High rate of translocation-based gene birth on the Drosophila Y chromosome

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The Y chromosome is a unique genetic environment defined by a lack of recombination and male-limited inheritance. The Drosophila Y chromosome has been gradually acquiring genes from the rest of the genome, with only seven Y-linked genes being gained over the past 63 million years (0.12 gene gains per million years). Using a next-generation sequencing (NGS)-powered genomic scan, we show that gene transfers to the Y chromosome are much more common than previously suspected: at least 25 have arisen across three Drosophila species over the past 5.4 million years (1.67 per million years for each lineage). The gene transfer rate is significantly lower in Drosophila melanogaster than in the Drosophila simulans clade, primarily due to Y-linked retrotranspositions being significantly more common in the latter. Despite all Y-linked gene transfers being evolutionarily recent (<1 million years old), only three showed evidence for purifying selection (ω ≤ 0.14). Thus, although the resulting Y-linked functional gene acquisition rate (0.25 new genes per million years) is double the longer-term estimate, the rate of most new Y-linked genes is defined by rapid degeneration and pseudogenization. Our results show that Y-linked gene traffic, and the molecular mechanisms governing these transfers, can diverge rapidly between species, revealing the Drosophila Y chromosome to be more dynamic than previously appreciated. Our analytical method provides a powerful means to identify Y-linked gene transfers and will help illuminate the evolutionary dynamics of the Y chromosome in Drosophila and other species.

Y chromosome | evolution | Drosophila | retrocopies | transposition

To validate our analytical pipeline, we performed a combination of in vitro and in silico tests. First, we estimated the false discovery rate (FDR) of our pipeline by rerunning it in full after reversing the role of the two sexes (SI Methods). Because no transfers were detected in this sex-reversed pipeline, the estimated FDR in the present study is indistinguishable from zero. Second, we reasoned that the donor regions of the Y-linked transfers should have significantly elevated coverage in males relative to females of the same strain, after weighting the coverage to account for variation across samples and chromosomes (SI Methods). Indeed, the male:female weighted coverage ratio (WCR) was consistently higher in the detected transfer regions than expected according to the empirical WCR distribution (and this was significant for more than half of the incipient transfers; Dataset S1), suggesting our pipeline accurately identifies transfers.

**Significance**

Using a powerful method that uses inexpensive short reads to detect Y-linked transfers, we show that gene traffic onto the Drosophila Y chromosome is 10 times more frequent than previously thought and includes the first Y-linked retrocopies discovered in these taxa. All 25 identified Y-linked gene transfers were relatively young (<1 million years old), although most appear to be pseudogenes because only three of these transfers show signs of purifying selection. Our method provides compelling evidence that the Drosophila Y chromosome is a highly challenging and dynamic genetic environment that is capable of rapidly diverging between species and promises to reveal fundamental insights into Y chromosome evolution across many taxa.

**Results**

**Y-Linked Transfer Properties and Pipeline Validation.** Our method detected numerous putative Y-specific sequences that mapped to feminized reference genomes from three Drosophila species (Fig. 1). Consistent with the high repeat content of the Y chromosome, many clusters of SNPs were located in or near heterochromatic regions that overlapped transposable elements (TEs) (Fig. 1). Restricting our analysis to regions that lacked TEs and contained at least five Y-specific SNPs resulted in 66 incipient Y-linked transfers. After combining incipient transfers from closely neighboring regions, we obtained 45 unique Y-linked consensus transfers across the three species (Table I and Datasets S1 and S2). Twenty-five of these consensus transfers were GeTYs, including six that were shared between D. simulans and D. mauritiana (Fig. 2). The set of donor genes underlying the Y-linked transfers were broadly dispersed over the genome and were not enriched with respect to functional category or male-biased expression (Datasets S3 and S4).

