Hybrid incompatibility is stronger in the presence of transposable elements in the *Drosophila virilis* clade

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Abstract

Mismatches between parental genomes in selfish elements are frequently hypothesized to underlie hybrid dysfunction and drive speciation. However, because the genetic basis of most hybrid incompatibilities is unknown, testing the contribution of selfish elements to reproductive isolation is difficult. Here we evaluated the role of transposable elements (TEs) in hybrid incompatibilities between *Drosophila virilis* and *D. lummei* by experimentally comparing hybrid incompatibility in a cross where active TEs are present in *D. virilis* (TE+) and absent in *D. lummei*, to a cross where these TEs are absent from both *D. virilis* (TE-) and *D. lummei* genotypes. Using genomic data, we confirmed copy number differences in TEs between the *D. virilis* (TE+) strain and a *D. virilis* (TE-) strain and *D. lummei*. We observed F1 postzygotic reproductive isolation specifically in the interspecific cross involving TE+ *D. virilis* but not in the cross involving TE- *D. virilis*. A series of backcross experiments, designed to account for alternative models of hybrid incompatibility, showed that expression of this F1 hybrid incompatibility is consistent with the action of TEs rather than other, genic, interactions. A further Y-autosome interaction contributes to additional, sex-specific, inviability in one direction of this cross combination. These experiments demonstrate that TEs can increase reproductive isolation between closely related lineages, thereby adding to the processes that accelerate speciation.

Introduction

Selfish elements have been proposed to play a key role in the evolution of species isolating barriers. However, most evidence for this role in both plants and animals comes from empirical studies that indirectly correlate differences in repetitive elements between species with both increased TE transcription and phenotypic dysfunction in hybrids (Labrador *et al.* 1999; Martienssen 2010; Brown *et al.* 2012; Dion-Cote *et al.* 2014). In comparison, there have been few attempts to directly assess the role of TEs in the expression of reproductive isolation, especially in comparison to genic differences between lineages. The proposal that transposable elements (TEs) contribute to reproductive isolation was met with early skepticism, largely due to a failure to detect their proposed mutational effects—including increases in the mutation rate—in species crosses that have marked postzygotic isolation (Coyne 1986; Hey 1988; Coyne 1989). However, we now understand that phenotypes related to 'dysgenesis'—a specific incompatibility phenotype caused by TEs—instead reflect more complex effects of de-repression of TE transcription (Martienssen 2010; Khurana *et al.* 2011) and genome wide DNA damage (Khurana *et al.* 2011), rather than strictly mutational effects on essential genes (Petrov *et al.* 1995; Blumenstiel and Hartl 2005). This potential to cause negative genetic interactions via TE de-repression in hybrids, places TEs within the classical Dobzhansky-Muller framework for the evolution of incompatibilities (Dobzhansky 1937; Muller 1942)—whereby postzygotic isolation results from negative epistasis among divergent loci in hybrid genomes (Johnson 2010; Castillo and Moyle 2012; Crespi and Nosil 2013). Accordingly, the relative contribution of TEs to reproductive isolation phenotypes should be amenable to dissection with experimental crosses, just as has been shown with standard genic incompatibilities (Johnson 2010; Presgraves 2010; Castillo and Barbash 2017).

The observation that TE content frequently differs between closely-related lineages, both between species and among lineages within a species (Kidwell and Lisch 1997; Stuart et al. 2016; Petersen et al. 2019) also provides an experimental mechanism for addressing the contribution of TEs to species reproductive barriers. In particular, where there is within-species polymorphism for the presence and absence of active TEs, inter-specific crosses are predicted *a priori* to differ in their magnitude of hybrid dysfunction such that stronger barriers are expected in crosses involving lineages containing active TEs compared to those without active TEs. Of known instances of intraspecific TE polymorphism, dysgenic systems in *Drosophila* are also among the best characterized in terms of phenotypic effects of TEs that could be relevant to reproductive isolation. In particular, the dysgenic syndrome within *Drosophila virilis* and the *P-element* system within *D. melanogaster* typically exhibit gonad atrophy in offspring when a strain carrying transposable elements is crossed with a strain lacking these elements (Kidwell 1985; Lozovskaya *et al.* 1990). The dysgenic phenomenon depends both on the direction of the cross—dysgenesis occurs when the female parent lacks the TE elements—and on the copy number in the paternal parent (Srivastav and Kelleher 2017; Serrato-Capuchina *et al.* 2020b). These intraspecific dysgenic systems can be used to evaluate the contribution of TEs to reproductive isolation with other species, when there are two genotypes/lines within a species that differ in the presence of TEs known to cause intraspecific dysgenesis as well as a second closely related species that lacks these TEs. If the TEs responsible for hybrid dysfunction within a species also influence reproductive isolation, interspecific crosses involving the TE-carrying line should exhibit greater hybrid incompatibility than crosses involving the lines in which TEs are absent.

In this study, we evaluate whether TEs affect interspecific reproductive isolation, using the *D. virilis* dysgenic system and the closely related species *D. lummei*. Species in the *D. virilis* clade are closely related, easily crossed with one another, and vary in transposable element abundance. Dysgenesis within *D. virilis* was first described by Lozovskaya and colleagues (Lozovskaya *et al.* 1990) when they observed gonadal atrophy and sterility in males and females in crosses between strains that vary in transposable elements; as expected, *D. virilis* is polymorphic for the abundance of active TE elements (below). In contrast, *D. lummei* lacks full length active TEs such as *Penelope* (Zelentsova *et al.* 1999; Evgen'ev *et al.* 2000) and other elements (see below), and is not reported to exhibit intraspecific dysgenesis.

Using the features of this system and the established role of hybrid dysgenesis among *D. virilis* strains we contrast a cross where dysgenic TEs are present in the *D. virilis* (TE+) parent and absent in the *D. lummei* parent to a cross where dysgenic TEs are absent from both *D. virilis* (TE-) and *D. lummei* parental genotypes, to evaluate the connection between postzygotic reproductive isolation and differences in TE composition. In intraspecific crosses between female *D. virilis* that lack relevant TEs (TE-) and male *D. virilis* that carry these TEs (TE+), strong dysgenesis is observed. Therefore, in interspecific crosses we expect the strongest reproductive isolation specifically between *D. lummei* females that lack dysgenic inducing TEs and *D. virilis* males that are TE+. With these expectations we show, using a series of directed crosses and backcrosses, that patterns of reproductive isolation observed in our study are consistent with a causal role for TEs in the expression of increased postzygotic species barriers.

Materials and Methods

Fly stocks

Our experiments used two *D. virilis* stocks that are known to differ in their abundance of TEs, and one stock of the closely related species *D. lummei*. We chose stocks that mated readily to facilitate genetic analysis. The *D. virilis* Strain 9 is a wild type strain that was collected in Georgia, USSR in 1970 and is consistently used as a parent in dysgenic crosses because it lacks inducing repetitive elements (Lozovskaya *et al.* 1990; Blumenstiel and Hartl 2005). Here we refer to this strain as TE-. The reference genome strain was used as our inducer strain (UCSD stock number 15010-1051.87) because it is known to induce dysgenesis (Blumenstiel 2014), and we refer to this strain as TE+. The genome strain was used instead of Strain 160, which is also commonly used in dysgenic studies, because we experienced significant premating isolation between Strain 160 and *D. lummei* in single pair crosses. The reference genome strain and Strain 160 are closely related; both were originally created by combining several phenotypic markers to make multiply marked lines (genome strain genotype *b; tb, gp-L2; cd; pe*) (Lozovskaya *et al.* 1990). Our strain of *D. lummei* was acquired from the UCSD stock center (15010-1011.07).

Estimating copy number of dysgenic causing elements

To examine the copy number of potentially dysgenic causing elements, we used publicly available genomes from several *D. virilis* strains and other species in the virilis clade, including two *D. lummei* strains. The SRA accession numbers and strain information for all samples are listed in Supplemental Table 1. We focused on candidate TEs that are associated with dysgenesis (Funikov *et al.* 2018) but mapped reads to all inserts from a previously compiled TE library (Erwin *et al.* 2015) to prevent bias that might arise from mapping reads from the other species to a TE library generated from *D. virilis*. All copy number estimates were made using deviaTE (Weilguny and Kofler 2019), which estimates haploid copy number based on single-copy

"control" genes. For our control genes we chose the *D. virilis* homologs of Beta-tubulin60D and alpha-tubulin84B as essential single copy genes in the *Drosophila virilis* genome.

