusted total. In some crosses, the females were heterozygous for *pol*. In those cases, diplo-*4* exceptions could not be scored. Since the frequency of diplo-*4* exceptions is much lower than that of haplo-*4* exceptions, the frequency of fourth chromosome nondisjunction was taken directly as the frequency of nullo-*4* exceptions. Therefore, the frequencies present a slight underestimate of the actual rate of fourth chromosome nondisjunction.

Meiosis II nondisjunction: In females heterozygous for a balancer chromosome, female progeny bearing two identical maternal *X* chromosomes were scored as meiosis II exceptions. In females with two regular sequence *X* chromosomes and heterozygous for *car*, meiosis II exceptions were detected as daughters that were homozygous for *car*, a marker 3.5 map units from the centromere. However, there is a small probability that such diplo-*X* females arose from a crossover proximal to *car* followed by nondisjunction at meiosis I. When the tested females were heterozygous for *pol*, all *pol* progeny that were not phenotypically Minute (haplo-*4*) were scored as the products of fourth chromosome nondisjunction at meiosis II.

Measurement of mitotic *X* chromosome loss: Mitotic *X* chromosome loss was detected by the occurrence of gynandromorphs. The frequency of gynandromorphs is calculated as the total number of gynandromorphs divided by the total number of regular and exceptional females. Because only those loss events that result in visible sexual mosaics are recognizable, the measured frequencies of mitotic loss underestimate the actual frequency of early chromosome loss.

Measurement of cold sensitivity: All experiments to assess cold sensitivity were carried out at 16.5°. Individual males carrying the *X* chromosome being tested were mated to several $C(1)DX,y\ f/B^S Y$ females in vials on day 0. At day 5 the parents were removed and 22–24 hr later the vials were moved to 23.5°, and scored until 10 days after the first eclosion (approximately day 13). These "upshift" conditions caused the same degree of lethality as continuous incubation at the lower temperature (WRIGHT 1974; this study).

Molecular techniques: Genomic DNA was prepared by the method of BENDER, SPIERER and HOGNESS (1983), and further purified using Elu-Tips (Schleicher & Schuell). An *Eco*RI genomic library was constructed from nod^{DTW} males in lambda gt10 using standard procedures (SAMBROOK, FRITSCH and MANIATIS 1989). Procedures for screening this library and isolating clones corresponding to the *nod* locus are as described in ZHANG *et al.* (1990). It was necessary to clone certain regions by polymerase chain reaction (PCR)-mediated amplification. Primers containing external *Bam*HI sites were synthesized based on the published *nod* sequence (ZHANG *et al.* 1990) in the Albert Einstein College of Medicine Oligonucleotide Synthesis Facility. The products of PCR amplification were phenol/chloroform extracted and purified using GeneClean (Bio101) or Elu-Tip before cloning into pBluescript SK (Stratagene). Purified PCR amplification products were cloned using either *Bam*HI sites in the PCR primers or internal *Eco*RI sites. Alternatively, some products were cloned by digesting with *Xho*I, which cuts the product internally, and ligating into vector digested with the appropriate enzyme and *Eco*RV. Double-stranded templates were sequenced with Sequenase (USB) according to the manufacturer's specifications.

TABLE 1

X chromosome recombination in nod^{DTW}/+ heterozygous females

Maternal genotype	NCO	SCO			DCO			Total progeny	Map length for region			Tetrad frequency		
		I	II	III	I, II	I, III	II, III		I	II	III	E_0	E_1	E_2
+/y cv v f car	1457	252	517	625	14	99	97	3061	11.9	20.5	27.5	0.10	0.60	0.30
nod^{DTW} *f/y cv v f car*	329	65	120	136	2	21	26	699	12.6	21.2	26.2	0.08	0.64	0.28

Females of the indicated *X* chromosome genotype were mated to *y cv v f car*/y^+ *Y* males, and the resulting regular daughters were scored. Recombination was measured in three intervals: I (*y-cv*), II (*cv-v*), and III (*v-car*). Tetrad frequencies were calculated as described by WEINSTEIN (1936). NCO = noncrossover, SCO = single crossover, DCO = double crossover.

RESULTS

Meiotic exchange and nondisjunction in nod^{DTW}/+ females

In this section we examine in detail the meiotic effects of the nod^{DTW} mutation. For purposes of clarity, the effects on each chromosome are considered separately.

The *X* chromosome: As shown in Table 1, we have confirmed WRIGHT's (1974) observation that *X* chromosome exchange is not affected by heterozygosity for nod^{DTW}; both the map distances as well as the tetrad distribution for nod^{DTW}/+ females are close to control values. However, the frequency of *X* chromosome nondisjunction in nod^{DTW}/+ females is elevated 10–20-fold compared to that of +/+ females (Table 2).

Half-tetrad analysis of matroclinous daughters produced by nod^{DTW}/+ females indicates that most of the nondisjunction induced by nod^{DTW} occurs at meiosis I. Among the progeny of nod^{DTW}+/+ *car* females carrying isosequential chromosomes, 84 out of 86 diplo-*X* exceptional daughters recovered arose from meiosis I nondisjunction (the marker *car* is 3.5 map units from the centromere and thus can be used reliably as a centromere marker for assaying nondisjunction at meiosis I *vs.* meiosis II). The other two diplo-*X* exceptions were homozygous for *car*, and may have resulted either from meiosis II nondisjunction or from a crossover between *car* and the centromere followed by nondisjunction at meiosis I. These data demonstrate that heterozygosity for nod^{DTW} affects meiosis I, and that there is at most, a weak effect on meiosis II.

Nondisjunction at meiosis I increases more than 15-fold when nod^{DTW}/+ females are also heterozygous for *X* chromosome rearrangements (*In(1)dl-49* or *FM7a*) that reduce the frequency of *X* chromosome recombination (see Table 2). Moreover, in all three crosses involving females heterozygous for nod^{DTW}, the observed frequency of *X* nondisjunction is approximately half of the estimated frequency of E_0 tetrads. As noted by both CARPENTER (1973) and ZITRON and HAWLEY (1989), this phenotype is characteristic of mutations that specifically affect distributive segregation. In the absence of a functional distributive system, nonexchange homologs segregate at random with respect to one another. As a consequence, half of the time two chromosomes go to the same pole giving rise to nondisjunctional gametes.

The hypothesis that nod^{DTW}/+ females are specifically defective in distributive segregation predicts that in females with isosequential chromosomes only those chromosomes that fail to undergo exchange will nondisjoin. To determine the exchange status of the nondisjunctional *X* chromosomes in such females, we performed a half-tetrad analysis of the diplo-*X* exceptional progeny (see Table 3). We determined the *X* chromosome genotype of 86 of 92 diplo-*X* exceptional daughters recovered from approximately 25,000 progeny (Table 3A; the remaining six died or failed to produce sufficient progeny). Of those females, 80 were identical in genotype to their mothers, bearing two noncrossover chromosomes (*f/y cv v f car*). As discussed above, two daughters were homozygous for *car* and most likely arose from nondisjunction at meiosis II. The remaining four of the 86 females resulted from exchanges followed by nondisjunction at meiosis I (that is, they carried at least one crossover chromosome and were also heterozygous for *car*). In all four cases, the exchange was in the most distal region (*y-cv*). This unusual distribution of exchanges, also noted by CARPENTER (1973) with *nod/nod* females, will be addressed in DISCUSSION.

