# The *Drosophila melanogaster sir2*$^{+}$ Gene Is Nonessential and Has Only Minor Effects on Position-Effect Variegation

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## ABSTRACT

Five *Drosophila melanogaster* genes belong to the highly conserved *sir2* family, which encodes NAD$^{+}$-dependent protein deacetylases. Of these five, *dsir2*$^{+}$ (CG5216) is most similar to the *Saccharomyces cerevisiae SIR2* gene, which has profound effects on chromatin structure and life span. Four independent Drosophila strains were found with *P*-element insertions near the *dsir2* transcriptional start site as well as extraneous linked recessive lethal mutations. Imprecise excision of one of these *P* elements (*PlacW*07223) from a chromosome freed of extraneous lethal mutations produced *dsir2*$^{17}$, a null intragenic deletion allele that generates no DSIR2 protein. Contrary to expectations from the report by Rosenberg and Parkhurst on their *P*-mobilization allele *dSir2*$^{ex10}$, homozygosity for *dsir2*$^{17}$ had no apparent deleterious effects on viability, developmental rate, or sex ratio, and it fully complemented *sir2*$^{ex10}$. Moreover, through a genetic test, we ruled out the reported effect of *dSir2*$^{ex10}$ on *Sex-lethal* expression. We did observe a modest, strictly recessive suppression of *white*$^{m4}$ position-effect variegation and a shortening of life span in *dsir2* homozygous mutants, suggesting that *dsir2* has some functions in common with yeast *SIR2*.

GENES belonging to the conserved silent information regulator 2 (*SIR2*) gene family encode NAD$^{+}$-dependent protein deacetylases (Imai *et al.* 2000; Landry *et al.* 2000; Smith *et al.* 2000). These proteins also possess an ADP-ribosylase activity (Frye 1999; Tanny *et al.* 1999; Tanner *et al.* 2000). *SIR2*-like genes are present in all three kingdoms (Brachmann *et al.* 1995) and have attracted considerable attention for the variety of regulatory processes they control. The best-studied member of this family is Sir2p from *Saccharomyces cerevisiae*. Mutations in the gene encoding ScSir2p lead to the inability to form heterochromatin, resulting in an altered chromatin structure at loci exhibiting position effects, such as the cryptic mating-type loci and telomeres (Ivy *et al.* 1986; Rine and Herskowitz 1987; Aparicio *et al.* 1991). Histones H3 and H4 are substrates of ScSir2p, which probably contributes to the histone acetylation pattern observed in silent chromatin (Braunstein *et al.* 1993; Rusche *et al.* 2002). *S. cerevisiae* contains four additional genes that are homologous to *SIR2*, and these homologs (*HST1–HST4*) are involved in silencing, cell-cycle progression, and chromosome stability (Brachmann *et al.* 1995). On the basis of the sequence identities between Sir2p and Hst1p to Hst4p, three classes of Sir2p family members were suggested. Sir2p and Hst1p belonged to one class, Hst3p and Hst4p to another, and Hst2p uniquely to a third. These family members also appear to function in different subcellular compartments since Sir2p and Hst1p are nuclear, but Hst2p is cytosolic (Perrod *et al.* 2001). Hst1p is involved in repressing sporulation-specific genes during vegetative growth (Xie *et al.* 1999), demonstrating that Sir2p family members also have a role in regulating euchromatic genes.

Fission yeast Hst4p is required for both centromeric and telomeric silencing (Freeman-Cook *et al.* 1999). *Kluyveromyces lactis* Sir2p is required for silencing of the cryptic mating-type loci in this organism (Åström and Rine 1998), indicating that silencing mechanisms are partly conserved between distantly related yeasts. For bacterial *SIR2* proteins, substrates are known only for *Sulfolobus solfaticus* in which an archaeal chromatin protein, Alba, is a substrate for Sir2 (Bell *et al.* 2002). In mammals, human Sir2 can deacetylate p53 and is suggested to regulate the activity of p53 and thus the apoptotic response (Luo *et al.* 2001; Vaziri *et al.* 2001; Langley *et al.* 2002).