We first compared copy number differences between the genomes of the inducing strain *D. virilis* Strain 160 and *D. virilis* Strain 9 (TE-), the two strains typically used in studies of dysgenesis. We determined the reliability of our estimates for identifying potentially causal TEs, by comparing our estimate for the enrichment of TE copy number in Strain 160 to other studies that have used read mapping abundance from genomic data and read abundance for piRNA from ovaries of each strain (Erwin *et al.* 2015; Funikov *et al.* 2018). Although the majority of work on dysgenesis in *D. virilis* has focused on *Penelope*, new evidence suggests that *Penelope* does not play a role in dysgenesis (Rozhkov *et al.* 2013; Funikov *et al.* 2018). Therefore, we looked at relationships between candidate TEs and *Penelope* across *D. virilis* genomes, to determine which of these TEs had similar copy number distribution as *Penelope*. We specifically focused on *Penelope, Polyphemus, Paris, Helena, Skippy,* and *Slicemaster,* given their greater mapping abundance in inducer vs non-inducer strains (Funikov *et al.* 2018). We used hierarchical clustering to determine the correspondence of copy number of each candidate TE and *Penelope* across strains. A strong correspondence suggests a causal role for elements that cluster with *Penelope* in the dysgenic studies that have previously focused on *Penelope*.

We next determined which candidates had a higher average copy number in the two inducing strains (the genome strain TE+ and 160) compared to the non- inducing Strain 9 (TE-) as well as the copy number average of two *D. lummei* strains for which we had genome data. We retained the threshold of two-fold increase in the average TE copy number, based on the analysis above. We also examined the differences in allele frequencies and polymorphic sites in the *D. lummei* strains compared to *D. virilis* strains. We filtered SNPs using default settings in deviaTE

to calculate the average major allele frequency at each site as well as the number of sites that were polymorphic. TEs that are homogenous (have an average major allele frequency close to one) reflect either new invasions or active TEs, whereas evidence for the accumulation of variable sites indicates older inactive elements (Beall *et al.* 2002; Erwin *et al.* 2015; Weilguny and Kofler 2019).

Intra- and interspecies crosses to determine the nature of reproductive isolation

To compare intra- and interspecific hybrid phenotypes, we used a design that was parallel for all experimental crosses done within and between species. For all our crosses, virgin males and females from each stock were collected as they eclosed and aged for seven days prior to matings. All crosses involved single pairs of the focal female and male genotype combination (a minimum of 20, range 20-23, replicates were completed for a given female × male genotype combination). We performed three intra-strain crosses (TE- × TE-, TE+ × TE+, and *D. lummei* × *D. lummei*) to account for intrinsic fecundity differences in subsequent analyses of inter-strain crosses. As expected, in the three intra-strain crosses we did not see any evidence of male or female gonad atrophy (classical dysgenic phenotypes) or skewed sex ratios. There were differences in the total number of progeny produced, hatchability, and proportion of embryos that had reached the pre-blastoderm stage (hereafter 'proportion fertilized'). We used these values as baseline estimates of maternal effects which might influence these phenotypes in subsequent inter-strain analyses (Table 1; Table 2).

For inter-strain comparisons, we performed all reciprocal crosses between the three strains to analyze reproductive isolation in F1 progeny. These crosses fell into three categories. **I-Intraspecific Crosses Polymorphic for TEs:** Crosses between TE- and TE+ were used to

confirm the expression of intraspecific male dysgenesis when the inducing (TE+) strain was used as a father. **II-Interspecies crosses with no TEs present:** Crosses between TE- and *D. lummei* were used to determine the nature of reproductive isolation between the species, in the absence of dysgenic inducing TEs. **III-Interspecies crosses with TEs present:** Crosses between TE+ and *D. lummei* were used to determine the additional contribution, if any, of TEs to interspecific post-mating reproductive isolation (Supplemental Fig 1). This contrast assumes that the two *D. virilis* strains, TE- and TE+, do not differ from each other in their genic Dobzhansky-Muller incompatibilities with *D. lummei*, an assumption that we could also evaluate with data from subsequent crosses performed using hybrid genotypes (described further below).

Backcross design to test models of hybrid incompatibility

The phenotypes that we observed for postzygotic reproductive isolation could be the product of several non-mutually exclusive genetic mechanisms, including both TEs and genic interactions. Therefore we used a set of backcross experiments to compare the fit of several models of hybrid incompatibility to our data for interspecific reproductive isolation and *D. virilis* hybrid dysgenesis. For all backcross experiments we used F1 males from TE+ and *D. lummei* crosses. We used these specific F1 males because this genotype was used previously to infer the genetic basis of hybrid dysgenesis within *D. virilis* (Lovoskaya et al. 1990; see below); however interspecific backcross genotypes generated using these males are also the most informative for identifying any additional genic incompatibilities between the TE+ and *D. lummei* parents. To evaluate interspecific isolation and hybrid dysgenesis in parallel, these F1 males were backcrossed individually to either *D. lummei* or TE- females. We evaluated two main classes of models with fitness data from the resulting BC hybrids. Both classes broadly describe

Dobzhansky-Muller incompatibilities, because they involve negative epistatic interactions between loci in the two parental strains, however they differ in whether they indicate that the interactions occur between genes or between TEs and TE suppressors. Note that we expected that observed hybrid dysgenesis within *D. virilis* would correspond to the TE model of incompatibility, based on previous work (Lovoskaya et al. 1990; Vieira et al. 1998). Nonetheless, we recapitulated and analyzed data from the dysgenic crosses in parallel to our interspecific crosses, to confirm those prior results.

Comparing observed patterns to theoretical expectations

Here we describe the unique predictions that are made by different incompatibility models, focusing on two major classes: models that assume interactions between genes from each parental genome (Genic models) and models that assume an asymmetric copy number of TEs between the parental genomes (TE models).

Genic models of hybrid incompatibilities

The first set of models we compared to our empirical observations assume that alternative alleles at incompatible loci have diverged in each lineage and have a dominant negative interaction in F1 and in backcross progeny that carry these alleles (Coyne and Orr 2004). When two unlinked loci are involved we expect to see discrete classes of backcross (BC1) genotypes that exhibit hybrid incompatibilities, because half of the progeny should carry the incompatible genotype and half should not (Supplemental Fig. 2). If the hybrid incompatibility is not fully penetrant then, on average, any specific replicate BC1 cross would have half the number of progeny exhibiting the hybrid incompatibility as were observed in F1s from the cross between

the parental species. This provides an expectation for us to compare observed levels of incompatibility between F1 and BC1 crosses (see Statistical Analysis below).

We also expanded this general model to include X-autosome interactions among two or more loci. In particular, we considered an X-autosome model where there is a negative interaction between the X chromosome of the TE- or *D. lummei* parent and an autosome of the TE+ parent (Supplemental Fig. 3). We restricted ourselves to this specific X-autosome interaction because this generates dysgenesis/incompatibility in both sexes for the canonical intraspecific dysgenic cross (TE- × TE+), but no male gonad dysgenesis/incompatibility in the reciprocal cross, consistent with previous observations of the intraspecific dysgenesis phenotype (Lozovskaya *et al.* 1990). For models in which higher order (greater than 2 loci) interactions are responsible for the incompatibility observed in the F1s, we would expect to observe a smaller proportion of affected individuals in the backcross compared to the F1 cross (Supplemental Table 2); the specific expected degree of hybrid incompatibility in the backcross depends on the total number of loci required for hybrid incompatibility and the number of loci carried by each parental strain.

TE models of hybrid incompatibility

To contrast with genic models of hybrid incompatibility we assessed the fit of our observations to a model that is based on transposable element copy number. Under this model we expect asymmetric postzygotic reproductive isolation in crosses between parental species when the female parent lacks the production of piRNAs from dysgenic eliciting TEs (Lovozkaya et al 1990; Blumenstiel and Hartl 2005). We also predict reduced hybrid incompatibility in the backcross as a result of reducing the dosage of dysgenic causing TEs (Lovoskaya et al. 1990;

Vieira et al. 1998). In particular, because F1s have an intermediate copy number of dysgenic causing TEs, when these F1s are used as the male parent in a backcross to TE- females, TE copy number in the resulting BC offspring can fall below a minimum number necessary to elicit dysgenesis. This dosage/copy number effect for the *D. virilis* system was first suggested by Vieira et al. (Vieira *et al.* 1998) and has been supported in the *P*-element system in *D. melanogaster* and *D. simulans* (Serrato-Capuchina *et al.* 2020b). The specific prediction of low or no hybrid incompatibility in backcrosses comes from previous observations that the progeny from crosses between TE- females and males heterozygous for dysgenic factors (i.e., F1 males from TE+ × *D. lummei* crosses) are not dysgenic (Lozovskaya et al. 1990). We interpret this observation as indicating that the copy number of dysgenic factors (TEs) in the male parent is insufficiently high to cause a dysgenic phenotype in their offspring. This expectation is not predicted from the genic hybrid incompatibility models.