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identified Y-linked transfers. Finally, we confirmed the existence of all Y-linked transfers from *D. mauritiana* and *D. simulans* by determining that the associated Y-linked sequences generated PCR amplicons in males only ([Dataset S5](#) and [SI Methods](#)). Notably, our results suggested that a handful of Y-linked transfers involved in additional bouts of duplication on the Y chromosome ([Table 1](#) and [SI Methods](#)). One of these GeTYs (Dsim_2R_9.41) also showed evidence for subsequent transfer onto the autosome or X chromosome ([Fig. S1](#) and [SI Methods](#)), supporting a previous report that the Y chromosome is also an occasional source for gene transfers to other chromosomes in *Drosophila* ([8]).

**Y-Linked Transfer Haplotype Assembly.** To facilitate additional analyses of the Y-linked transfers, we reconstructed the Y-linked haplotype for each of the incipient transfers by extracting all reads mapping to the donor region that contained putative Y-linked alleles, then using these reads to de novo assemble the translocated sequence ([SI Methods](#)). We checked the quality of our de novo assemblies by looking in more detail at the reconstructed haplotype for GeTY Dmel_3R_20.35, for which a 200-kb contig bearing the full translocation is publically available ([9](#)). GeTY Dmel_3R_20.35 was previously reported to be a DNA translocation that contains the youngest functional Y-linked gene described for *D. melanogaster* (FDY; parental ortholog: vig2) ([9](#)). The original DNA translocation also included additional genes that show evidence of pseudogenization (Moc2/CG42503, Caliban, and Bili) ([9](#)). Our four Y-linked haplotypes from this region produced near-perfect alignments with the published 200-kb Y-linked haplotype for each incipient transfer. No anno, no recorded annotation; TE, transposable element. ([D](#)) The Integrative Genomics Viewer (IGV) screen shot for the read alignment ([Bottom](#)) and subsequent de novo assembled Y-linked haplotype [green (Velvet) and red (Transabyss) bars; [Top](#)], relative to the donor gene annotation for GeTY (blue bars; [Top](#)). The final step of the pipeline involved the iterative aggregation of incipient transfers lying within 200 kb of one another into a single consensus transfer.

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**Fig. 1.** Schematic overview of the Y-linked transfer detection pipeline. In step 1, two separate Cochran–Mantel–Haenszel (CMH) tests were performed and then combined to identify outlier SNPs; the first CMH test contrasted sexes with the strains (different colored flies) as replicates ([A](#)), and the second CMH test contrasted the strains with sexes as replicates. For all three species, more outlier SNPs (StdDiff > 2) were detected for male-specific variants (M) than for female-specific variants (F) ([B](#)), indicating that our pipeline was accurately identifying Y transfers. In step 2, male-specific outlier SNPs are grouped into clusters ([C](#)). The annotation of genomic regions containing outlier SNPs is indicated by different color codes. Although GeTYs are broadly dispersed across the genome of each species, TE peaks typically cluster within heterochromatic regions. In step 3, reads containing Y-linked variants were used in the de novo assembly of the Y-linked haplotype for each incipient transfer. No anno, no recorded annotation; TE, transposable element. ([D](#)) The Integrative Genomics Viewer (IGV) screen shot for the read alignment ([Bottom](#)) and subsequent de novo assembled Y-linked haplotype [green (Velvet) and red (Transabyss) bars; [Top](#)], relative to the donor gene annotation for GeTY (blue bars; [Top](#)). The final step of the pipeline involved the iterative aggregation of incipient transfers lying within 200 kb of one another into a single consensus transfer.
Table 1. Summary of Y-linked transfers

