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Rapid evolution of a Y-chromosome heterochromatin protein underlies sex chromosome meiotic drive

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Sex chromosome meiotic drive, the non-Mendelian transmission of sex chromosomes, is the expression of an intragenomic conflict that can have extreme evolutionary consequences. However, the molecular bases of such conflicts remain poorly understood. Here, we show that a young and rapidly evolving X-linked heterochromatin protein 1 (HP1) gene, *HP1D2*, plays a key role in the classical Paris sex-ratio (*SR*) meiotic drive occurring in *Drosophila simulans*. Driver *HP1D2* alleles prevent the segregation of the Y chromatids during meiosis II, causing female-biased sex ratio in progeny. *HP1D2* accumulates on the heterochromatic Y chromosome in male germ cells, strongly suggesting that it controls the segregation of sister chromatids through heterochromatin modification. We show that Paris *SR* drive is a consequence of dysfunctional *HP1D2* alleles that fail to prepare the Y chromosome for meiosis, thus providing evidence that the rapid evolution of genes controlling the heterochromatin structure can be a significant source of intragenomic conflicts.

meiotic drive | intragenomic conflict | heterochromatin | sex chromosomes

Meiosis is a fundamental step underlying sexual reproduction, because it allows equal segregation of chromosomes and alleles. However, various genetic parasitic elements can take advantage of this process by promoting their own transmission at the expense of other components of the genome. Among them, segregation distorters acting in heterozygous individuals are known in a variety of organisms (1). They disrupt meiosis or kill the alternative meiotic products and therefore, end up in a majority, if not all, of the functional gametes, often with deleterious effects on fertility. When segregation distorters are sex-linked and expressed in the heterogametic sex, they cause a bias in offspring sex ratio (2). X-linked distorters in *Drosophila*, called *sex-ratio* (hereafter *SR*), typically cause a Y-bearing sperm shortage, with impact on male sterility. *SR* males sire a large excess of females (90–100%) carrying the X^{SR} chromosome. The spread of X^{SR} in populations triggers an extended genetic conflict between the X chromosome and the rest of the genome over the skewed sex ratio. Y-resistant chromosomes and autosomal suppressors are, thus, predicted to arise and counteract the distorters (3, 4). Such a scenario has occurred recurrently in *Drosophila simulans*, which harbors at least three independent *SR* systems (Paris, Winters, and Durham) that are all cryptic or nearly cryptic because of the evolution of suppressors (5). This finding supports the hypothesis of an endless arms race between X^{SR} chromosomes and the rest of the genome (6, 7), with potential important consequences on sex chromosomes and genome evolution (5, 8), mating system (9), or even speciation through the evolution of hybrid incompatibilities (10, 11).

The Paris *SR*, which results from the missegregation of Y chromatids in anaphase II (12) (Fig. 1A), involves the cooperation of two X-linked driver loci (Fig. 1B). The first locus colocalizes with the tandem duplication of a 37-kb segment spanning six genes [hereafter duplication *SR* (Dp^{SR})]. The second locus (*Wlasta*) was mapped about 110 kb away from Dp^{SR} within a 42-kb interval showing no large rearrangements (13, 14). Here, we show

that *Wlasta* is heterochromatin protein 1 D2 (*HP1D2*), a young member of the HP1 gene family, and we characterize *HP1D2* alleles that cause the drive.

Results and Discussion

Genetic Identification of *HP1D2* as *Wlasta*. To identify *Wlasta*, we performed an ultrafine genetic mapping using recombination between a strong distorter X^{SR4} chromosome (~93% of daughters on average) and a standard (ST) X chromosome carrying the *singed* (*sn*) and *lozenge* (*lz*) markers ($X^{sn\ lz}$; ~50% of daughters on average) that frame the two driver loci (Fig. 1B). We generated 1,740 (+ *lz*) recombinant chromosomes, and among them, 10 have a breakpoint within the candidate region showing either an *SR* phenotype ($X^{+lz}_{[SR]}$; 80–100% of daughters on average) or an ST phenotype ($X^{+lz}_{[ST]}$; 45–60% of daughters on average). The positions of the recombination breakpoints enabled us to map *Wlasta* within a 4.5-kb interval in X^{SR4} (Fig. 1C) overlapping three genes: *Spirit*, *CG12065*, and *HP1D2* (*GD16106*). The last one, a member of the HP1 gene family, is possibly involved in heterochromatin organization (15), and two members of this gene family have been shown to affect chromosome segregation (16, 17). *HP1D2* is predicted to encode a chromatin-interacting protein with an N-terminal chromo domain that enables interactions with histones and a C-terminal chromo shadow domain (CSD) mediating protein–protein interactions. Interestingly, only *HP1D2* was entirely included within the 4.5-kb region (Fig. 1C). We sequenced and compared the

Significance

Intragenomic conflict between the sex chromosomes is a strong evolutionary force. It can arise through the evolution of sex chromosome meiotic drive, where selfish genes located on the X chromosome promote their own transmission at the expense of the Y chromosome. Sex chromosome drive occurs in *Drosophila simulans*, where Paris drive results from segregation failure of the heterochromatic Y chromosome during meiosis II. Here, we show that Paris drive is caused by deficient alleles of the fast-evolving X-linked heterochromatin protein 1 D2 (*HP1D2*) gene. Our results suggest that dysfunctional *HP1D2* alleles promote their own transmission, because they do not prepare the Y chromosome for meiosis. This finding shows that the rapid evolution of genes involved in heterochromatin structure can fuel intragenomic conflict.

