Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

Variation in mutation, recombination, and transposition rates in *Drosophila melanogaster* and *Drosophila simulans*

Yiguan Wang, Paul McNeil, Rashidatu Abdulazeez, et al.

Genome Res. 2023 33: 587-598 originally published online April 10, 2023
Access the most recent version at doi:10.1101/gr.277383.122

References This article cites 97 articles, 19 of which can be accessed free at:
http://genome.cshlp.org/content/33/4/587.full.html#ref-list-1

Open Access Freely available online through the *Genome Research* Open Access option.

Creative Commons License This article, published in *Genome Research*, is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at http://creativecommons.org/licenses/by-nc/4.0/.

Email Alerting Service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or **click here.**

To subscribe to *Genome Research* go to:
https://genome.cshlp.org/subscriptions

© 2023 Wang et al.; Published by Cold Spring Harbor Laboratory Press

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

Research

Variation in mutation, recombination, and transposition rates in *Drosophila melanogaster* and *Drosophila simulans*

Yiguan Wang,[1] Paul McNeil,[1] Rashidatu Abdulazeez,[2] Marta Pascual,[3] Susan E. Johnston,[1] Peter D. Keightley,[1] and Darren J. Obbard[1]

[1]*Institute of Ecology and Evolution, University of Edinburgh, Edinburgh EH9 3FL, United Kingdom;* [2]*Department of Zoology, Ahmadu Bello University, 810107 Zaria, Nigeria;* [3]*Departament de Genètica, Microbiologia i Estadística and IRBio, Universitat de Barcelona, 08028 Barcelona, Spain*

The rates of mutation, recombination, and transposition are core parameters in models of evolution. They impact genetic diversity, responses to ongoing selection, and levels of genetic load. However, even for key evolutionary model species such as *Drosophila melanogaster* and *Drosophila simulans*, few estimates of these parameters are available, and we have little idea of how rates vary between individuals, sexes, or populations. Knowledge of this variation is fundamental for parameterizing models of genome evolution. Here, we provide direct estimates of mutation, recombination, and transposition rates and their variation in a West African and a European population of *D. melanogaster* and a European population of *D. simulans*. Across 89 flies, we observe 58 single-nucleotide mutations, 286 crossovers, and 89 transposable element (TE) insertions. Compared to the European *D. melanogaster*, we find the West African population has a lower mutation rate (1.67×10^{-9} site^{-1} gen^{-1} vs. 4.86×10^{-9} site^{-1} gen^{-1}) and a lower transposition rate (8.99×10^{-5} copy^{-1} gen^{-1} vs. 23.36×10^{-5} copy^{-1} gen^{-1}), but a higher recombination rate (3.44 cM/Mb vs. 2.06 cM/Mb). The European *D. simulans* population has a similar mutation rate to European *D. melanogaster*, but a significantly higher recombination rate and a lower, but not significantly different, transposition rate. Overall, we find paternal-derived mutations are more frequent than maternal ones in both species. Our study quantifies the variation in rates of mutation, recombination, and transposition among different populations and sexes, and our direct estimates of these parameters in *D. melanogaster* and *D. simulans* will benefit future studies in population and evolutionary genetics.

[Supplemental material is available for this article.]

Mutation is the ultimate source of all genetic variation, and the mutation rate consequently plays a key role in evolutionary processes (Lynch 2010; Lynch et al. 2016). Although the germline mutation rate is thought to be lower than the somatic mutation rate (Milholland et al. 2017), only germline mutations can be inherited, and so have greater importance in population and evolutionary genetics. It has been hypothesized that the germline mutation rate in multicellular eukaryotes may be explained by an equilibrium between natural selection to minimize it and the power of genetic drift to overcome the effect of selection (Lynch et al. 2016). However, to date, there are relatively few estimates of germline mutation rate from multicellular eukaryotes, and those estimates that we do have are patchily distributed across the tree of life (for review, see Yoder and Tiley 2021; but see Bergeron et al. 2023; Wang and Obbard 2023). On the one hand, mutation rates appear to differ markedly between some distantly related species. For example, primate mutation rates are around 10×10^{-9} site^{-1} gen^{-1} (Rahbari et al. 2016; Jónsson et al. 2017; Lindsay et al. 2019), whereas insect mutation rates are generally lower than 6×10^{-9} site^{-1} gen^{-1} (Keightley et al. 2014, 2015; Yang et al. 2015; Liu et al. 2017). On the other hand, some very distantly related lineages, such as *Drosophila* and *Heliconius* that are separated by ∼290 million years of evolution (Misof et al. 2014; Suvorov et al. 2022), have estimates that are not significantly different from each other (Keightley et al. 2014, 2015).

The apparent variation in mutation rate may, in part, reflect variation within species combined with very limited sampling. For example, in humans—for which many direct estimates are available—estimates range from 9.6×10^{-9} (Campbell et al. 2012) to 21.7×10^{-9} site^{-1} gen^{-1} (O'Roak et al. 2012), and parental age at conception is positively correlated with the number of mutations in the offspring (Kong et al. 2012; Kaplanis et al. 2022). For *Drosophila melanogaster* there are relatively few estimates, ranging from 2.8×10^{-9} to 6.0×10^{-9} site^{-1} gen^{-1} (Haag-Liautard et al. 2007; Keightley et al. 2009, 2014; Schrider et al. 2013; Huang et al. 2016; Sharp and Agrawal 2016; Assaf et al. 2017), but there has been no formal attempt to quantify variation in mutation rate between the sexes or among wild individuals or populations.

Apparent variation in mutation rate may also be partly attributable to a lack of precision in the estimates, as the challenge of accurately estimating the mutation rate can be formidable when one or fewer de novo mutations is expected per offspring, and when there are millions of segregating sites—as in *D. melanogaster* (Keightley et al. 2014). As a consequence of this, most studies in model species have used a "mutation accumulation" (MA) approach, in which selection is relaxed for multiple generations in fully inbred sib-mated lines, and multiple mutations are counted

Corresponding author: yiguan.wang@ed.ac.uk
Article published online before print. Article, supplemental material, and publication date are at https://www.genome.org/cgi/doi/10.1101/gr.277383.122. Freely available online through the *Genome Research* Open Access option.

© 2023 Wang et al. This article, published in *Genome Research*, is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at http://creativecommons.org/licenses/by-nc/4.0/.

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

at the end of the experiment (Sung et al. 2015; Uchimura et al. 2015; Oppold and Pfenninger 2017). Unfortunately this approach cannot detect recessive lethal mutations and may conceivably be biased by the accumulation of deleterious variants that themselves affect mutation rates (Baer et al. 2007). In contrast, pedigree or parent-offspring trio studies, such as those widely used for humans and other large mammals, give a direct and relatively unbiased estimate of the mutation rate (Ségurel et al. 2014). This approach has rarely been applied in *Drosophila* (but see Keightley et al. 2014; Krasovec 2021), despite the fact that it provides the opportunity to obtain less biased estimates of the mutation rate and how it varies within and between species.

Recombination also plays a central role in creating variation, by breaking linkage between segregating mutations and facilitating their independent fixation or loss (Webster and Hurst 2012). However, in contrast to mutation rates, recombination rates have frequently been estimated in mapping studies. These have shown that recombination rates vary within and between chromosomes, individuals, sexes, populations, and species, and the variation can be attributed to both genetic factors (e.g., rate and landscape modifier loci, genome architecture, and chromatin structure) and environmental factors (e.g., age, temperature, and pathogen infection) (Stapley et al. 2017). Genome-wide estimates of recombination rate are available for several *Drosophila* species, including a *D. serrata* estimate of ~ 1.4 cM/Mb (Stocker et al. 2012), *D. pseudoobscura* of 3.3–4.6 cM/Mb, *D. miranda* of 4.9–6.1 cM/Mb (Heil et al. 2015), *D. persimilis* of 4.1 cM/Mb for autosomes and 5.0 cM/Mb for X Chromosome (Stevison and Noor 2010), *D. virilis* of 4.6 cM/Mb (Hemmer et al. 2020), and *D. melanogaster* of 2.5 cM/Mb (Comeron et al. 2012). However, experimental studies of *Drosophila* have rarely looked at variation in genome-wide recombination rates in natural populations of outbred individuals, and wild populations may differ systematically from populations that have been strongly selected by adaptation to the laboratory (Aggarwal et al. 2021).

Transposition by mobile genetic elements (transposable elements; TEs), although perhaps less widely considered by evolutionary studies as an important source of variation, can occur at a rate similar to that of the nucleotide mutation rate (Adrion et al. 2017). New TE insertions can be a major source of loss-of-function mutations (Hirsch and Springer 2017), including those that directly disrupt genes and those that indirectly lead to ectopic chromatin formation (Wells and Feschotte 2020), and over evolutionary time TEs have often been "domesticated" into important roles in the host genome via various mechanisms (Chuong et al. 2017; for review, see Almeida et al. 2022). Using in situ hybridization in *D. melanogaster*, some early studies revealed that the transposition rate varied by several orders of magnitude, ranging from 10^{-5} copy^{-1} gen^{-1} to 10^{-2} copy^{-1} gen^{-1} (Nuzhdin and Mackay 1995; Pasyukova et al. 1998; Maside et al. 2000; Díaz-González et al. 2011). This variation is partly caused by differences in activity between TE families; for example, *INE-1* has been inactive for millions of years in *D. melanogaster*, whereas *Transib* is recently active (Kapitonov and Jurka 2003). TE activity also varies among populations or chromosomes (Adrion et al. 2017; Ho et al. 2021), and is affected by factors such as stress and aging (Capy et al. 2000; Guerreiro 2012; Chuong et al. 2017; Horváth et al. 2017). It has also been claimed that colonization of new habitats could induce transposition in some *Drosophila* species, for example in *D. buzzatii* and *D. subobscura* (Guerreiro et al. 2008; Guerreiro and Fontdevila 2011), highlighting the potential influence of environmental stress and emphasizing differences in TE activity between laboratory and wild conditions.

Here, we directly estimate the de novo mutation rate, recombination rate, and rate of TE insertion in low-complexity regions by sequencing parents and offspring from 18 families of full-sib *D. melanogaster* and *D. simulans*. These direct estimates in fully wild fly genomes not only highlight the variation in rates among populations and individuals, but also provide improved parameter estimates for future evolutionary studies.

