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16. Blue tits bred in nestboxes at densities of 0.5 to 2 pairs per hectare. First egg and hatching dates and clutch and brood size were determined by routine nest inspections. When nestlings were 13 to 14 days of age (here used to define breeding date), adults were captured in mist nets, then weighed to ±0.1 g, banded, and injected with 60 μl of doubly labeled water ($^2H_2{}^{18}O$). After a 30-min equilibration period, an initial blood sample (20 to 30 μl) was drawn from the brachial vein and sealed in a capillary tube. We then released adults and weighed nestlings. We recaptured adults in the nest after 24 ± 0.5 hours and drew a second blood sample. Blood samples were later microdistilled, and ^2H and ^{18}O abundances in the water were determined by isotope ratio mass spectrometry at the University of Aberdeen. See (*24*) for detailed descriptions of doubly labeled water applications and methodology.
17. We calculated $FMR_{24\text{-}h}$ for 13 Corsican and 13 continental adults using the single pool equation (equation 7.17) from Speakman (*24*). $FMR_{24\text{-}h}$ did not differ significantly between males and females in either population, so sexes were pooled. Because only daytime (sunrise to sunset) FMR is directly related to nest provisioning, we subtracted nighttime metabolic rates from $FMR_{24\text{-}h}$ to leave FMR_{day}. We estimated nighttime metabolic rate (mW) as $609.64 - 17.41 \cdot T_a$, where T_a is average ambient temperature recorded during the measurement night (*21*). BMR was 15.5 mW/g and 17.5 mW/g for birds from the continental and Corsican populations, respectively (D. W. Thomas, J. Blondel, P. Perret, *Zoology*, in press).
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Sterility of *Drosophila* with Mutations in the Bloom Syndrome Gene—Complementation by *Ku70*

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The *Drosophila Dmblm* locus is a homolog of the human Bloom syndrome gene, which encodes a helicase of the RECQ family. We show that *Dmblm* is identical to *mus309*, a locus originally identified in a mutagen-sensitivity screen. One *mus309* allele, which carries a stop codon between two of the helicase motifs, causes partial male sterility and complete female sterility. Mutant males produce an excess of XY sperm and nullo sperm, consistent with a high frequency of nondisjunction and/or chromosome loss. These phenotypes of *mus309* suggest that *Dmblm* functions in DNA double-strand break repair. The mutant *Dmblm* phenotypes were partially rescued by an extra copy of the DNA repair gene *Ku70*, indicating that the two genes functionally interact in vivo.

The human recessive disorder, Bloom syndrome, is characterized by an elevated risk for a wide variety of cancers, as well as immunodeficiency, slow growth, and partial sterility (*1*). Cells from Bloom syndrome patients exhibit greatly enhanced rates of sister chromatid exchange. The causative mutations are in *BLM* (*2*), which encodes a RECQ helicase. Mutations in two other RECQ helicase genes, *WRN* and *RecQ4*, are responsible for Werner syndrome and Rothmund-Thomson syndrome, which are both associated with cancer predisposition and premature aging (*3*, *4*).

In mice, one *BLM* mutant line is viable and exhibits Bloom syndrome phenotypes (*5*). Our previous work showed that the *Drosophila Dmblm* gene is closely related to *BLM* (*6*). Here, we show that *Dmblm* corresponds to *mus309*, which had been originally identified in a mutagen-sensitivity screen (*7*).

In an earlier study, Beall and Rio (*8*) reported that *mus309* corresponds to a *Drosophila* homolog of *Ku70*, whose gene product binds to the ends of double-stranded DNA breaks. Their conclusion was based on low-resolution mapping information and a preliminary indication of partial complementation by a *Ku70* transgene of the mutagen sensitivity of *mus309*. However, Szabad (*9*) found that deficiency *Df(3R)T–7*, abbreviated *Df T7*, failed to complement the sterility of *mus309* mutations. This deficiency lies in polytene chromosome region 86F1-2 (*10*, *11*) as opposed to 86E2-3 where *DmKu70* had been placed (*11*). These considerations suggested that *Dmblm*, which maps to 86F1-4 (*6*), is a better candidate for *mus309* than *DmKu70*.