Author contributions: Q.H., P.R.G., R.D., B.L., and C.M.-M. designed research; Q.H., P.R.G., R.D., D.O., and B.P. performed research; Q.H., P.R.G., R.D., B.L., and C.M.-M. analyzed data; and Q.H., P.R.G., R.D., B.P., B.L., and C.M.-M. wrote the paper.

The authors declare no conflict of interest.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. KU527662–KU527680).

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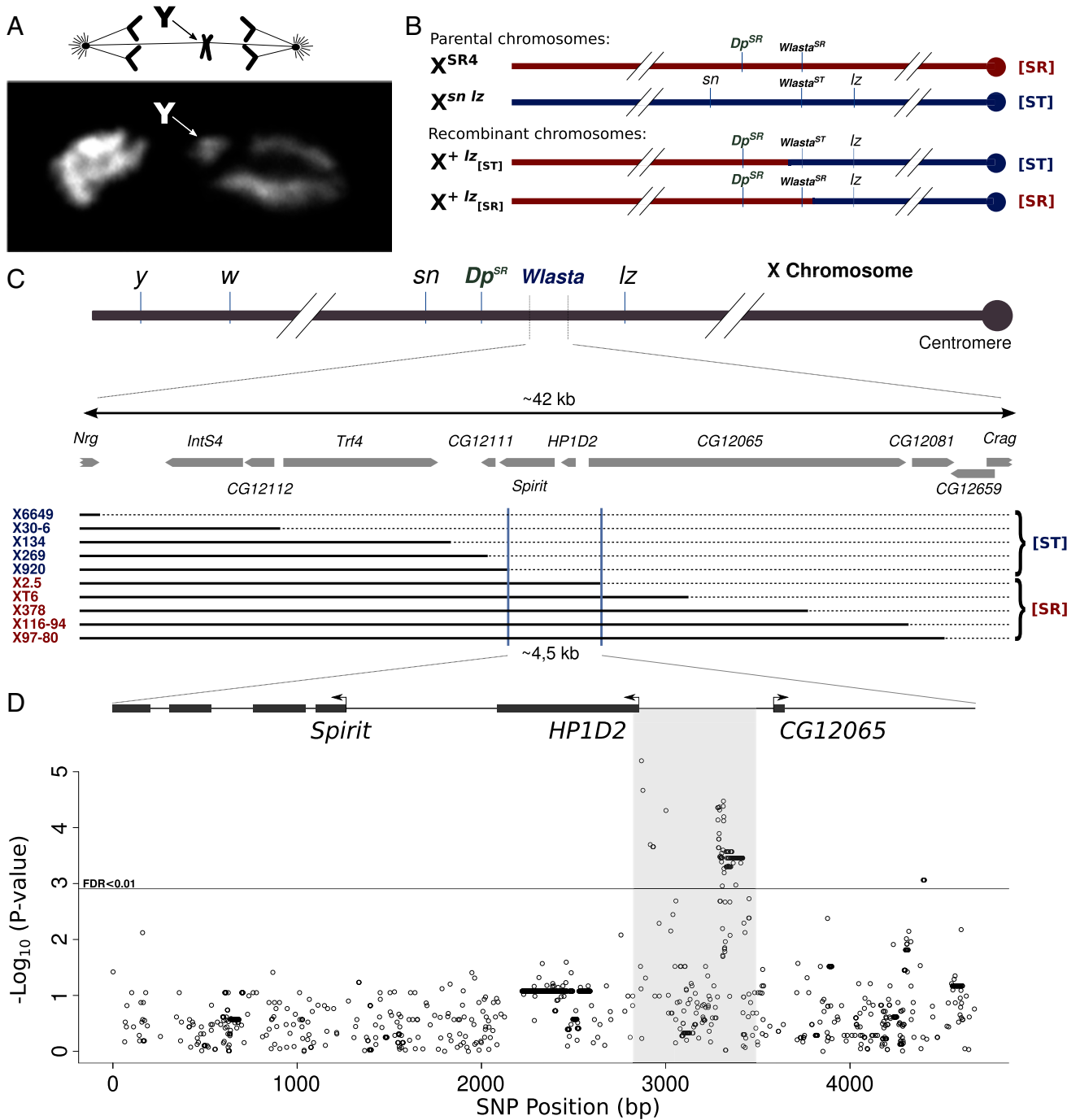