Results

We sequenced parents and offspring of six unrelated outbred sibships from a West African population of *D. melanogas*ter, a European population of *D. melanogaster*, and a European population of *D. simulans*. In total, we generated 9.5 billion 150 bp paired-end Illumina sequencing reads, and after removing duplicates, the median genome coverage was above 30-fold for all but one of the 125 flies (range 28- to 108-fold; Supplemental Fig. S1). Based on the simulation of synthetic mutations in raw reads (Supplemental Code), we estimate the median proportion of callable sites was 83.1% (range 78.3%–86.0%) for SNMs, and 78.3% (range 73.5%–80.1%) for short indels.

Based on our wild-collected flies, the overall synonymous diversity (π_S) was 1.27% in the West African *D. melanogaster*, 0.89% in the European *D. melanogaster*, and 2.25% in the European *D. simulans*, similar to previously reported estimates (Parsch et al. 2010; Campos et al. 2017). The nonsynonymous to synonymous diversity ratio (π_A/π_S) was very similar between the three populations (0.106, 0.107, and 0.097, respectively). Examination of a genomic relatedness matrix suggests that all the parents were unrelated except one pair from West African *D. melanogaster* (dmeA_15_F0 vs. dmeA_23_F0) and one pair from European *D. melanogaster* (dmeE_27_M0 vs. dmeE_30_F0), which were potentially third-degree relatives (i.e., first cousins; Supplemental Fig. S2). However, as these flies appeared in different families in our study, no further inbreeding occurred. Using published breakpoint markers, we tested for the presence of seven cosmopolitan chromosomal inversions in the two populations of *D. melanogaster*, and discovered 20 In(2L)t, three In(2R)Ns, six In(3L)P, six In(3R)K, and seven In(3R)Payne inversions, but no In(3R)C inversions segregating among the 48 haplotypes (Supplemental Table S2).

To test the performance of our pipeline for SNM identification, we applied the whole pipeline to trio data from rhesus macaques, as recently used by the "Mutationathon" study (Bergeron et al. 2022). Of the 33 SNMs there validated by polymerase chain reaction (PCR), our pipeline recovered 27 (i.e., an ~20% underestimate), which was an improvement on the other presented pipelines (Supplemental Fig. S3). Of the six false-positive SNMs that were mistakenly identified by some other pipelines, we only identified one. However, our pipeline also identified a further six candidates that have not been examined by PCR (i.e., an ~20% overestimate if all proved to be false positives). Thus, at least in terms of sensitivity, our pipeline shows a performance at least as good as equivalent state-of-the-art approaches.

Mutation rates differ between populations and sexes

In total, we identified 58 SNMs across an estimated 17.45 billion callable sites, giving a crude overall de novo mutation rate across sexes and populations of 3.32×10^{-9} site^{-1} gen^{-1}, with 95% binomial bounds of 2.52×10^{-9} to 4.30×10^{-9} (Fig. 1A; Supplemental Fig. S4; Supplemental Table S1). However, the number of

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

mutations appeared to vary among populations, sexes, and individuals (analysis below). Overall, SNMs were significantly biased toward transitions (overall 60.3%, binomial *P*-value = 3.31×10^{-5}; Fig. 1B), but the mutational spectrum did not vary among populations (Fisher's exact test; *P*-value = 0.82). We also identified six short deletions and three insertions, giving an overall short indel rate of 5.00×10^{-10} site^{-1} gen^{-1}, with 95% binomial bounds of 2.29×10^{-10} to 9.50×10^{-10}; Table 1). Unlike the SNMs, the number of indels did not appear to differ markedly between populations or sexes, with six of the attributable indel mutations occurring in the father.

Where the larger number of SNMs permitted, we used a Bayesian binomial linear mixed model to analyze variation in the mutation rate, fitting sex and population as fixed effects and parental identity as a random effect. Some mutations could not be attributed to one parent or the other, and we chose to assign half of these to males and half to females (underestimating the difference between sexes). Our model estimates are presented in Figure 1C, and alternative models are presented in Supplemental Material (Supplemental Fig. S5), although the results were not qualitatively different. Throughout, we treat the proportion of the posterior density overlapping zero as a "*P*-value," and we make no correction for multiple tests. We found the SNM rate of the West African *D. melanogaster* was significantly lower than that of the European populations of *D. melanogaster* or *D. simulans*, at 1.67×10^{-9} (95% HPD CI 0.54–3.14) versus 4.86×10^{-9} (2.11–8.02) and 4.51×10^{-9} (1.94–7.75), respectively (*P*-value = 0.035, *P*-value = 0.048; no correction for multiple testing), but that the European *D. melanogaster* and *D. simulans* populations did not differ significantly from each other (*P*-value = 0.863). Overall, we found that the mutation rate was higher in males than females, at 5.24×10^{-9} (2.96–7.83) versus 2.05×10^{-9} (0.94–3.26; *P*-value = 0.010)—although this was no longer significant if all of the unphased mutations were assigned to females, thereby minimizing the possible difference (*P*-value = 0.108). Despite an apparently large range in the number of mutations seen per parent, after accounting for the fixed effects we found no strong support for substantial variation in mutation rate among individuals (65.48% of the remaining variance being attributable to among-individual variation, but with a 95% credibility interval of 5.00%–99.96%).

Recombination rates vary among populations and chromosomes

We phased the parental haplotypes using the offspring data, and then used phase switches in the offspring to infer meiotic crossover breakpoints. Preliminary examination of the haplotype plots (Supplemental Fig. S6) identified a small number of large-scale

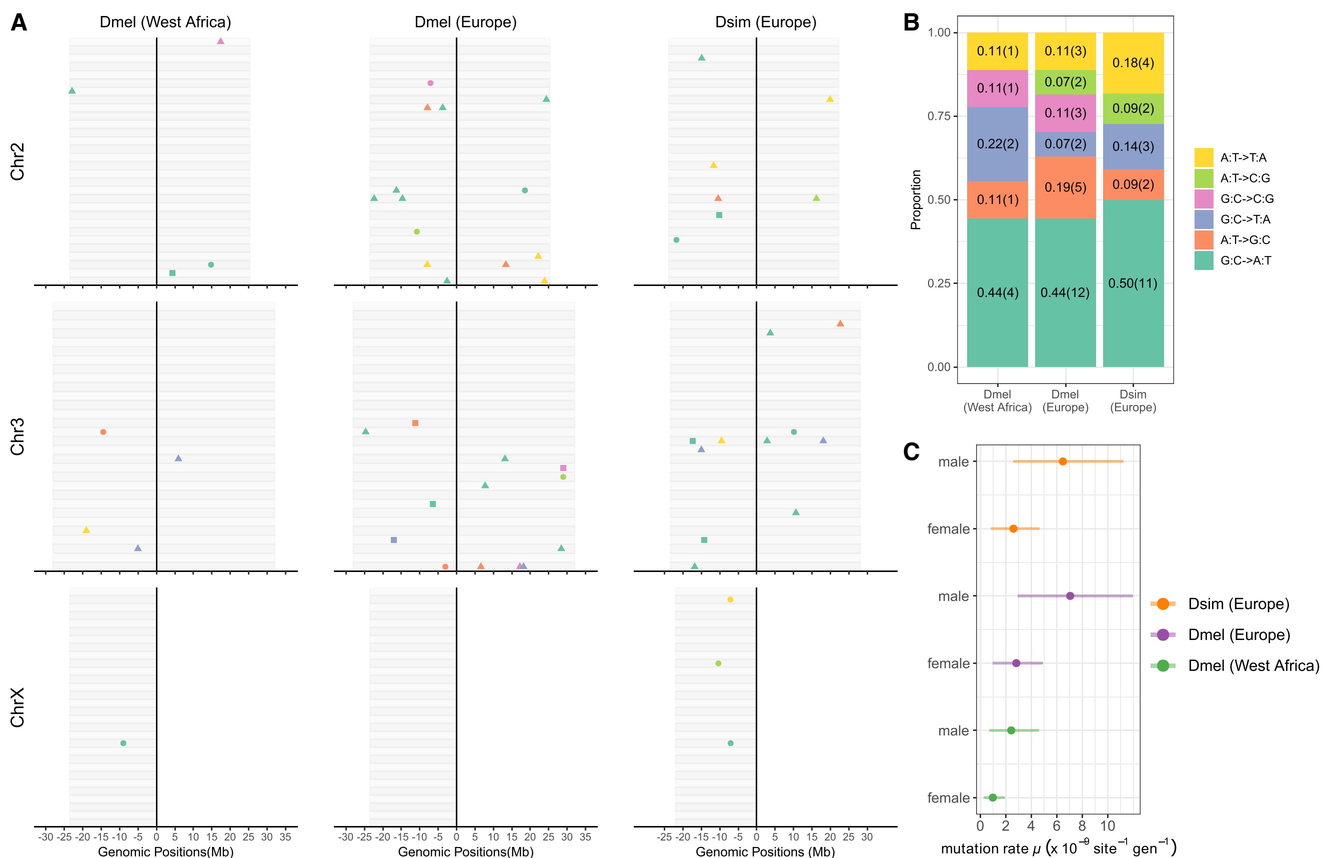

Figure 1. A summary of de novo SNMs identified in a West African and a European population of *Drosophila melanogaster*, and a European population of *Drosophila simulans*. (*A*) The genomic positions of the SNMs on chromosomes. Each gray bar represents the genome of one offspring. Point color represents mutation type and point shape represents the parental origin of the mutation (triangle: paternal; circle: maternal). Square points are used to denote the SNMs with unknown parental origin caused by the lack of informative surrounding SNP markers. (*B*) The SNM spectrum. The *y*-axis shows the proportion of each mutation type, and the numbers in brackets are the counts. (*C*) The mutation rate of SNMs and 95% CI estimated from our main Bayesian GLMM in which "population" and "sex" were fixed effects and parental ID was a random effect.