Interestingly, in both *S. cerevisiae* and *C. elegans*, Sir2p is involved in regulation of life span (Kaeberlein *et al.* 1999; Tissenbaum and Guarente 2001). Lack of Sir2p leads to a shorter life span, whereas extra copies of the *SIR2* gene extend life span. Since Sir2p depends upon NAD$^{+}$ for its activity, and levels of NAD$^{+}$ vary depending on the metabolic activity of the cell, Sir2p was suggested to couple longevity to nutrient availability/calorie restriction in many eukaryotic organisms (Guarente 2000; Lin *et al.* 2000). In yeast, however, it was recently suggested

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Sequence data from this article have been deposited with the EMBL/GenBank Data libraries under accession no. AF068758.

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that caloric restriction and Sir2p affected life span by independent pathways (Jiang *et al.* 2002).

Of the five Drosophila *SIR2* family members (CG5216, CG5085, CG3187, CG6284, and CG11305), *dsir2* (CG5216) encodes the protein most similar to ScSir2p (data not shown). dSIR2 protein deacetylates labeled histone peptides and intact histone H4 (Barlow *et al.* 2001; Rosenberg and Parkhurst 2002). The claim that strong *dSir2* mutants are recessive embryonic lethal (Rosenberg and Parkhurst 2002) was notable in light of the fact that all *Sir2* family members characterized to date in other organisms are nonessential genes. Indeed, even strains lacking multiple *sir2* paralogs in *S. cerevisiae* are viable (Brachmann *et al.* 1995). The Drosophila *dsir2* gene was also implicated in regulating both euchromatic and heterochromatic gene expression with striking effects on segmentation, position-effect variegation (PEV), and sex determination (Rosenberg and Parkhurst 2002). The latter two effects were dominant, but with an apparent maternal-effect component. In addition, both genetic and physical interactions were reported between DSIR2 and HAIRY, a sequence-specific DNA-binding repressor.

Unfortunately, Rosenberg and Parkhurst did not establish that the phenotypes they observed were actually due to mutations in *dsir2*, nor did they describe genetic crosses adequately to substantiate the genetic behavior claimed. Moreover, to show that the distorted sex ratios they observed were due to misexpression of *Sex-lethal* (*Sxl*), they relied on potentially problematic immunostaining of embryos rather than on a straightforward and unambiguous genetic test using null *Sxl* alleles. Below we present our own independent analysis of *dsir2*, which contradicts their genetic analysis in essentially every respect. We found that loss of *dsir2* alone had only very subtle effects on the fly, a result consistent with the existence of functional overlap among the members of the Sir2 family in this species.

## MATERIALS AND METHODS

Nucleic acid preparations and manipulations followed standard protocols (Ausubel *et al.* 2002). Oligonucleotides for nested, degenerate PCR were 5′-ggnat(act)ccnga(ct)tttag-3′ (GIPDFRS); 5′-gtcgatgttctg(agc)gtgta-3′ (YTQNID); and a vector primer 5′-gcggccgcattcg-3′ complementary to the template cDNA library (Brown and Kafatos 1988). The *dsir2*$^+$ cDNA was isolated from a third instar λADH library (from S. Elledge, Baylor College, Houston) using the degenerate PCR fragment as probe. Positive plaques were purified and converted into a plasmid in *Escherichia coli* strain BNN132, resulting in plasmid p196. The p196 insert was sequenced on both strands using an ABI 373 sequencer and a Prism sequencing kit. The cDNA contained the 2472-bp *dsir2*$^+$ open reading frame (ORF), preceded by a 391-bp 5′ untranslated region and followed by a 910-bp 3′ untranslated region. The GenBank accession number for *dsir2*$^+$ is AF068758. A construct expressing a maltose-binding protein-DSIR2 fusion was generated by cloning a *sir2*$^+$ fragment corresponding to amino acids 318–732 into pMAL$^{c2}$, and fusion protein was produced and purified according to the manufacturer's instructions (New England Biolabs, Beverly, MA). A polyclonal antiserum was raised in rabbits using standard procedures. For immunoblots the affinity-purified α-DSIR2 antiserum was preabsorbed against total protein from the *dsir2*$^{17}$ strains and then used at a 1:50 dilution. For the RNA blot, the probe used was p196, labeled with [$^{32}$P]dATP. For the DNA blot a plasmid (p204), corresponding to pGEM5 (Promega, Madison, WI) with a 2428-bp *Xho*I fragment corresponding to the 3′ end of the *dsir2*$^+$ cDNA was labeled with [$^{32}$P]dATP and used as probe.

