Letter to the Editor

Trans-silencing by *P* Elements Inserted in Subtelomeric Heterochromatin Involves the Drosophila Polycomb Group Gene, *Enhancer of Zeste*

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REGARDING the article that Siobhan Roche and I published in the August 1998 issue of Genetics (vol. 149, pp. 1839–1855): In the course of trying to extend the observations in the article, we have found that we cannot reproduce the results concerning the role of the polycomb group gene, *Enhancer of zeste* [*E(z)*], in the *trans*-silencing of *P*-element transgenes by *P* elements in subtelomeric heterochromatin. [We have not retested the effect of the *E(z)* alleles on repression of gonadal dysgenic (GD) sterility.] We have spent the past 6 months doing controls, repeating crosses and checking, remaking, or getting new copies of stocks used in these studies. Outlined below, in detail, is a summary of our findings since the article was published.

In our original article, seven different alleles of *E(z)* were tested for their effects on *trans*-silencing of *P*-element transgenes. We observed a loss of *trans*-silencing in the presence of four different *E(z)* alleles.

1. The original observation that *E(z)* is required for the *trans*-silencing of *P*-element transgenes was made with the $E(z)^{61}$ allele. For this experiment, the $E(z)^{61}$ allele (which is marked with the recessive *ebony* mutation) was crossed into an *Lk-P*(1A);+ / *TM3* background and black-bodied progeny were selected. The presence of the *Lk-P*(1A) *P* elements was confirmed by DNA blot hybridization (Southern blotting). The $E(z)^{61}$ allele no longer affects the *trans*-silencing of an *hsp83*-IVS3-*LacZ* reporter transgene. The assumption now is that there was originally a modifier mutation present in the stock that contributed to the loss of *trans*-silencing and that has since (over the past 2 years) been lost from the stock.
2. The second set of data in which we observed a loss of *trans*-silencing involved three additional *E(z)* alleles [$E(z)^{28}$, $E(z)^{32}$, and $E(z)^{60}$]. For these tests, the *E(z)* alleles were crossed into an *Lk-P*(1A);*CxD* / *TM3* background. The *Lk-P*(1A);*CxD* / *TM3* strain was generated by crossing the *CxD* mutation in to the *Lk-P*(1A);+ / *TM3* background and was shown to repress *P*-element transposition as effectively as the *Lk-P*(1A);+ / *TM3* strain. We first used the *Lk-P*(1A);*CxD* / *TM3* strain to test the effect of the polycomb allele, Pc^{16}, on *trans*-silencing and did not observe a derepression of the reporter transgene. However, the three additional *E(z)* alleles that affected *trans*-silencing were also tested in the *Lk-P*(1A);*CxD* / *TM3* background. We have tried unsuccessfully for the last year to repeat these results and made the unfortunate discovery that over time, the *Lk-P*(1A) *P* elements became unstable in the *Lk-P*(1A);*CxD* / *TM3* strain and its $E(z)^{-}$ derivatives. This effect seems to be peculiar to this stock, since normally the *Lk-P*(1A) *P* elements are extremely stable. While we had tested for the presence of the *Lk-P*(1A) *P* elements in the *Lk-P*(1A); $E(z)^{-}$ / *TM3* strains by Southern blotting at the time the genetic experiments were performed, subsequent PCR analysis of single flies from samples frozen at that time (8/96) indicated that approximately half of the individuals in the population had lost the *Lk-P*(1A) *P* elements. This *P*-element instability explains the apparent loss of *trans*-silencing that was observed in 1995–96 and reported in the article.
3. Finally, the reported observation that *P*-element enhancer traps at 1A or 100F also exhibit *trans*-silencing is correct, but the observation that the $E(z)^{32}$ allele reduces this effect cannot be repeated. We know that our inability to repeat these results cannot be due to instability of the *P*-element enhancer traps but may also be a result of background modifiers or changes in the strength of the *trans*-silencing, which we had observed before.

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