As shown in Table 3B, the distribution of tetrads giving rise to the exceptional females was $E_0 = 0.94$ and $E_1 = 0.06$, a distribution that is markedly different from that of mono-*X* progeny produced by +/+ or nod^{DTW}/+ females. Clearly, in nod^{DTW}/+ females, the vast majority of nondisjunctional segregations involve nonexchange tetrads. By comparing the frequency of *X* nondisjunction (3%) in the cross of nod^{DTW} *f/y cv v f car* females reported in Table 3 with the estimated frequency of E_0 tetrads (8.2%), it may be concluded that nonexchange *X* chromosomes fail to disjoin properly at least 37% of the time. If the nondisjunctional gametes are the result of random *X* segregation at meiosis I, and if the frequency of *X* nondisjunction reflects only half of the affected segregations, then the *X* chromosomes segregate randomly in 74% of the meioses in which they do not undergo exchange. These observations show that heterozygosity for nod^{DTW} induces a specific defect in distributive segregation, since nearly all the nondisjunctional progeny

TABLE 2

X and fourth chromosome nondisjunction in nod^{DTW} heterozygotes

Gametes		Maternal genotype[a]					
Mother	Father	*y/y*	*y nod^{DTW}/y*	*y/FM7*	*y nod^{DTW}/FM7*	*y/dl-49*	*y nod^{DTW}/dl-49*
Regular							
X 4	$\widehat{XY}$ $\widehat{44}$	626	1,834	1,690	913	1,350	598
X 4	O $\widehat{44}$	740	2,250	1,396	690	2,165	865
X nondisjunctional							
O *4*	$\widehat{XY}$ $\widehat{44}$	0	16	4	152	1	130
XX 4	O $\widehat{44}$	0	30	2	160	1	181
4 nondisjunctional							
X 44	$\widehat{XY}$ O	0	944	0	238	4	252
X O	$\widehat{XY}$ $\widehat{44}$	1	2,073	0	896	0	645
X O	O $\widehat{44}$	0	2,445	1	566	5	967
X 44	O O	0	784	0	313	2	204
X, 4 nondisjunctional							
O *44*	$\widehat{XY}$ O	0	2	0	57	0	29
O O	$\widehat{XY}$ $\widehat{44}$	0	23	0	279	0	275
XX O	O $\widehat{44}$	0	12	0	82	0	116
XX 44	O O	0	13	1	113	1	94
Total		1,367	10,426	3,094	4,559	3,529	4,356
Adjusted total		1,367	10,522	3,101	5,402	3,532	5,181
% *X* nondisjunction		0	1.8	0.5	31.2	0.2	31.8
% *4* nondisjunction		0.1	60.1	0.1	51.7	0.4	59.8
E_0 Tetrad frequency		10%[b]	8%[b]	67%[c]	ND	60%[c]	ND

[a] *pol/pol* females of the indicated genotype were crossed to $\widehat{XY}$, *v f B*/O; *C(4)RM, ci ey^R*/O males.
[b] Data taken from Table 3.
[c] Data taken from ZITRON and HAWLEY (1989).

TABLE 3

Diplo-X exceptional females recovered from a cross of nod^{DTW} f/ y cv v f car; pol/+ females to $\widehat{XY}$, y B/O; C(4)RM, ci ey^R/O males

A. Genotypes of diplo-X exceptional females[a]

Genotype	Number	Genotype	Number
$\frac{y\ cv\ v\ f\ car}{+\ +\ +\ f\ +}$	80	$\frac{y\ cv\ v\ f\ car}{y\ +\ +\ f\ +}$	3
$\frac{y\ cv\ v\ f\ car}{y\ cv\ v\ f\ car}$	2	$\frac{+\ cv\ v\ f\ car}{y\ +\ +\ f\ +}$	1
			Total 92[b]

B. Tetrad distribution in mono and diplo-X-bearing ova produced by nod^{DTW}/+ females[c]

	Mono-X ova[d]		Diplo-X ova	
Tetrad rank	+/+	nod^{DTW}/+	nod/nod[e]	nod^{DTW}/+
E_0	0.10	0.08	(0.97)	0.94
E_1	0.60	0.64	(0.03)	0.06
E_2	0.30	0.28	(0)	0

[a] Recovered from among approximately 25,000 progeny. In a subset of this cross (n = 6958), *X* nondisjunction was 3% and *4* nondisjunction was 72%. To determine genotypes, exceptional females were crossed to *y cv v f car/y$^+$Y* males and resulting daughters were scored.
[b] This total includes six females that were sterile, died or failed to produce enough progeny to allow classification.
[c] Tetrad frequencies for mono-X ova calculated as described by WEINSTEN (1936) and for diplo-X ova as described by MERRIAM and FROST (1964).
[d] From data in Table 1.
[e] Data were taken from CARPENTER (1973).

are derived from nonexchange tetrads and the majority of nonexchange tetrads fail to disjoin properly.

The fourth chromosome: In wild-type females, the small fourth chromosomes never undergo exchange and thus are always segregated by the distributive system. However, in females heterozygous for nod^{DTW}, the level of fourth chromosome nondisjunction is approximately 60% (Table 2). There is a significant excess of nullo-*4* gametes compared to diplo-*4* gametes. For instance, in *y nod^{DTW}/y* females the frequency of nullo-*4* gametes is 72% while the frequency of diplo-*4* gametes is 28%, indicative of chromosome loss.

As with *X* chromosome nondisjunction, most fourth chromosome nondisjunction events occur at meiosis I. This was demonstrated by measuring fourth chromosome nondisjunction in nod^{DTW}/+; *pol*/+ females for a subset of the cross reported in Table 3 (n = 6958). In this cross, diplo-*4* meiosis I exceptions are phenotypically wild type and thus indistinguishable from regular progeny. Half of the diplo-*4* exceptions resulting from meiosis II nondisjunction would be readily detected as *pol* progeny, but only one *pol* exception was observed among 6958 progeny. Thus, as with the *X* chromosome, meiosis II nondisjunction occurs infrequently.

These effects on fourth chromosome nondisjunction are consistent with the results of our analysis of *X* chromosome nondisjunction: nonexchange chro-

TABLE 4

Chromosome 2 nondisjunction in *nod*^{DTW}/+; *al dp b pr cn*/+ females

Maternal X chromosome genotype	Ova composition with respect to X and 2						Total progeny	No. of female parents
	X; O[a]	X; 2/2	O; O[a]	O; 2/2	X/X; O[a]	X/X; 2/2		
y/y	5	3	1	1	0	0	10	900
y *nod*^{DTW}/y	210	1463[b]	5	9	0	6	1695	1150

[a] The tester males used in these crosses transmit the *C(2)* chromosomes at low efficiency. Thus the number of nullo-2 progeny is low.
[b] 59 were homozygous for 1 or more second chromosome marker.
Females of the indicated X chromosome genotype were mated to *C(2L)dp*; *C(2R)px* males.

mosomes in *nod*^{DTW}/+ females nondisjoin at high frequencies at the first meiotic division.

The second chromosome: *nod*^{DTW}/+ females show an increase in second chromosome nondisjunction as measured by crossing *nod*^{DTW}/+; +/*al dp b pr cn* females to +/*Y*; *C(2)L*; *C(2)R* males (Table 4). Because the only progeny recoverable from this cross result from second chromosome nondisjunction, the rate of nondisjunction can only be estimated by the number of progeny per female. Females heterozygous for *nod*^{DTW} show at least a 100-fold increase in second chromosome nondisjunction.

To determine whether the observed increase in nondisjunction was due to a *nod*^{DTW}-induced reduction in exchange, we measured the effect of *nod*^{DTW} on second chromosome exchange (Table 5). Although exchange is increased in both *nod*^{DTW}/+ and *nod*^{DTW}/*nod*^{DTW} females in interval IV (*pr-cn*) which spans the centromere, it is reduced in the three distal intervals spanning the length of the left arm. This effect is most pronounced in interval III (*b-pr*) where exchange is reduced to 42% and 53% of control values in *nod*^{DTW}/+ and *nod*^{DTW}/*nod*^{DTW} females, respectively. The medial interval II (*dp-b*) shows only weak reductions (80% of control in *nod*^{DTW}/+ and 78% of control in *nod*^{DTW}/*nod*^{DTW}), while the distalmost interval I (*al-dp*) displays an intermediate level of reduction (67% and 65% of control in heterozygotes and homozygotes, respectively).