Drosophila strains were grown at 25° on a standard cornmeal, yeast, sucrose, molasses medium and the *P*-element insertion strains were as previously described (Torok *et al.* 1993; Spradling *et al.* 1999). Fly strains containing *P*-element insertions in the 34A region of the genome were screened by PCR for *P*-element insertions in or adjacent to *dsir2*$^+$. For this purpose we used one primer complementary to the *P*-element terminal repeat (5′-cgggaccaccttatgttatttcatc-3′) and one gene-specific primer complementary to *dsir2*$^+$. In this way we found four independent strains [*l(2)05327*, *l(2)07223*, *l(2)k06008*, and *l(2)14153*] with *P*-element insertions ~400 bp upstream of the *dsir2*$^+$ start codon. White-eyed, non-Tufted offspring from the cross (*w*$^{1118}$; *P(lacW07223)/CyO; mus309*$^{D3}$, *Δ2-3/mus309*$^{D2}$ × *w*$^{1118}$/*w*$^{1118}$; *Tft/CyO*) were screened for small imprecise excisions by PCR using one primer in the 3′ untranslated region and another primer in the 5′ region of *dsir2*$^+$, upstream of the *P*-element insertion point. *mus309* increases the yield of imprecise excisions (Beall and Rio 1996). The extent of the deletion in *dsir2*$^{17}$ was determined by sequencing a PCR fragment generated from mutant template DNA by primers flanking the *dsir2*$^+$ locus. *dsir2*$^{17}$ was deleted for 2360 bp from $-62$ to $+2298$ (cDNA start at $+1$), hence missing the first 579 amino acids (full length = 823 amino acids). A total of 13 bp of unknown origin was inserted at the deletion endpoint.

Life-span measurements were performed with 20 adults/vial for a total of 120 newly eclosed flies/strain. Adults were transferred to new food and counted without anesthetization every second day. Statistical significance was determined by a Wilcoxon rank-sum test.

## RESULTS AND DISCUSSION

The *SIR2* gene family present in many organisms is characterized by a 250-amino-acid core domain with 40–60% sequence similarity among homologs, which contains two signature motifs: GIPDFRS and YTQNID. Degenerate oligonucleotides corresponding to these amino acid sequences were used to amplify a 200-bp fragment from a Drosophila *sir2*$^+$ gene and subsequently to isolate a 3.8-kb complementary DNA clone from a Drosophila λ-library. Sequencing of the cDNA revealed a 2472-bp open reading frame with 31% similarity to Saccharomyces *SIR2* and 39% similarity to a *Caenorhabtidis elegans sir2* homolog (Figure 1). The homology to ScSir2p and the *C. elegans* protein was greatest in the central region of the peptide (amino acids 200–470), whereas the N- and C-terminal domains were unique to the Drosophila protein (Figure 1). Homology searches of the Drosophila genome revealed four other related genes of the *SIR2* gene family, all more distantly related to Saccharomyces *SIR2* than the original gene that we call *dsir2*$^+$ (rather than the previously published