To test this possibility, we used P element–induced male recombination (*12–14*) to create a series of deletions in the 86E-F region. These deletions, along with *Df T7*, were tested against the mutant allele $mus309^{D2}$, and four P element–insertion mutations in the area, to generate a more detailed deletion map of 86E-F (Fig. 1A). The *mus309* phenotypes tested were mutagen sensitivity and female sterility. The results ruled out the reported identity between *DmKu70* and *mus309*, but were consistent with identity between *Dmblm* and *mus309*.

We next sequenced the *Dmblm* genes from the two existing mutant alleles of *mus309*, and found that both carry mutational changes that could potentially impair or abolish the function of *Dmblm* (Fig. 1B). The less severe allele, $mus309^{D3}$, encodes lysine instead of glutamic acid within a conserved region of helicase motif II, plus another amino acid substitution near the COOH-terminus. The more severe allele, $mus309^{D2}$, has a stop codon between helicase motifs III and IV, and is expected to yield a severely truncated polypeptide. Both *mus309* alleles were induced by ethylmethane sulfonate (*7*), which generates predominantly G → A transitions (*15*), and notably, the two suspect changes in the helicase region are both G → A transitions (Fig. 1B).

We constructed a P element–borne transgene carrying the *Dmblm* cDNA driven by an *hsp70* heat shock promoter (*16*), and we tested its ability to rescue *mus309* phenotypes. The methylmethane sulfonate (MMS) sensitivity was rescued, at least partially, by each of two transgene insertions tested (Fig. 1C). Position effects normally preclude complete rescue by transgenes of this kind. Both mutant *mus309* alleles were rescued by the *hsp-Dmblm* transgene, and the degree of complementation was enhanced by heat shock for both transgene insertions (Fig. 1C). The sterility phenotype of *mus309* males and females was also complemented by the *Dmblm* transgene (Fig. 2) (*17*).

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To examine the potential interaction of *Dmblm* and *Ku70*, we obtained two stocks carrying *Ku70* transgenes. One had a genomic *Drosophila* sequence, *DmKu70*, and the other carried a *hsp70*-driven human cDNA, *hsp-HsKu70*. Both the *Drosophila* and human *Ku70* transgenes partially rescued the MMS sensitivity and the sterility of $mus309^{D2}$/*Df T7* mutants (Figs. 2 and 3) (*17*). This finding is consistent with a previous report that the *Drosophila* genomic *Ku70* transgene partially rescues MMS sensitivity in $mus309^{D2/D3}$ heterozygotes (*8*). We can rule out the possibility that the two *mus309* alleles, as well as *Df T7*, carry a second lesion at the *DmKu70* locus because all three were induced independently on unrelated chromosomes (*7*, *10*). Our interpretation, therefore, is that a third copy of *Ku70* partially compensates for a mutation at *Dmblm*.

Chromosome loss and nondisjunction are potential phenotypes of mutations affecting DNA repair. We measured the frequency of these events in *mus309* mutant males by scoring for XY and nullo-X sperm. The results revealed that both nondisjunction and chromosome loss increased more than tenfold in $mus309^{D2}$/*Df T7* males relative to the wild-type and heterozygous controls (Table 1). Partial rescue was again seen with the *hsp-Dmblm* transgene. These events are apparently independent of meiotic recombination, which does not occur in *Drosophila* males. The absolute frequency of XY sperm was considerably greater than that of nullo-X sperm in the *mus309* mutant males. This disparity is expected if nondisjunction occurs in the premeiotic germ cells where the nullo-X cells would be inviable.