These effects on second chromosome exchange are unusual in several respects. First, *X* chromosome exchange is unaffected (Table 1). Thus, if *nod*^{DTW} does affect exchange, it must do so in a chromosome-specific fashion. Such specificity has been reported for only one other meiotic mutation in *D. melanogaster*, *mei-1* (VALENTIN 1973), which specifically reduces exchange medially on the *X* chromosome. Second, the reductions of second chromosome exchange observed in both *nod*^{DTW}/+ and *nod*^{DTW}/*nod*^{DTW} females are neither uniform, as in recombination-defective mutations like *mei-9*, nor are they polar as in the vast majority of mutations that reduce the frequency of crossing over by altering the preconditions for exchange (BAKER and HALL 1976). Rather, exchange is reduced in the distalmost interval I and in the proximal interval III, but nearly normal in the intervening region II. Third, loss-of-function mutations at the *nod* locus have no effect on second chromosome exchange (CARPENTER 1973). Finally, unlike the disjunctional and mitotic phenotypes associated with the *nod*^{DTW} lesion (WRIGHT 1974; M. GATTI and B. S. BAKER, in preparation; see below), the effect on exchange is not dosage sensitive: the same reduction of exchange is seen in both *nod*^{DTW}/+ and *nod*^{DTW}/*nod*^{DTW} females.

It is possible that the effect on second chromosome exchange results from some unrelated cause, such as genetic background. Whatever the cause, the effect on exchange is weak and so cannot be responsible for the high rate of second chromosome nondisjunction in *nod*^{DTW}/+ females.

To determine whether the *nod*^{DTW}/+-induced second chromosome nondisjunction preferentially involves nonexchange chromosomes, diplo-2 sons (derived from crossing *nod*^{DTW}/+; +/*al dp b pr cn* females to +/*Y*; *C(2)L*; *C(2)R* males) were subjected to half-tetrad analysis (Table 6). The tetrad distributions for these nondisjunctional offspring are presented in Table 6b. The proportion of E_0 tetrads is increased in diplo-2 exceptions compared to their mono-2 sibs. Sixty-six percent of the observed nondisjunction involves bivalents without an exchange on the left arm of chromosome 2. It seems likely that most of these exceptions result from meioses in which chromosome 2 was entirely achiasmate.

Nevertheless, 34% of the diplo-2 exceptions result from the nondisjunction of exchange tetrads (see Table 6b). The distribution of crossover events in these diplo-2 exceptions is unusual. As shown in Table 7, there is nearly 3-fold more exchange in the distalmost *al-dp* region among diplo-2 exceptions compared to mono-2 ova. This unusual distribution is similar to that noted above for diplo-*X* exceptions. Thus, not only do nonexchange chromosomes fail to segregate properly in *nod*^{DTW}/+ females, but the disjunction of bivalents with distal exchanges also fails frequently. The data suggest that the ability of an exchange to guarantee disjunction depends on its location on the chromosome arm.

Given that a substantial fraction of chromosome 2 nondisjunction in *nod*^{DTW}/+ females involves ex-

TABLE 5

Second chromosome recombination in nod^{DTW} females

Maternal X chromosome genotype	NCO	SCO				DCO					Total progeny	Map distance for region			
		I	II	III	IV	I, II	I, III	I, IV	II, III	II, IV		I	II	III	IV
$\frac{y}{y}$	2071	472	1036	172	2	12	17	2	18	3	3805	13.2	28.1	5.4	0.2
$\frac{y}{y\ nod^{DTW}}$	2566	317	963	83	12	12	4	2	4	6	3969	8.4	24.8	2.3	0.5
$\frac{y\ nod^{DTW}}{y\ nod^{DTW}}$	1566	188	496	51	9	6	9	1	8	3	2337	8.7	22.0	2.9	0.6

X/X; *al dp b pr cn*/+ females were crossed to *al dp b pr cn*/*al dp b pr cn* males. Map distances were calculated for four regions: *al-dp* (I), *dp-b* (II), *b-pr* (III) and *pr-cn* (IV).

TABLE 6

Diplo-2 exceptional males recovered from cross of nod^{DTW}/+; *al dp b pr cn*/+ females to *C(2L) dp*; *C(2R) px* males

A. Genotypes of exceptional males

Genotype	Number[a]	Genotype	Number[a]	Genotype	Number
$\frac{al\ dp\ b\ pr\ cn}{+\ +\ +\ +\ +}$	478	$\frac{+\ +\ b\ pr\ cn}{al\ dp\ +\ +\ +}$	27	$\frac{al\ dp\ b\ pr\ cn}{al\ +\ +\ +\ +}$	18
$\frac{+\ dp\ b\ pr\ cn}{al\ +\ +\ +\ +}$	50	$\frac{+\ +\ b\ pr\ cn}{+\ +\ +\ +\ +}$	20	$\frac{al\ dp\ b\ pr\ cn}{al\ dp\ +\ +\ +}$	6
$\frac{+\ dp\ b\ pr\ cn}{+\ +\ +\ +\ +}$	41	$\frac{+\ +\ +\ pr\ cn}{al\ dp\ b\ +\ +}$	2	$\frac{al\ dp\ b\ pr\ cn}{+\ +\ b\ +\ +}$	1
					Total = 643

B. Tetrad distribution in mono and diplo-2-bearing ova produced by nod^{DTW}/+ females[b]

Tetrad rank	Mono-2 ova[c]		Diplo-2 ova	
	+/+	nod^{DTW}/+	*nod/nod*[d]	nod^{DTW}/+
E_0	0.12	0.31	(0.39)	0.66
E_1	0.83	0.66	(0.38)	0.33
E_2	0.05	0.03	(0.19)	0.01

[a] A total of 618 diplo-2 wild-type males were recovered from the cross of nod^{DTW}/*y* females shown in Table 4. Genotypes of 274 phenotypically wild-type diplo-2 male exceptions were determined by crossing to *al dp b pr cn*/*al dp b pr cn* females. The number shown in the table is an extrapolation calculated by multiplying (observed) (274/618). 25 diplo-2 exceptional males homozygous for one or more recessive marker were recovered from the cross of nod^{DTW}/*y* females shown in Table 4. Seven of 18 phenotypically *al* males were testcrossed and all were of the genotype shown. None of the six phenotypically *al dp* males was testcrossed successfully, but all were assumed to be of the genotype shown. The single *b* male was testcrossed and the genotype is shown.

[b] Tetrad frequencies for mono-2 ova were calculated as described by WEINSTEIN (1936) and for diplo-2 ova as described by MERRIAM and FROST (1964).

[c] Data from Table 5.

[d] Data from *nod/nod*, shown for comparison, were taken from CARPENTER (1973). Note, however, that in this case markers spanned both arms of chromosome 2, whereas the other crosses reported only detected exchange on the left arm. Thus, the tetrad frequencies reported for the mono-2 ova and nod^{DTW}/+ diplo-2 ova are underestimates of the actual frequencies of exchange tetrads.

change bivalents, and that more distal exchanges are more likely to nondisjoin, it is tempting to speculate that the reductions in second chromosome exchange in distal regions observed in nod^{DTW}/+ females reflect the loss of distal crossovers rather than a direct effect on the frequency or distribution of exchanges. However, the distribution of exchanges among the diplo-2 exceptions (Table 7) is different from the pattern of reduction of exchange in mono-2 ova (Table 5). While the lengths of region I (*al-dp*), region II (*dp-b*) and region III (*b-pr*) are all reduced 3–4 map units in mono-2 ova (Table 5), there is substantially more nondisjunction of chromosomes with exchanges in region I (*al-dp*) than of those with exchanges in region II, and virtually no nondisjunction of chromosomes with exchanges in region III (*dp-b*) (Tables 6 and 7). Thus the reduction in map distance observed among mono-2 ova from nod^{DTW} females cannot result entirely from nondisjunction of chromosomes with distal exchanges.