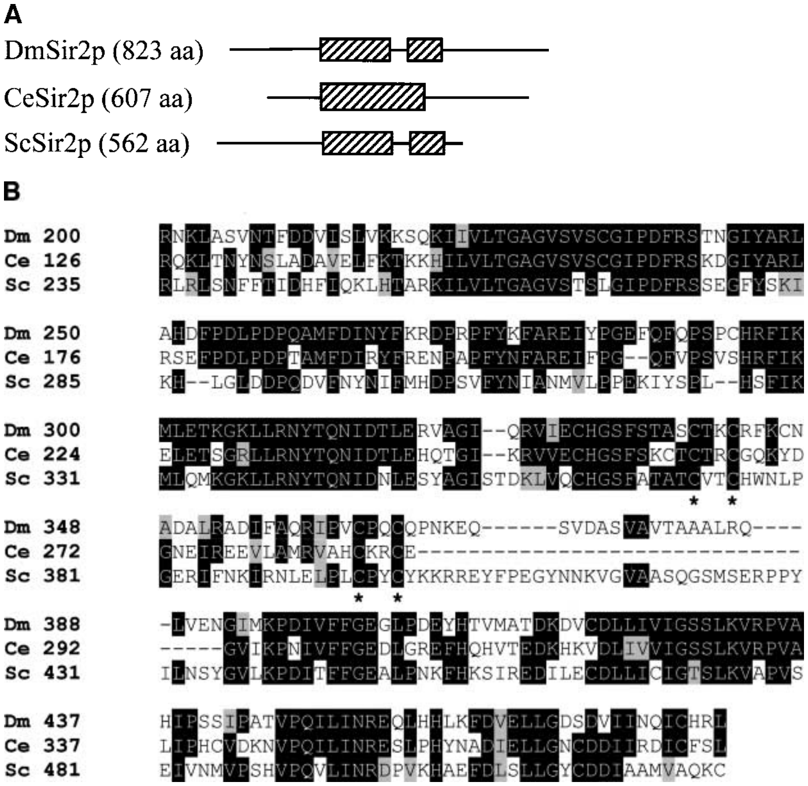

Figure 1.—*D. melanogaster* has a gene highly homologous to *S. cerevisiae SIR2*. (A) Schematic drawing of the homologous regions between the fly (DmSir2p), worm (CeSir2p), and yeast (ScSir2p) Sir2 proteins. Boxes correspond to regions of homology and the length of the proteins is indicated. (B) Sequence alignment of the Sir2p core domain of DmSir2p (accession no. AAC79684), CeSir2p (NP501912), and ScSir2p (CAA25667) using the Clustal method. Identical amino acids (solid boxes) and similar amino acids (shaded boxes) are indicated. Asterisks correspond to the conserved cysteines of the potential $Zn^{2+}$ finger.

*dSir2*, since we found no dominant mutant phenotypes).

We mapped *dsir2*$^+$ to chromosome 2L band 34A by *in situ* hybridization to polytene chromosomes (data not shown) and identified four independent strains with *P*-element insertions at nearly identical positions ~400 bp upstream of the *dsir2*$^+$ start codon. All four mutant chromosomes contained recessive lethal mutations, which in principle could have suggested that *dsir2* was a vital gene. However, our subsequent finding that various pairs of these mutants fully complemented each other indicated that lethality was likely due to extraneous recessive lethal mutations. This possibility was confirmed when we allowed the *l(2)07223* chromosome to recombine with the wild-type chromosome and recovered a lethal-free chromosome still carrying the *P*-element insertion near *dsir2*, which we refer to as *P(lacW)7223*.

**Generation of a *dsir2* null allele:** To ascertain the phenotype of flies devoid of SIR2 protein, we generated a *dsir2* null allele via imprecise excision of the *P(lacW)7223* transposon just upstream of *dsir2* in our now lethal-free chromosome (see MATERIALS AND METHODS). Of six independent imprecise excision strains recovered, five were fully viable, including *dsir2*$^{17}$, which was chosen for genetic analysis because its deletion eliminated most of the *dsir2* ORF without disrupting neighboring genes (Figure 2). We found *dsir2*$^{17}$ to be lacking coding information for the first 579 amino acids of dSIR2, which include the entire conserved *SIR2* family core domain (Figure 2). As an ideal *dsir2*$^+$ control for the comparisons that follow, we also recovered a precise excision of the *P(lacW)7223* transposon, as determined by blots of genomic DNA (Figure 2B).