| Species       | Transfer ID | Transfer type | Donor(s)                  | ω   | Expression | Age (Ky) |
|---------------|-------------|---------------|---------------------------|-----|------------|----------|
| D. mauritiana | Dmau_2L_6.65| Ambig         | Hrb27C                    | 1.02| Filt, NA   | 673 [309,1885] |
|               | Dmau_2L_9.1 | Ambig         | numb                      | NA  | norf       | 460 [211,1287] |
|               | Dmau_2L_12.3| DNA           | CG5787;PhD1               | NA  | Filt, Filt, Filt | 609 [280,1706] |
|               | Dmau_2L_15.71| RNA           | Cyp1                      | 1.38| NA         | 517 [247,1446] |
|               | Dmau_2R_0.15*| DNA           | NA                        | NA  | NA         | 429 [197,1201] |
|               | Dmau_2R_4.35| RNA           | 14-3-zeta                 | nORF| NA         | 382 [176,1072] |
|               | Dmau_2R_8.65| RNA           | Lttv;LamC                 | nORF| Filt, Filt, Filt | 153 [240] [70(110),429(672)] |
|               | Dmau_2R_9.41*| RNA           | SRPK                      | NA  | Filt       | 429 [197,1201] |
|               | Dmau_3R_4.35| RNA           | 14                       | NA  | NA         | 30 [13,78] |
|               | Dmau_3R_0.06| DNA           | NA                        | NA  | NA         | 267 [122,747] |
|               | Dmau_3R_0.16| RNA           | Sdtd                      | 0.30| NA         | 158 [73,443] |
|               | Dmau_3R_0.25| RNA           | Sod                       | 0.30| NA         | 98 [45,273] |
|               | Dmau_3L_7.55| DNA           | CG7492;Ank2               | nORF| Filt, Filt | 213 [98,597] |
|               | Dmau_3L_22.2| DNA           | NA                        | NA  | NA         | 957 [439,2679] |
|               | Dmau_3R_0.34*| DNA           | NA                        | NA  | NA         | 380 [174,1064] |
|               | Dmau_3R_1.23| DNA           | Nmdar1;dmau_PGO0479       | nORF| nORF      | 282 [130,790] |
|               | Dmau_3R_2.13| DNA           | NA                        | NA  | NA         | 28 [13,78] |
|               | Dmau_3R_7.55| DNA           | Cyp1                      | 1.38| NA         | 697 [320,1951] |
|               | Dmau_3L_2.15| RNA           | Sdt                       | 0.30| NA         | 305 [140,854] |
|               | Dmau_3L_2.15| RNA           | Sod                       | 0.30| NA         | 98 [45,273] |
|               | Dmau_3L_7.55| DNA           | CG7492                    | nORF| Filt, Filt | 213 [98,597] |
| D. melanogaster| Dmel_2L_4.46| DNA           | Gs11;RpL27A               | nORF| nORF      | 45 [21,125] |
|               | Dmel_2L_12.86| DNA           | NA                        | NA  | NA         | 174 [80,487] |
|               | Dmel_2L_19.94| RNA           | sick                      | nORF| NA         | 559 [256,1564] |
|               | Dmel_2L_22.75| DNA           | NA                        | NA  | NA         | 697 [320,1951] |
|               | Dmel_2R_0.09| DNA           | NA                        | NA  | NA         | 624 [287,1748] |
|               | Dmel_2R_0.57| DNA           | NA                        | NA  | NA         | 303 [139,848] |
|               | Dmel_2R_2.32| DNA           | NA                        | NA  | NA         | 315 [145,883] |
|               | Dmel_3L_23.41| DNA           | NA                        | NA  | NA         | 443 [203,1241] |
|               | Dmel_3L_24.3| DNA           | NA                        | NA  | NA         | 343 [158,962] |
|               | Dmel_3R_17.04| Ambig         | CR43975                   | nORF| NA         | 516 [237,1444] |
|               | Dmel_3R_20.95| DNA           | vig2;Moca2;CG42530;ClbrnBili| 0.53;0.45;0.64;Filt | 463 [97] [213(391),1297(3191)] |
|               | Dmel_3R_17.04| DNA           | ade5;CG12717              | nORF| Filt      | 725 [333,2031] |
|               | Dmel_3R_12.86| DNA           | NA                        | NA  | NA         | 403 [197,1204] |
| D. simulans   | Dsim_2L_11.91| DNA           | bru1                      | Filt| 2/11 [3]  | 169 [77,472] |
|               | Dsim_2L_12.3| DNA           | CG5787;PhD1               | Filt;nORF | 141/17 [4.9;4.6] [1.2] | 813 [373,2275] |
|               | Dsim_2L_15.71| RNA           | Cyp1                      | nORF| NA         | 197 [90,551] |
|               | Dsim_2L_19.34| DNA           | NA                        | NA  | NA         | 351 [161,983] |
|               | Dsim_2R_0.06| DNA           | NA                        | NA  | NA         | 267 [122,747] |
|               | Dsim_2R_4.35| RNA           | 14-3-zeta                 | Filt| 0/2 [18.5] | 90 [41,252] |
|               | Dsim_2R_9.41*| RNA           | SRPK                      | Filt| 25/29 [575.6] | 204 [94,573] |
|               | Dsim_3L_7.55| DNA           | CG7492;Ank2               | nORF| 0.63;0.62 | 89 [11.2;32/36 [6.6] | 383 [176,1072] |
|               | Dsim_3L_10.87| RNA           | Sod                       | 0.09  | 1/8 [10.4] | 182 [477] [84(219),509(1335)] |
|               | Dsim_3L_22.2| DNA           | NA                        | NA  | NA         | 408 [117,842] |
|               | Dsim_3R_4.18| DNA           | NA                        | NA  | NA         | 161 [74,452] |
|               | Dsim_3R_12.73| DNA           | NA                        | NA  | NA         | 96 [44,269] |
|               | Dsim_3R_13.94*| DNA           | Tctp                      | nORF| 0/30 [17.4] | 197 [90,551] |
|               | Dsim_3L_7.55| RNA           | Cyp1                      | 0.78 | 0/18 [4.5] | 222 [102,623] |
|               | Dsim_3R_20.2| DNA           | CG17450;CG32819;CG32820   | Filt| 0/8 [19.5] | 268 [123,750] |