Fig. 1. Mapping of the *Wlasta* locus from the Paris SR system. (A) Cellular phenotype of the Paris SR trait: lagging Y chromosomes during anaphase of meiosis II. (B) Parental chromosomes used to map the *Wlasta* locus: X^{SR4} carries Dp^{SR} and a driver allele at the *Wlasta* locus and shows a strong and stable SR phenotype. $X^{sn\ lz}$ carries the *sn* and *lz* mutations and is associated with an ST phenotype (equal sex ratio). The phenotype of the recombinant chromosomes that retain Dp^{SR} only depends on the *Wlasta* allele; they are named $X^{+ lz}_{[ST]}$ for recombinants with an ST phenotype and $X^{+ lz}_{[SR]}$ for recombinants with an SR phenotype. (C) Phenotype of recombinant chromosomes $X^{+ lz}$ with breakpoints located within the 42-kb candidate region. Solid lines indicate X^{SR4} chromosome, and dashed lines indicate $X^{sn\ lz}$. (D) Association mapping using 56 X chromosomes from natural populations, all carrying Dp^{SR} . Circles correspond to \log_{10} P value scores from a Spearman correlation test between the SR phenotype and the frequency of each SNP. The horizontal line indicates the significance threshold at a false discovery rate of 0.01 (52). The region rich in significant associated polymorphisms is in gray.

Wlasta candidate region between the two parental chromosomes and observed that X^{SR4} and $X^{sn\ lz}$ diverged by 88 SNPs and 16 small (<100-bp) insertions or deletions. Intriguingly, two larger deletions were detected on X^{SR4} . The first one spans 143 bp in the noncoding sequence between *HP1D2* and *CG12065*, and the second one removes one-half (371 bp) of the *HP1D2* coding sequence, resulting in a frameshift that prevents the translation of the CSD (Fig. 2B).

We then performed an association mapping using a collection of 56 X chromosomes from various geographic locations (SI Appendix, Table S1). All of them carried Dp^{SR} , but they showed different drive strengths, with a sex ratio ranging from 50% to 96% females in progeny. We sequenced the 4.5-kb *Wlasta* candidate region and analyzed the association between nucleotide polymorphism and the SR phenotype. Surprisingly, the noncoding