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

Table 1. The summary of indel mutations

Populations	FamilyID	Chromosome	Reference	Alternate	Parental	μ (site^{-1} gen^{-1})
Dmel (West Africa)	01	3R	AAAAATATATGCCAGCTTCAGTTGGCCATATTTTAATGGG	A	paternal	3.56×10^{-10}
	18	2R	TGTCAAAGA	T	paternal	
Dmel (Europe)	28	2L	T	TGCTA	paternal	5.31×10^{-10}
	29	3L	T	TG	paternal	
	29	3R	CCAGGAAGG	C	unknown	
Dsim (Europe)	26	2L	TA	T	paternal	7.76×10^{-10}
	31	3R	CAAATA	C	paternal	
	27	X	GTTTTCCATTTTT	G	maternal	
	28	X	T	TATCCA	maternal	

phasing errors in the parents that were detectable through multiple sibling F1 individuals sharing identical breakpoints, for example on the X Chromosome in the European *D. melanogaster* "fam30," 2R in "fam27," X in "fam28," and 2R in "fam29" in the European *D. simulans*. We manually corrected these errors by switching the parental phases. Consistent with an absence of recombination in male *Drosophila* (Morgan 1912), no recombinant haplotypes strongly supportive of crossover were detected in males. However, we did identify shorter apparent recombinant regions with maximum length of up to ~1 Mbp in males and ~4 Mbp in females (corresponding to 1190 SNP markers). We believe these most likely represented genotyping errors (longer regions) or gene conversion events (shorter regions). After excluding these regions, we identified 103 recombination events in female West African *D. melanogaster*, 74 in European *D. melanogaster*, and 109 in European *D. simulans* (Fig. 2; Supplemental Table S2; Supplemental Fig. S4). This corresponds to 3.43, 2.55, and 3.63 detectable crossovers per individual, resulting in raw recombination rates of 2.59 cM/Mb (95% binomial confidence interval: 2.11–3.14), 1.92 cM/Mb (1.51–2.42), and 3.03 cM/Mb (2.49–3.66), respectively. To examine the robustness of our inference, we explored the impact of marker thresholds lower than 1190 and found that the number of recombination events remained stable even if we varied the threshold (Supplemental Fig. S7). We could not detect any correlation between the number of crossovers detected and the number of available SNP markers in a chromosome arm (P-value = 0.126; Supplemental Fig. S8), suggesting our ability to detect crossovers is not strongly affected by variation in the number of SNPs.

As expected, there were few recombination breakpoints close to centromeres and telomeres. In *D. melanogaster*, the nearest breakpoint to a centromere was identified at a distance of 6.8 Mb on 2L (Fig. 2A). In *D. simulans*, all the breakpoints were >3.7 Mb away from centromere except one identified at 1.3 Mb from the centromere on 3R. Among the crossovers, we observed 45 double crossovers, in which two crossovers were detectable on the same chromosomal arm in one meiosis, with 18 in West African *D. melanogaster*, six in European *D. melanogaster*, and 21 in European *D. simulans*. Double crossovers were particularly common for the X Chromosome in West African *D. melanogaster*, in which nearly half of the crossovers occurred in doubles, and there were three triple crossovers and three quadruple crossovers (Fig. 2B). Although recombination can be mutagenic (McVey et al. 2016), no breakpoint was inferred closer than 149 kbp to an SNM (West African *D. melanogaster* family15, the maternal X Chromosome inherited by offspring M1). Given that 14 SNMs and 286 recombination breakpoints were confidently assigned to mothers, this suggests an upper 95% binomial bound of <1.2% for the proportion of crossovers that result in SNMs, and an upper 95% binomial bound of <23% for the proportion of maternal SNMs that result from crossover recombination.

To quantify maternal recombination rate more robustly, and to test if recombination rates varied among populations, individuals, and chromosome arms, we applied MCMCglmm using models analogous to those used above for mutation rate. We fitted population and chromosome arm as fixed effects, and individual as a random effect. In addition, because large inversions are expected to suppress recombination, we fitted a fixed effect of "inverted" for maternal chromosome arms in individuals inferred to be heterozygous for any of the seven large inversions we examined. In the absence of large inversions, we found that the recombination rate in flies from the West African population of *D. melanogaster* was significantly higher than that in flies from the European populations of *D. melanogaster* at 3.44 (95% HPD CI 2.72–4.18) cM/Mb versus 2.06 (1.57–2.57) cM/Mb (P-value = 0.004), but not higher than the European *D. simulans* 3.04 (2.45–3.73) cM/Mb (P-value = 0.430). The European *D. melanogaster* had a lower recombination rate than *D. simulans* (P-value = 0.019). We did not see any recombination on chromosome arms that were heterozygous for large cosmopolitan inversions in *D. melanogaster*, and the presence of an inversion significantly suppressed recombination (P-value $< 2 \times 10^{-4}$). Because marker-based inversion detection may miss those in less well-studied African populations, we may underestimate the impact of inversions on the recombination rate. However, only one of the 23 putatively uninverted chromosome arms in the African population failed to show evidence of recombination, suggesting the impact of this effect is likely to be very small. If the presence or absence or large inversions is not considered, the estimated recombination rates are 2.61 (2.02–3.27) cM/Mb in the West African population and 1.94 (1.42–2.47) cM/Mb in European population of *D. melanogaster* (Fig. 2C).

Using a threshold P-value of 0.05 without correcting for multiple testing, we detected a significantly higher recombination rate on arm 2L (3.62 [2.71–4.63] cM/Mb) and the X Chromosome (3.57 [2.74–4.46] cM/Mb) than that on arms 2R (2.43 [1.70–3.20] cM/Mb) and 3L (2.05 [1.49–2.67] cM/Mb). The recombination rate on arm 3R was estimated at 2.70 (2.00–3.47) cM/Mb and was not significantly different from other chromosomal arms. It is possible that differences in recombination rate among chromosomes, such as a slightly higher rate on the X, may be at least partly attributable to differences in chromosome length (Jensen-Seaman et al. 2004) as the average number of chiasmata per chromosome arm was relatively constant within populations. After accounting for all of the fixed effects, we found little evidence for variation among individuals (48.6% of the remaining variance being attributable to among-individual variation; 95% CI of 0%–96.2%).

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

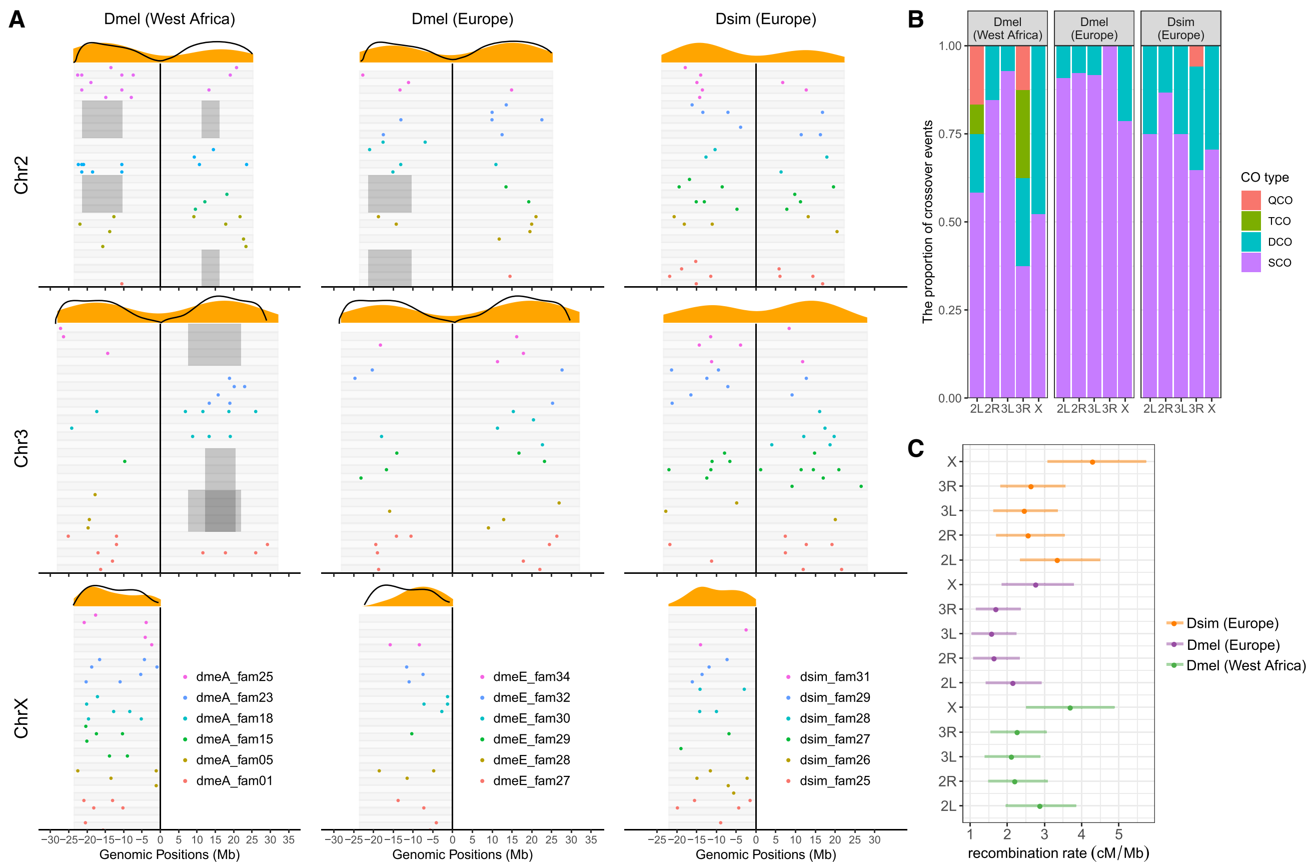

Figure 2. A summary of recombination (crossovers) in the females of three populations of *Drosophila*. (*A*) The genomic positions of breakpoints on chromosomes. Each gray bar represents an offspring individual and colors denote the breakpoints in different families. Orange density plots *above* represent the density of breakpoint along each chromosome and the black curve lines represent recombination rates from Comeron et al. (2012). The classical inversions of *D. melanogaster* (In(2L)t, In(2R)NS, In(3R)K, and In(3R)Payne) heterozygous in the parents are shown as gray areas. (*B*) The proportion of crossover types; SCO: one crossover event on a chromosomal arm during one meiotic process; DCO: two crossover events in one meiotic process; TCO: three crossover events; QCO: four crossover events. (*C*) The recombination rate and 95% CI estimated from a Bayesian GLMM in which "population" and "chromosome" were fitted as fixed effects and parental ID as a random effect, but with no effect of inversion fitted (see the main text for alternative estimates).

TE insertion rates differ between populations and sexes

We used TEFLoN (Adrion et al. 2017) to annotate TEs in parents and offspring, and we identified new insertions as those that were heterozygous in a single F1 offspring, but absent from all other individuals in the study. In total, TEFLoN identified ~ 200,000 TE copies across the 24 parental *D. melanogaster* genomes. The 15 most abundant TEs in the parents are shown in Supplemental Figure S9. As expected, the rankings of TE abundance were very similar between the two populations of *D. melanogaster*, with *Gypsy*, *Helitron*, and *Jockey* elements being the most abundant, followed by *Pao*, *P*, *TcMar-Tc1*, *CR1*, *R1*, *CMC-Transib*, and *INE-1*. In total we identified ~ 138,000 TE copies across the 12 *D. simulans* parents. The ranking was slightly different in the *D. simulans* population, as predicted from the known differences in the TE community between these species (Mérel et al. 2020), with *Helitron* elements being the most common TE, followed by *Gypsy* and *Jockey*. DNA transposons made up 39% of all TEs in *D. melanogaster* and 44% in *D. simulans*; long-terminal repeats (LTRs) made up 36% and 34% in the two species, respectively; non-LTRs made up 25% and 22%, respectively. In all populations, most individual TE insertions were rare—with 45.7% being singletons and 17.8% being doubletons—and in general retrotransposons were more frequent than DNA transposons.

