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Repression of hybrid dysgenesis in *D. melanogaster* males by the X-linked telomeric *P* element *NA-P(1A)*.

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Repression of hybrid dysgenesis is anchored in *P* elements that have inserted in the Telomere Associated Sequences (TAS) at the left end of the X chromosome. These telomeric *P* elements confer the P cytotype, a regulatory state that is mediated by small RNAs that interact with the Piwi class of proteins; the RNAs are, therefore, called Piwi-interacting, or "pi", RNAs. *NA-P*(*1A*) is a telomeric *P* element that produces *P*-specific piRNAs (Brennecke *et al.*, 2008). This element, hereafter denoted simply as *NA*, is inserted at the junction of the distal retrotransposon array and the TAS of chromosome XL (Marin *et al.*, 2000). The *NA* element is structurally incomplete, lacking the first 871 base pairs of the canonical *P*-element sequence, including the *P* promoter, the first *P* exon, the first *P* intron, and half of the second *P* exon; consequently, it cannot encode the transposase that catalyzes *P*-element activity or a truncated polypeptide that might interfere with this activity. The discovery that this element represses hybrid dysgenesis was a strong indication that cytotype regulation does not involve *P*-encoded repressor polypeptides.

Marin *et al.* (2000) demonstrated that *NA* represses hybrid dysgenesis in females. To determine if it also represses dysgenesis in males, we used a genetic test that detects excisions of the *P* elements inserted in sn^w, a weak mutant allele of the X-linked *singed* bristle locus (Engels, 1979). Two incomplete *P* elements are inserted in the 5' untranslated region of sn^w (Roiha *et al.*, 1988). Excision of the upstream element converts sn^w into sn^e, an allele with an extreme mutant phenotype. Excision of the downstream element converts sn^w into $sn^{(+)}$, a pseudo-wild allele. The extreme mutant and pseudo-wild phenotypes are easily distinguished from the weak mutant phenotype. We screened for *P*-element excisions from sn^w that occurred in the germ lines of males carrying sn^w and $H(w^+, \Delta 2\text{-}3)6$, a hobo transgene that produces the P transposase (Merriman and Simmons, 2013). These males were crossed to females with attached-X chromosomes so that sn^w or its sn^e or $sn^{(+)}$ derivatives would be inherited patroclinously. The sons of these crosses were then scored on days 14 and 17 for the three bristle phenotypes (weak, extreme, and pseudo-wild), and the frequency of the extreme and pseudo-wild sons was used to estimate the germ-line sn^w excision rate. A reduced rate indicates that *P* excisions have been repressed.

The $H(w^+, \Delta 2\text{-}3)6$ transgene contains a terminally truncated *P* element that lacks the last intron of the transposase gene—the one between exons 2 and 3 in a complete *P* element; hence its designation as $\Delta 2\text{-}3$. This transgene, inserted on chromosome 3, produces the P transposase in the soma as well as in the germ line. Genetic analyses have shown that like the widely used P transposase source $P(ry^+, \Delta 2\text{-}3)99B$ (Robertson *et al.*, 1988), $H(w^+, \Delta 2\text{-}3)6$ does not transmit

transposase activity through the egg independently of the element itself—that is, *H*(w^+, Δ*2-3*)*6* does not induce *P*-element excisions through a strictly maternal effect (Merriman and Simmons, 2013). The absence of such an effect allowed us to ascertain if *NA*-mediated regulation of *P* activity has a maternal component.

The experiment was similar to one that studied the regulatory abilities of *TP5* and *TP6*, two other X-linked telomeric *P* elements (Simmons *et al.*, 2004). It consisted of "reciprocal" crosses between *y w* flies heterozygous for the *H*(w^+, Δ*2-3*)*6* transgene and flies hemizygous or homozygous for an X chromosome carrying *NA* and the markers *w* and sn^w. The *NA w* sn^w; *H*(w^+, Δ*2-3*)*6*/+ F_1 males from these crosses were mated individually to *C*(1)*DX*, *y f* females with attached-X chromosomes. Due to somatic production of the P transposase by the *H*(w^+, Δ*2-3*)*6* transgene, these F_1 males were all bristle mosaics; sn^w and $sn^{(+)}$ or sn^e bristles were present on every fly. The *NA* element—like other telomeric *P* elements—therefore does not repress transposase activity in the somatic tissues. The sons of these F_1 males were scored for their bristle phenotype to estimate the frequency of *P*-element excisions from the sn^w allele that had occurred in each F_1 male's germ line. To permit these sons to be scored unambiguously, the somatic mosaicism caused by the segregating *H*(w^+, Δ*2-3*)*6* transgene had to be repressed by using attached-X females from a special strain in the F_1 matings (Robertson and Engels, 1989; Simmons *et al.*, 2004); this strain carries *P* elements that produce polypeptide repressors of somatic *P* activity.