Phenotypes measured to infer dysgenesis and post-zygotic reproductive isolation

The phenotypes that we measured tracked the developmental time course of hybrid individuals. We first broadly estimated prezygotic isolation to capture potential mating differences or post-copulatory pre-fertilization defects in our crosses; this was measured by comparing the proportion of fertilized and unfertilized embryos from large cage experiments. Our measures of postzygotic isolation included inviability (hatchability, progeny production, and sex ratio) and sterility (testes dysgenesis) in hybrid offspring. The testes dysgenesis phenotype in F1s is well established within the intraspecific dysgenic system, but for interspecies incompatibilities we wanted to be agnostic to the type of incompatibility that we might observe, including earlier stages of F1 inviability. These phenotypes were measured in both F1 and backcross experiments

and could assess both germline and somatic effects of TEs. Since hatchability and progeny production is the result of both prezygotic success and offspring viability, we could leverage our prezygotic measures to better interpret these measures of inviability in our hybrids.

Prezygotic isolation and early stage inviability:

From preliminary crosses we knew that the chosen all strains mated and premating isolation was not complete, so we chose to use large crosses in cages to estimate prezygotic isolation and early stage inviability (i.e. hatchability). We set up cages consisting of 25 males and 25 females for each genotype combination (3 replicate populations cages were set up per cross type). Males and females were left in the cages for one week and were given grape agar plates supplemented with yeast culture daily. After the acclimation period, a fresh grape agar plate was given to each cage for embryo collection. We allowed females in the cage to oviposit on this plate for 24 hours and then prepared embryos for staining. Embryos were collected, fixed, and stained with DAPI following basic protocols (Rothwell and Sullivan 2000; Rothwell and Sullivan 2007; Ferree and Barbash 2009). Briefly, embryos were collected in a nitex basket using 0.1% Triton X-100 Embryo Wash (7g NaCl, 3mL Triton X, 997 mL H20). Embryos were dechorionated using 50% bleach for 3 minutes and rinsed well with water. Embryos were fixed with 37% formaldehyde and n-Heptane and then vitelline membrane was removed by serially shaking and washing in methanol. To stain embryos, they were first rehydrated by rinsing with PBS and then blocked by incubating with PBTB, followed by rinsing with PBS before staining with PBTB+ 1:1000 DAPI for 5 minutes. Embryos were visualized using a fluorescent scope immediately after staining to determine if development had progressed. Embryos were considered to have begun development if we could see nuclei patterns typical of preblastoderm, blastoderm, and gastrula stages. Since we did not score fertilization by co-staining and looking for presence of sperm or whether the

second phase of meiosis (formation of polar bodies) had been initiated, we may have missed any very early stage embryo lethality and classified these eggs as unfertilized. Thus our estimates of embryo lethality are conservative (they potentially underestimate embryo lethality). All females were dissected after this last embryo collection to confirm they had mated by looking for the presence of sperm stored in the reproductive tract.

To determine if there were differences in viability in early stage embryos we estimated hatchability. After allowing females to oviposit on a fresh grape agar plate for 24 hours, we removed the plate and counted all the eggs. Seventy-two hours later (Day 4) all eggs and larvae on the plate were counted. We calculated hatchability as the proportion of eggs that failed to hatch/total number of eggs. This was confirmed by counting larvae on the plates. Each hatchability plate was paired with a plate used for the test of fertilization (above)

Hybrid progeny production, sex ratio, and sterility:

The remaining phenotypes were estimated with progeny generated from individual pairings. After each male by female pairing was set up, parent individuals were kept for seven days. On day 4 they were transferred to a new fresh vial. On day 7 the parents were transferred to a vial that had been previously dyed with blue food coloring to assist counting of eggs. All vials were cleared and parental individuals discarded at day 8. To ensure that we compared progeny of the same age among different crosses, we did not use any progeny from the first three days of the cross because both intra-strain and inter-strain crosses involving *D. lummei* females would often produce few or no progeny within this time. To be used in further analyses, an individual cross must have produced at least 10 progeny over the 4 day period (days 4-7).

To estimate the total number of progeny produced and their sex ratio we counted the number of male and female progeny from days 4-7. We determined the viability of individuals

for each cross by counting the number of eggs in the day 7 vial as this was the day when females were laying sufficient numbers of eggs to accurately determine hatching rates. We then allowed these eggs to hatch and develop and counted the number of pupae and adults. The number of progeny produced and egg to adult viability gave the same results; since the total number of progeny produced was collected from more replicates and could be summarized by normal distributions we only present those data.

To estimate male gonad atrophy we collected all flies that eclosed from days 4-6 of the cross and saved males into new vials that we supplemented with extra yeast paste, with <10 males per vial. We aged these flies on yeast for five days before scoring gonad atrophy on a dissecting scope by looking for the presence of atrophied testes (Blumenstiel and Hartl 2005). In crosses between Strain 9 and Strain 160 it has been noted that both, one, or zero testes can be atrophied (Blumenstiel and Hartl 2005). Our estimate for analysis was the proportion of testes atrophied for the total number of testes scored. We also examined female ovary atrophy for a small sample from each interspecies cross but did not observe any atrophy, so we focus exclusively on male gonad atrophy.

Statistical analysis

Comparison of embryo development and hatchability measurements

To quantify prezygotic isolation and early embryo lethality (postzygotic isolation), we compared our estimates of embryo development and hatchability using contingency tables. We used the proportion of eggs that had begun development past the pre-blastoderm stage as our measure of prezygotic isolation. Since there were few eggs for a given embryo collection replicate, we pooled across all three replicates. We then compared the proportion of eggs hatching to the

proportion of eggs fertilized using a Chi-square test of independence. We could also compare differences in the proportion of eggs fertilized between crosses that shared a common female genotype, to infer prezygotic (fertilization) isolation.

F1 reproductive isolation

All analyses were performed in R v3.3.2. For traits measured in the crosses used to generate F1s we performed three separate analyses, based on the identity of the female used in the cross. Since most of these traits are potentially influenced by female fertility, all comparisons were made using the same female parent genotype. To determine if there were differences in trait values based on the male parent in the cross we used linear and generalized linear models. All models had the form

$$y = \mu + \beta_i x_i + \beta_j x_j$$

μ represents the trait value for the intra-strain cross. β_i and β_j represent the effect sizes for males that were from different strains/species than the female in the specific analysis. For example, when we analyzed data where TE- was the female parent, β_i could represent the deviation caused by having the TE+ strain as the father and β_j could represent the deviation caused by having *D. lummei* as the father. For a given phenotype there was significant reproductive isolation when β_i or β_j was significantly less than zero, indicating lower trait value than the intra-strain cross. In the case of sex-ratio, any deviation of the correlation coefficient from zero could be interpreted as sex specific effects. The dysgenesis and total progeny produced variables were analyzed with simple linear regressions. Since the sex ratio data are more accurately represented by binomial sampling we analyzed these data using binomial regression (a generalized linear model).

To examine whether there was asymmetry in reproductive isolation for the *D. lummei* and TE+ strain cross, we converted these phenotypes into relative values based on the intra-strain cross. For example, TE+ female × *D. lummei* male measurements can be converted to relative values by finding the difference from the TE+ intra-strain cross average value. These relative measurements were compared in pairwise t-tests.

Backcross reproductive isolation

The results for the backcross experiment were analyzed in the same way as the F1 crosses. We compared phenotypes for crosses based on the female parent. Specifically, we compared the phenotype values for the control TE- (*D. virilis*) intra-strain cross with the crosses between TE- females and TE+ or F1 male genotypes (*D. virilis* comparisons). Similarly, we compared the phenotype values for the control *D. lummei* intra-strain cross with the crosses involving *D. lummei* females mated to TE+ or F1 male genotypes (*D. lummei* comparisons).