By comparing the parents and offspring in each family, we identified 89 new TE insertions across the three population samples, with 18 in West African *D. melanogaster*, 45 in European *D. melanogaster*, and 26 in European *D. simulans* (Supplemental Fig. S4). Although these numbers probably represent lower bounds for the true number of insertions, as those falling in repetitive hard-to-map regions or within other TEs are unlikely to be detectable, our estimates are likely to be a good estimate of the number of transpositions into nonrepetitive, gene-rich, euchromatin. The 18 new insertions in the West African *D. melanogaster* came from just two TE superfamilies: *CMC-Transib* and *Gypsy*, with the largest contributor being *CMC-Transib* (17 insertions) (Fig. 3A,B; Supplemental Fig. S10). The five active superfamilies appeared to be different in Europe (Fisher's exact test; *P*-value = 0.0005), with 19 *Gypsy*, 16 *CMC-Transib*, 6 *Pao*, 3 *Jockey*, and 1 *hAT-hobo* insertions. The highly active *CMC-Transib* elements in *D. melanogaster* (3.09×10^{-3} insertions copy^{-1} gen^{-1}) were less active in *D. simulans*, only showing three insertions with an insertion rate of 5.67 $\times 10^{-4}$ copy^{-1} gen^{-1}. We observed 6 *Copia* insertions and 8 *TcMar-Mariner* insertions in *D. simulans*, but none in

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

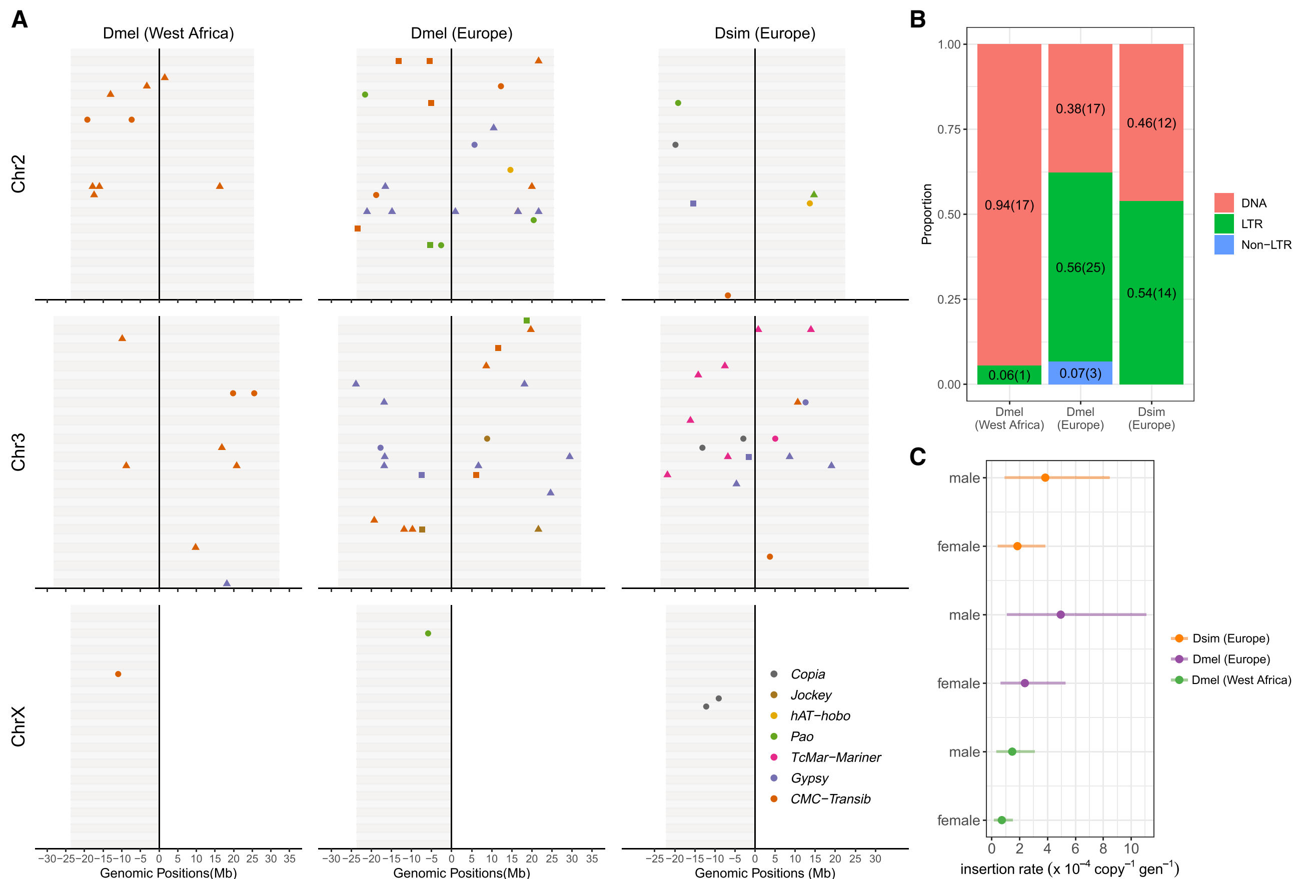

Figure 3. A summary of TE insertions in the three populations of *Drosophila*. (*A*) The genomic positions of the new TE insertions on chromosomes. Each gray bar represents an offspring individual, point color represents TE superfamilies, and point shape represents the parental origin of the insertion (triangle: paternal; circle: maternal; square: unknown). As above, insertions with unknown parental origin are shown as squares. (*B*) The proportion of new insertions of DNA elements, LTRs, and non-LTRs in the three populations. (C) The insertion rate (per copy per generation) and 95% CI estimated from a Bayesian GLMM in which "population" and "sex" were fixed effects and parental ID was a random effect.

D. melanogaster. The insertion rates for *Copia* and *TcMar-Mariner* in *D. simulans* were estimated as high as 2.71×10^{-3} copy^{-1} gen^{-1} and 3.06×10^{-2} copy^{-1} gen^{-1}, respectively. We did not observe new *Jockey* insertions in *D. simulans*. Underlying these raw counts is substantial variation among individuals; for example, all 11 *Nomad* insertions (*Gypsy* superfamily) in European *D. melanogaster* occurred in the same individual parent. There were also potential differences between the sexes, as among those TE insertions that could be attributed to the parent of origin in *D. melanogaster* the majority occurred in males (38 vs. 15), although in *D. simulans* the males and females had a similar number of TE insertions (12 vs. 11).

As above, we used MCMCglmm to quantify transposition rates and their variation between populations and sexes. Transposition rates can be quantified relative to the number of TE copies in the parent or relative to the number of sites available in the genome, depending on whether one is interested directly in the insertion activity of TEs or instead in their impact on the genome. Accordingly, we fitted models with either the number of parental element copies as the "trials" in the binomial model, or the number of callable sites. Similar to the SNM rate, insertion rates were significantly lower in West African *D. melanogaster* than in European *D. melanogaster*, both when quantified per parental element (P-value = 0.026) (Fig. 3C) and per base (P-value = 0.035). The European population of *D. simulans* had an intermediate rate that was not significantly different from the two *D. melanogaster* populations. Males tended to have slightly more insertions than females, but this effect was not significant (P-value = 0.118 for per-copy rate, P-value = 0.071 for per-site rate). Overall, per parental copy, we estimate 8.99×10^{-5} (5.33–14.21) insertions copy^{-1} gen^{-1} for the West African population versus 23.36×10^{-5} (17.04–31.26) insertions copy^{-1} gen^{-1} in the European *melanogaster*, and 18.70×10^{-5} (12.25–27.48) insertions copy^{-1} gen^{-1} in the European *simulans* (Supplemental Table S3). Per callable base, the rates were 2.95×10^{-9} (1.73–4.62) site^{-1} gen^{-1}, 7.54×10^{-9} (5.50–10.09) site^{-1} gen^{-1}, and 4.38×10^{-9} (2.86–6.42) site^{-1} gen^{-1} for the three populations, respectively. Note, however, that our estimate of "callable sites" here depends on the number of sites passing quality filters, not on simulation, and thus may be upwardly biased—leading to a potential downward bias in estimated rate of TE insertions.

Discussion

Here, we used parent-offspring sequencing to directly estimate the mutation, recombination, and transposition rates in two populations of *D. melanogaster* and one population of *D. simulans*. By crossing unrelated wild-collected or first-generation flies, we were

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

able to avoid many of the biases that can arise from inbreeding in mutation-accumulation and mapping studies, such as selection against recessive deleterious mutations or the direct impact of new mutations on mutation, recombination, or transposition rates. In addition, because we could identify the parent of origin for the majority of events, we were able to quantify differences in rate between the sexes and between individuals.

Overall mean rates of mutation, recombination, and transposition

Previous estimates of the de novo SNM rate for *D. melanogaster* span the range from 2.8×10^{-9} site^{-1} gen^{-1} to 6.0×10^{-9} site^{-1} gen^{-1} (Keightley et al. 2014; Sharp and Agrawal 2016), and our overall mean estimate of 3.3×10^{-9} site^{-1} gen^{-1} across *D. melanogaster* and *D. simulans* is in close agreement. This is at the lower end of the range seen for metazoa (Wang and Obbard 2023), which range from 2.0×10^{-9} to 16.6×10^{-9} (Yoder and Tiley 2021), but is very similar to other insects, including bumblebees at 3.6×10^{-9} (Liu et al. 2017), honeybees at 3.4×10^{-9} (Yang et al. 2015), and butterflies at 2.9×10^{-9} (Keightley et al. 2015). Our estimates correspond to an average of just 0.8 new SNMs present in each new female embryo.