Dosage and temperature influence the nod^{DTW} meiotic phenotype: As noted by WRIGHT (1974),

TABLE 7

Relative distribution of chromosome 2 exchanges in mono and diplo-2 ova from nod^{DTW}/+ females

Maternal genotype	Ova type	Relative map length of chromosome 2 regions[a] I	II	III
y/*y*	Mono-2[b]	28.1	59.9	11.5
y nod^{DTW}/*y*	Mono-2[b]	23.3	68.8	6.4
y nod^{DTW}/*y*	Diplo-2[c]	65.7	32.5	1.8

[a] For each ova type, the map length of each region was divided by the total map length of the chromosome arm.
[b] Data from Table 5.
[c] Data from Table 6B.

homozygous nod^{DTW} females exhibit much higher levels of *X* chromosome nondisjunction than do nod^{DTW}/+ heterozygotes (see Table 8). As shown in Table 8, temperature also affects the meiotic phenotype of nod^{DTW} (see also WRIGHT 1974). In nod^{DTW} heterozygotes, the rate of *X* nondisjunction rises nearly an order of magnitude as the temperature is decreased from 25° to 18°. Since the frequency of exchange is the same in nod^{DTW}/nod^{DTW} and nod^{DTW}/+ females (Table 5), the high rates of nondisjunction are therefore the result of exchange chromosome nondisjunction.

Interestingly, while the rate of fourth chromosome nondisjunction remains constant, the proportion of nullo-*4* gametes among fourth chromosome exceptional progeny rises at lower temperatures. This indicates that there is progressively more fourth chromosome loss at lower temperatures, even though the total rate of fourth chromosome misbehavior remains constant.

In summary, although the meiotic defect in nod^{DTW} heterozygotes at 25° preferentially affects distributive segregation, both dosage and temperature appear to broaden the mutant phenotype.

The nod^{DTW} mutation is a dominant allele of *nod*

Cosegregation of the nod^{DTW} meiotic and cold-sensitive phenotypes: WRIGHT (1974) originally mapped the nod^{DTW} mutation to 37.1 on the *X* chromosome. We further localized nod^{DTW} by crossing *y* nod^{DTW}/*ptg v m g sd f* females to wild-type males. Sons of all possible genotypes were recovered, established as stocks and tested for cold sensitivity and a dominant meiotic effect. Seventy-two recombinants in the *v-m* interval were recovered, none of which separated the dominant meiotic effect from the cold-sensitive lethality. Of these, 43 were *v* nod^{DTW} m^+, 15 were v^+ nod^{DTW+} *m*, 4 were v^+ nod^{DTW} *m*, and 10 were *v* nod^{DTW+} m^+, placing nod^{DTW} at 35.5 on the genetic map, between *v* (33) and *m* (36.1). Thus both phenotypes associated with the nod^{DTW} mutation co-map.

Originally, the similarities between the nod^{DTW} mutation and alleles of the *nod* locus in phenotype and map position led WRIGHT (1974) to suggest that nod^{DTW} might be a poisonous or antimorphic allele of *nod*, but that "the magnitude of the dominant effects" of nod^{DTW} would make it "difficult to establish allelism." In order to demonstrate allelism, we isolated revertants of the zygotic lethality or of the dominant meiotic phenotype of nod^{DTW}. We reasoned that since all existing *nod* alleles, including a deletion of the locus, are recessive (ZHANG and HAWLEY 1990) and only affect distributive segregation, the nod^{DTW} dominant allele must be a gain-of-function allele. Thus, revertants of nod^{DTW} might be loss-of-function or amorphic alleles, which could then be tested for complementation with existing *nod* alleles. Two different screens were used to find revertants of nod^{DTW}; each is described in detail below.

Revertants of the dominant nod^{DTW} meiotic phenotype: The first screen was designed to find revertants of the dominant meiotic phenotype, that is, chromosomes that do not induce high levels of meiotic sex chromosome nondisjunction when heterozygous with *FM7a* (Figure 1A). Mutagenized nod^{DTW} f/B^SY males were mated to *FM7a/FM7a;pol/pol* females. The resulting nod^{DTW}* *f/FM7a;pol*/+ females (the asterisk indicates a mutagenized *X* chromosome) were mated individually to $FM7a/B^SY$ males. Generally, this cross yields a high proportion of exceptional *XXY* daughters, readily identified as y^+ B^S females. However, revertant/*FM7a* females should produce few, if any, such daughters (Figure 1A). One *X*-linked revertant of the dominant meiotic phenotype, nod^{DR1} (*DR1*), was recovered from over 5000 mutagenized chromosomes screened. As shown in Tables 9 and 10, *DR1/FM7a* and *DR1*/+ females show only background levels of *X* and fourth chromosome nondisjunction.

DR1 was examined with respect to the two other phenotypes associated with nod^{DTW}, cold sensitivity and abnormal mitotic chromosome behavior. Flies hemizygous for the *DR1* chromosome are not cold sensitive; the survival of *DR1* males at low and normal temperatures is identical (see Table 11). In addition, when the mitotic phenotype of *DR1* at 18° was examined cytologically, no anaphase bridges or other mitotic abnormalities were found (M. GATTI, personal communication). Thus, all three nod^{DTW} phenotypes were reverted by the same lesion.

Revertants of nod^{DTW} cold-sensitive zygotic lethality: A second screen based on survival at 16.5° was undertaken to isolate nod^{DTW} revertants (Figure 1B). Mutagenized nod^{DTW} f/B^SY males were mated to attached-*X* (*C(1)DX,y f*/B^SY) females at 16.5° under the conditions described in MATERIALS AND METHODS. All sons bear a mutagenized nod^{DTW} chromosome, and only revertants and a few (approximately 7%) nod^{DTW} *f* sons survive to adulthood under these conditions (WRIGHT 1974) (Table 11). To distinguish

TABLE 8

Effect of dosage and temperature on the nod^{DTW} meiotic phenotype

Maternal genotype	Temperature (°C)[a]	% *X* nondisjunction	% *4* nondisjunction	Fraction nullo-*X*	Fraction nullo-*4*	Total progeny
y/y	25	0	0.1			1,367
y nod^{DTW}/*y*	25	1.8	60.3	0.43	0.72	10,426
y nod^{DTW}/*y* nod^{DTW}	25	25.7	78.0	0.77	0.94	3,091
y/y	21	0	0			1,212
y nod^{DTW}/*y*	21	7.4	56.1	0.72	0.78	5,818
y/y	18	0	0.1			1,415
y nod^{DTW}/*y*	18	16.8	55.8	0.68	0.81	3,826

[a] Temperature at which animals were reared and crossed. Females of the indicated genotype were mated to *XY, v f B*/O; *C(4)RM, ci* ey^R/O males.

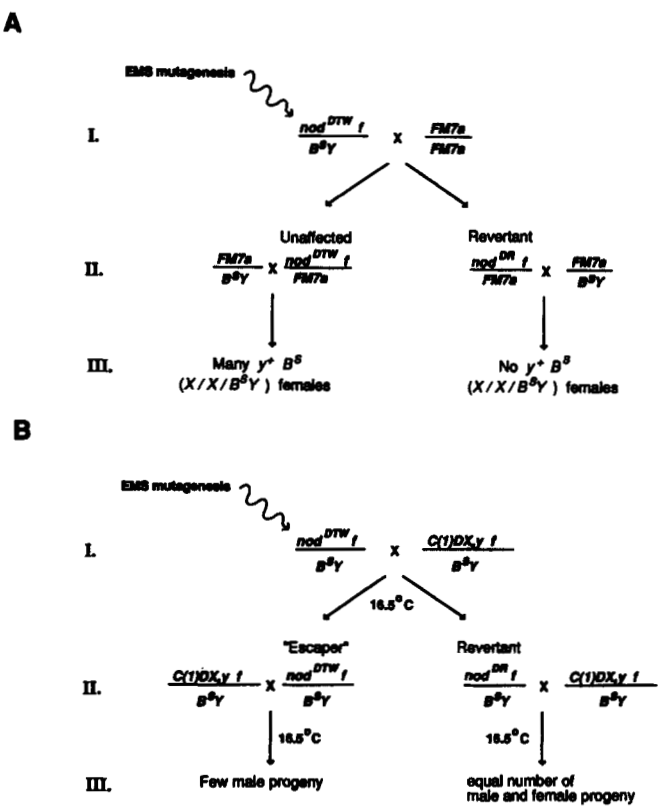

FIGURE 1.—Mating schemes for recovery of nod^{DTW} revertants. (A) Screen for revertants of dominant meiotic phenotype. In generation I, EMS-mutagenized males were mated to females homozygous for the balancer *X* chromosome *FM7a*. The resulting daughters (generation II) were screened for the production of exceptional XXB^SY progeny, identified as y^+ B^S females in generation III (see text for details). Chromosomes bearing a putative nod^{DR} locus were recovered by mating nod^{DR}/B^SY males from generation III to *C(1)DX,y f*/B^SY females. (B) Screen for revertants of the cold-sensitive phenotype. In generation I, EMS-mutagenized males were mated to females at 16.5° using upshift conditions (see MATERIALS AND METHODS). The few resulting male progeny (generation II) were retested under identical conditions to distinguish escapers from revertants. More than 90% of the escaper males in generation II were sterile. Chromosomes bearing a putative nod^{DR} locus were recovered as nod^{DR}/B^SY males in generation III and mated to *C(1)DX,y f*/B^SY females to establish stocks.