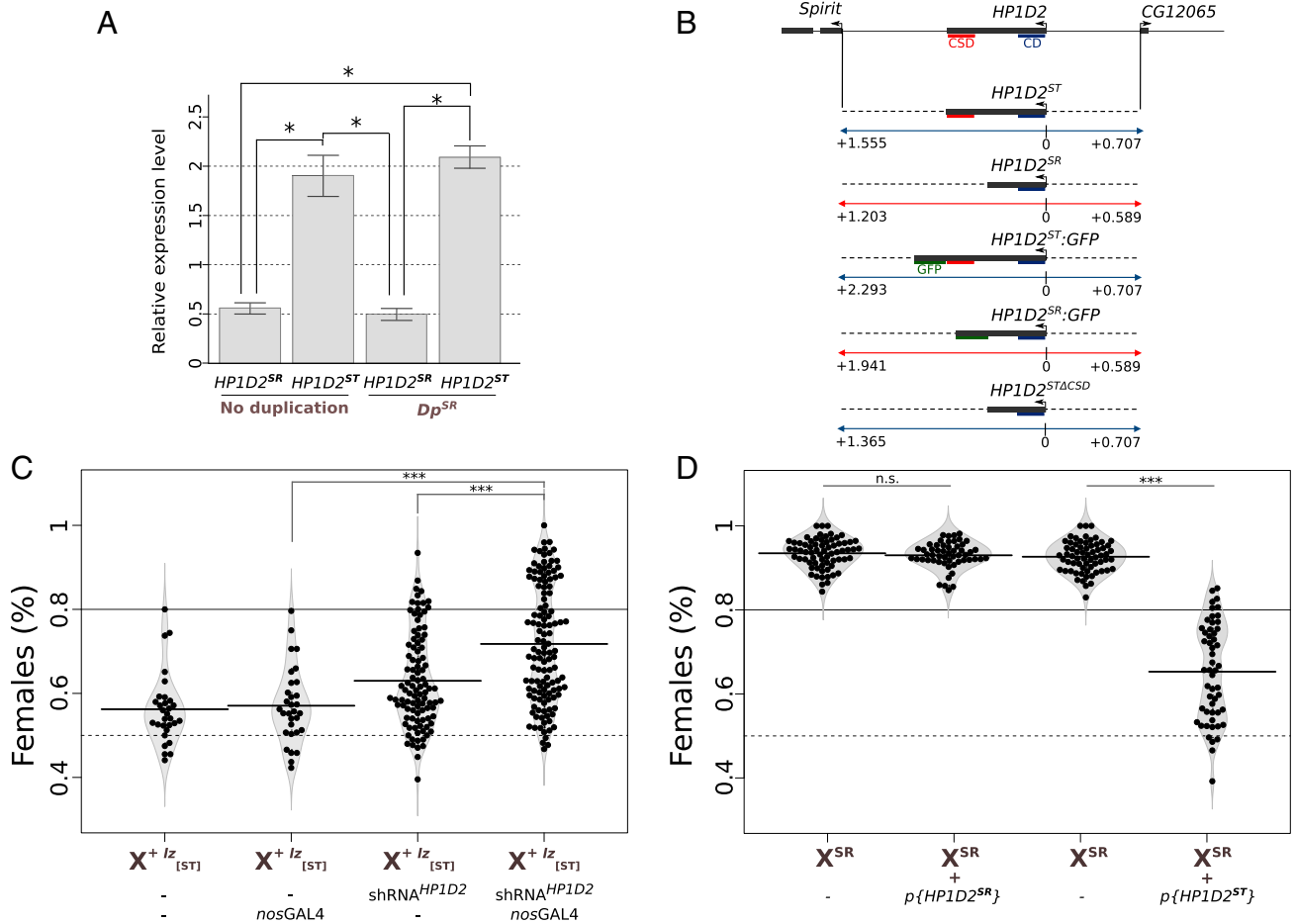


Fig. 2. *HP1D2* is involved in the Paris *SR* system. (A) Quantitative RT-PCR analysis of *HP1D2*ST and *HP1D2*^{SR} testicular expression in males carrying or not carrying *Dp*^{SR} (Materials and Methods). Error bars represent SEMs. **P* < 0.05 (Wilcoxon posthoc test using the Bonferroni correction). (B) Structure of the *HP1D2* transgenes. CSD is in red, Chromo Domain (CD) is in blue, and GFP is in green. Based on the first nucleotide of the coding sequence (indicated as position 0) of each allele, the lengths of upstream and downstream sequences are indicated (kilobases). (C) *SR* produced by X⁺Iz_[ST] male siblings that carry or do not carry an shRNA targeting *HP1D2* with or without the *nos*-Gal4 driver. Black bars represent the means. The horizontal dashed line indicates an equal sex ratio (0.5), and the black line indicates an arbitrary limit between strong and moderate sex ratio bias (0.8). ****P* < 0.001 (Wilcoxon test). (D) Sex ratios produced by X^{SR4} male siblings carrying or not carrying an extra autosomal copy of an *HP1D2*^{SR} or an *HP1D2*ST allele. n.s., not significant. ****P* < 0.001 with the Wilcoxon test.

sequence between *HP1D2* and *CG12065* was particularly enriched with highly significant associated polymorphisms (Fig. 1D). This finding suggested that *SR* drive might be caused by a variation in expression of one of the adjacent genes.

Drive Results from *HP1D2* Dysfunction. We measured, by quantitative RT-PCR, their testicular expression in males of different genotypes carrying X chromosomes from the recombination mapping experiment (Materials and Methods). We did not observe any significant variation in transcription level of *CG12065* regardless of the allele analyzed and the presence/absence of *Dp*^{SR} (Materials and Methods and SI Appendix, Fig. S1A). By contrast, the *HP1D2* allele from X^{SR4} was significantly less transcribed than the allele from X^{snIz}, regardless of the presence of *Dp*^{SR} (Fig. 2A).

To test the possibility that the *SR* phenotype could result from a reduction of *HP1D2* expression, we knocked down *HP1D2* through male germ-line expression of a specific shRNA using the upstream activation sequence system (UAS)/Gal4 (SI Appendix, Table S2). Although this knockdown only caused a slight reduction of *HP1D2* expression in the testis (SI Appendix, Fig. S1B), it resulted in a highly variable but clearly significant *SR* phenotype in males carrying a recombinant X⁺Iz_[ST] chromosome (Fig. 2C).

Then, to provide definitive evidence of the role of *HP1D2* in Paris *SR*, we introduced a transgenic copy of either *HP1D2*ST

(from X^{snIz}) or *HP1D2*^{SR} (from X^{SR4}) alleles into X^{SR4} males and measured progeny sex ratios. Strikingly, the presence of the *HP1D2*ST transgene strongly reduced the drive phenotype produced by X^{SR4} males (Fig. 2D). For its part, the *HP1D2*^{SR} transgene, with expression that was attested by GFP fluorescence, had no effect (SI Appendix, Fig. S2A). These results show that the *HP1D2*ST allele is dominant over the *HP1D2*^{SR} allele and can prevent the distortion. In addition, after deletion of its CSD coding sequence, which is lacking in *HP1D2*^{SR}, the modified allele *HP1D2*^{STΔCSD} (Fig. 2B) became unable to reduce the drive phenotype, suggesting that this domain is required to restore a proper segregation of Y chromatids (SI Appendix, Figs. S1C and S2B). Among the X chromosomes used in the association study, those carrying an *HP1D2* allele lacking CSD exhibit, on average, a higher but not significantly different drive ability (Wilcoxon rank sum test *W* = 273; *P* = 0.05188) (SI Appendix, Fig. S3A and B). Because both groups of chromosomes show large variation in drive strength, it is possible that the impact of ΔCSD is masked by the greater association in the regulatory sequence of *HP1D2*.