As with previous studies, we observed a mutational spectrum that is biased toward transitions, particularly G:C → A:T (Schrider et al. 2013), and we observed one complex mutational event, with two mutations occurring only 2 bp apart. This one event out of 57 is consistent with a previous MA study of two *D. melanogaster* lines that suggested around 2% of SNMs in *Drosophila* are complex (Schrider et al. 2013), likely as result of the error-prone polymerases used to bypass some DNA lesions during synthesis (Ségurel et al. 2014). Compared to SNMs, new short indels are scarce in *Drosophila*, and we only detected nine in total. This implies an indel rate of 0.5×10^{-9} site^{-1} gen^{-1}, which is about half that estimated by Keightley et al. (2014) who estimated 1.2×10^{-9}, but similar to the estimate of Schrider et al. (2013) at ~ 0.4×10^{-9}. Indel rates vary across multicellular eukaryotes, from 0.3×10^{-9} site^{-1} gen^{-1} in mouse to 1.8×10^{-9} site^{-1} gen^{-1} in humans (for review, see Sung et al. 2016), placing *Drosophila* at the lower end of the range.

Recombination rates are known to vary among taxa, from 0.03 cM/Mb to 28.10 cM/Mb among multicellular plants and animals (Stapley et al. 2017). Although our parent-offspring approach lacks power, at 2.50 cM/Mb our raw estimate of the overall recombination rate in females is very close to a previous estimate of 2.45 cM/Mb (Comeron et al. 2012). As expected, recombination events appeared to be suppressed near centromeres and in the presence of large chromosomal inversions (Crown et al. 2018). Previous studies have reported the variation of recombination rate between chromosomes, for example the higher rate on X Chromosome in African *D. melanogaster* (Chan et al. 2012), which is in line with our study after excluding the impact of large chromosomal inversions.

Although TE insertions provide an unusually disruptive form of mutation (Wells and Feschotte 2020), transposition rates are more difficult to estimate directly and have not been widely studied. Here we estimate an overall rate of at least 16.7×10^{-5} insertions per parental TE copy per generation, corresponding to 4.93×10^{-9} insertions per site per generation, or approximately one new insertion in each new female embryo. Our estimates are 2.3-fold higher than a previous estimate (Adrion et al. 2017) in *Drosophila* after ~150 generations of mutation accumulation (2.11×10^{-9} site^{-1} gen^{-1}), and it is tempting to speculate that this could be as a result of very strong selection against new insertions, even in MA lines. It is notable that our estimate of the TE insertion rate is ~1.5-fold higher than the rate of de novo SNMs. This suggests that the impact of deleterious mutation on the genome from TE insertions is likely to be substantially higher, especially given their likely effects, than the impact of deleterious SNMs.

Rate differences between populations, sexes, and individuals

Underlying these rates of mutation, recombination, and TE insertion was substantial variation among populations, sexes, and individuals. We observed an SNM rate that was nearly threefold (95% HPD CI: 1.07- to 6.58-fold) higher in the European population than in the West African population of *D. melanogaster* (4.8×10^{-9} site^{-1} gen^{-1} vs. 1.6×10^{-9} site^{-1} gen^{-1}), with European *D. simulans* being more similar to European *D. melanogaster* (4.5×10^{-9} site^{-1} gen^{-1} vs. 4.8×10^{-9} site^{-1} gen^{-1}). We note that our low estimate for West African *D. melanogaster* is close to that of the only other African estimate (2.8×10^{-9} site^{-1} gen^{-1})—which was similarly based on parent-offspring sequencing from a single family collected in Ghana (Keightley et al. 2014)—whereas our estimate for European *D. melanogaster* is closer to those from inbred North American isolates (Schrider et al. 2013; Huang et al. 2016; Assaf et al. 2017). If the mutation rate is minimized by selection (Lynch et al. 2016), then a higher rate in the *D. melanogaster* diaspora could reflect reduced efficacy of selection, perhaps caused by smaller N_e and/or bottle-necking effects. However, there was no appreciable difference in the π_A/π_S ratio between the European and African populations, and this would be expected to be higher when constraint is ineffective.

We also saw a substantial (2.5- to 4.3-fold) difference in the SNM rates between male and female flies, approaching that seen in primates (three- to eightfold) (Taylor et al. 2006; Arnheim and Calabrese 2009; Ségurel et al. 2014). Direct observation of sex differences in mutation rate has not been reported previously for *Drosophila*, as other studies have not assigned mutations to the parent of origin. However, Schrider et al. (2013) examined differences in mutation accumulation on the X Chromosome and autosomes within MA lines, suggesting a potential difference between the two sexes, and a study of neo-sex chromosomes in *D. miranda* suggested twice as many mutations in males as females (Bachtrog 2008). Finally, although we did observe many more mutations in some individuals than in others (up to nine among the offspring of one *D. melanogaster* male, in which the expected number was 3.9), and previous studies have suggested substantial differences between inbred lines (Schrider et al. 2013), after accounting for the fixed effects of population and sex we had little power to quantify the level of among-individual variation (95% CI: 0 to 0.79). This is in contrast to an MA study on the green alga *Chlamydomonas reinhardtii* that found a sevenfold variation among the strains (Ness et al. 2015), an order difference among genotypes in *Daphnia magna* (Ho et al. 2020) and the presence of hypermutation in some individual humans (Kaplanis et al. 2022).

As expected, we saw no compelling evidence for crossovers in male *Drosophila* (Morgan 1912), but we did see some evidence for variation in female recombination rate between populations, with the model estimates for female African and European *D. melanogaster*, and European *D. simulans* of 3.4 cM/Mb, 2.1 cM/Mb, and 3.0 cM/Mb, respectively. This is comparable to previous estimates from *D. melanogaster* (Comeron et al. 2012; Miller et al. 2016), and is consistent with Chan et al. (2012) who suggested the North American *D. melanogaster* had a lower recombination rate than the African populations. Our estimate for *D. simulans* is in close agreement with the observation that the linkage map

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

length in *D. simulans* is around 1.3-fold longer than that in *D. melanogaster* (True et al. 1996).

As with mutation and recombination rate, we identified substantial variation among populations in the rate of transposition. Notably, the West African population of *D. melanogaster* had a 2.6-fold lower insertion rate than the European population, with the rate in the *D. simulans* population more similar to that of European *D. melanogaster*. We observed some TEs were highly active in specific families, consistent with observations from MA lines of *D. melanogaster* (Adrion et al. 2017).

Causes and consequences of rate variation

Per generation mutation rates are generally thought to be determined by DNA replication fidelity, DNA repair efficiencies, mutagen exposure, and the number of germline cell divisions per generation (Baer et al. 2007). Consequently, sex and age are often found to be critical factors, and male mutation rates are usually severalfold higher than female rates in older primates (Taylor et al. 2006; Arnheim and Calabrese 2009; Kong et al. 2012; Ségurel et al. 2014; Thomas et al. 2018; Kaplanis et al. 2022). The difference between the sexes has historically been attributed to the larger number of cell divisions required in the production of sperm, but recently studies on humans suggest that DNA damage and maternal age are also critical in determining the germline mutation rate (Gao et al. 2019). However, environmental factors such as temperature and exposure to mutagenic chemicals or ultraviolet radiation are also likely to play a key role—perhaps especially so in small terrestrial invertebrates (Pfeifer et al. 2005; Baer et al. 2007; Waldvogel and Pfenninger 2021; Kaplanis et al. 2022).

The differences we see here between males and females, and perhaps also between West African and European populations, might therefore be attributable to a higher male mutation rate per se, or to differences between wild-collected (European males) and first-generation laboratory flies (females and West African flies) in age, stress, or mutagen (ultraviolet) exposure. Given our sample sizes, we were unable to test this possibility. More complex models that fitted either a sex-by-population interaction or an additional "wild-collected" term were not supported over our simpler model, but were not substantially worse. However, both models give point estimates for the sex difference and the Africa-Europe difference that were similar to (or more extreme than) the presented estimates—although the only remaining "significant" term was the higher male rate in the interaction model.

Recombination rates in *Drosophila* not only depend on genetic background, with up to a twofold difference between North American genotypes (Hunter et al. 2016), but also depend on female age, number of matings, nutrition, temperature, and pathogen exposure (Redfield 1966; Hunter and Singh 2014; Stapley et al. 2017; Aggarwal et al. 2021). However, as all the female flies used in this experiment were first-generation laboratory flies, and were thus similar in age and environmental exposure, the difference in recombination rate more likely resulted from genetic differences.

As for recombination, high rates of transposition have been associated with variation in temperature, irradiation, chemical agents, and pathogens (Capy et al. 2000; Guerreiro 2012). The worldwide colonization by *D. melanogaster* and *D. simulans* has exposed the migrants to new environmental conditions—which has been claimed to reactivate TEs—and may have exposed them to invasion by new TEs from sympatric species (Guerreiro 2012; Schwarz et al. 2021). All these factors could come into play in explaining the variation we see here, as some of the male flies used in this study were F0 wild-collected flies whereas some were first-generation flies maintained in the laboratory conditions at a constant temperature. Thus, as for the SNM rate above, the apparent difference between populations could partly result from differences in environmental exposure, in addition to differences in the TE families that are active. Nevertheless, our estimates should be closer to the rates in the wild than studies that use laboratory inbred or MA lines (Roles and Conner 2008).

Regardless of the causes of this variation in mutation, recombination, and transposition, it has potentially important implications for studies of *Drosophila* evolution. Most notably, *D. melanogaster* mutation rate estimates are widely used to derive estimates of N_e from measures of diversity (Kimura 1991), and to apply timescales to the evolutionary process in *Drosophila* (Obbard et al. 2012; Kapopoulou et al. 2018) and even beyond (e.g., Miles et al. 2017). On the one hand, our finding of a broadly similar rate in European *D. melanogaster* and *D. simulans* is reassuring; it may suggest that rates are usefully transferable between species. On the other hand, the possibility of a nearly threefold difference in mutation rate between African populations and the out-of-Africa diaspora could have substantial implications for estimates of N_e or timescales of *Drosophila* history.

Methods

Fly crosses and sequencing

West African *D. melanogaster* were collected in Zaria, northern Nigeria (28 September 2020; 11.1611 N, 7.6471 E). European *D. melanogaster* were collected in Sussex, southeast England (4–10 August 2020; 51.0998 N, 0.1644 E). European *D. simulans* were collected in Gimenells, Northern Spain (21 August 2020; 41.6564 N, 0.3885 E). For the West African flies, we crossed the virgin male and female offspring from different wild-collected (i.e., wild-mated) females. For the European flies, we crossed virgin female offspring of wild-collected females with wild-collected males. All "parental" flies for sequencing were therefore unrelated and genetically wild, that is, no more inbred or related than naturally occurring flies. All progeny groups comprised outbred full-sibships. In total, we sequenced six families comprising two parents and five F1 offspring (males) from each of the three populations (West African and European *D. melanogaster*, European *D. simulans*), giving 125 flies in total (36 parents and 89 offspring; one offspring individual failed sequencing).