For a two locus genic model of incompatibility with an autosome-autosome interaction, no sex specific effects are expected in F1 progeny (Supplemental Fig. 2). When F1 males are used as backcross parents an intermediate number of progeny, regardless of sex, should exhibit the hybrid incompatibility compared to the parental and F1 crosses. If the epistatic interaction does not have complete penetrance then the effect size in this BC should be ½ compared to the effect size in the parental cross. For example if 30% of F1 progeny are dysgenic then only 15% of the backcross progeny are expected to be dysgenic. Under a genic model with an X-autosome interaction between the X chromosome of the TE- parent and an autosome of the TE+ parent, sex specific effects will only be observed in the F1 progeny and not in the backcross (Supplemental Fig. 3). Lastly, for the TE model, there are no expected sex-specific effects and the level of

incompatibility in the backcross will be the same as the intra-strain control, and significantly less than predicted from the genic models of incompatibility. For the gonad atrophy data, we analyzed only the data from the F1 male that carried the X chromosome from TE+ since the other genotype was already determined to not be significantly different from zero (see results). For the data on numbers of progeny produced, we pooled all backcrosses for this analyses since they produced the same number of progeny on average (see results).

Data availability

All data and R code used is available through dryad (doi to be determined).

Results

Estimates of copy number and divergence in dysgenic causing TEs

With our copy number estimates from genomic data, we identified two elements, *Polyphemus* and *Slicemaster*, but not *Penelope*, that had higher average copy number in the inducing *D. virilis* strains—Genome Strain (TE+) and Strain 160—compared to the non-inducing *D. virilis* Strain 9 (TE-) and two *D. lummei* strains. Interestingly, we also found copies of *Penelope* in the *D. lummei* genome despite previous reports of the absence of this element (Zelentsova *et al.* 1999). We confirmed that our approach for estimating copy number was consistent with previous approaches that analyzed differences between Strain 160 and Strain 9 (Erwin *et al.* 2015; Funikov *et al.* 2018). Specifically, we found that *Penelope, Polyphemus, Paris, Helena, Skippy,* and *Slicemaster* were enriched in Strain 160 compared to Strain 9 (Fig 1A). Using hierarchical clustering across the *D. virilis* strains we determined *Polyphermus* had a similar copy number distribution as *Penelope* and formed a cluster that did not include the other

candidate TEs (Fig. 1B), indicating that *Polyphermus* is the element whose distribution most closely matches that of *Penelope*. The correlation in copy number between *Penelope* and *Polyphemus* across strains was r=0.68 (Fig. 1C, P=0.0073). When one outlier (Dvir87) was removed the correlation increased to r=0.866 (P=0.0001). Either correlation indicates a strong relationship between copy number of these two elements.

We used the patterns of allele frequency to assess the likelihood that *Polyphemus* was active in inducing strains but not in non-inducing strains. We expected active elements to have homogenous insertions with very few polymorphic sites (Erwin et al. 2015). The pattern for *Polyphemus* was consistent with our expectation for the inducing *D. virilis* strains and non-inducing *D. virilis* strain, although there was a difference in the estimates between the *D. lummei* samples (Supplemental Fig. 4)—one *D. lummei* sample was nearly identical to the non-inducing Strain 9 (TE-), while the other strain had a higher copy number and average major allele frequency. Nonetheless, *Polyphemus* was still lower in both *D. lummei* samples than in the inducing strains (Supplemental Fig. 4). We also assessed evidence for activity of *Slicemaster* in inducing strains. In contrast to *Polyphemus*, the major allele frequency data for *Slicemaster* was consistent with a recent broad invasion the *D. virilis* clade because all strains, with the exception of one *D. lummei* strain, had high average major allele frequency. As a result, for *Slicemaster* there was no consistent pattern differentiating inducing *D. virilis* and non-inducing *D. virilis*, making it an unlikely candidate contributing to dysgenesis.

Combined, our observations from within *D. virilis* and comparison between *D. virilis* and *D. lummei* suggest that copy number differences in *Polyphemus* have the potential to play important roles in hybrid dysgenesis. This is consistent with analyses that suggest *Polyphemus* induced DNA breaks in dysgenic progeny (Hemmer *et al.* 2019).

No postzygotic isolation occurs in interspecies crosses when TEs are absent

We did not observe inviability or gonad atrophy in males or females in either reciprocal cross between TE- and *D. lummei* (Fig.2; Fig. 3.). We did observe an 18% reduction in progeny production in the TE- female × *D. lummei* male cross compared to the intra-strain cross; the former produced, on average, 22 fewer adult progeny than the TE- intra-strain control cross (μ=123.318; P<0.0001; β=-22.509; P=0.0341). However, two comparisons indicate that this reduction in progeny production results from prezygotic isolation (specifically reduced fertilization) in this particular direction of the cross, rather than reduced F1 embryo viability. First, the proportion of fertilized embryos did not differ from the proportion of embryos hatching within this cross, indicating no evidence for post-fertilization embryo lethality (χ_1^2 = 0.90, P=0.34). Second, the estimated proportion of fertilized embryos was significantly less for the TE- × *D. lummei* cross compared to the TE- intra-strain control cross (χ_1^2 = 130.60, P<0.001; Table 2), consistent with a prezygotic fertilization barrier.

The reciprocal cross, *D. lummei* female × TE-, had no evidence of prezygotic isolation. We actually observed a higher proportion of hatched embryos compared to our estimate for proportion fertilized. This observation likely reflects differences in attrition between the techniques used to estimate each stage; in particular, fertilization rate relies on handling embryos with multiple washing and fixing steps which can lead to loss of embryos in the final sample (Rothwell and Sullivan 2007b).

Strong F1 postzygotic isolation occurs in interspecies crosses when TEs are present

In contrast to the TE- interspecific crosses, we observed significantly fewer progeny in both directions of cross between the TE+ strain and *D. lummei* (Fig 3), compared to intra-strain

(control) crosses. In the *D. lummei* female × TE+ male cross, where we would expect to see the effects of TEs, 17 fewer progeny were produced compared to the *D. lummei* intra-strain cross (μ =34.50; P<0.001; β=-16.95; P=0.002). This was also significantly lower than the number of progeny produced in the *D. lummei* female × TE- male interspecific cross, where TEs are absent (*D. lummei* female × TE+ male 95% Confidence Interval= -27.86, -6.04; *D. lummei* female × TE- male 95% Confidence Interval = -10.25, 11.82).

We found that the reciprocal TE+ female × *D. lummei* male cross also produced fewer progeny compared to the intra-strain (TE+) control cross (mu=50.52; P<0.0001; β=-31.37; P<0.0001), in this case 19 fewer progeny. Taking into account intra-strain and maternal effects, for the TE+ female × *D. lummei* male cross, the relative reduction in progeny production was 62%; whereas for the reciprocal cross (*D. lummei* × TE+) progeny production was reduced by 50%. We tested whether the relative reduction in viability differed between these reciprocal crosses but found they were not significantly different from one another (t=1.547; df=41;P=0.1527). In both reciprocal crosses involving *D. lummei* and TE+, the proportion of embryos hatched was significantly lower than the proportion of embryos fertilized, indicating that the reduced number of progeny was due to early embryo lethality in both cases (*D. lummei*×TE+, χ_1^2 = 6.15, P=0.01; TE+×*D. lummei*, χ_1^2=4.76, P=0.02; Table 2). In only one cross direction did we observe some level of sex specific inviability. In the TE+ female × *D. lummei* male cross, inviability was also specifically increased for males, resulting in a significant excess of females and a sex ratio that was 63% female (β=0.1171; P<0.0001; Fig 3). Finally, the cross between TE+ females and *D. lummei* males produced rare male gonad atrophy, that was significantly different than zero (μ=0.00, β=0.063, t=3.33, P=0.001). This frequency is similar to the intraspecies non-dysgenic cross, where the TE+ female is also crossed with a male that does

not carry inducing TEs. We saw no atrophy in the reciprocal *D. lummei* × TE+ cross where gonad atrophy might be expected based on intraspecific dysgenic phenotypes (Fig 2).

Backcrosses indicate that both TEs and genic interactions contribute to F1 hybrid incompatibility phenotypes

Together our data for F1s suggested that there are possibly two mechanisms contributing to hybrid incompatibilities in these crosses. In particular, the TE model predicts asymmetric reproductive isolation, specifically reduced F1 fitness, in the TE+ female × *D. lummei* male cross. Therefore, our observation of F1 inviability in both crossing directions suggests an additional mechanism contributing to postzygotic isolation in the *D. lummei* female × TE+ male cross. Alternatively, the observed F1 hybrid inviability could be due to a genic autosome-autosome mechanism acting in both cross directions, without a contribution from TEs. We were able to differentiate between these (and more complex) models using a series of backcrosses.