To explore the effect of the imprecise excision mutations on the production of dSIR2 proteins, we raised and affinity purified an anti-dSIR2 antiserum. On immunoblots of extracts from the control *dsir2*$^+$ adults mentioned above, this antiserum recognized proteins of estimated molecular weights of 125 and 105 kD (Figure 2). Only the 125-kD species appeared to correspond to SIR2, since only it was absent in extracts prepared from all five homozygous viable, imprecise excision lines (Figure 2; data not shown). Since the antiserum was raised against the central and carboxyl-terminal parts of SIR2, it should have detected N-terminally truncated forms of SIR2 were any generated, but no truncated products were observed, even after long exposures. Post-translational modification of dSIR2 *in vivo* was suggested by the difference between the observed molecular weight of 125 kD on the immunoblot *vs.* the predicted 92 kD. The breadth of the SIR2 band suggested that more than one modified form might be generated.

The *dsir2*$^{17}$ allele, as expected, generated no wild-type transcript (Figure 2D). An RNA blot of total RNA from mutant and *dsir2*$^+$ control flies revealed a transcript of the expected size (3.8 kb) for the wild type, but none from the *dsir2*$^{17}$ strain. Another unknown transcript hybridized with the probe in this experiment; possibly as a result of that, the probe also contained vector sequences and served as a loading control. In summary, by DNA sequencing, RNA blots, and immunoblots, *dsir2*$^{17}$ appeared to be a null allele.