Table 1. *NA*-mediated repression of *P* excisions from the sn^w allele in the male germ line.

Stock	Cross A: *NA* inherited maternally			Cross B: *NA* inherited paternally		
	No. vials	No. flies	Excision rate [a]	No. vials	No. flies	Excision rate [a]
w sn^w	32	928	0.632 ± 0.029	32	1063	0.470 ± 0.032
NA w sn^w #1	32	1163	0.250 ± 0.038	32	1210	0.498 ± 0.029
NA w sn^w #2	31	1117	0.209 ± 0.033	32	1111	0.482 ± 0.032
NA w sn^w #3	31	949	0.194 ± 0.028	32	1158	0.471 ± 0.026

To obtain these data, (*NA*) *w* sn^w; *H*(w^+, Δ2-3)*6*/+ F_1 males were mated at 25°C to *C*(1)*DX*, *y f* females from a somatic *P* repressor strain and their sons were scored for the three bristle phenotypes—sn^w, $sn^{(+)}$, and sn^e. The F_1 males were obtained from "reciprocal" crosses at 18 °C; cross A was (*NA*) *w* sn^w females x *y w*; *H*(w^+, Δ2-3)*6*/+ males and cross B was (*NA*) *w* sn^w males x *C*(1)*DX*, *y w f*; *H*(w^+, Δ2-3)*6*/+ females. The *H*(w^+, Δ2-3)*6*-bearing flies for crosses A and B were obtained by mating *C*(1)*DX*, *y w f* females to *y w*; *H*(w^+, Δ2-3)*6* males at 25 °C. The three *NA w* sn^w stocks that were tested had been independently generated by recombining an *NA* w^{sp} X chromosome with a *y w* sn^w X chromosome and then making each *NA w* sn^w recombinant chromosome homozygous.

[a] Unweighted excision rate, computed by averaging the proportion of $sn^{(+)}$ and sn^e males among all the males in each culture; the standard error was calculated from the empirical variance among cultures.

Three independently generated stocks with the *NA w* sn^w X chromosome were tested along with a *w* sn^w control stock that did not carry *NA*. In cross A, the *NA* element was inherited maternally and the *H*(w^+, Δ*2-3*)*6* transgene was inherited paternally. In cross B, the derivations of *NA* and *H*(w^+, Δ*2-3*)*6* were reversed. The results of the experiment are summarized in Table 1. For the control stock, the excision rate was 0.632 in cross A and 0.470 in cross B. These rates indicate that *H*(w^+, Δ*2-3*)*6* induces a high frequency of germ-line *P*-element excisions in the male germ line. We do not

know why the excision rate in cross A is significantly higher than in cross B. For the three *NA* stocks, the excision rates in cross A were significantly lower than the corresponding control rate (and also significantly lower than the control rate in cross B); however, the excision rates for the *NA* stocks in cross B were not lower than the rate for the cross B control. These observations indicate that *P*-element excisions in the male germ line are repressed, but only when the *NA* element is inherited maternally. Thus, regulation of *P* activity by *NA*, as by other telomeric *P* elements, involves a maternal effect, and an *NA* element that passes patroclinously from father to son loses its ability to repress hybrid dysgenesis.

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Influence of age on mating and fitness of *Drosophila melanogaster*.

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Abstract

The effect of age on mating time, remating duration, quantity of ACP transferred, fecundity, and productivity was studied within and between 7 and 26 days old *Drosophila melanogaster*. Mating time, accessory gland secretory protein transferred, fecundity, and productivity of 1st mating is more than the 2nd mating irrespective of age. The remating duration among young and old flies is almost similar, whereas it is more in different aged flies mating. Mating time, fecundity, and productivity are more in young flies compared to other combinations. In old flies mating time, fecundity, and productivity are more than the different aged flies. The fecundity is more if the female is young and productivity is more if the male is young in different aged flies mating. Key words: *Drosophila*, mating behavior, fecundity, productivity, age.

Introduction

Sexual reproduction occurs in a wide range of organisms. Reproductive capacity is particularly a good index of fitness in organisms that go through repeated cycles of rapid population growth, and it has evolved as a way for species to maximize their potential of survival. Mating is the most important and fundamental process of reproduction. Male fitness depends largely on the