In these experiments, we examined progeny production and sex ratio in backcrosses between *D. lummei* females and F1 males that were created in both reciprocal directions between *D. lummei* and the TE+ strain. For the TE copy number model we expect rescue of progeny viability, and no sex specific lethality, when F1 males from either initial crossing direction are used as the male parent. For an autosome-autosome model or X-autosome model, we expect ~26 progeny to be produced, which is intermediate in progeny production compared to the *D. lummei* female × TE+ male control cross (where ~17.5 progeny were produced) and the intrastrain control cross (where ~34.5 progeny were produced). Using a one-sample *t-test* we determined that both the F1 male carrying the *D. lummei* X chromosome (μ=26, $\bar{X}$ =39.7, t=2.61, df=16, P=0.009) and the F1 male carrying the X chromosome from the TE+ (μ=26, $\bar{X}$ =38.4, t=2.22,

df=11, P=0.023) produced significantly more progeny when crossed with the *D. lummei* female than the expected 26 progeny under the two-locus incompatibility model (Table 3). Instead, in both backcrosses the observed number of progeny was not significantly different than the *D. lummei* × *D. lummei* control cross, and represented a complete rescue of viability. The magnitude of this rescue is consistent with the TE copy number model but not the two-locus incompatibility model, whether based on X-autosome or autosome-autosome interactions (Table 3; Fig 3).

Two-locus genic incompatibility models might be an oversimplification when considering hybrid incompatibilities, so we also considered models with higher order interactions (greater than two independent/freely-recombining loci). For a three-locus autosomal interaction the greatest expected rescue would be 1/4 of the original effect (therefore 9.5% dysgenesis with TE- females and ~30.26 progeny produced with D. *lummei* females), while for a four-locus autosomal interaction the largest rescue would be 1/8 the original effect (4.75% dysgenesis with TE- females and 32.38 progeny produced with *D. lummei* females) (Supplemental Table 2). Using a one sample t-test we rejected the hypothesis that our data can be explained by a three-locus incompatibility for gonadal dysgenesis (H0: μ<9.5; t= -627.2, df = 10, P< 0.0001) and for number progeny produced (H0: μ>30.26; t=2.36, df=28, P=0.0126). We also rejected the hypothesis for the four-locus incompatibility for gonadal dysgenesis (H0: μ<4.75; t= -311.48, df = 10, P<0.0001) and number progeny produced (H0: μ >32.38; t=1.802, df=28, P=0.041). We did not explore interactions beyond four loci because *D. virilis* only has four major autosomes and, since we used F1 males that lack recombination, each chromosome is transmitted as a single locus in our crosses.

In addition to progeny production, we also had data on sex ratio in our backcrosses. Interestingly, there was a significant deviation from 50% female sex ratio (β =0.25; p=0.037) in

the cross involving *D. lummei* females and F1 males that carried the X chromosome from TE+. This deviation could be caused by a hybrid incompatibility, however neither our TE incompatibility model nor the specific X-autosome incompatibility model predict sex-specific effects past the F1 generation (see methods). Since we had already observed a pattern of sex-specific inviability from the cross between TE+ females and *D. lummei* males (see above, Fig. 3), we also used the backcross data to determine the genetic factors responsible for this additional male-specific hybrid inviability (Fig. 4). The only two other crosses where we observed a skewed sex ratio were also female-biased and involved F1 males that carried the X chromosome from TE+ and the Y chromosome from *D. lummei,* crossed with *D. lummei* (β=-0.252, z=-2.08, P=0.037) or TE- females (β=-0.273, z=-3.491, P<0.001). In each of these three crosses, males had different X chromosome genotypes but consistently had the *D. lummei* Y chromosome. From this, we conclude that this additional incompatibility is caused by an autosome-Y interaction.

The classic dysgenesis system conforms to the TE model of incompatibility

We expected that the classic dysgenic cross (TE- female × TE+ male) within *D. virilis* would be consistent with a TE model of incompatibilities, based on previous work (Lozovskaya et al. 1990 Vieira et al. 1998). Nonetheless, we recapitulated the originally reported crosses and interspecific backcrosses to confirm this expectation and to compare our inferences in parallel with interspecific crosses.

In the F1, we expected to observe dysgenesis only when the TE- strain was used as the female parent and the genome strain was used as the TE+ parent. As expected, we observed 38% testes atrophy (Fig. 2), a frequency that was significantly greater than the TE- intra-strain cross

(β=0.3807; P<0.0001); in the reciprocal non-dysgenic cross (TE+ female × TE- male) we observed rare gonad atrophy events at a frequency not significantly different from zero. We did not observe inviability in either reciprocal cross. The non-dysgenic cross produced significantly more offspring than the TE- female × TE- male intra-strain comparison (β=26.732; P=0.0136); this increased productivity may reflect some outbreeding vigor between these long-established lab lines. There was no deviation in the proportion of eggs hatching compared to the proportion fertilized in either cross, indicating there was no embryo inviability (TE-×TE, χ_1^2 = 1.62, P=0.20; TE+×TE-, χ_1^2=0.00, P=1.00; Table 2).

As with our interspecific crosses, to differentiate whether the observed patterns of dysgenesis were more consistent with a TE model or genic incompatibility models we used a directed backcross experiment using the same F1 male genotypes used in previous analyses (Lovavskaya et al. 1990) and in our interspecific experiments. For the TE model we expected (see Methods) to observe rescue of gonad atrophy in a backcross using F1 males because these males will have fewer TEs than required to cause dysgenesis (Supplemental Fig. 2 and 3). For the two-locus genic incompatibility models we expected this backcross to have half the amount of gonad atrophy observed in the F1s, because only 1/2 of their progeny would carry both incompatible alleles. Our baseline level of gonad atrophy—i.e., what we observed in F1 males of the classic intraspecific dysgenic cross (TE- × TE+ cross)—was 38%. We found that both backcrosses had significantly less than half (19%) this amount of gonad atrophy, results that are inconsistent with the two locus genic models. When F1 males carried the X chromosome from *D. lummei* the level of dysgenesis was not significantly different than zero (β=0.0264; P=0.1366). When F1 males carried the X chromosome from TE+ the level of dysgenesis was ~6%, significantly less than the TE+ parent (β=0.0638; P=0.0011; CI= 0.0261, 0.1016) and less

than 19% atrophy. Therefore, data for gonad atrophy in our backcrosses are consistent with strong rescue of fertility predicted from the TE incompatibility model.

Discussion

In this study, we used a powerful set of crosses to test whether the presence of TEs influences the magnitude of reproductive isolation. Using the *D. virilis* dysgenic system we predicted that the *D. virilis* strain carrying dysgenic-inducing TEs should show greater postzygotic reproductive isolation when crossed with *D. lummei* lacking these TEs, compared to the cross involving the *D. virilis* strain that lacks dysgenic-inducing TEs. We indeed observed this pattern of elevated postzygotic isolation specifically in the TE+ interspecific cross, along with other more nuanced differences between our focal crosses. Taken together, our genetic data from both F1 and backcross experiments support the inference that TEs contribute to elevated reproductive isolation observed in the interspecific cross that involves dysgenic TEs, and are inconsistent with alternative hybrid incompatibility models to explain this elevated isolation. In addition, with genomic data we also assessed which candidate TE might be responsible for observed dysgenesis patterns among inducing *D. virilis* strains compared to non-inducing *D. virilis* and *D. lummei*, and confirmed previous reports that *Polyphemus* is a strong candidate for causing dysgenesis (Funikov *et al.* 2018; Hemmer *et al.* 2019).

A role for accumulated selfish genetic elements in the expression of postzygotic reproductive isolation is consistent with the Dobzhansky-Muller model of hybrid incompatibility (Johnson 2010; Castillo and Moyle 2012; Crespi and Nosil 2013), but little emphasis has been placed on the difficulty of disentangling the effects of these selfish elements from the effects of other hybrid incompatibilities. For example, one way TEs are thought to contribute to

reproductive isolation is through transcriptional misregulation that causes sterility or inviability (Martienssen 2010; Michalak 2010; Dion-Cote *et al.* 2014). However, this is not a unique feature of TE-based hybrid incompatibility; divergence in trans and cis-regulatory elements are common and can also cause misregulation and hybrid incompatibilities, via ordinary genic effects (reviewed in Mack and Nachman 2017). To disentangle TE-specific from these other effects it would therefore also be necessary to differentiate whether TE misregulation is symptomatic of more general misregulation in hybrids or if TE divergence itself drives global misregulation. Instead of focusing on analyzing patterns of misregulation, here we took the approach of isolating phenotypic effects due to TEs by using strains with defined differences in TE abundance, and potential activity, making explicit predictions about which hybrid classes should exhibit increased reproductive isolation, and evaluating these using classical genetic crosses.