males carrying a nod^{DTW} revertant from "escapers," groups of five or fewer surviving males from each bottle were mated to *C(1)DX,y f*/B^SY females under the same conditions. Males bearing revertant chromosomes produced abundant male progeny, while nod^{DTW} "escaper" males produced very few (Figure 1B).

Two cold-resistant, sex-linked mutations, nod^{DR2} and nod^{DR3} (*DR2* and *DR3*) were recovered from 17,000 progeny screened (Table 11). The first of these, *DR2*, has no dominant meiotic effect, either as *DR2/FM7a* (Table 9) or as *DR2/+* (Table 10). Its phenotype is very similar to that of the first revertant, *DR1* (Tables 9 and 10).

DR3 has an intermediate mutant phenotype. Although its survival at 16.5° is completely wild type (see Table 11), *DR3* exhibits a slight dominant effect on *X* and fourth chromosome nondisjunction; *DR3/FM7a* females exhibit 8% *X* and 3% fourth chromosome nondisjunction (Table 9). These frequencies are low compared to those of nod^{DTW}/*FM7a* females, but are at least twice as high as those observed in *DR1/FM7a* or *DR2/FM7a* females. Thus, *DR3* retains a weak dominant meiotic phenotype.

In summary, three revertants were selected on the basis of either loss of the nod^{DTW} dominant meiotic effect or loss of cold sensitivity. All three co-reverted or greatly ameliorated both phenotypes, and one, *DR1*, was also shown to be completely normal with respect to a third phenotype, anaphase bridge formation and subsequent chromosome breakage during mitosis at low temperatures. The simplest explanation of these results and the mapping data presented above is that the nod^{DTW} mutation is a single lesion with pleiotropic effects on both meiotic and mitotic chromosome behavior.

***DR1, DR2* and *DR3* are recessive meiotic mutations that fail to complement *nod*:** All three revertants when homozygous exhibited a dramatic effect on female meiotic chromosome segregation. This phenotype was characterized by very high rates of fourth chromosome nondisjunction, and lower, but clearly

TABLE 9

Frequency of nondisjunction in revertant/*FM7a nod* females

Gametes						
Mother	Father	*FM7a/+*	*FM7a/ nod^{DTW}*	*FM7a/DR1*	*FM7a/DR2*	*FM7a/DR3*
Regular						
X 4	$\hat{XY}$ $\hat{44}$	1460	668	1032	3825	1132
X 4	O $\hat{44}$	1336	434	715	3457	868
X Nondisjunctional						
O *4*	$\hat{XY}$ $\hat{44}$	5	341	2	27	22
XX 4	O $\hat{44}$	5	314	5	78	55
4 Nondisjunctional						
X O	$\hat{XY}$ $\hat{44}$	5	501	4	34	30
X O	O $\hat{44}$	2	413	1	21	8
X,4 nondisjunctional						
O O	$\hat{XY}$ $\hat{44}$	1	666	3	25	17
XX O	O $\hat{44}$	0	144	0	4	2
Total		2814	3481[a]	1761	7471[a]	2134[a]
Adjusted total		2825	4946	1770	7605	2230
% *X* nondisjunction		1	59	1.0	3.5	8.6
% *4* nondisjunction		0	51	0.8	1.5	3.4

[a] Twenty-two gynandromorphs recovered in *nod^{DTW}/FM7a*; 2 in *DR2/FM7a*; 1 in *DR3/FM7a*.

+ chromosomes are marked with *y cv v f car*. *DR* and *nod^{DTW}* chromosomes are marked with *f*. *pol/+* females of the indicated genotype were crossed to $\hat{XY}$, *v f B*/O; *c(4)RM, ci ey^{R}*/O males.

TABLE 10

Rates of nondisjunction in heterozygous and homozygous revertant females

Gamete types		Maternal genotype								
Mother	Father	+/+	*nod^{DTW}/+*	*DR1/+*	*DR2/+*	*DR3/+*	*nod^{DTW}/nod^{DTW}*	*DR1/DR1*	*DR2/DR2*	*DR3/DR3*
Regular										
X 4	$\hat{XY}$ $\hat{44}$	1164	1106	1118	894	1217	85	129	231	221
X 4	O $\hat{44}$	979	828	974	963	1454	116	168	322	294
X nondisjunctional										
O *4*	$\hat{XY}$ $\hat{44}$	0	17	0	1	0	34	1	4	30
XX 4	O $\hat{44}$	2	22	1	2	1	7	3	7	10
4 nondisjunctional										
X O	$\hat{XY}$ $\hat{44}$	0	2172	0	3	19	169	306	655	301
X O	O $\hat{44}$	0	2745	0	0	20	229	426	1017	484
X,4 nondisjunctional										
O O	$\hat{XY}$ $\hat{44}$	2	56	0	0	0	55	9	10	42
XX O	O $\hat{44}$	0	12	0	0	0	20	9	5	28
Total		2148	6958	2093	857	2711	715	1051	2251	1410[a]
Adjusted total		2152	7065	2094	860	2713	831	1073	2277	1520
% nondisjunction										
X		0.4	3	0.1	0.3	0.1	28	4.1	2.3	14.5
4		0	71.5	0	0.2	1.4	66	71.6	74.7	60.9

[a] One gynandromorph recovered.

pol/+ females of the indicated genotype were crossed to $\hat{XY}$,*y B*/O; *C(4)RM, ci ey^{R}*/O males. + chromosomes are marked with *y cv v f car*. *DR* and *nod^{DTW}* chromosomes are marked with *f*.

elevated rates of *X* nondisjunction (Table 10). In fact, the rates of *X* and fourth chromosome nondisjunction were comparable to those of *nod/nod* females. This supports the hypothesis that the revertants are amorphic (loss-of-function) alleles of the *nod* locus.

To test this directly, we performed a series of complementation tests using the *nod^{DTW}* revertants and an existing *nod* allele. Each revertant was mated to *FM7a,nod^{b27}/+* females. *FM7a,nod^{b27}* is a recessive, amorphic *nod* allele induced by γ-ray mutagenesis of the balancer chromosome *FM7a* (ZHANG and HAWLEY 1990). In each case, the resulting *nod^{DR}/FM7a,nod^{b27}* females were tested for *X* and fourth chromosome nondisjunction, using *nod^{a}/FM7a,nod^{b27}* females as a control. *nod^{a}* is the original recessive *nod* allele induced by EMS mutagenesis of a wild-type chromo-

TABLE 11

Cold resistance of revertants

Male	16.5° upshift: Regular females	16.5° upshift: Regular males	16.5° upshift: B^+ males[a]	23.5°: Regular females	23.5°: Regular males	23.5°: B^+ males	% survival[b]
nod^{DTW} *f*/B^S*Y*[c]	218	21	3		NA		6.6%
nod^{DTW} *f*/B^S*Y*	256	28	0	131	191	9	7.5%
DR1 f/B^S*Y*	154	179	9	433	528	33	95%
DR2 f/B^S*Y*	494	549	23	589	671	18	97%
DR3 f/B^S*Y*	222	289	12	675	882	32	99%

[a] Exceptional sons resulting from nullo-*X* ova.