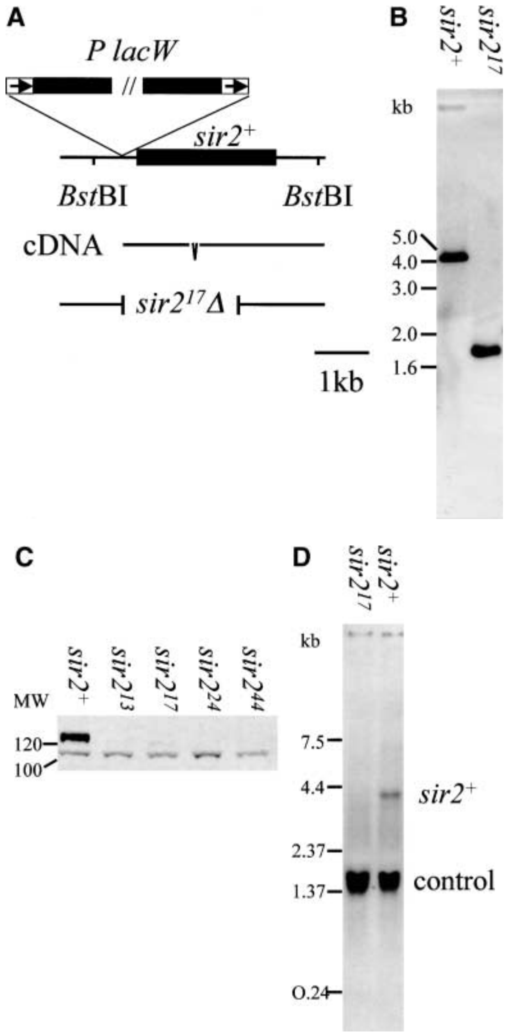

Figure 2.—Generation of *dsir2* null alleles in *D. melanogaster*. (A) Schematic drawing of the *dsir2*$^+$ genomic locus, which contains a single short intron, located at polytene band 34 A and the *P{lacW,w*$^{+mC}$*}* insertion present in a homozygous viable derivative of the *l(2)07223* chromosome 400 bp upstream of the *sir2*$^+$ ORF. *Bst*BI restriction sites, the isolated cDNA, and the extent of the *dsir2*$^{17}$ deletion are indicated. (B) DNA blot analysis of genomic DNA, digested with *Bst*BI, from adult flies homozygous for the indicated *sir2* allele. *dsir2*$^+$ indicates an extract of flies homozygous for a precise excision of the parental *P{lacW}* element, whereas *dsir2*$^{17}$ indicates an extract of flies homozygous for the deletion shown in A. Size markers (in kilobases) are indicated on the left. The probe corresponded to the carboxyl-terminal part of the *sir2*$^+$ cDNA. (C) Immuno-blot of whole-fly extracts from the indicated strains separated on a 7.5% SDS-polyacrylamide gel. Protein was prepared from flies homozygous for the indicated *sir2* allele. Size markers (in kilodaltons) are indicated on the left. The 105-kD protein is a cross-reacting species of unknown identity. (D) RNA-blot hybridization of total *D. melanogaster* RNA prepared from the indicated strains. Positions of the *dsir2*$^+$ mRNA and the cross-hybridizing band serving as control are indicated on the right. Size markers (in kilobases) are indicated on the left. A plasmid containing the *dsir2*$^+$ cDNA was used as a probe.

**The null allele *dsir2*$^{17}$ was homozygous viable and fully complemented *dsir2*$^{ex10}$:** Animals homozygous for *dsir2*$^{17}$ were essentially fully viable (Table 1, A). Moreover, young mutant adults were as fecund as their heterozygous balanced siblings (data not shown). Since this result contradicted the claims of Rosenberg and Parkhurst (2002) based on their finding of recessive lethality for a far less disrupted allele, *dsir2*$^{ex10}$, it was important to know whether *dsir2*$^{17}$ complemented *dsir2*$^{ex10}$. The *dsir2*$^{ex10}$ chromosome indeed carried a recessive lethal mutation, but *dsir2*$^{17}$ fully complemented this recessive lethality (Table 1, B and C, respectively). Hence the observed lethality in the other report was due to an extraneous lethal on that chromosome, a possibility not explored in that study.

The previous study also reported that *dsir2* was required for sex determination through its involvement in the regulation of *Sxl*, a female-specific gene that serves as the master regulator of Drosophila sexual dimorphism and X-chromosome dosage compensation (reviewed in Cline and Meyer 1996). Males (haplo-X animals) that express Sxl in its female-specific (diplo-X) mode die during development as a consequence of dosage compensation upsets. In the previous report, *sir2*$^{ex10}$ was said to exhibit a dominant combined maternal and zygotic effect that was sex-specifically lethal to 95% of sons (Rosenberg and Parkhurst 2002). Anti-SXL antibody staining of embryos indicated that ectopic expression of female-specific SXL protein in males caused their death.

In exploring the basis for the male-lethal effect, a simpler and definitive genetic test of their hypothesis is available that takes advantage of males not requiring Sxl. If the male-lethal effect of *dsir2*$^{ex10}$ were indeed caused by inappropriate expression of *Sxl*, it would be fully suppressed by a null *Sxl* mutation. In such a test, we found that among the *dsir2*$^{ex10}$/+ sons of *dsir2*$^{ex10}$/+ daughters, the viability of *Sxl*$^+$ and *Sxl*$^-$ sons was the same, a result indicating no misexpression of *Sxl* (Table 1, D). On the other hand, this negative result was not entirely unexpected. Indeed, when we used the *dsir2*$^{ex10}$ strain supplied by the authors of the previous work, we saw no indication of the male-specific lethal effect reported (Table 1, B). The reported lethal effect failed to turn up even after many generations and after repeated outcrosses (data not shown).

**Phenotypic effects of the *sir2* null allele:** To investigate if Drosophila SIR2 had a role in genomic silencing like ScSir2p, we assayed the effect of *dsir2*$^{17}$ on PEV of the *white* gene observed in flies carrying the *white mottled 4* (*w*$^{m4}$) inversion. This inversion places *white*$^+$ close to centric heterochromatin, resulting in variegated expression due to spreading of the adjacent condensed heterochromatin. Since the requirement for *white*$^+$ in eye pigmentation is cell autonomous, variegated expression generates mosaic eyes with red patches of cells express-