A total of 45 unique Y-linked transfers were detected, arising either as retrocopies (RNA), as DNA translocations (DNA), or via an undetermined mode (Ambig). Twenty-five of the Y-linked transfers harbored at least one gene—i.e., were GetYs [donor gene names in donor(s) column]—with six GetYs being shared between species (italicized rows). In all columns, GetYs comprising several genes have each gene name separated by semicolons, with those having identical gene models being separated by a forward slash. Purifying selection was detected for three GetYs (ω column; genes having more than one detected ORF being further delineated by a comma), whereas others showed evidence of degeneration (ω column; nORF = no ORF; Filt = Y-linked or donor CDS lacked either a start or stop codon, contained an inactivating mutation, or >10% linked codons were missing in donor alignment; Dataset S5). Two public available testes-specific RNA-Seq datasets (23, 24) revealed weak evidence for Y-linked GetY expression, with most GetYs having low relative expression (i.e., the fraction of diagnostic exonic sites where the Y-linked allele contributed >1% of the total coverage for that site; fraction shown in expression column) and low absolute expression (i.e., mean coverage of Y-linked alleles mapping to diagnostic exonic sites; values in square brackets in expression column). Point estimates of transfer times revealed evolutionarily recent origins, with the oldest transfer arising around 1 Mya (see age column; error margins in square brackets, age estimates for putatively functional genes after correcting for purifying selection shown in standard parentheses; Dataset S7). For some Y-linked transfers, multiple haplotypes were detected in male-specific short read data suggesting that these transfers likely underwent subsequent duplication on the Y chromosome, with GetTY Dsim_2R_9.41 also showing signs of an additional autosomal/X-linked duplication. Age and expression estimates may be unreliable for these Y-linked transfers.

*Putative duplicated transfers.

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Y-Linked Gene Transfers Are Recent but Show Limited Evidence of Purifying Selection. The general lack of shared Y-linked transfers across all three species suggests that the observed Y-linked transfers were relatively recent. We estimated the age of each transfer using a method that minimizes the influence of ancestral polymorphisms by ignoring Y-linked substitutions that are still segregating in the donor copy in large female samples (Methods and SI Methods). Although coarse, our estimates indicate that the Y-linked transfers are evolutionarily recent—with all arising within the past 1 My (Table 1 and Dataset S7)—and that the age distribution of GeTYs and nongenic transfers were not significantly different ($P = 0.58$, two-sided Kolmogorov–Smirnov test; $P = 0.74$ after GeTYs adjusted for the effects of purifying selection and putative duplicated Y-linked transfers removed; SI Methods). Although the latter result implies that GeTYs were effectively behaving like neutral loci, the fact that the only shared Y-linked transfers between species were GeTYs suggests they were subject to stronger Y-linked purifying selection than nongenic transfers in general. Further, many of these shared transfers had estimated ages that were younger than the recorded split between the two species, which may have resulted from purifying selection removing new mutations in the Y-linked copies. Thus, we performed two additional analyses to determine if any of the GeTYs were functional.