[b] Calculated as percentage of regular sons surviving under upshift conditions divided by expected. The expected was calculated by multiplying regular females surviving under upshift conditions by (regular males at 23.5°/regular females at 23.5°).

[c] Cross carried out entirely at 16.5°, as a control for upshift conditions.

Males of the indicated genotype were mated to *C(1)DX, y f*/B^S*Y* females at the indicated temperature. For upshift, parents were discarded after 5 days and the vials moved to 23.5° after 24 hr (see MATERIALS AND METHODS).

TABLE 12

Complementation test between revertants and *FM7a*, *nod*^{b27}

Gametes: Mother	Gametes: Father	Maternal genotype: *nod*/*nod*[a]	nod/DR1	nod/DR2	nod/DR3
Regular					
X 4	$\hat{X}\hat{Y}$ $\hat{4}\hat{4}$	136	111	184	82
X 4	O $\hat{4}\hat{4}$	88	56	142	62
X nondisjunctional					
O 4	$\hat{X}\hat{Y}$ $\hat{4}\hat{4}$	64	81	89	54
XX 4	O $\hat{4}\hat{4}$	73	53	94	32
4 nondisjunctional					
X O	$\hat{X}\hat{Y}$ $\hat{4}\hat{4}$	534	450	599	253
X O	O $\hat{4}\hat{4}$	409	255	423	182
X,4 nondisjunctional					
O O	$\hat{X}\hat{Y}$ $\hat{4}\hat{4}$	337	422	372	241
XX O	O $\hat{4}\hat{4}$	187	155	212	89
Total		1828[b]	1583[b]	2115[b]	995[b]
Adjusted total		2489	2294	2882	1411
% *X* nondisjunction		53	62	53	59
% 4 nondisjunction		82	81	76	78

[a] Data from ZHANG and HAWLEY (1990).

[b] Twenty-five gynandromorphs recovered from *nod*^{a}/*FM7a*, *nod*^{b27}; 14 from *DR1*; 19 from *DR2*; 9 from *DR3*.

pol/+ females of the indicated genotype were mated to $\hat{X}\hat{Y}$, *v f B*/O; *C(4)RM, ci ey*^{R}/O males. The *nod*^{DTW} and *DR* chromosomes are marked with *f*.

some (BAKER and CARPENTER 1972; CARPENTER 1973). All of the revertant/*FM7a*,*nod*^{b27} females had rates of *X* and fourth chromosome nondisjunction that are similar to those observed in *FM7a*,*nod*^{b27} /*nod*^{a} females (Table 12). Thus, all three revertants fail to complement *nod*, behaving as loss-of-function *nod* alleles. We conclude that *nod*^{DTW} is a dominant allele of the *nod* locus.

Dosage studies of *nod*^{DTW}: Although *nod*^{DTW} is an allele of the *nod* locus, it behaves as a dominant, pleiotropic mutation, while all previously recovered *nod* alleles are recessive, loss-of-function, mutations whose effects are primarily limited to distributive disjunction (CARPENTER 1973; ZHANG and HAWLEY 1990). Dominant alleles often are gain-of-function mutations, either by producing higher levels of the normal product (hypermorphs), or by encoding a protein with novel functions (neomorphs), or by encoding a protein that is antagonistic to the wild-type function (antimorphs).

We characterized the nature of the *nod*^{DTW} mutation, by varying the number of mutant and wild-type alleles in dosage experiments. Both *nod*/*nod* and *nod*/deficiency females are fully viable at low temperature, demonstrating that lack of *nod* expression is not responsible for the *nod*^{DTW} cold-sensitive phenotype. Furthermore, *nod*^{DTW} /*nod* females and *nod*^{DTW} females heterozygous for one of two deficiencies (*Df(1)v*^{65b} or *Df(1)N71*) spanning the *nod* region (10C2-3) are fully viable at 16.5° (data not shown). Thus, a single dose of *nod*^{DTW} in females is not sufficient to cause cold sensitivity, whether or not wild-type protein is present.

The effects of duplications of the *nod* locus were also examined. *nod*^{DTW} males bearing duplications of the *nod* region (*Dp(1;3)v*$^{+74C}$, *Dp(1;2)v*$^{+65b}$, and *v*$^+$*B*^{S}*Yy*$^+$) are viable at low temperature (data not shown). Thus, in males, a dosage-compensated copy of wild-type suppresses the effect of one dosage-compensated copy of *nod*^{DTW}.

The *nod*^{DTW} mutation therefore behaves as an antimorphic mutation. The more mutant protein product present, the more severe the phenotype. In the case of cold sensitivity, the presence of wild-type protein can titrate the mutant protein, and moderate the mutant phenotype.

The molecular nature of *nod*^{DTW}, *DR2* and *DR3*: In order to understand the molecular basis of the *nod*^{DTW} mutation, we cloned and sequenced the coding region of the *nod* locus on a *nod*^{DTW}-bearing chromosome. The first three exons were sequenced using a PCR-generated clone (see MATERIALS AND METHODS and Figure 2) as a template. To eliminate the possibility of a PCR-generated sequence artifact, several independent clones were sequenced. The remainder of the coding region was sequenced using a cloned 6.0-kb fragment as a template.

The sequencing revealed a single G to A change at base-pair 352 compared to the Oregon R wild-type sequence (the parental strain in which the *nod*^{DTW} mutation was originally isolated was not available) (ZHANG *et al.* 1990). This mutation predicts the substitution of asparagine for serine at position 94 (Figure 3A). The change is in the mechanochemical domain of the kinesin-like *nod* protein, and affects the highly conserved putative ATP-binding/hydrolysis domain (Figure 3A). All nine kinesin-like proteins analyzed to

FIGURE 2.—The *nod* locus. The exons of the *nod* locus are shown as thick boxes, while the surrounding untranscribed region is shown as thin rules. The precise transcription initiation and termination sites are not known (ZHANG *et al.* 1990) and so the end of the 3′ untranslated region is shown as a dashed line. Position 0 is at the second internal *Eco*RI (E) site, following the notation of ZHANG *et al.* (1990). Relevant *Xho*I sites (X) are also indicated. The positions of the primers used for PCR amplification are shown as triangles. The site of the mutation in the *nod*^{DTW} allele is indicated by an asterisk.

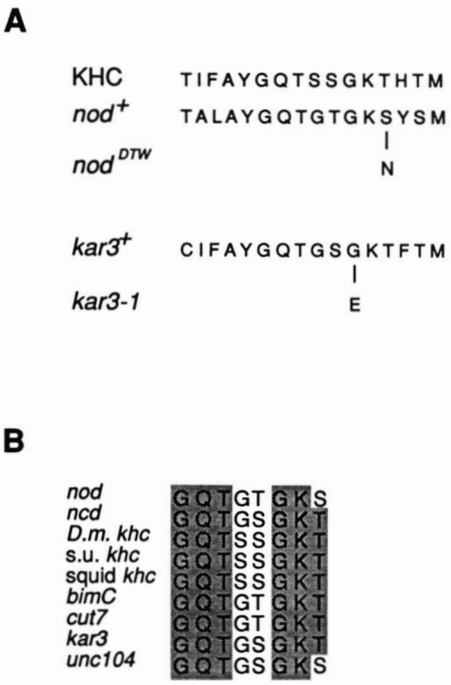

FIGURE 3.—The mutation in the *nod*^{DTW} allele. (A) Positions 84–99 of the wild-type *nod* protein are shown with the corresponding region from *D. melanogaster* kinesin heavy chain (KHC) (ZHANG *et al.* 1990). The *nod*^{DTW} mutation is shown below. For comparison, a mutation in the corresponding region of the yeast *kar3* locus is shown (MELUH and ROSE 1990). *kar3* also encodes a kinesin-like protein and the *kar3-1* mutation has similar properties to the *nod*^{DTW} mutation (see DISCUSSION). (B) The predicted sequence of the consensus ATP-binding/hydrolysis domain (GX_4GKT/S) (WALKER *et al.* 1982) from nine members of the kinesin superfamily of proteins is shown. The comparison includes the sequences of the *D. melanogaster* kinesin-like proteins, *nod* (ZHANG *et al.* 1990) and *ncd* (ENDOW, HENIKOFF and NIEDZIELA 1990), kinesin heavy chain (khc) from *D. melanogaster* (YANG, LAYMON and GOLDSTEIN 1989), sea urchin (s.u.) (WRIGHT *et al.* 1991), and squid (KOSIK *et al.* 1990), as well as three fungal kinesin-like proteins, *bimC* (ENOS and MORRIS 1990), *cut7* (HAGAN and YANAGIDA 1990) and *kar3* (MELUH and ROSE 1990), and a *Caenorhabditis elegans* kinesin-like protein, *unc104* (OTSUKA *et al.* 1991).

date have the consensus GX_4GKT/S ATP-binding domain (Figure 3B).