The strength of this approach relies on our ability to make and test predictions that are exclusive to a TE incompatibility model, compared to alternative genic models, in parallel for two crosses. We observed that postzygotic incompatibility occurred only in crosses involving the TE+ carrying *D. virilis* strain, and not in the cross between TE- *D. virilis* and *D. lummei,* clearly implicating the presence of TEs in this postzygotic isolation. We were able to support this inference by using backcrosses, and comparing these results to parallel crosses examining intraspecies dysgenesis. Our ability to disentangle the effects of TEs vs non-TE incompatibilities in backcross offspring rested on the observation that F1 males failed to produce dysgenic sons when crossed to TE- females (Lovoskaya et al. 1990), leading to a hypothesis specific to the TE incompatibility model. The mechanism we propose based on previous studies (Vieira *et al.* 1998; Srivastav and Kelleher 2017; Serrato-Capuchina *et al.* 2020b), is that F1s have sufficiently low copy number of TEs such that dysgenesis does not occur when F1 males are themselves used as

fathers. This pattern allowed us to directly compare the TE incompatibility model with genic incompatibility models because they make contrasting predictions about the level of incompatibility expected, irrespective of the specific incompatibility phenotype observed. We found that crosses between F1 males and TE- females and F1 males and *D. lummei* females produced largely concordant results. Both incompatibility phenotypes—atrophied testes in crosses between TE- females and F1 males, and viability in crosses between *D. lummei* females and F1 males—were completely rescued, which is consistent with the TE incompatibility model. Significantly greater rescue was observed in these crosses than predicted by any of the genic incompatibility models. Results from these backcrosses also enabled us to determine the basis of additional, non-TE, interactions contributing to the incompatibility observed specifically in the TE+ × *D. lummei* cross. This inviability was male specific, suggested by a female biased sex ratio, and results from an autosome-Y incompatibility. Together these observations support the operation of two distinct mechanisms of hybrid inviability in our crosses, one of which directly implicates TEs in the expression of this postzygotic isolating barrier.

A second inference from our results is that TEs can cause different hybrid incompatibility phenotypes—that is, sterility or inviability—depending on context. Because TE misregulation can cause substantial cellular or DNA damage, it has the potential to produce multiple different incompatibility phenotypes (Tasnim and Kelleher ; Phadnis N. *et al.* 2015). Although the mechanistic link between damage and hybrid incompatibility needs further exploration, here our observations suggest that the occurrence of hybrid sterility vs hybrid inviability in different (intra- versus inter-specific) TE+ crosses could result from misregulation of the same TEs at different developmental stages. While transposable elements have typically been thought to restrict their damage to the germline, this is not always the case, including in *D. virilis* system

where germline and somatic expression have been demonstrated for *Penelope* (Blumentstiel and Hartl 2005). Direct evidence that TEs that can cause inviability phenotypes when active in somatic tissue comes from a *P-element* mutant engineered to have expression in somatic cells (Engels *et al.* 1987); crosses involving this somatically-expressed *P-element* mutant line showed inviability that was dependent on *P*-element copy number. These observations indicate it is mechanistically plausible that dysgenic elements can be responsible for both inviability and sterility phenotypes. The involvement of TEs in both germline and somatic effects in hybrids could provide a direct mechanistic connection (via TE activity) between intraspecies dysgenesis and interspecies reproductive isolation.

Finally, our observations also identify a mechanism that could contribute to variation among intraspecific strains in the strength of their isolation from other species. Evidence for polymorphic incompatibilities is typically inferred from the observation of among-population variation in isolation phenotypes when crossed with a second species. Although the specific alleles underlying this variation are usually unknown (Reed and Markow 2004; Kozlowska *et al.* 2012), these polymorphic incompatibility loci are typically assumed to be based on genic Dobzhansky-Muller incompatibilities (Cutter 2012). In this study, we show that the presence/absence of TEs segregating in populations and species can also contribute to intraspecific variation in the strength of isolation between species. Moreover, the rapid pace with which TE abundance and identity can change within a lineage also indicates that such differences could rapidly accumulate between species; this suggests that this mechanism is most likely to influence species pairs in which there is dynamic turnover in the identity of their active TEs.

Overall, here we provide evidence for a role of TEs in increased reproductive isolation between lineages that differ in the presence of specific active TE families, by leveraging known

TE biology to differentiate TE vs non-TE effects in a set of targeted crosses. The generality of these results could be addressed by focusing on other *a priori* cases where species are known to vary in the number or identity of TEs. For instance, increased reproductive isolation caused by transposable elements was recently reported in crosses between *D. simulans*, that are polymorphic for *P-elements*, and a sister species, *D. sechellia*, that completely lack *P-elements* (Serrato-Capuchina et al. 2020a). Similar studies that examine lineages at a range of stages of TE differentiation would be particularly valuable for addressing the contribution of TEs to the accumulation of hybrid incompatibilities over evolutionary time, especially in comparison to 'ordinary' genic incompatibilities. At present, our and other recent (Serrato-Capuchina et al. 2020a), findings suggest that the rapid lineage-specific evolution of TEs could potentially explain the frequent observation of polymorphic incompatibilities in species that experience dynamic changes in selfish genetic elements, and could therefore represent one of the earliest arising mechanisms of postzygotic reproductive isolation in these groups.

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Table 1. Average trait means for control intrastrain crosses. These values are used as baseline in statistical models testing for increased reproductive isolation in interspecis crosses. For male dysgensis (n) refers to the number of male progeny scored. se=standard error. The number of replicates used to calculate the standard error are n=22 (TE-), n=23 (TE+), and n=20 (*D. lummei*)

Female	Male	Dysgenesis (n)	Sex Ratio (se)	Viability (se)
TE-	TE-	0.0 (1398)	0.488 (0.016)	0.844 (0.055)
TE+	TE+	0.0 (575)	0.516 (0.014)	0.571 (0.057)
D. lummei	*D. lummei*	0.0 (335)	0.521 (0.015)	0.411 (0.067)

Table 2. F1 postzygotic reproductive isolation, as observed in crosses between TE+ and *D. lummei,* is a product of embryo inviability. Inviability is inferred when fewer eggs hatch than expected based on the proportion of eggs that initiated development (proportion fertilized). The samples sizes (n) are the pooled number of embryos examined across replicate crosses. † represents a significant difference in the proportion fertilized for an interspecific cross compared to the control cross of the maternal strain (P<0.05).

Female	Male	Proportion Fertilized (n)	Proportion Hatched (n)	χ_1^2 (P-value)
TE-	TE-	0.85 (179)	0.79 (344)	2.25 (0.13)
TE+	TE+	0.58 (155)	0.55 (224)	0.25 (0.61)
D. lummei	*D. lummei*	0.61 (218)	0.67 (260)	1.75 (0.21)
TE-	TE+	0.85 (180)	0.80 (283)	1.62 (0.20)
TE+	TE-	0.57 (153)	0.57 (206)	0.00 (1.00)
TE-	*D. lummei*	0.33† (389)	0.39 (79)	0.90 (0.34)
D. lummei	TE-	0.60 (161)	0.75 (337)	10.76 (0.001)
TE+	*D. lummei*	0.55 (126)	0.42 (235)	4.76 (0.02)
D. lummei	TE+	0.43† (212)	0.31 (208)	6.15(0.01)

Table 3. Progeny production is rescued when F1 males are crossed to *D. lummei* females. For the backcrosses we tested whether progeny rescue was greater than the expectation for two locus genic incompatibility models (26 progeny produced). F1-1 are males from the *D. lummei* × TE+ cross and F1-2 males are from the reciprocal cross. For the progeny produced we report the number of replicate crosses (n) and the standard error (se). *indicates a significant difference in progeny production from the intrastrain control.

Female	Male	Mean Progeny Produced (n, se)	t statistic (df)	P-value
D. lummei	*D. lummei*	34.50 (20, 4.28)	NA	NA
D. lummei	TE+	17.54* (22, 2.78)	NA	NA
D. lummei	F1-1	39.76 (17,5.26)	2.61 (16)	0.009
D. lummei	F1-2	38.41 (12, 5.56)	2.22 (11)	0.023

Figure 1. Identification of dysgenic causing transposable elements in *D. virilis*. A) Candidate TEs have higher copy number in the inducing Strain 160 compared to the noninducing Strain 9 (TE-). Red dashed line represents 2-fold increase in copy number. B) Clustering of candidate TEs across strains of *D. virilis*. C) Positive relationship between the copy number of *Penelope* and *Polyphemus* across *D. virilis* strains.