DNA was extracted from single flies using Qiagen Blood and Tissue kits (Qiagen Ltd.) according to the manufacturer's protocol, and provided to the Centre for Genomic Research at the University of Liverpool (UK) for library preparation and sequencing. Libraries were prepared using NEBNext Ultra II FS Kits (New England Biolabs, Ltd.) with an insert size of ∼350 bp. Paired-end (2 × 150 bp) sequencing was performed using Illumina NovaSeq with S4 chemistry, and raw FASTQ files were trimmed for adapter sequences using cutadapt-version 1.2.1 (Martin 2011). Reads were further trimmed for quality using Sickle (https://github.com/najoshi/sickle, version 1.200), with a minimum window quality score of 20. The median coverages were all above 30-fold (except one with 28-fold) and the X Chromosome coverages in males were half of that in autosomes. For further details, see the Supplemental Material.

Read mapping and variant calling

We used BWA-MEM (version 0.7.17-r1188; Li 2013) to map the trimmed reads to the reference genomes of *D. melanogaster* (version r6.42, FlyBase.org) and *D. simulans* (version

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

GCF_016746395.2_Prin_Dsim_3.1, NCBI) using default parameters. We used Picard MarkDuplicates (https://github.com/broadinstitute/picard, version 2.26.2) to identify and remove duplicate reads, and BCFtools-version 1.11 (Li 2011) to call variants for all the samples in each family. We used the GCTA (version 1.93.2; Yang et al. 2011) to examine the relatedness of the parental flies using a genetic relatedness matrix (GRM) between pairs of individuals, and seqinr (version 4.2-5; Charif and Lobry 2007) to estimate the genome-wide synonymous and nonsynonymous nucleotide diversity from the inferred parental haplotypes (π_S and π_A). For a detailed description, see the Supplemental Material.

Filtering of candidate single-nucleotide and short indel mutations

The central challenge in calling rare heterozygous de novo mutations in highly diverse diploid genomes is to distinguish true mutations from read-mapping errors (Keightley et al. 2014; Bergeron et al. 2022). For each family, we used GATK-version 4.2.2 (McKenna et al. 2010) select single-nucleotide variants (SNVs), and we filtered the SNVs to identify sites of candidate de novo mutations according to (e.g.) depth, genotype quality, allelic balance, absolute read number, and "purity" (the absence of nonreference reads), as described in detail in the Supplemental Material. To be considered a candidate, a site could only be heterozygous in a single individual in the sample. To filter candidate short indel mutations (those <50 bp), we recalled indels for each of the families using platypus-version 0.8.1.2 (Rimmer et al. 2014), then we used VCFtools-version 0.1.16 (Danecek et al. 2011) to extract biallelic indels from the VCF files and filter these candidates as described in detail in the Supplemental Material.

Finally, for each family, we manually examined all of the candidate SNMs and short indel mutations using the Integrative Genomics Viewer (IGV) (Robinson et al. 2011). To be accepted, a candidate mutation had to be absent from all parental reads, and satisfy all the described criteria in the nonfocal offspring. The candidate mutation and its surrounding genomic markers (such as SNP or indel alleles, if present) had to be phased into only two haplotypes if on autosomes, or one haplotype if on sex chromosomes in a male, and this step also allowed us to trace the parent of origin for the majority of mutations. The approach is described in detail in the Supplemental Material and all scripts are available (see Data access).

Simulation and defining "callable" sites

Unevenness in mapped read depth means that some fraction of the genome will not be accessible to mutation detection. This can be accounted for by using only the "callable" sites as the denominator when calculating per-site rates, but the complex filtering required to achieve high specificity (see Supplemental Material) can reduce detection sensitivity in ways that are hard to capture. One solution to this has been to simulate "synthetic mutations" in the empirical reads, and rerun the mapping and mutation-detection pipeline to estimate its overall sensitivity (Keightley et al. 2014) and use this to "correct" the overall rate estimate. Here we take this approach one step further and use these simulations to directly define the proportion of the genome that is "callable." We did this for both SNMs and short indels and we tested our filtering and mutation-calling pipeline using the rhesus macaque trio data provided by the recent "Mutationathon" study (Bergeron et al. 2022). For details of our simulation and mutation-calling approach, see the Supplemental Material.

Recombination detection

To infer crossover recombination in the parents, we used VCFtools to select biallelic SNPs and then applied "phase_by_transmission" from the Python package *scikit-allel* (version 1.3.5) to phase the two haplotypes in each offspring (Miles et al. 2017). We visualized the haplotypes of informative SNPs in the offspring using R, and manually resolved a small number of visible errors, by switching the parental phase at the shared breakpoint. The resulting phased genomes should permit a direct count of crossover breakpoints, and thus an estimate of the recombination rate. However, substantial "noise" was visible in the phasing at a scale of hundreds to thousands of bases (Supplemental Fig. S6). This noise could reflect sequencing errors, genotyping errors, or short phasing errors, but may also reflect gene-conversion events (Comeron et al. 2012). We, therefore, selected a length threshold, based on the presumed absence of recombination in males, for the detection of true crossovers. When applied to the maternal haplotypes, this threshold allowed us to exclude 31,123 likely erroneous short haplotype blocks (median length 2 kbp, 95% quantile = 188 kbp).

Finally, given the potential for suppression of recombination by genomic inversions in heterozygotes, we used inversion-specific markers in *D. melanogaster* to detect cosmopolitan inversions in the parental samples and examined whether the inversions, if present, affect the occurrence of recombination (Kapun et al. 2014). The seven canonical inversions we examined were In(2L)t, In(2R)Ns, In(3L)P, In(3R)C, In(3R)K, In(3R)Mo, and In(3R)Payne.

Detection of transposable element insertions

The robust detection of TE insertions into repetitive regions is extremely challenging, and rarely possible using short-read data alone. However, a number of approaches can reliably detect TE insertions into low-complexity and gene-rich regions (Ewing 2015; Chen et al. 2023). We used TEFLoN (Adrion et al. 2017) to identify TE insertions in our data, and we detected new insertions by comparing the TE locations between parents and offspring. To confidently identify novel TE insertions, we then followed a similar logic to our SNM and indel filtering above (see the Supplemental Material). To estimate the transposition rate per parental element, we additionally counted the number of TEs in both parents of each family.

Statistical analyses

We used binomial generalized linear mixed models to test for rate differences between populations and sexes and to quantify potential rate variation among individuals, using the Bayesian mixed-model package MCMCglmm (version 2.32; Hadfield 2010) in R (R Core Team 2021). To do this, we treated mutations (or recombination breakpoints, or insertions) as outcomes from a Bernoulli trial, with the estimated number of callable sites (or equivalent) as the number of trials. This approach naturally accounts for variation in power across the experiment (genome size, coverage, family size) and provides a robust and well-established framework for statistical testing. By transforming estimates of the latent rate parameter back on to the data scale, it also provides direct estimates of the rates and their credibility intervals.

To quantify differences in mutation rate among our three "populations" (i.e., West African *D. melanogaster*, European *D. melanogaster*, *D. simulans*; termed "POPULATION" in the model) and between the two parental sexes ("SEX"), we fitted these terms as fixed effects. To quantify the variation among parents, we fitted parental id ("PID") as a random effect. MCMCglmm also fits a residual variance, such as might be caused by differences among observations of offspring that are not attributable to parents. We

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

similarly used MCMCglmm to estimate the recombination rate and to quantify variation in the recombination rate, but excluding sex (because recombination is absent in males), including chromosomal arm identity as a fixed effect, and taking the number of Bernoulli trials to be the genome length rather than the number of "callable sites." For transposable elements, we considered transposition rate in two contexts, transposition rate per base and transposition rate per parental element; the first used the length of host genome size that passed depth filtering as a proxy for "callable sites," and the second used the inferred copy number of TEs in host genomes to characterize the transposition rate. For full details of the models, see the Supplemental Material.

Data access

All raw sequencing data generated in this study have been submitted to the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena/browser/home) under accession number PRJEB51956. The phased parental genomes are provided at Figshare (https://doi.org/10.6084/m9.figshare.19733860.v1). All scripts are available via GitHub (https://github.com/Yiguan/muDmelDsim) and as Supplemental Code.

Competing interest statement

The authors declare no competing interests.

Acknowledgments

This work was funded by the UK Biotechnology and Biological Sciences Research Council through Grant No. BB/T007516/1 to D.J.O., P.D.K., and S.E.J. We thank Mallam Adamu Shuaibu, and Keith and Sue Obbard for assistance in collecting the *Drosophila*, and Brian Charlesworth for encouraging us to examine the impact of inversions on recombination rate.

Author contributions: D.J.O., P.D.K., and S.E.J. designed the project. Y.W. and D.J.O. wrote the manuscript with comments from all authors. Y.W. wrote the pipeline and analyzed the data. P.M. reared the flies and extracted genomic DNA. D.J.O., R.A., and M.P. collected the wild flies. All authors read and approved the final manuscript.

References

Adrion JR, Song MJ, Schrider DR, Hahn MW, Schaack S. 2017. Genome-wide estimates of transposable element insertion and deletion rates in *Drosophila melanogaster*. *Genome Biol Evol* **9:** 1329–1340. doi:10.1093/gbe/evx050

Aggarwal DD, Rybnikov S, Sapielkin S, Rashkovetsky E, Frenkel Z, Singh M, Michalak P, Korol AB. 2021. Seasonal changes in recombination characteristics in a natural population of *Drosophila melanogaster*. *Heredity (Edinb)* **127:** 278–287. doi:10.1038/s41437-021-00449-2

Almeida MV, Vernaz G, Putman AL, Miska EA. 2022. Taming transposable elements in vertebrates: from epigenetic silencing to domestication. *Trends Genet* **38:** 529–553. doi:10.1016/j.tig.2022.02.009

Arnheim N, Calabrese P. 2009. Understanding what determines the frequency and pattern of human germline mutations. *Nat Rev Genet* **10:** 478–488. doi:10.1038/nrg2529

Assaf ZJ, Tilk S, Park J, Siegal ML, Petrov DA. 2017. Deep sequencing of natural and experimental populations of *Drosophila melanogaster* reveals biases in the spectrum of new mutations. *Genome Res* **27:** 1988–2000. doi:10.1101/gr.219956.116

Bachtrog D. 2008. Evidence for male-driven evolution in *Drosophila*. *Mol Biol Evol* **25:** 617–619. doi:10.1093/molbev/msn020

Baer CF, Miyamoto MM, Denver DR. 2007. Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nat Rev Genet* **8:** 619–631. doi:10.1038/nrg2158