Overall, our results indicate that Paris *SR* drive is a consequence of dysfunctional *HP1D2* alleles that are less transcribed and/or lack CSD.

HP1D2 Is Expressed in Spermatogonia and Specifically Binds the Y Chromosome. Through their relatively conserved domains and functions, HP1 proteins are usually enriched in heterochromatin (18). To determine the localization of HP1D2 in the testis, we established transgenic lines expressing HP1D2^{SR} or HP1D2ST, with the GFP fused in their C termini (Fig. 2B). We observed that both alleles are expressed specifically in spermatogonia (Fig. 3A). Interestingly, both HP1D2:GFP proteins were not uniformly distributed on the chromatin but instead, were highly enriched on a single, highly heterochromatinized chromosome, most likely the Y chromosome (Fig. 3B). We then stained HP1D2:GFP transgenic testes with an antibody recognizing D1, an Adenine-Thymine (AT)-hook domain DNA-binding protein that is specifically enriched on the Y chromosome and to a lesser extent, the fourth chromosome in *D. simulans* (19). Very clearly, HP1D2:GFP was systematically associated with the main D1 nuclear foci in male germ cells (Fig. 3B). We, thus, conclude that HP1D2 specifically binds the Y chromosome in premeiotic male germ cells. This remarkable localization suggests a role for HP1D2 in organizing at least certain regions of the Y chromosome in preparation of meiosis, such as satellite DNA or other repetitive elements. In the Paris *SR* system, Y chromatids fail to segregate normally during the second meiotic division and frequently exhibit chromatin bridges (12). In the absence of meiotic recombination in *Drosophila* males, this phenotype probably indicates the presence of incompletely replicated or aberrantly organized regions of the Y chromosome at the onset of meiotic divisions.

In any case, it suggests that HP1D2^{SR} proteins are unable to prepare the Y chromosome for meiosis.

HP1D2 Is a Young and Fast-Evolving Gene. According to the estimated age of *Dp*^{SR} (<500 y) (14), the spread of the Paris drivers is recent, and their evolution in populations is very fast (20, 21). Through its own spreading, together with *Dp*^{SR}, the *HP1D2*^{SR} allele carried by *X*^{SR4} (lacking CSD) became the most frequent allele in the Malagasy populations (based on the selective sweep studied in refs. 20 and 22) (*SI Appendix*, Fig. S3). The observation that *SR* drive is caused by a reduced expression or a loss of function of *HP1D2* alleles suggests a scenario where *HP1D2* is running headlong toward its degeneration through the spread of increasingly deficient alleles. However, because HP1D2^{SR} localizes to the Y chromosome as well, we cannot exclude that CSD-lacking HP1D2 proteins cause drive through aberrant interactions with other chromatin proteins. If it is the case, CSD-lacking HP1D2 may evolve a new function, which has been described for another HP1 (23).

In any case, the way forward for the species is in the evolution of Y chromosomes able to manage without the ancestral *HP1D2* and/or the recruitment of new genes to make up for it.

HP1D2 is a young intronless gene that originates from a duplication of *HP1D/Rhino* around 15–22 Mya (15). Age duplication estimation based on ref. 24. We investigated *HP1D2* evolution in the *Sophophora* clade (Fig. 4 and *SI Appendix*, Figs. S5 and S6 and Tables S3–S5). We observed a cis-duplication of *HP1D2* that occurred in an ancestor of the takahashii and suzukii subgroups. Another duplication happened in the melanogaster subgroup (in the ancestor of *Drosophila orena* and *Drosophila erecta*). We also found that it has been recurrently lost, like in *Drosophila melanogaster* (15) and *Drosophila elegans*, or suffers potential pseudogenization, like in *D. erecta* and *D. orena* (Fig. 4 and *SI Appendix*, Fig. S5). The complex and rapid evolution of *HP1D2* in the *melanogaster* species group suggests that it is involved in recurrent genetic conflicts.

Conclusion

It has been previously hypothesized that genes that encode proteins interacting strongly with heterochromatin can be potential actors of genetic conflicts (10, 15, 19, 23). We show here for the first time, to our knowledge, that such a gene, expressed in the male germ line, plays a key role in an extended intragenomic conflict in *D. simulans*.