Figure 2. Gonad atrophy characteristic of the classic dysgenic phenotype. A) Intrastrain control crosses, intraspecific crosses, and interspecific crosses demonstrating the lack of dysgenesis except in the intraspecific cross. B) Dysgenesis in backcross progeny compared to TE- × TE+ cross showing that the F1 males do not induce dysgenesis to the same extent as the pure TE+ males. F1-1 are males from the *D. lummei* × TE+ cross and F1-2 males are from the reciprocal cross. The thick red line is the median, and thin red lines the inter-quartile range for each distribution * indicates significantly different from zero ($P<0.05$). ** indicates significant difference between TE+ and F1 males ($P<0.05$). The dashed line represents the expected effect size based on the two-locus incompatibility models.

Figure 3. The number of progeny produced for each cross demonstrating significantly fewer progeny produced in crosses between *D. lummei* and TE+ in both directions. A) Intrastrain control crosses, intraspecific crosses, and interspecific crosses. B). Backcross progeny compared to the *D. lummei* intrastrain cross showing that the F1 males do not induce progeny lethality, and progeny production is rescued. F1-1 are males from the *D. lummei* × TE+ cross and F1-2 males are from the reciprocal cross. The thick red line is the median, and thin red lines the inter-quartile range for each distribution. * after a specific letter indicates significantly different from the intrastrain cross of the maternal line ($P<0.05$).

Figure 4. The sex ratio of each cross used to examine deviations from a 50:50 sex ratio caused by sex specific lethality. A) Intrastrain control crosses, intraspecific crosses, and interspecific crosses. B). The sex ratio of backcross progeny showing sex biased progeny ratios occur when *D. lummei* Y is in combination with some complement of TE+ autosomes. F1-1 are males from the *D. lummei* × TE+ cross and F1-2 males are from the reciprocal cross. The thick red line is the median, and thin red lines the inter-quartile range for each distribution * indicates significant deviation from 50:50 sex ratio ($P<0.05$).

Figure 1

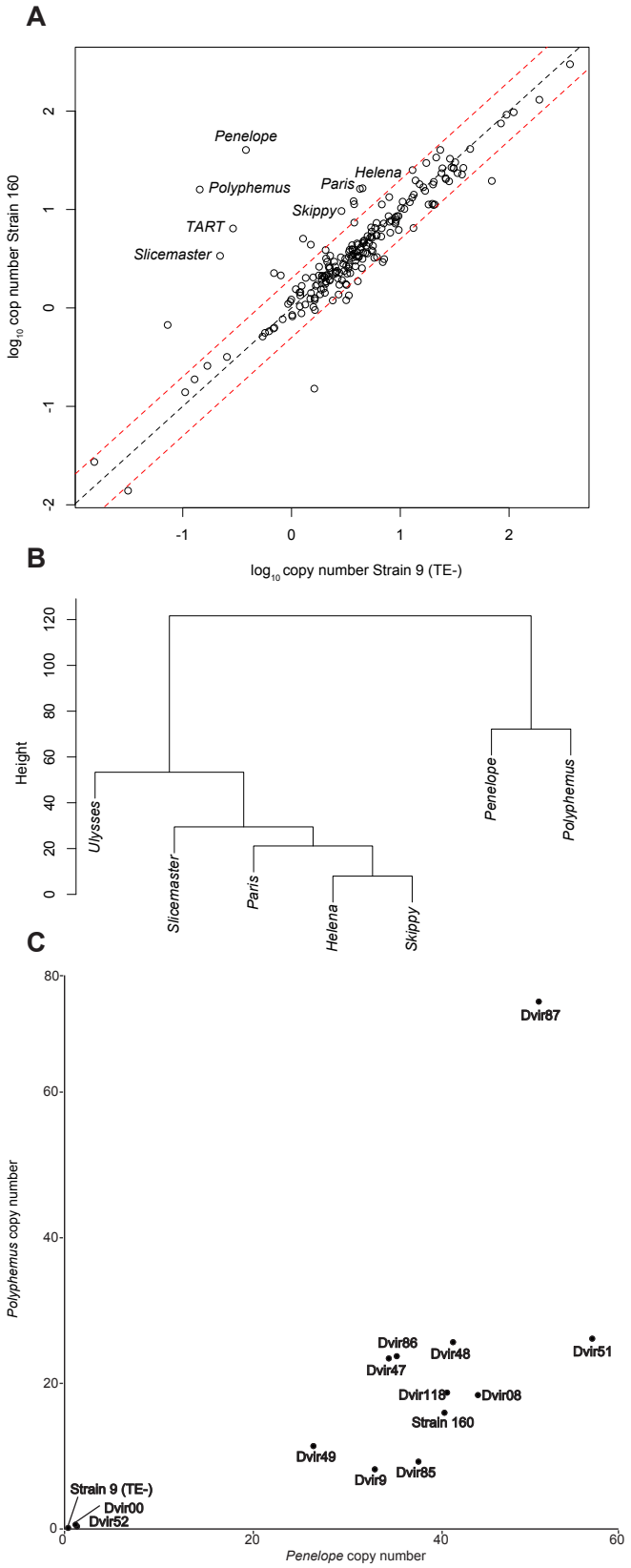

A
Penelope
Polyphemus
Helena
Paris
Skippy
TART
Slicemaster
log10 cop number Strain 160
log10 copy number Strain 9 (TE-)
B
Height
Ulysses
Slicemaster
Paris
Helena
Skippy
Penelope
Polyphemus
C
Polyphemus copy number
Penelope copy number
Dvir87
Dvir86
Dvir48
Dvir51
Dvir47
Dvir118
Dvir08
Strain 160
Dvir49
Dvir9
Dvir85
Strain 9 (TE-)
Dvir00
Dvir52