We also observed an elevated frequency of sperm in which one of the two dominant markers on the Y chromosome had been lost in the germ line of the *mus309* mutant males. This effect was also partially rescued by the *hsp-Dmblm* transgene (Table 1). These two markers [y^+ and B^S (*11*)] were present on opposite ends of the Y chromosome. We postulate that these events represent chromosome breakage and partial loss of the Y chromosome. To investigate this possibility, we recovered 23 such sons from $mus309^{D2/D3}$ fathers and tested them for fertility. If part of the Y chromosome had been lost, it is likely that one or more of the male fertility factors located on the Y chromosome would be lost as well. All but one of the 23 males proved

Table 1. Nondisjunction and chromosome loss in *mus309* mutant males, and *Dmblm* complementation. We used a marked Y chromosome, designated $y^+ YB^S$, with dominant markers translocated to each end (*11*) to detect nondisjunction and chromosome loss events. Males carrying this Y chromosome and the indicated genotypes on chromosomes 2 and 3 were crossed to *y cv f* homozygous females (*11*). XY sperm were detected as y^+ cv^+ B^S f^+ daughters. Nullo-X sperm were indicated by *y cv f* sons. Cases of marker loss were identified by progeny, usually sons, that inherited one of the two Y-borne markers, y^+ and B^S, without the other. All crosses were set up with individual males so that clustered events could be detected. Out of 10 XY nondisjunctional offspring females from $mus309^{D2}$/*Df T7* fathers without the transgene, six were from one father.

Father's genotype		Total progeny scored	XY sperm (%)	Nullo-X sperm (%)	Marker loss (% of Y-bearing sperm)
Transgene	Third chromosome genotype				
hsp-Dmblm	$mus309^{D2}$/*Df T7*	8018	0.274	0.050	0.729
None	$mus309^{D2}$/*Df T7*	937	1.067	0.427	1.923
None	+/*Df T7*	5697	0.000	0.018	0.164
None	$mus309^{D2}$/+	3986	0.000	0.050	0.000
None	+/+	5440	0.092	0.018	0.000