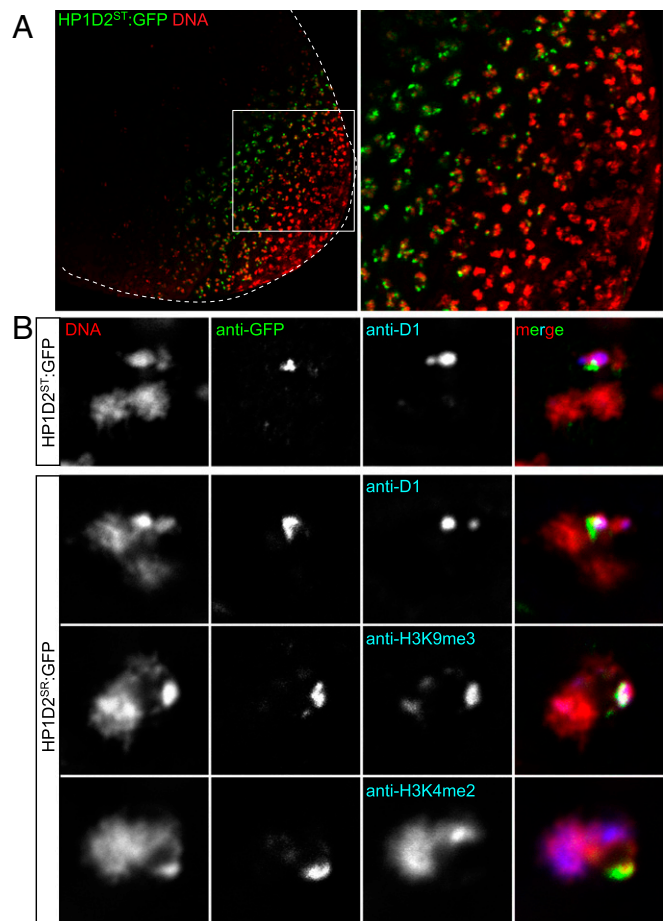


Fig. 3. Localization of HP1D2 in testes. (A) Confocal images showing the expression of the native GFP-tagged HP1D2ST protein (HP1D2ST:GFP; green) in a testis stained for DNA (red). In *Left*, the white dashed line delineates the edge of the testis. *Right* shows a magnification of *Left*, *Inset*. HP1D2ST:GFP is detected in nuclei that are located close to the tip of the testis. These cells correspond to spermatogonia, which are rapidly dividing premeiotic germ cells. The same localization was observed for HP1D2^{SR}:GFP (Fig. S4). (B) Confocal images of testes from HP1D2ST:GFP or HP1D2^{SR}:GFP males stained for GFP (green), DNA (red), and (rows 1 and 2) the AT-rich satellite binding protein D1 (blue), which specifically binds the Y and fourth chromosomes in *D. simulans* (19), or the histone marks (row 3) H3K9me3, which is specifically enriched in heterochromatin, or (row 4) H3K4me2, which is enriched in actively transcribed genomic regions. The fusion proteins HP1D2ST:GFP or HP1D2^{SR}:GFP are located in close proximity to D1. Moreover, HP1D2^{SR}:GFP colocalizes with H3K9me3 signal but not H3K4me2 signal.

HP1D2 is specifically enriched on the Y chromosome, which is, like most old Y chromosomes, heterochromatic and rich in repeated sequences (25). Other meiotic drivers are known to favor their own transmission through DNA condensation perturbation, and sometimes the interaction with a specific satellite DNA (26–28). Similarly, the discrete distribution of HP1D2 on the Y chromosome suggests an impact of this protein on the chromatin organization of specific repeated sequences. The Paris *SR* system, thus, brings definitive evidence that the coevolution of highly repeated sequences and heterochromatin interacting proteins can generate unstable interactions, which ultimately end in genetic conflict.

Materials and Methods

Recombination Mapping. The stocks used were previously described in refs. 22, 29, and 30. ST8 is the reference ST stock free of distorters and suppressors. *X*^{SR4} is an *SR* X chromosome. *X*^{sn/lz} is an ST (nondriving) X chromosome carrying *sn* and *lz* mutations (22). The crossing scheme used to produce recombinants is

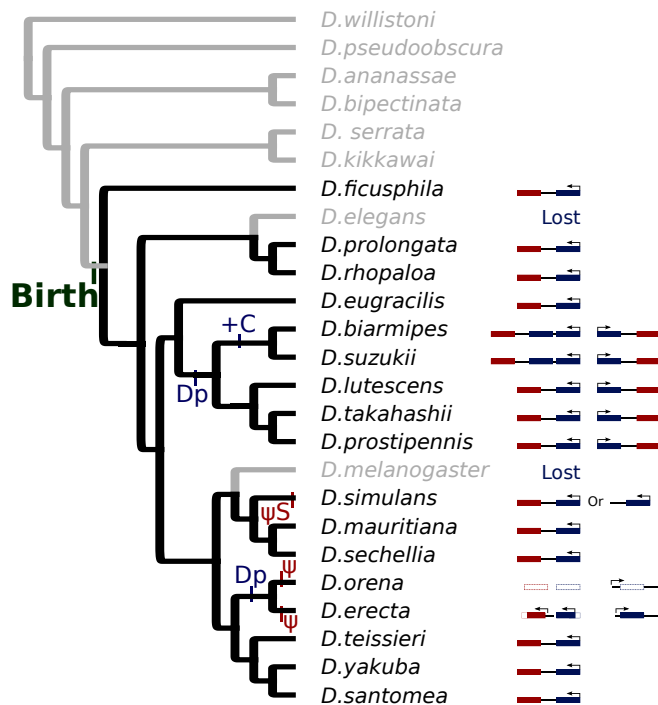


Fig. 4. Evolution of *HP1D2* homologs and paralogs in the *Sophophora* clade. The species phylogeny is from the work in ref. 24 and suggests that *HP1D2* was lost at least two times. Canonical CD and CSD are pictured in green and red, respectively. +C indicates a new CD, Ψ indicates potential pseudogenization, and ΨS indicates potential CSD pseudogenization.