TABLE 1

**Relative viability of *sir2* mutant animals**

| Cross[a] | Zygotic genotype | Viability relative to reference class |
|---|---|---|
| A. The null allele $sir2^{17}$ is homozygous viable in both sexes | | |
| 1 | $sir2^{17}/sir2^{17}$ males | 79% (181) |
| 1 | $sir2^{17}/sir2^{17}$ females | 85% (194) |
| 1 | $sir2^{17}/CyO,sir2^{+}$ males | 89% (406) |
| 1 | $sir2^{17}/CyO,sir2^{+}$ females | Reference (456 flies) |
| B. The $sir2^{ex10}$ chromosome is recessive lethal but displays no dominant, male-specific lethal effect | | |
| 2 | $sir2^{ex10}/sir2^{ex10}$ males | 0% |
| 2 | $sir2^{ex10}/sir2^{ex10}$ females | 0% |
| 2 | $sir2^{ex10}/CyO,sir2^{+}$ males | 95% (668) |
| 2 | $sir2^{ex10}/CyO,sir2^{+}$ females | Reference (705 flies) |
| C. The null allele $sir2^{17}$ complements $sir2^{ex10}$ chromosome lethality | | |
| 3 | $sir2^{ex10}/sir2^{17}$ males | 107% |
| 3 | $sir2^{ex10}/sir2^{17}$ females | 105% |
| 3 | $sir2^{ex10\ \underline{OR}\ 17}/CyO,sir2^{+}$ males | 90% |
| 3 | $sir2^{ex10\ \underline{OR}\ 17}/CyO,sir2^{+}$ females | Reference (643) |
| D. The *Sxl* genotype has no effect on $sir2^{ex10}/+$ male viability | | |
| 4 | $Sxl^{+}/Y;\ sir2^{ex10}/CyO,sir2^{+}$ males | 102% (470) |
| 4 | $Sxl^{null}/Y;\ sir2^{ex10}/CyO,sir2^{+}$ males | Reference (460) |

[a] In all crosses, the expected number of *sir2* homozygotes if fully viable is half the number of heterozygotes. 1: $w;\ sir2^{17}/CyO$ ¡¡ X ¢¢ $w;\ sir2^{17}/CyO$. 2: $sir2^{ex10}/CyO;\ ry/ry$ ¡¡ X ¢¢ $sir2^{ex10}/CyO;\ ry/ry$. 3: $sir2^{ex10}/CyO;\ ry/ry$ ¡¡ X ¢¢ $y\ w/Y;\ sir2^{17}/CyO$. 4: $w/y\ w\ cm\ Sxl^{f1}\ ct;\ CyO/sir2^{ex10};\ \pm ry/+$ ¡¡ X ¢¢ $w/Y;\ sir2^{ex10}/CyO;\ \pm ry/+$. For cross 4, only sons nonrecombinant for the 20-cM *y-ct* region that includes *Sxl* are listed: $y^{+}$ $ct^{+}$ males are $Sxl^{+}$ while y ct are $Sxl^{null}$. As expected, no $sir2^{ex10}$ homozygotes were recovered.

ing the gene and with white patches where the gene is silent. Several dominant modifiers of $w^{m4}$ have been found, some suppressing and others enhancing PEV, whose protein products are thought either to interact directly with chromatin or to regulate higher-order chromatin structure (Karpen 1994).

We found *dsir2* to be a mild suppressor of PEV. The eyes of $w^{m4}$ flies lacking dSIR2 were significantly more pigmented than those of *dsir2*$^{+}$ controls; hence, dSIR2 appeared to participate in genomic silencing in *Drosophila melanogaster* (Figure 3). Although there was overlap in the eye phenotypes of mutant and control flies, ~79% of the flies lacking SIR2 had more pigmented eye cells (less variegation) than did the controls (Figure 3). The other four independent homozygous viable *dsir2* mutant alleles had comparable effects. PEV is very sensitive to variations in genetic background. Our confidence in the significance of these differences is based upon the availability of a closely matched *dsir2*$^{+}$ control. Most known modifiers of PEV are dominant because of the nature of the genetic screens in which they were recovered. In contrast, the effect of *dsir2* mutants on $w^{m4}$ PEV was strictly recessive (data not shown).