Figure 2

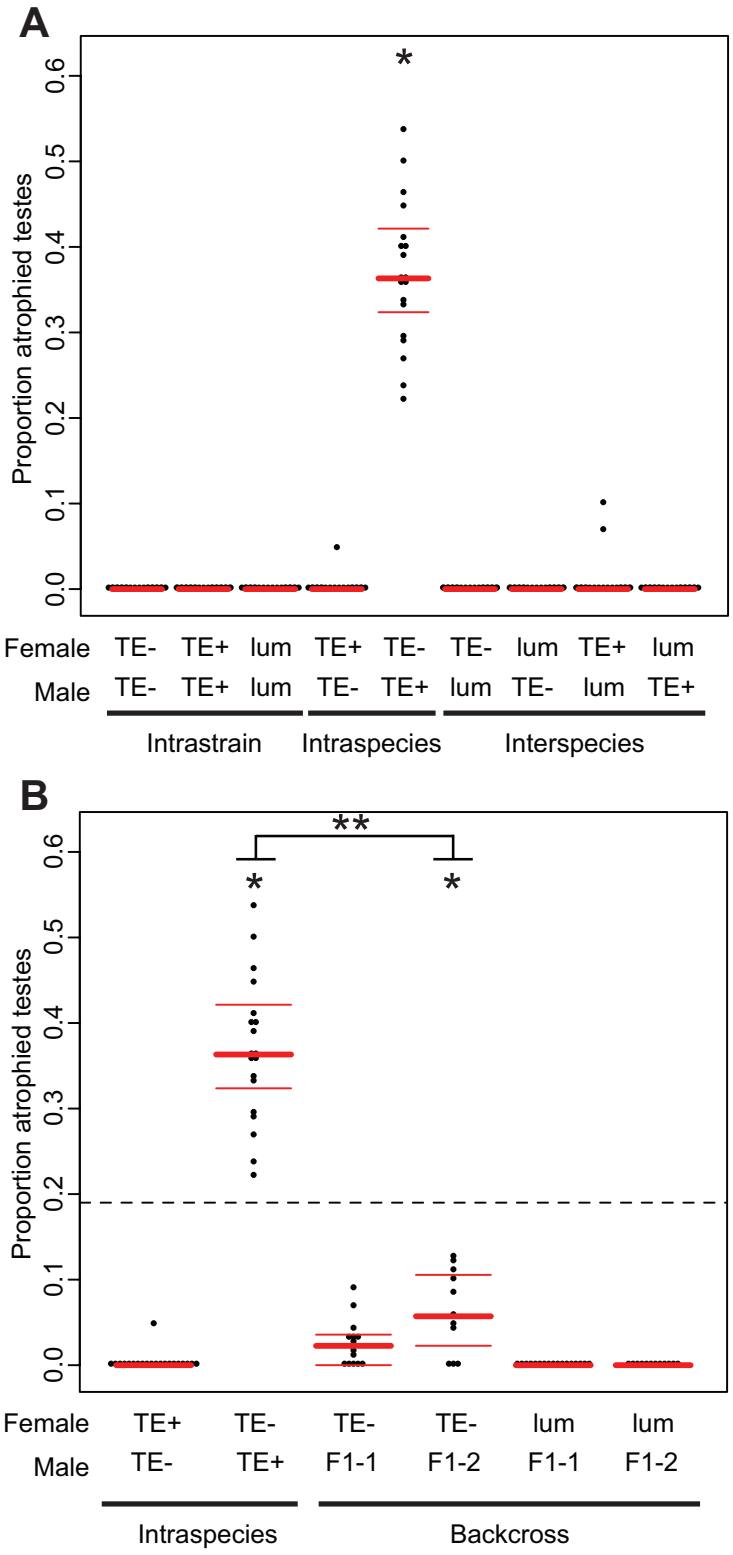

A
Proportion atrophied testes
0.0
0.1
0.2
0.3
0.4
0.5
0.6
*
Female TE- TE+ lum TE+ TE- TE- lum TE+ lum
Male TE- TE+ lum TE- TE+ lum TE- lum TE+
Intrastrain
Intraspecies
Interspecies
B
Proportion atrophied testes
0.0
0.1
0.2
0.3
0.4
0.5
0.6
**
*
*
Female TE+ TE- TE- TE- lum lum
Male TE- TE+ F1-1 F1-2 F1-1 F1-2
Intraspecies
Backcross

Figure 3

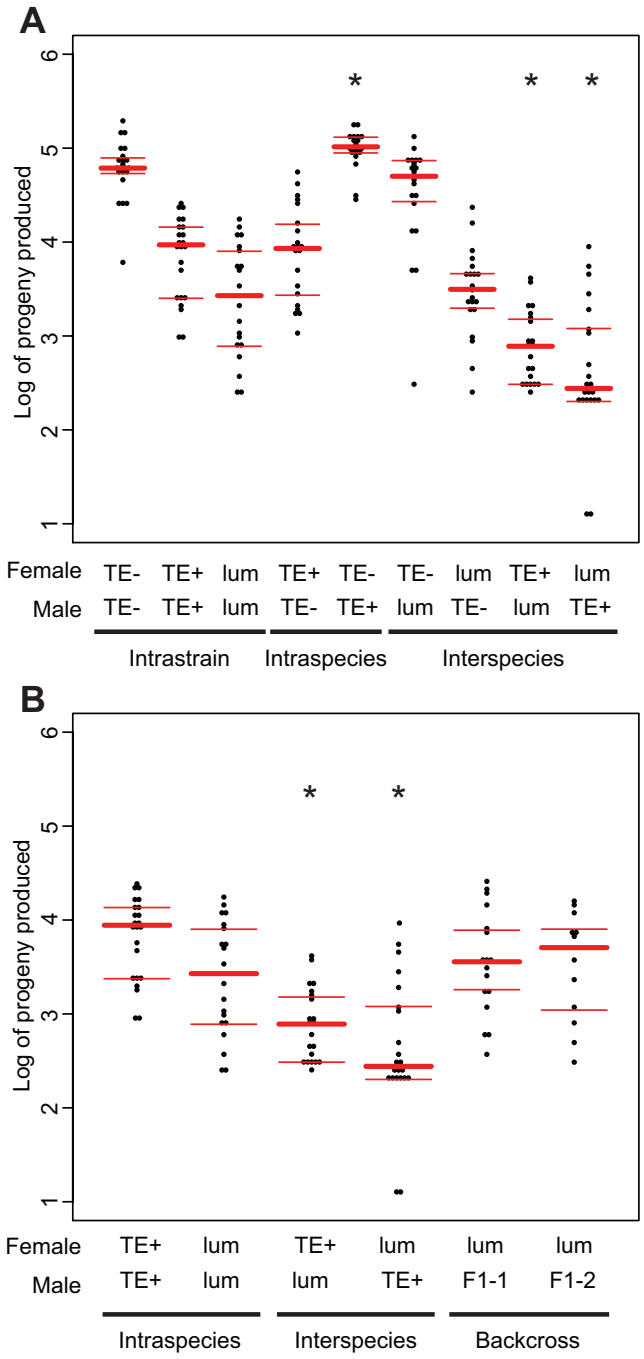
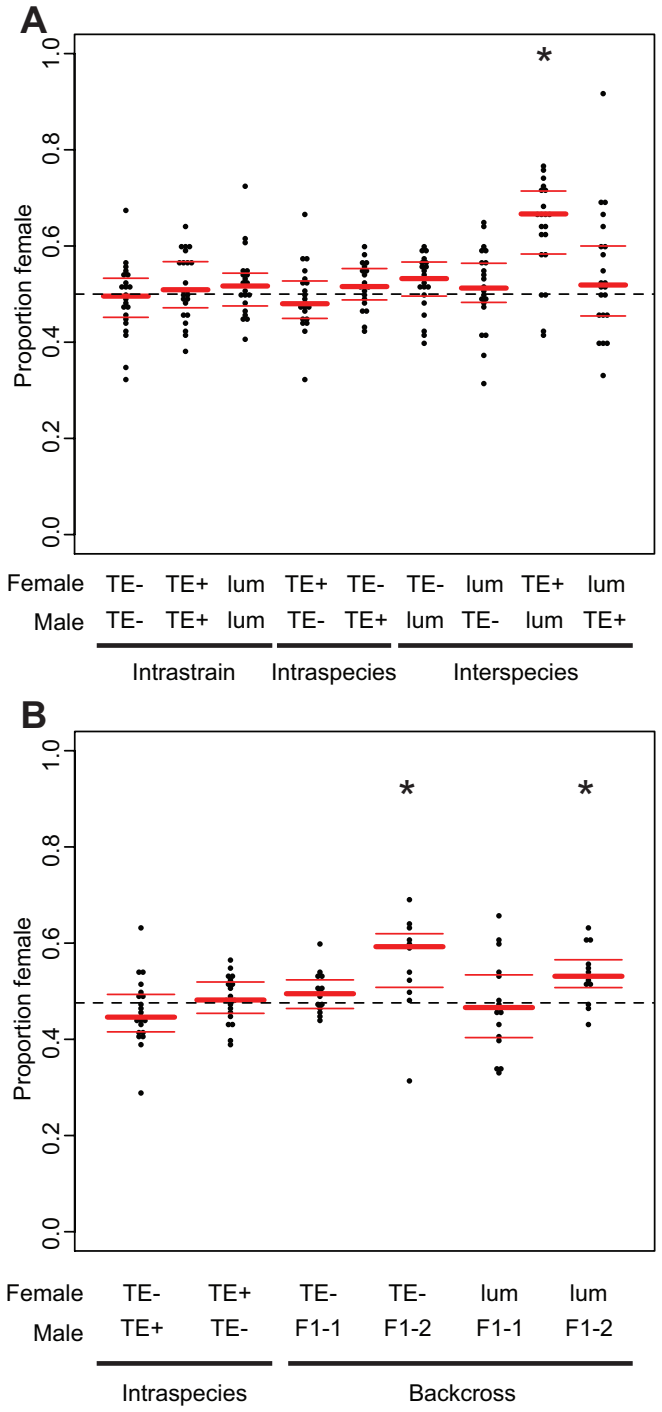

A
Log of progeny produced
1
2
3
4
5
6
*
*
*
Female
Male
TE-
TE-
TE+
TE+
lum
lum
TE+
TE-
TE-
TE+
TE-
lum
lum
TE-
TE+
lum
lum
TE+
Intrastrain
Intraspecies
Interspecies
B
Log of progeny produced
1
2
3
4
5
6
*
*
Female
Male
TE+
TE+
lum
lum
TE+
lum
lum
TE+
lum
F1-1
lum
F1-2
Intraspecies
Interspecies
Backcross

Figure 4


A
Proportion female
0.0
0.2
0.4
0.6
0.8
1.0
*
Female TE- TE+ lum TE+ TE- TE- lum TE+ lum
Male TE- TE+ lum TE- TE+ lum TE- lum TE+
Intrastrain
Intraspecies
Interspecies
B
Proportion female
0.0
0.2
0.4
0.6
0.8
1.0
*
*
Female TE- TE+ TE- TE- lum lum
Male TE+ TE- F1-1 F1-2 F1-1 F1-2
Intraspecies
Backcross