described in *SI Appendix, Fig. S6A* (additional information is in ref. 22). Parental and recombinant X chromosomes are kept in male lineage through repeated backcrosses to C(1)RM,y,w females with ST8 background. We designed eight molecular markers to map the recombination sites within the 42-kb candidate region (*SI Appendix, Table S6*).

SR Test. The SR phenotype for each X chromosome was measured by individual crosses of at least five single 1- to 5-d-old males with ST8 virgin females. Females were allowed to lay eggs for 4–6 d. The progeny were counted and sexed until no more flies emerged. Only crosses producing at least 50 flies were considered. All experiments were carried out at 25 °C. All of the flies were reared on axenic medium (31) at 25 °C.

Association Mapping. The 4.5-kb candidate region for *Wlsta* was sequenced on 56 different X chromosomes (*SI Appendix, Table S1*) using the primers listed in *SI Appendix, Table S7*. We checked the presence of *Dp^{SR}* using primers listed in *SI Appendix, Table S6*.

Measure of Testicular Gene Expression. For each condition, at least 30 testes pairs were dissected in PBS from males less than 5 d old. RNA extractions were performed using the NucleoSpin RNA Xs Kit as indicated by the manufacturer (Macherey-Nagel). Two different reverse transcriptases were used depending on the experiment: Maxima First-Strand cDNA Synthesis Kit for RT-qPCR (Life Technologies) and iScript cDNA Synthesis Kit (BioRad). cDNA quantification was performed with iQ SYBR Green Supermix (BioRad) in a CFX-96 Thermocycler (BioRad). For each condition, gene expression was measured using at least two biological and two technical replicates.

We selected three different reference genes [*RPII140*, *eIF2B-β*, and *Light* (*SI Appendix, Table S8*)] all recommended by four different softwares [Best-Keeper (32), NormFinder (33), Genorm (34), and the comparative ΔCt (cycle threshold) method (35) (www.leonxie.com/referencegene.php)]. We then used at least two of them in each experiment.

Difference in expression level was tested with the Wilcoxon rank sum test for pairwise comparisons. We used the Kruskal-Wallis rank sum test for multiple tests followed by the posthoc test (pairwise comparisons using the Wilcoxon rank sum test) corrected by the Bonferroni method. Statistical tests have been executed using R (36).

Males Used to Measure Testicular Expression of *HP1D2* and *CG12065*. Males carrying parental and recombinant chromosomes from the recombination mapping experiment (Fig. 1 B and C and *SI Appendix, Fig. S7A*) were used to measure the expression of both candidate genes (*HP1D2* in Fig. 2A and *CG12065* in *SI Appendix, Fig. S1A*):

Three recombinant $X^{sn+}_{[ST]}$ without *Dp^{SR}* and with the X^{SR4} sequence in the 4.5-kb candidate region;

Two recombinant $X^{sn+}_{[ST]}$ without *Dp^{SR}* and with the X^{snLz} sequence in the 4.5-kb candidate region and the parental X^{snLz} chromosome;

Two recombinant $X^{+Lz}_{[SR]}$ (X16 and X378) with *Dp^{SR}* and the X^{SR4} sequence in the 4.5-kb candidate region and the parental X^{SR4} chromosome; and

Three recombinant $X^{+Lz}_{[ST]}$ (X134, X269, and X6649) with *Dp^{SR}* and the X^{snLz} sequence in the 4.5-kb candidate region.

Cloning and Injection. We amplified *HP1D2^{SR}* and *HP1D2ST* alleles, with their upstream and downstream sequences, respectively, at one time with the Phusion DNA Polymerases (Life Technologies); then, we made the modified alleles (*HP1D2ST* without its CSD: ΔCSD and both alleles with a GFP domain) with a fusion PCR technique (37, 38) using the primers described in *SI Appendix, Table S2*. Constructs were cloned in a pCaSpeR4 plasmid. shRNA was designed using the DSIR algorithm (39) (sequence is in *SI Appendix, Table S2*) and the microRNA-1 loop (40) and cloned in a pUASi plasmid. All inserts have been checked by sequencing before transgenesis. The injection protocol was the same as described in ref. 41, and it was carried out on flies carrying X chromosomes from the *w501* stock and Y chromosome and autosomes from the ST8 stock. For each construction, at least three independent transformed lines with autosomal insertions were kept and tested.