First, we measured $\omega$, the ratio of nonsynonymous to synonymous substitutions that had accumulated in each Y-linked copy following the transfer, and tested whether this ratio differed from neutral expectations (i.e., $\omega$ significantly less than 1; Methods and SI Methods). To avoid bias toward high $\omega$ values due to incorrect gene models, we performed de novo gene predictions for each GeTY and only retained instances where the predicted Y-linked ORF included the start and stop codons (should the donor gene contain exons) and will lie within donor gene boundaries. In contrast, DNA translocations will include intrinsic sequences, and the translocated regions need not coincide with parental gene boundaries. Based on these characteristics, 8 of the 25 GeTYs were Y-linked retrocopy insertions (5 in $D. mauritiana$, 6 in $D. simulans$, 3 shared; 0 in $D. melanogaster$) and 13 were DNA translocations (7 in $D. mauritiana$, 5 in $D. simulans$, 3 shared; 4 in $D. melanogaster$) (Table 1, Figs. S2 and S3, and Dataset S5). The transfer mechanism of the remaining four GeTYs (three in $D. mauritiana$ and one in $D. melanogaster$) could not be unambiguously determined (SI Methods). Three lines of evidence suggest that the observed Y-linked transfers are probably fixed within each species: (i) the effective population size of the Y chromosome is relatively small (25% that of autosomes), (ii) two $D. simulans$ Y-linked retrocopies analyzed in a PCR assay were fixed in a global sample of 25 males (Dataset S6 and SI Methods), and (iii) some gene transfers are shared between species.

Although the number of nongenic transfers is similar across the three species (8 in $D. melanogaster$, 7 in $D. mauritiana$, and 5 in $D. simulans$), the 5 GeTYs in $D. melanogaster$ are significantly fewer than the 20 independent transfers observed in the $D. simulans$ clade ($P = 0.011$, Poisson test; Methods). This discrepancy appears to be largely driven by a significantly elevated Y-linked retrocopy insertion rate in the $D. simulans$ clade ($P = 0.004$, Poisson test), a result that is even more remarkable given the absence of evidence for Y-linked retrocopies in $Drosophila$ to date. Notably, $D. melanogaster$ has the most complete gene annotation of the three species in this study, implying that interspecies differences in the quality and quantity of gene annotations did not bias our Y-linked retrocopy detection. These findings suggest that Y-linked gene transfer rates, and the underlying molecular mechanisms driving the translocations, can undergo significant divergence over relatively brief evolutionary time spans in $Drosophila$. Y-Linked Gene Transfers Are Recent but Show Limited Evidence of Purifying Selection. The general lack of shared Y-linked transfers across all three species suggests that the observed Y-linked transfers were relatively recent. We estimated the age of each transfer using a method that minimizes the influence of ancestral polymorphisms by ignoring Y-linked substitutions that are still segregating in the donor copy in large female samples (Methods and SI Methods). Although coarse, our estimates indicate that the Y-linked transfers are evolutionarily recent—with all arising within the past 1 My (Table 1 and Dataset S7)—and that the age distribution of GeTYs and nongenic transfers were not significantly different ($P = 0.58$, two-sided Kolmogorov–Smirnov test; $P = 0.74$ after GeTYs adjusted for the effects of purifying selection and putative duplicated Y-linked transfers removed; SI Methods). Although the latter result implies that GeTYs were effectively behaving like neutral loci, the fact that the only shared Y-linked transfers between species were GeTYs suggests they were subject to stronger Y-linked purifying selection than nongenic transfers in general. Further, many of these shared transfers had estimated ages that were younger than the recorded split between the two species, which may have resulted from purifying selection removing new mutations in the Y-linked copies. Thus, we performed two additional analyses to determine if any of the GeTYs were functional.