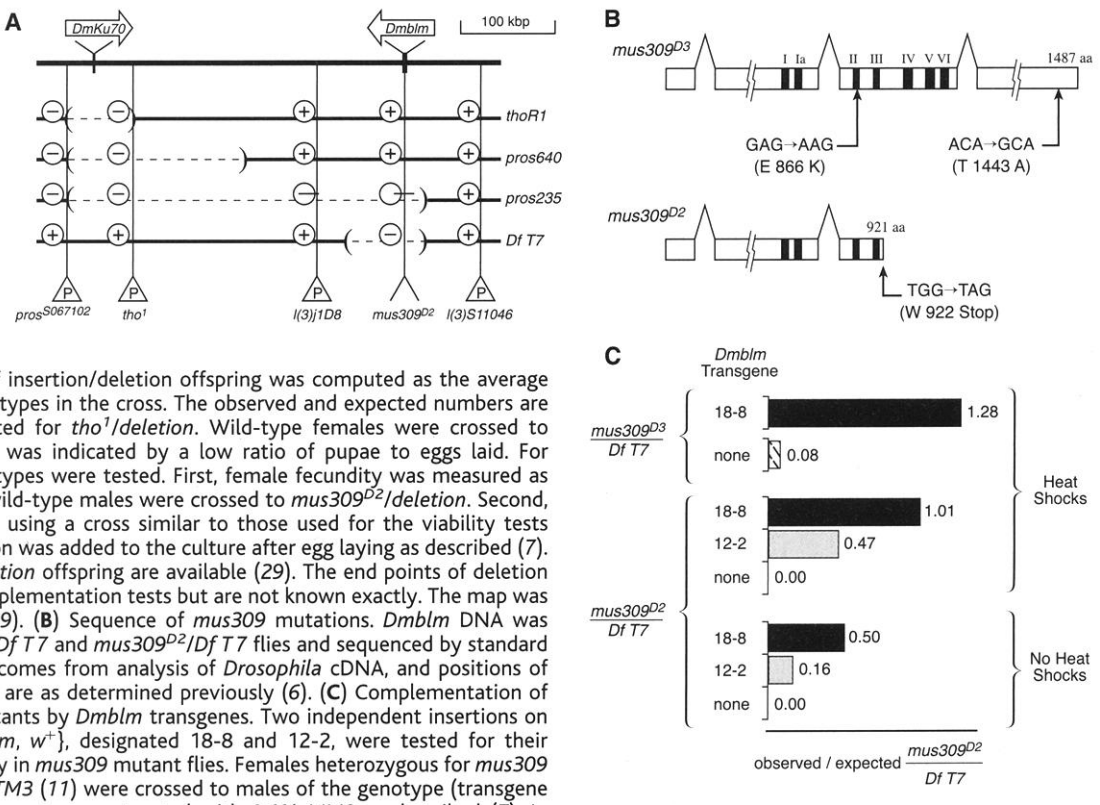

Fig. 1. (**A**) Deletion mapping and complementation tests. Each of four deletions was tested against each of four insertion and $mus309^{D2}$ mutations. Complementation is indicated by "+" and failure to complement by "−". Flies heterozygous for the mutation and a balancer chromosome were crossed to balancer heterozygotes for each deletion (*11*, *13*). Viability was tested for $pros^{S067102}$, *l(3)j1D8*, or *l(3)S11046/deletion*. The expected number of insertion/deletion offspring was computed as the average number of all other viable genotypes in the cross. The observed and expected numbers are in (*29*). Male fertility was tested for tho^1/*deletion*. Wild-type females were crossed to tho^1/*deletion*, and the fertility was indicated by a low ratio of pupae to eggs laid. For $mus309^{D2}$/*deletion*, two phenotypes were tested. First, female fecundity was measured as pupae per eggs laid (*29*) after wild-type males were crossed to $mus309^{D2}$/*deletion*. Second, MMS sensitivity was measured using a cross similar to those used for the viability tests except that a 0.1% MMS solution was added to the culture after egg laying as described (*7*). These counts of $mus309^{D2}$/*deletion* offspring are available (*29*). The end points of deletion *Df T7* are bracketed by our complementation tests but are not known exactly. The map was drawn to scale as described (*29*). (**B**) Sequence of *mus309* mutations. *Dmblm* DNA was cloned by PCR from $mus309^{D3}$/*Df T7* and $mus309^{D2}$/*Df T7* flies and sequenced by standard methods. The intron structure comes from analysis of *Drosophila* cDNA, and positions of the helicase motifs (black bars) are as determined previously (*6*). (**C**) Complementation of MMS sensitivity in *mus309* mutants by *Dmblm* transgenes. Two independent insertions on chromosome 2 of P{*hsp–Dmblm*, w^+}, designated 18-8 and 12-2, were tested for their ability to rescue MMS-sensitivity in *mus309* mutant flies. Females heterozygous for *mus309* and the balancer chromosome *TM3* (*11*) were crossed to males of the genotype (transgene or +)/*CyO*; *Df T7/TM3*. The progeny were treated with 0.1% MMS as described (*7*). In crosses with heat shock treatments, larvae were placed at 37°C for 1 hour on days 5, 6, and 7 after the eggs were laid. The numbers reported are the ratios of observed to expected *mus309/Df T7* adult progeny, where the expected numbers were computed as the average of the two heterozygous classes, *mus309/TM3* and *TM3/Df T7*, which are approximately equally frequent. We scored an average of 242 progeny per cross.

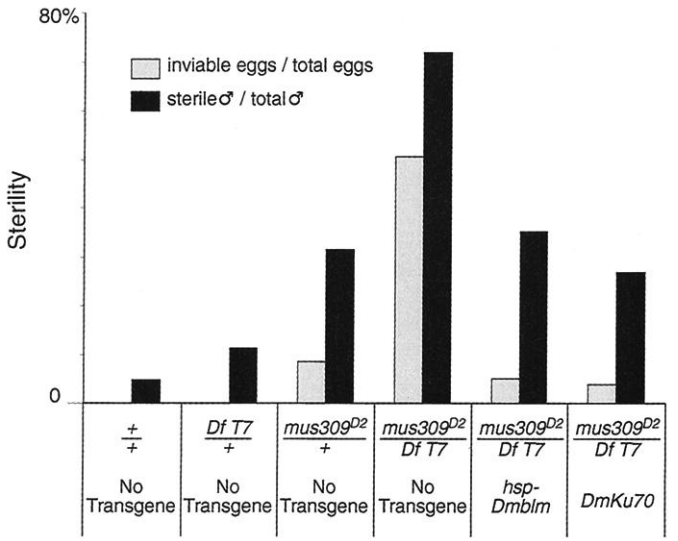

Fig. 2. Complementation of *mus309* male sterility by *Dmblm* and *Ku70* transgenes. Each male of the indicated genotype was crossed to two wild-type females (*14*). After 2 days of egg laying, the number of embryos produced in each cross was scored, and 5 to 8 days later, the number of pupae generated was scored. Two measures of sterility were used: the percent of inviable offspring, calculated as (inviable eggs)/(total eggs), and the percent of males that were completely sterile. The numbers of eggs counted (left to right) were 544, 520, 1197, 3452, 1553, and 1542. The numbers of males tested were 20, 20, 35, 75, 39, and 26. The *Dmblm* transgene was insertion 18-8.