Bergeron LA, Besenbacher S, Turner T, Versoza CJ, Wang RJ, Price AL, Armstrong E, Riera M, Carlson J, Chen HY, et al. 2022. The mutationathon highlights the importance of reaching standardization in estimates of pedigree-based germline mutation rates. *eLife* **11:** e73577. doi:10.7554/eLife.73577

Bergeron LA, Besenbacher S, Zheng J, Li P, Bertelsen MF, Quintard B, Hoffman JI, Li Z, St. Leger J, Shao C. 2023. Evolution of the germline mutation rate across vertebrates. *Nature* **615:** 285–291. doi:10.1038/s41586-023-05752-y

Campbell CD, Chong JX, Malig M, Ko A, Dumont BL, Han LD, Vives L, O'Roak BJ, Sudmant PH, Shendure J, et al. 2012. Estimating the human mutation rate using autozygosity in a founder population. *Nat Genet* **44:** 1277–1281. doi:10.1038/ng.2418

Campos JL, Zhao L, Charlesworth B. 2017. Estimating the parameters of background selection and selective sweeps in *Drosophila* in the presence of gene conversion. *Proc Natl Acad Sci* **114:** E4762–E4771. doi:10.1073/pnas.1619434114

Capy P, Gasperi G, Biémont C, Bazin C. 2000. Stress and transposable elements: co-evolution or useful parasites? *Heredity (Edinb)* **85:** 101–106. doi:10.1046/j.1365-2540.2000.00751.x

Chan AH, Jenkins PA, Song YS. 2012. Genome-wide fine-scale recombination rate variation in *Drosophila melanogaster*. *PLoS Genet* **8:** e1003090. doi:10.1371/journal.pgen.1003090

Charif D, Lobry JR. 2007. Seqinr 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. In *Structural approaches to sequence evolution* (ed. Bastolla U, et al.), pp. 207–232. Springer, Berlin, Heidelberg.

Chen J, Basting PJ, Han S, Garfinkel DJ, Bergman CM. 2023. Reproducible evaluation of short-read transposable element detectors and species-wide data mining of insertion patterns in yeast. bioRxiv doi:10.1101/2023.02.13.528343

Chuong EB, Elde NC, Feschotte C. 2017. Regulatory activities of transposable elements: from conflicts to benefits. *Nat Rev Genet* **18:** 71–86. doi:10.1038/nrg.2016.139

Comeron JM, Ratnappan R, Bailin S. 2012. The many landscapes of recombination in *Drosophila melanogaster*. *PLoS Genet* **8:** e1002905. doi:10.1371/journal.pgen.1002905

Crown KN, Miller DE, Sekelsky J, Hawley RS. 2018. Local inversion heterozygosity alters recombination throughout the genome. *Curr Biol* **28:** 2984–2990.e3. doi:10.1016/j.cub.2018.07.004

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools. *Bioinformatics* **27:** 2156–2158. doi:10.1093/bioinformatics/btr330

Díaz-González J, Vázquez JF, Albornoz J, Domínguez A. 2011. Long-term evolution of the *roo* transposable element copy number in mutation accumulation lines of *Drosophila melanogaster*. *Genet Res* **93:** 181–187. doi:10.1017/S0016672311000103

Ewing AD. 2015. Transposable element detection from whole genome sequence data. *Mob DNA* **6:** 24. doi:10.1186/s13100-015-0055-3

Gao ZY, Moorjani P, Sasani TA, Pedersen BS, Quinlan AR, Jorde LB, Amster G, Przeworski M. 2019. Overlooked roles of DNA damage and maternal age in generating human germline mutations. *Proc Natl Acad Sci* **116:** 9491–9500. doi:10.1073/pnas.1901259116

Guerreiro MPG. 2012. What makes transposable elements move in the *Drosophila* genome? *Heredity (Edinb)* **108:** 461–468. doi:10.1038/hdy.2011.89

Guerreiro MPG, Fontdevila A. 2011. *Osvaldo* and *Isis* retrotransposons as markers of the *Drosophila buzzatii* colonisation in Australia. *BMC Evol Biol* **11:** 111. doi:10.1186/1471-2148-11-111

Guerreiro MPG, Chávez-Sandoval BE, Balanyà J, Serra L, Fontdevila A. 2008. Distribution of the transposable elements *bilbo* and *gypsy* in original and colonizing populations of *Drosophila subobscura*. *BMC Evol Biol* **8:** 234. doi:10.1186/1471-2148-8-234

Haag-Liautard C, Dorris M, Maside X, Macaskill S, Halligan DL, Charlesworth B, Keightley PD. 2007. Direct estimation of per nucleotide and genomic deleterious mutation rates in *Drosophila*. *Nature* **445:** 82–85. doi:10.1038/nature05388

Hadfield J. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J Stat Softw* **33:** 1–22. doi:10.18637/jss.v033.i02

Heil CSS, Ellison C, Dubin M, Noor MAF. 2015. Recombining without hotspots: a comprehensive evolutionary portrait of recombination in two closely related species of *Drosophila*. *Genome Biol Evol* **7:** 2829–2842. doi:10.1093/gbe/evv182

Hemmer LW, Dias GB, Smith B, Van Vaerenberghe K, Howard A, Bergman CM, Blumenstiel JP. 2020. Hybrid dysgenesis in *Drosophila virilis* results in clusters of mitotic recombination and loss-of-heterozygosity but leaves meiotic recombination unaltered. *Mob DNA* **11:** 10. doi:10.1186/s13100-020-0205-0

Hirsch CD, Springer NM. 2017. Transposable element influences on gene expression in plants. *Biochim Biophys Acta Gene Regul Mech* **1860:** 157–165. doi:10.1016/j.bbagrm.2016.05.010

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

Ho EKH, Macrae F, Latta LC, McIlroy P, Ebert D, Fields PD, Benner MJ, Schaack S. 2020. High and highly variable spontaneous mutation rates in *Daphnia*. *Mol Biol Evol* **37:** 3258–3266. doi:10.1093/molbev/msaa142

Ho EK, Bellis ES, Calkins J, Adrion JR, Latta LC IV, Schaack S. 2021. Engines of change: transposable element mutation rates are high and variable within *Daphnia magna*. *PLoS Genet* **17:** e1009827. doi:10.1371/journal.pgen.1009827

Horváth V, Merenciano M, González J. 2017. Revisiting the relationship between transposable elements and the eukaryotic stress response. *Trends Genet* **33:** 832–841. doi:10.1016/j.tig.2017.08.007

Huang W, Lyman RF, Lyman RA, Carbone MA, Harbison ST, Magwire MM, Mackay TFC. 2016. Spontaneous mutations and the origin and maintenance of quantitative genetic variation. *eLife* **5:** e14625. doi:10.7554/eLife.14625

Hunter CM, Singh ND. 2014. Do males matter? Testing the effects of male genetic background on female meiotic crossover rates in *Drosophila melanogaster*. *Evolution (N Y)* **68:** 2718–2726. doi:10.1111/evo.12455

Hunter CM, Huang W, Mackay TFC, Singh ND. 2016. The genetic architecture of natural variation in recombination rate in *Drosophila melanogaster*. *PLoS Genet* **12:** e1005951. doi:10.1371/journal.pgen.1005951

Jensen-Seaman MI, Furey TS, Payseur BA, Lu Y, Roskin KM, Chen C-F, Thomas MA, Haussler D, Jacob HJ. 2004. Comparative recombination rates in the rat, mouse, and human genomes. *Genome Res* **14:** 528–538. doi:10.1101/gr.1970304

Jónsson H, Sulem P, Kehr B, Kristmundsdottir S, Zink F, Hjartarson E, Hardarson MT, Hjorleifsson KE, Eggertsson HP, Gudjonsson SA, et al. 2017. Parental influence on human germline *de novo* mutations in 1,548 trios from Iceland. *Nature* **549:** 519–522. doi:10.1038/nature24018

Kapitonov VV, Jurka J. 2003. Molecular paleontology of transposable elements in the *Drosophila melanogaster* genome. *Proc Natl Acad Sci* **100:** 6569–6574. doi:10.1073/pnas.0732024100

Kaplanis J, Ide B, Sanghvi R, Neville M, Danecek P, Coorens T, Prigmore E, Short P, Gallone G, McRae J, et al. 2022. Genetic and chemotherapeutic influences on germline hypermutation. *Nature* **605:** 503–508. doi:10.1038/s41586-022-04712-2

Kapopoulou A, Pfeifer SP, Jensen JD, Laurent S. 2018. The demographic history of African *Drosophila melanogaster*. *Genome Biol Evol* **10:** 2338–2342. doi:10.1093/gbe/evy185

Kapun M, van Schalkwyk H, McAllister B, Flatt T, Schlötterer C. 2014. Inference of chromosomal inversion dynamics from Pool-Seq data in natural and laboratory populations of *Drosophila melanogaster*. *Mol Ecol* **23:** 1813–1827. doi:10.1111/mec.12594

Keightley PD, Trivedi U, Thomson M, Oliver F, Kumar S, Blaxter ML. 2009. Analysis of the genome sequences of three *Drosophila melanogaster* spontaneous mutation accumulation lines. *Genome Res* **19:** 1195–1201. doi:10.1101/gr.091231.109

Keightley PD, Ness RW, Halligan DL, Haddrill PR. 2014. Estimation of the spontaneous mutation rate per nucleotide site in a *Drosophila melanogaster* full-sib family. *Genetics* **196:** 313–320. doi:10.1534/genetics.113.158758

Keightley PD, Pinharanda A, Ness RW, Simpson F, Dasmahapatra KK, Mallet J, Davey JW, Jiggins CD. 2015. Estimation of the spontaneous mutation rate in *Heliconius melpomene*. *Mol Biol Evol* **32:** 239–243. doi:10.1093/molbev/msu302

Kimura M. 1991. The neutral theory of molecular evolution: a review of recent evidence. *Jpn J Genet* **66:** 367–386. doi:10.1266/jjg.66.367

Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, et al. 2012. Rate of *de novo* mutations and the importance of father's age to disease risk. *Nature* **488:** 471–475. doi:10.1038/nature11396

Krasovec M. 2021. The spontaneous mutation rate of *Drosophila pseudoobscura*. *G3 (Bethesda)* **11:** jkab151. doi:10.1093/g3journal/jkab151

Li H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* **27:** 2987–2993. doi:10.1093/bioinformatics/btr509

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997 [q-bio.GN]. doi:10.48550/arXiv.1303.3997

Lindsay SJ, Rahbari R, Kaplanis J, Keane T, Hurles ME. 2019. Similarities and differences in patterns of germline mutation between mice and humans. *Nat Commun* **10:** 4053. doi:10.1038/s41467-019-12023-w