We did not sequence the upstream regions of either the wild-type or *nod*^{DTW} genes, so it is possible that there is an additional mutation in the *nod* regulatory region on *nod*^{DTW}-bearing chromosomes. However, restriction analysis does not reveal any gross rearrangements of the *nod* upstream region in the *nod*^{DTW} chromosome (data not shown). Furthermore, Northern analysis of transcripts in *nod*^{DTW}/*FM7a,Df(1)nod*^{219} females (*Df(1)nod*^{219} is a γ-ray induced deficiency; ZHANG and HAWLEY 1990) reveals no difference in the size or amount of *nod* mRNA (data not shown).

Sequencing data on two revertants *DR2* and *DR3*, reveals that each mutation is associated with a single G to A change in addition to the original *nod*^{DTW} mutation. In *DR2*, the mutation is at base-pair 523, predicting an amino acid change from aspartate to arginine at position 151. In *DR3*, the mutation is at base-pair 649, predicting that the amino acid at position 194 is changed from arginine to histidine (R. S. RASOOLY, P. ZHANG, A. K. TIBOLTA and R. S. HAWLEY, manuscript in preparation). These mutations both lie within the putative microtubule binding-domain of *nod* as deduced by comparison with *D. melanogaster* kinesin heavy chain (YANG, LAYMON and GOLDSTEIN 1989). Thus, both these revertants are second-site intragenic suppressors of the *nod*^{DTW} mutation.

DISCUSSION

The *nod*^{DTW} mutation is semidominant and pleiotropic. Depending on both dosage and temperature, the phenotypes of *nod*^{DTW} mutation include a specific effect on distributive segregation, effects on exchange chromosome segregation during female meiosis, and a defect in mitotic chromosome behavior that results in cold-sensitive lethality in both sexes. All three phenotypes appear to result from a single lesion: they are inseparable by recombination, and revertants selected on the basis of either cold resistance or loss of the dominant meiotic phenotype show reversion of the other phenotype.

All three revertants of nod^{DTW} behave as loss-of-function alleles of *nod*. The nod^{DTW} mutation is therefore an allele of the *nod* locus. Reversion of the antimorphic nod^{DTW} mutation occurs by gene knockout, generating loss-of-function alleles.

Sequence analysis of nod^{DTW} reveals a single base-pair change that predicts a change from serine-94 to asparagine. As shown in Figure 3b, this mutation alters a residue within the putative ATP-binding/hydrolysis domain (GX_4GKT/S), which is completely conserved among all nine members of the kinesin superfamily of proteins (McDONALD and GOLDSTEIN 1990; WALKER *et al.* 1982; ZHANG *et al.* 1990). ATP is not required for the binding of kinesin microtubules, but is required to release the microtubules once bound (VALE, REESE and SHEETZ 1985). We propose that the nod^{DTW} mutant protein is able to bind microtubules, but is unable to release them because of the defect in the ATP-binding region.

A similar mutation in a yeast kinesin-like protein, *kar3*, has been analyzed (MELUH and ROSE 1990). The semidominant *kar3-1* mutation in yeast prevents karyogamy, and like nod^{DTW}, alters the consensus ATP-binding domain of the protein (Figure 3). Ultrastructural studies have shown that the mutant protein appears to bind more tenaciously to cytoplasmic microtubules, consistent with a mutant "rigor-binding" protein (MELUH and ROSE 1990). MELUH and ROSE (1990) speculate that "the mutant protein is expected to physically impede the progress of the wild-type motors; such a mutation would be dominant."

Females heterozygous for nod^{DTW} exhibit a specific defect in distributive segregation: Previous work has shown that loss-of-function alleles of *nod* have a specific defect in distributive segregation (CARPENTER 1973; ZHANG and HAWLEY 1990; this report). $nod^{DTW}/+$ females have a similar effect on distributive segregation. Because the phenotype of $nod^{DTW}/+$ females at 25° is similar to that of *nod/nod*, we argue that nod^{DTW} is antimorphic, antagonizing the wild-type nod^{+} gene product.

Our model for nod^{+} function is that the *nod* kinesin-like protein is a plus-end directed microtubule motor that acts in the early stages of female meiosis. We suggest that along with the chiasmata on exchange chromosomes, the *nod* protein holds chromosomes at the metaphase plate, preventing premature segregation. This model is consistent with the observation that nondisjunction in $nod^{DTW}/+$ females primarily involves nonexchange chromosomes, because such chromosomes depend entirely on nod^{+} function to ensure proper disjunction. However, as noted in the Results, nondisjunction of bivalents with distal exchanges is also observed at a low frequency in $nod^{DTW}/+$ females and in *nod/nod* females (CARPENTER 1973). We interpret this result to mean that heterozygosity for nod^{DTW} affects both those bivalents in which there is either no exchange and those in which a distal exchange is insufficient to insure disjunction.

This suggests that not all exchanges are sufficient to guarantee proper disjunction, a result that has been documented previously. First, GRELL (1963) showed that interarm exchanges in attached-*X* chromosomes do not prevent them from segregating distributively from a *Y* chromosome. Second, NOVITSKI (1975) showed that the *Y* chromosome arms of an *T(2;Y)* translocation can still orient the disjunction of an attached-*X* chromosome even when the autosomal material connected to the *Y* is involved in exchange. Third, although most of the secondary nondisjunction ($XX \leftrightarrow Y$ segregation) that is observed in wild-type *XXY* females involves nonexchange *X* chromosomes, there is a low frequency of secondary nondisjunction among *X* chromosomes with very distal exchanges (CARPENTER 1973). Finally, CARPENTER (1973) observed that in *nod/nod* females, the few exchange bivalents that do nondisjoin have undergone distal exchanges. We interpret these data to mean that not all exchanges are capable of ensuring proper chiasma function, perhaps because they are too distal to properly co-orient two centromeres, and in such cases, the chromosomes segregate distributively.

We observed a higher rate of exchange chromosome nondisjunction for chromosome *2* than for *X* in $nod^{DTW}/+$ females. CARPENTER (1973) made the same observation in *nod/nod* females. Although we do not completely understand this disparity, it is possible that the ability of distal exchanges to co-orient two centromeres properly depends in part on the size of the chromosome.

Thus, as suggested by KNOWLES and HAWLEY (1991), we propose that distributive and chiasma-mediated segregation are not wholly distinct, but that the proteins that mediate distributive segregation act on all chromosomes regardless of their exchange status. Because most exchanges are sufficient to ensure disjunction, the distributive segregation proteins, such as nod^{+}, are functionally redundant for most bivalents. However, in the absence of exchange, or in the presence of distal exchanges that are not sufficient to ensure disjunction, the distributive system becomes the primary means of segregation. As a result, in the absence of wild-type *nod* protein or in the presence of a single dose of nod^{DTW} protein both nonexchange chromosomes and a fraction of chromosomes with distal exchanges fail to segregate properly.

nod^{DTW}/nod^{DTW} females exhibit a general effect on meiotic chromosome segregation: During meiosis in nod^{DTW}/nod^{DTW} females, and presumably during meiosis in $nod^{DTW}/+$ females raised at 18°, both exchange and nonexchange chromosomes nondisjoin at very high frequencies. The meiotic phenotype of the nod^{DTW} mutation at low temperature, or when homozygous, is more similar to that exhibited by loss-of-

function alleles of *ncd* (*non-claret disjunctional*), than to that of *nod/nod* or nod^{DTW}/+ females. Homozygous *ncd* females exhibit high rates (>30%) of *X* nondisjunction during meiosis, involving both exchange and nonexchange chromosomes (CARPENTER 1973; DAVIS 1969).