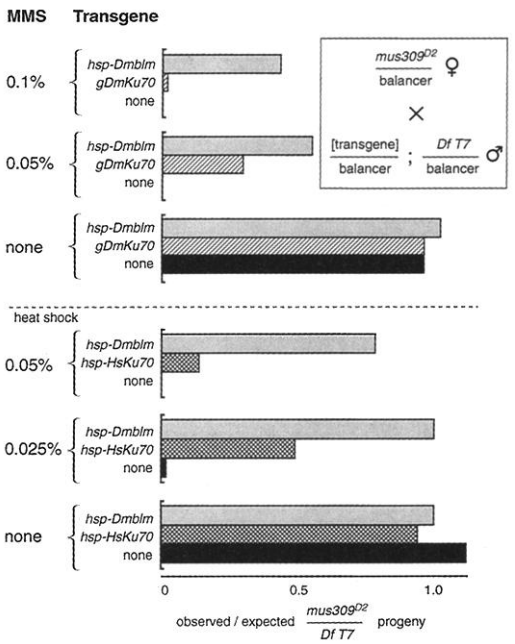

Fig. 3. Complementation of *mus309* MMS sensitivity by *Dmblm* and *Ku70* transgenes. Progeny from the indicated cross were grown with MMS (or water) as described (*7*). The balancer chromosomes were *CyO*, *Roi* and *TM3*, *Sb e* (*11*). The numbers plotted are the ratios of observed to expected *mus309^{D2}/Df T7* progeny, where the expected numbers are as in Fig. 1C with other surviving genotypes being in approximately equal frequencies. Heat shocks were applied only to the lower three groups as described in Fig. 1C. The *Dmblm* transgene used was 18-8 in the upper three groups and 12-2 for the lower three. Each of the 18 crosses was set up with 8 males and 20 females, and yielded an average of 687 progeny.

to be sterile, consistent with the chromosome breakage hypothesis.

The identification of *mus309* as the *Drosophila* Bloom syndrome gene opens up new avenues for studying its function. First is the unexpected finding that *Dmblm* and *Ku70* interact. One possible basis for this interaction is that an extra copy of *Ku70* facilitates use of alternative pathways of double-strand break repair. However, more direct interactions cannot be ruled out. Indeed, biochemical interactions between Ku70 and another *RECQ* family member, WRN, have been observed (*18*, *19*). In addition, both Ku70 and BLM have DNA helicase activity (*20*, *21*), which suggests possible redundancy.

The elevated frequency of chromosomal nondisjunction and chromosome loss in the germ line is likely to promote frequent germ cell loss during development. This process could explain the sterility seen in *mus309* mutants, as well as in humans with Bloom syndrome. In addition, previous studies have shown that mutant alleles of *mus309*, as well as those of *mei41*, a *Drosophila* homolog of the *ataxia telangiectasia* gene, increase sensitivity to chromosome breakage (*8*, *22*, *23*). However, *mei41*, but not *mus309*, mutants display defects in a DNA damage checkpoint assay (*24*, *25*). In contrast to a recent proposal that BLM functions to prevent chromosome breakage during replication (*26*, *27*), our data, combined with previous studies of *mus309* phenotypes, suggest that the *Drosophila BLM* homolog functions in a repair role after double-strand break formation. BLM could perform this role during replication, as well as in response to incidental DNA damage.

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17. The percentages of *mus309^{D2}/Df T7* females that were completely sterile were 100% (18/18), 15% (3/20), and 26% (5/19) without transgene, with *hsp-Dmblm*, and with *DmKu70*, respectively.
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