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an order of magnitude [0.12 per My (12)]. Despite being much higher than previous estimates, the primary Y-linked gene acquisition rate inferred here appears to be up to an order of magnitude lower than for the rest of the genome for Drosophila (13–15), although the inclusion of different transfer categories in previous studies (e.g., de novo genes and intrachromosomal transfers) complicates direct comparisons. Further, despite a handful of GeTYs showing evidence for functionality [4/25 = 16%, including F8 in D. melanogaster (9)]—which lead to a functional Y-linked gene acquisition rate that is approximately double the previous estimate (12) (four functional GeTYs/5.4 My/three species = 0.25 new genes/species/My)—many of the GeTYs did not have complete ORFs, contained frameshift mutations, or showed no evidence of expression. This implies that the majority of the Y-linked transfers reported here have become pseudogenes and that the Y chromosome is a less hospitable genetic environment for new gene evolution than the rest of the genome in Drosophila.

The Dynamic and Challenging Genetic Environment of the Drosophila Y Chromosome. A recent study revealed that D. melanogaster has more Y-linked genes than Drosophila virilis, primarily due to the higher number of gene gains in the former since the two species shared a common ancestor (3). This result suggests that the elevated rate of functional Y-linked gene acquisition reported here may reflect a general acceleration in this rate across the Drosophila subgroup relative to their sister taxa. The mechanistic driver behind this putative lineage-specific change remains unknown, but possible factors include increased accessibility of the Y chromosome to transfers (e.g., due to more relaxed chromatin conformation) or improved efficacy of Y-linked selection (e.g., due to increased effective population size for the Y chromosome), among others. Alternatively, the rate of functional GeTY acquisition reported here could be a transient phenomenon, whereby the short-term rate (over ~1 My) eventually converges with the slower long-term rates (over ~60 My). The efficacy of selection on weakly deleterious mutations is reduced on the Drosophila Y chromosome relative to other chromosomes (16), whereby many of the newly transferred genes, including those currently under selection, could become pseudogenized over longer time periods. Consistent with this idea, many of the GeTYs displaying evidence for low levels of expression in D. simulans were also present in D. mauritiana; however, none of these shared GeTYs displayed significant purifying selection in either species. Additional testing on more Drosophila species is ultimately required to determine the temporal stability the Y-linked gene transfer rate, although the acquisition rate of duplicated genes on the autosomes and X chromosome appears to be relatively stable over long periods in Drosophila (15), particularly for retrocopies (14). Regardless of the underlying cause of the temporal disparity in Y-linked gene gains reported here, when combined with the significant differences in Y-linked retrocopy traffic across the D. melangaster subgroup, our study reveals that the Drosophila Y chromosome is an even more dynamic genetic environment than previously appreciated and is capable of undergoing significant changes over relatively short evolutionary time scales.

Conclusion

In contrast to many other species, the Drosophila Y chromosome is a highly dynamic genetic environment. For example, in D. pseudoobscura the Y chromosome is not homologous to the ancestral Drosophila Y chromosome but has arisen de novo (17). We have shown that Y-linked gene acquisition over the past million years is a highly dynamic feature of the Drosophila Y chromosome, with 10 times more gene traffic and twice the number of functional gene gains than are expected given the Y-linked gene acquisition rate recorded over the past 63 My (12). In addition to heterogeneous Y-linked gene acquisition dynamics, our method has revealed previously unknown properties of the Y chromosome, i.e., frequent retrocopy traffic onto the Y chromosome, which appears to have lineage-specific dynamics. Further research is required to determine whether this pattern reflects an actual elevation in the functional gene acquisition rate or represents the short-term evolutionary dynamics of the Y chromosome, which will eventually converge to the slower long-term rate. Similarly, we still do not know what the ancestral Y-linked retrocopy transfer rate was and how this is evolving in general across the Drosophila complex. These questions and many others can be empirically tested by applying the present method to the growing number of Drosophila species with reference genomes. Moreover, because our method can determine Y-linked gene transfers using inexpensive short reads and does not depend on a preassembled Y chromosome or associated contigs, it holds the potential reveal fundamental details of Y chromosome evolution in many other species at a hitherto unmatched level of resolution.