In both *S. cerevisiae* and *C. elegans*, SIR2 has been implicated in regulating life span (Kaeberlein *et al.* 1999; Tissenbaum and Guarente 2001). The same may be true for Drosophila (Figure 4). Flies lacking SIR2 had a significantly decreased life span compared to wild-type flies ($P = 0.006$ by a Wilcoxon rank-sum test). As before, because a matched *dsir2*$^{+}$ control was used for this comparison, it is unlikely that differences in genes other than *dsir2* were responsible for this difference in longevity. If overexpression of *sir2*$^{+}$ extends life span, as observed for the *C. elegans* ortholog, it will be harder to consider the reduced life span of the mutant as resulting from a nonspecific effect on vigor.

In summary, we showed that elimination of *dsir2* function by itself has only rather subtle effects, in contrast to previous claims (Rosenberg and Parkhurst 2002). If the weakness of these phenotypes is a consequence of overlapping functions among *SIR2* family members, one might be able to observe dominant enhancement of the homozygous *dsir2*$^{17}$ mutant phenotype by chromosomal deficiencies of one or more of these other loci. CG5085 is an obvious first choice for such studies, since it is the paralog most closely related to *dsir2*.

Extraneous mutations on their *dsir2* mutant chromosomes misled the earlier workers. Such mutations would not, however, account for our failure to confirm effects on *Sex-lethal* functioning or indeed even the basic claim

| | Low | Medium | High |
|---|---|---|---|
| *sir*$^+$ | 92% | 8% | 0% |
| *sir2*$^{17}$ | 15% | 72% | 13% |

Figure 3.—Flies lacking SIR2 showed increased expression of the $w^{m4}$ allele. Eye pigmentation was assessed for $w^{m4}$/Y males, whether wild type (*dsir2*$^+$) or mutant (*dsir2*$^{17}$). Males were assigned to three different classes, with respect to the amount of eye pigmentation (low, medium, and high), with representative examples of each class shown. At least 100 males of each genotype were assessed, generating the distributions shown.

of a dominant, male-specific lethal effect of the *dsir*$^{ex10}$ chromosome. In this connection, it should be noted that the immunostaining results reported in Table 2 of the earlier work to argue for effects on *Sxl* were not consistent with data in the same table on male-specific viability effects. In any event, recessive lethal *P*-insert lines are likely to harbor mutations in more than one gene. Although many standard genetic approaches can be used to avoid being misled by second-site mutations, a particular advantage of working with intact *P*-element-mediated transgene insertions (such as that used to generate *dsir2*$^{ex10}$) is the ability to establish unequivocally by precise *P*-element excision that the phenotype one observes is indeed caused by the gene one finds to be disrupted.

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*Note added in proof*: During review of our article, Newman *et al.* published a closely related study (B. L. Newman, J. R. Lundblad, Y. Chen and S. M. Smolik, 2002, A Drosophila homologue of Sir2 modifies position-effect variegation but does not affect life span. Genetics **162:** 1675–1685) whose conclusions generally agree with ours except with respect to the effects of *sir2* mutations on life span.

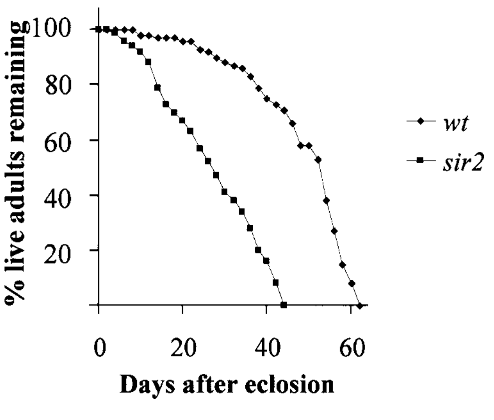

Figure 4.—Life-span analysis of flies lacking SIR2. A total of 120 newly eclosed wild-type (*wt*) or mutant (*sir2*$^{44}$) adults were transferred and counted every 2 days.

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