Like *nod*, *ncd* has recently been shown to encode a kinesin-like protein (ENDOW, HENIKOFF and NIEDZIELA 1990; MCDONALD and GOLDSTEIN 1990). KNOWLES and HAWLEY (1991) have found that the two loci do not fully complement; the double heterozygote exhibits elevated levels of *X* and fourth chromosome nondisjunction. The *nod* and *ncd* interaction is attributed to dosage sensitivity of essential proteins in the meiotic chromosome segregation apparatus, and not to a direct physical interaction between the two proteins.

In light of this observed interaction between *ncd* and *nod*, it seems possible that the severe effect of nod^{DTW} on female meiosis at low temperature, or when homozygous, results from interference with other proteins that ensure segregation, such as *ncd*. It is possible that the protein encoded by the nod^{DTW} allele interferes with an array of microtubule motors. Just as with the *kar3-1* mutant "rigor-binding" protein, the defective nod^{DTW} protein might bind tenaciously to microtubules, impeding the function of other microtubule motors such as *ncd*. At low concentrations (as in nod^{DTW}/+ females) the mutant nod^{DTW} protein may compete with the wild-type *nod* protein for binding sites, and thus produce a loss-of-function *nod* phenotype. At higher concentrations (as in homozygous females), additional nod^{DTW} mutant protein may block other microtubule motors as well, mimicking the meiotic phenotype of *ncd*.

nod^{DTW}/nod^{DTW} females and nod^{DTW} males exhibit a cold-sensitive lethality due to a defect in mitotic chromosome segregation: Although females heterozygous for nod^{DTW} are fully viable at low temperature, and display no mitotic anomalies, two doses of nod^{DTW} in females or one (dosage-compensated) dose in males results in cold-sensitive lethality. This cold-sensitive lethality apparently results from abnormal mitotic chromosome segregation at lower temperature. B. S. BAKER and M. GATTI (manuscript in preparation) have demonstrated that in neuroblasts of nod^{DTW} males at intermediate temperatures there is a high frequency of mitotic chromosome breakage, apparently due to the formation of anaphase bridges between homologous chromosomes.

Loss-of-function *nod* alleles have not been shown to affect mitotic chromosome segregation, and are not conditional lethals. Thus the nod^{DTW} cold-sensitive lethality does not reflect a requirement for wild-type *nod* product. Furthermore, the fact that nod^{DTW}/*Df(1)nod* females are viable at low temperatures argues that this lethality is not due to poisoning of the wild-type *nod* product. Instead, we argue that two doses of nod^{DTW} produce cold-sensitive lethality by poisoning other proteins involved in the mitotic segregation apparatus, analogous to the way two doses of nod^{DTW} appear to mimic the *ncd* phenotype in female meiosis. We predict that it will be possible to isolate these other proteins by looking for second-site noncomplementing mutations that give a mitotic phenotype in combination with *nod*.

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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., A. T. C. CARPENTER and P. RIPOLL, 1978 The utilization during mitotic cell division of loci controlling meiotic recombination and disjunction in *Drosophila melanogaster*. Genetics **90:** 531–578.

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

BENDER, W., P. SPIERER and D. S. HOGNESS, 1983 Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster*. J. Mol. Biol. **168:** 17–33.

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

CARPENTER, A. T. C., 1991 Distributive segregation: motors in the polar wind? Cell **64:** 1–6.

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

ENDOW, S. A., S. HENIKOFF and L. S. NIEDZIELA, 1990 Mediation of meiotic and early mitotic chromosome segregation in Drosophila by a protein related to kinesin. Nature **345:** 81–83.

ENOS, A. P., and N. R. MORRIS, 1990 Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. Cell **60:** 1019–1027.

GRELL, E. H., 1963 Distributive pairing of compound chromosomes in females of *Drosophila melanogaster*. Genetics **48:** 1217–1229.

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 *Genetics and Biology of Drosophila*, Vol. Ia, edited by E. NOVITSKI and M. ASHBURNER. Academic Press, New York.

HAGAN, I., and M. YANAGIDA, 1990 Novel potential mitotic motor protein encoded by the fission yeast *cut7*$^{+}$ gene. Nature **347:** 563–566.

HAWLEY, R. S., 1988 Exchange and chromosomal segregation in

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

KNOWLES, B. A., and R. S. HAWLEY, 1991 Genetic analysis of microtubule motor proteins in *Drosophila*: a mutation at the *ncd* locus is a dominant enhancer of *nod*. Proc. Natl. Acad. Sci. USA (in press).

KOSIK, K. S., L. D. ORECCHIO, B. SCHNAPP, H. INOUYE and R. L. NEVE, 1990 The primary structure and analysis of the squid kinesin heavy chain. J. Biol. Chem. **265:** 3278–3283.

LEWIS, E. B., and F. BACHER, 1968 Method of feeding ethyl methanesulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43:** 193.

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

LINDSLEY, D. L., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*, part 1: genes A-K. Drosophila Inform. Serv. **62.**

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*, part 3: rearrangements. Drosophila Inform. Serv. **65.**

LINDSLEY, D. L., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*, part 4: genes L-Z. Drosophila Inform. Serv. **68.**

MELUH, P. B., and M. D. ROSE, 1990 *KAR3*: a kinesin-related gene required for yeast nuclear fusion. Cell **60:** 1029–1041.

MERRIAM, J. R., and J. N. FROST, 1964 Exchange and nondisjunction of the *X*-chromosomes in female *Drosophila melanogaster*. Genetics **49:** 109–122.

MCDONALD, H. B., and L. S. B. GOLDSTEIN, 1990 Identification and characterization of a gene encoding a kinesin-like protein in Drosophila. Cell **61:** 991–1000.

NOVITSKI, E., 1975 Evidence for the single phase pairing theory of meiosis. Genetics **79:** 63–71.

OTSUKA, A. J., A. JEYAPRAKASH, J. GARCIA-ANOVEROS, L. Z. TANG, G. FISK, T. HARTSHORNE, R. FRANCO and T. BORN, 1991 The *C. elegans unc-104* gene encodes a putative kinesin heavy chain-like protein. Neuron **6:** 113–122.

SAMBROOK, K. J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

VALE, R. D., T. S. REESE and M. P. SHEETZ, 1985 Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell **42:** 39–50.

VALENTIN, J., 1973 Characterization of a meiotic control gene affecting recombination in *Drosophila melanogaster*. Hereditas **75:** 5–22.

WALKER, J. E., M. SARASTE, M. J. RUNSWICK and N. J. GAY, 1982 Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1:** 945–951.

WEINSTEIN, A., 1936 The theory of multiple-strand crossing over. Genetics **21:** 155–199.

WRIGHT, B. D., J. H. HENSON, K. P. WEDAMAN, P. J. WILLY, J. N. MORAND and J. M. SCHOLEY, 1991 Subcellular localization and sequence of sea urchin kinesin heavy chain: evidence for its association with membranes in the mitotic apparatus and interphase cytoplasm. J. Cell Biol. **113:** 817–833.

WRIGHT, T. R. F., 1973 The recovery, penetrance and pleiotropy of *X*-linked cold sensitive mutants in Drosophila. Mol. Gen. Genet. **122:** 101–118.

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.

YANG, J. T., R. A. LAYMON and L. S. B. GOLDSTEIN, 1989 A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analysis. Cell **56:** 879–889.

ZHANG, P., and R. S. HAWLEY, 1990 The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the *nod* locus. Genetics **125:** 115–127.

ZHANG, P., B. A. KNOWLES, L. S. B. GOLDSTEIN and R. S. HAWLEY, 1990 A kinesin-like protein required for distributive chromosome segregation in Drosophila. Cell **62:** 1053–1062.

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.

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