Functional Tests of Transgenes. To test *HP1D2* transgenes, we crossed males carrying $X^{+Lz}_{[SR]}$ or X^{SR4} with C(1)RM,y,w females homozygous for the tested transgene (Fig. 1B). F1 males were then crossed with C(1)RM,y,w females to obtain F2 male siblings with or without the *HP1D2* transgene. We measured the SR in the offspring of individual F2 males. The presence/absence of the *HP1D2* transgene in each male was then assessed by PCR amplification using a plasmid-specific primer (GTGGGCAAGAGACATCCACT) combined with an insert-specific primer that works on all *HP1D2* transgenes (AACGGACGCTCGTGTCTTTC).

To test the effect of UAS-shRNA^{*HP1D2*} in $X^{+Lz}_{[ST]}$ males, we crossed males with the X chromosome of interest with C(1)RM,y,w females homozygous for the *nos-Gal4* driver (*SI Appendix, Fig. S7C*). F1 males were then crossed with C(1)RM,y,w females homozygous for the UAS-shRNA^{*HP1D2*} transgene. We obtained two kinds of F2 males: males with the UAS transgene and the Gal4 driver and males with only the UAS transgene. SRs were measured in the offspring of individual F2 males. We also checked under UV light for the presence/absence of the GreenEye marker associated with the *nos-Gal4* driver (42). The presence of the UAS transgene was then assessed by PCR amplification (primers: AGG-CATTCCACCACGTCTCCCA and AACAAAGCGCAGCTGAACAAGC).

Immunostaining. Whole-mount testes were stained as previously described (43). Primary antibodies were mouse monoclonal anti-GFP (1:200; 11 814 460 001; Roche), rabbit anti-D1 (1:1,000) (19), rabbit anti-H3K9me3 (1:500; ref 07–442; Millipore) (44), and rabbit anti-K4me2 (1:500; 07–030; Millipore) (45). Secondary antibodies were DyLight-coupled goat anti-mouse and goat anti-rabbit antibodies (1:1,000; Jackson Immuno Research). After RNase treatment, tissues were mounted in mounting medium (Dako) containing 5 μg/mL propidium iodide (Sigma-Aldrich). For the observation of native GFP, testes were dissected and mounted without fixation in mounting medium containing 1 μM DRAQ 5 (BioStatus) to visualize DNA.

Evolutionary Analysis. *HP1D2* orthologs and paralogs (*HP1D2B*) were found by BLAST (*SI Appendix, Table S9*). They were resequenced in *D. erecta*, *Drosophila takahashii*, *Drosophila suzukii*, *Drosophila prolongata*, *Drosophila biarmipes*, *Drosophila yakuba*, *Drosophila sechellia*, and *D. melanogaster*. Based on the obtained sequences, we amplified and sequenced homologs in additional species: *Drosophila prostipennis*, *Drosophila orena*, *Drosophila teissieri*, *Drosophila santomea*, *Drosophila lutescens*, and *Drosophila mauritiana*.

HP1D2 coding sequence and protein sequences were compared between *D. simulans* lines and between *Drosophila* species. Sequences were aligned using Geneious (Geneious, version 7.1.7 developed by Biomatters) and checked manually. The phylogenetic tree used in the different evolutionary tests is based on the work in ref. 24. We used PAML (46, 47) (*SI Appendix, Table S3*) and HyPhy package (48, 49) (Datamonkey webserver) (*SI Appendix, Fig. S6 and Table S4*) to look for positive selection pressure on the Chromo Domains or the CSDs. The polymorphism of the hinge sequence is too high to allow proper alignment.

We executed typical analyses in PAML: free ratio model allowing variation of the ratio of nonsynonymous and substitutions to the number of synonymous substitutions (dN/dS) (ω) along different branches of the phylogeny to calculate dN/dS values between lineages compared with model = 0, which measures a global ω for all lineages (SI Appendix, Table S3).

We compared the polymorphism and divergence of *HP1D2* between the sister species *D. simulans*, *D. sechellia*, and *D. mauritiana* with the McDonald–Kreitman test (50) calculated on the web interface mkt.uab.es/mkt/MKT.asp (SI Appendix, Table S10). The *D. mauritiana* and *D. sechellia* lines used in this test are shown in SI Appendix, Table S8. For *D. simulans*, we used the following lines: Ch006, MP31, CE122, Ch019, Ma244, Ch005, Ma247, Rf47, FP3, Rf46, MP29, MP39, Mp7, SR6, Ch007, *snlz*, and MP45 (SI Appendix, Table S1).

They recapitulate all of the known polymorphisms for the coding sequence of *HP1D2* in *D. simulans*, excluding the deletions.

The phylogenetic tree in SI Appendix, Fig. S3C was constructed using PhyML (51) with the generalized time reversible (GTR) nucleotide substitution model. Support values was acquired from 1,000 bootstrap replicates.

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