Methods

Y-Linked Transfer Identification and Haplotype Assembly. Using Illumina paired end reads, we sequenced males and females of two strains from all three species and aligned them against the appropriate D. simulans reference genomes lacking Y chromosomes. SNPs with large allele frequency differences between the two sexes from the same strain were determined (see Dataset S11 for a list of all diagnostic SNPs) and then grouped into larger regions according to inter-SNP distances and gene boundaries. Haplotype sets for the resulting regions were de novo assembled with trans-ABYSS (Assembly by Short Sequences) (19) combining the subset of reads containing Y-linked alleles from both strains. A detailed explanation of the analytical pipeline and haplotype assembly is provided in SI Methods.

Estimating GeTY Age and Function. The age of each GeTY was estimated using the Y-specific nucleotide divergence from the parental ortholog scaled by the D. melanogaster base substitution rate (2.8 × 10^{-6}; ref. 20). Tests for purifying selection were performed by using AUGUSTUS (21) to predict Y-linked ORFs for each GeTY, then using codon-based phylogenetic analyses implemented in PAML (Phylogenetic Analysis by Maximum Likelihood) (22)) to estimate ω for each ORF relative to the reconstructed ancestral donor sequence and using likelihood ratio tests to determine whether these ω estimates significantly differed from 1. More details on the aging and function tests are provided in SI Methods.

Y-Linked Gene Transfer Rate. The transfer rate was calculated as the average number of GeTYs observed per lineage and three lineages divided by the estimated time of divergence between D. melanogaster and the D. simulans clade. To ensure phylogenetic independence in the D. simulans clade, we counted the transfers shared between species only once and added this number to the average of the remaining species-specific transfers in this clade [i.e., 6 + (5 + 9)/2 = 13]. Thus, the effective number of transfers, N_eff, is equal to N_{trans} + N_{sim\_clade} = 5 + 13 = 18. To get the average number of Y-linked transfers per lineage, we divided N_{eff} by the number of distinct lineages, L, and divided this value by the divergence time, $\Delta t$, to derive the average transfer rate: ($N_{eff}/(L\Delta t)$) = (18/2)/5.4 My = 1.67 novel GeTYs per lineage per My. Note that this serves as a lower bound to the true GeTY rate because transfers will be unobserved if they have degraded sufficiently to prevent read alignment or because they lack the required number of diagnostic divergent SNPs to be determined in our pipeline (i.e., ≤5 SNPs; SI Methods).

Lineage-Specific Rate Tests. We modeled gene transfers on the Y chromosome as a Poisson process where $\lambda$ is the Y-linked gene transfer rate. Differences in the Y-linked gene transfer rate between the D. melanogaster and D. simulans lineages were tested by determining the probability of observing at most the number of D. melanogaster-specific transfers given the average number of transfers specific to the two species in the D. simulans clade. Phylogenetic independence was accounted for in the same way as for the estimation of the gene transfer rate (see above). This method was applied to all gene transfers, and DNA translocations and retrocopies separately, resulting in the following probabilities: Poisson(X ≤ 5 | $\lambda$ = 13) = 0.011 for all gene transfers, Poisson(X ≤ 4 | $\lambda$ = 6) = 0.285 for DNA translocations, and Poisson(X ≤ 0 | $\lambda$ = 5.5) = 0.004 for retrocopies. Note that the latter
test remained significant when treating the ambiguous GeTY in D. melanogaster as a retrocopy: Poisson\(\lambda \leq 1 | \lambda = 5.5\) = 0.027. No significant differences were observed between D. melanogaster and the D. simulans clade for the detected functional GeTYs: Poisson\(\lambda \leq 1 | \lambda = 1.5\) = 0.56.

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