Liu H, Jia Y, Sun X, Tian D, Hurst LD, Yang S. 2017. Direct determination of the mutation rate in the bumblebee reveals evidence for weak recombination-associated mutation and an approximate rate constancy in insects. *Mol Biol Evol* **34:** 119–130. doi:10.1093/molbev/msw226

Lynch M. 2010. Evolution of the mutation rate. *Trends Genet* **26:** 345–352. doi:10.1016/j.tig.2010.05.003

Lynch M, Ackerman MS, Gout JF, Long H, Sung W, Thomas WK, Foster PL. 2016. Genetic drift, selection and the evolution of the mutation rate. *Nat Rev Genet* **17:** 704–714. doi:10.1038/nrg.2016.104

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J* **17:** 10–12. doi:10.14806/ej.17.1.200

Maside X, Assimacopoulos S, Charlesworth B. 2000. Rates of movement of transposable elements on the second chromosome of *Drosophila melanogaster*. *Genet Res* **75:** 275–284. doi:10.1017/S0016672399004474

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, et al. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* **20:** 1297–1303. doi:10.1101/gr.107524.110

McVey M, Khodaverdian VY, Meyer D, Cerqueira PG, Heyer W-D. 2016. Eukaryotic DNA polymerases in homologous recombination. *Ann Rev Genet* **50:** 393–421. doi:10.1146/annurev-genet-120215-035243

Mérel V, Boulesteix M, Fablet M, Vieira C. 2020. Transposable elements in *Drosophila*. *Mob DNA* **11:** 23. doi:10.1186/s13100-020-00213-z

Miles A, Harding NJ, Botta G, Clarkson CS, Antao T, Kozak K, Schrider DR, Kern AD, Redmond S, Sharakhov I, et al. 2017. Genetic diversity of the African malaria vector *Anopheles gambiae*. *Nature* **552:** 96–100. doi:10.1038/nature24995

Milholland B, Dong X, Zhang L, Hao XX, Suh Y, Vijg J. 2017. Differences between germline and somatic mutation rates in humans and mice. *Nat Commun* **8:** 15183. doi:10.1038/ncomms15183

Miller DE, Smith CB, Kazemi NY, Cockrell AJ, Arvanitakas AV, Blumenstiel JP, Jaspersen SL, Hawley RS. 2016. Whole-genome analysis of individual meiotic events in *Drosophila melanogaster* reveals that noncrossover gene conversions are insensitive to interference and the centromere effect. *Genetics* **203:** 159–171. doi:10.1534/genetics.115.186486

Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, et al. 2014. Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346:** 763–767. doi:10.1126/science.1257570

Morgan TH. 1912. Complete linkage in the second chromosome of the male of *Drosophila*. *Science* **36:** 719–720. doi:10.1126/science.36.934.719

Ness RW, Morgan AD, Vasanthakrishnan RB, Colegrave N, Keightley PD. 2015. Extensive de novo mutation rate variation between individuals and across the genome of *Chlamydomonas reinhardtii*. *Genome Res* **25:** 1739–1749. doi:10.1101/gr.191494.115

Nuzhdin SV, Mackay TFC. 1995. The genomic rate of transposable element movement in *Drosophila melanogaster*. *Mol Biol Evol* **12:** 180–181. doi:10.1093/oxfordjournals.molbev.a040188

Obbard DJ, Maclennan J, Kim KW, Rambaut A, O'Grady PM, Jiggins FM. 2012. Estimating divergence dates and substitution rates in the *Drosophila* phylogeny. *Mol Biol Evol* **29:** 3459–3473. doi:10.1093/molbev/mss150

Oppold AM, Pfenninger M. 2017. Direct estimation of the spontaneous mutation rate by short-term mutation accumulation lines in *Chironomus riparius*. *Evol Lett* **1:** 86–92. doi:10.1002/evl3.8

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, et al. 2012. Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations. *Nature* **485:** 246–250. doi:10.1038/nature10989

Parsch J, Novozhilov S, Saminadin-Peter SS, Wong KM, Andolfatto P. 2010. On the utility of short intron sequences as a reference for the detection of positive and negative selection in *Drosophila*. *Mol Biol Evol* **27:** 1226–1234. doi:10.1093/molbev/msq046

Pasyukova EG, Nuzhdin SV, Filatov DA. 1998. The relationship between the rate of transposition and transposable element copy number for *copia* and *Doc* retrotransposons of *Drosophila melanogaster*. *Genet Res* **72:** 1–11. doi:10.1017/S0016672398003358

Pfeifer GP, You YH, Besaratinia A. 2005. Mutations induced by ultraviolet light. *Mutat Res* **571:** 19–31. doi:10.1016/j.mrfmmm.2004.06.057

Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, Turki SA, Dominiczak A, Morris A, Porteous D, Smith B, et al. 2016. Timing, rates and spectra of human germline mutation. *Nat Genet* **48:** 126–133. doi:10.1038/ng.3469

R Core Team. 2021. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Redfield H. 1966. Delayed mating and the relationship of recombination to maternal age in *Drosophila melanogaster*. *Genetics* **53:** 593–607. doi:10.1093/genetics/53.3.593

Rimmer A, Phan H, Mathieson I, Iqbal Z, Twigg SRF, Wilkie AOM, McVean G, Lunter G, Consortium W. 2014. Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. *Nat Genet* **46:** 912–918. doi:10.1038/ng.3036

Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. *Nat Biotechnol* **29:** 24–26. doi:10.1038/nbt.1754

Downloaded from genome.cshlp.org on May 2, 2026 . Published by Cold Spring Harbor Laboratory Press

Roles AJ, Conner JK. 2008. Fitness effects of mutation accumulation in a natural outbred population of wild radish (raphanus raphanistrum): comparison of field and greenhouse environments. *Evolution (N Y)* **62:** 1066–1075. doi:10.1111/j.1558-5646.2008.00354.x

Schrider DR, Houle D, Lynch M, Hahn MW. 2013. Rates and genomic consequences of spontaneous mutational events in *Drosophila melanogaster*. *Genetics* **194:** 937–954. doi:10.1534/genetics.113.151670

Schwarz F, Wierzbicki F, Senti KA, Kofler R. 2021. Tirant stealthily invaded natural *Drosophila melanogaster* populations during the last century. *Mol Biol Evol* **38:** 1482–1497. doi:10.1093/molbev/msaa308

Ségurel L, Wyman MJ, Przeworski M. 2014. Determinants of mutation rate variation in the human germline. *Annu Rev Genomics Hum Genet* **15:** 47–70. doi:10.1146/annurev-genom-031714-125740

Sharp NP, Agrawal AF. 2016. Low genetic quality alters key dimensions of the mutational spectrum. *PLoS Biol* **14:** e1002419. doi:10.1371/journal.pbio.1002419

Stapley J, Feulner PGD, Johnston SE, Santure AW, Smadja CM. 2017. Variation in recombination frequency and distribution across eukaryotes: patterns and processes. *Philos Trans R Soc Lond B Biol Sci* **372:** 20160455. doi:10.1098/rstb.2016.0455

Stevison LS, Noor MAF. 2010. Genetic and evolutionary correlates of fine-scale recombination rate variation in *Drosophila persimilis*. *J Mol Evol* **71:** 332–345. doi:10.1007/s00239-010-9388-1

Stocker AJ, Rusuwa BB, Blacket MJ, Frentiu FD, Sullivan M, Foley BR, Beatson S, Hoffmann AA, Chenoweth SF. 2012. Physical and linkage maps for *Drosophila serrata*, a model species for studies of clinal adaptation and sexual selection. *G3 (Bethesda)* **2:** 287–297. doi:10.1534/g3.111.001354

Sung W, Ackerman MS, Gout JF, Miller SF, Williams E, Foster PL, Lynch M. 2015. Asymmetric context-dependent mutation patterns revealed through mutation-accumulation experiments. *Mol Biol Evol* **32:** 1672–1683. doi:10.1093/molbev/msv055

Sung W, Ackerman MS, Dillon MM, Platt TG, Fuqua C, Cooper VS, Lynch M. 2016. Evolution of the insertion-deletion mutation rate across the tree of life. *G3 (Bethesda)* **6:** 2583–2591. doi:10.1534/g3.116.030890

Suvorov A, Kim BY, Wang J, Armstrong EE, Peede D, D'Agostino ERR, Price DK, Waddell P, Lang M, Courtier-Orgogozo V, et al. 2022. Widespread introgression across a phylogeny of 155 *Drosophila* genomes. *Curr Biol* **32:** 111–123.e5. doi:10.1016/j.cub.2021.10.052

Taylor F, Tyekucheva S, Zody M, Chiaromonte F, Makova KD. 2006. Strong and weak male mutation bias at different sites in the primate genomes: insights from the human-chimpanzee comparison. *Mol Biol Evol* **23:** 565–573. doi:10.1093/molbev/msj060

Thomas GWC, Wang RJ, Puri A, Harris RA, Raveendran M, Hughes DST, Murali SC, Williams LE, Doddapaneni H, Muzny DM, et al. 2018. Reproductive longevity predicts mutation rates in primates. *Curr Biol* **28:** 3193–3197.e5. doi:10.1016/j.cub.2018.08.050

True JR, Mercer JM, Laurie CC. 1996. Differences in crossover frequency and distribution among three sibling species of *Drosophila*. *Genetics* **142:** 507–523. doi:10.1093/genetics/142.2.507

Uchimura A, Higuchi M, Minakuchi Y, Ohno M, Toyoda A, Fujiyama A, Miura I, Wakana S, Nishino J, Yagi T. 2015. Germline mutation rates and the long-term phenotypic effects of mutation accumulation in wild-type laboratory mice and mutator mice. *Genome Res* **25:** 1125–1134. doi:10.1101/gr.186148.114

Waldvogel AM, Pfenninger M. 2021. Temperature dependence of spontaneous mutation rates. *Genome Res* **31:** 1582–1589. doi:10.1101/gr.275168.120

Wang Y, Obbard DJ. 2023. Experimental estimates of germline mutation rate in eukaryotes: a phylogenetic meta-analysis. bioRxiv doi:10.1101/2023.01.24.525323

Webster MT, Hurst LD. 2012. Direct and indirect consequences of meiotic recombination: implications for genome evolution. *Trends Genet* **28:** 101–109. doi:10.1016/j.tig.2011.11.002

Wells JN, Feschotte C. 2020. A field guide to eukaryotic transposable elements. *Annu Rev Genet* **54:** 539–561. doi:10.1146/annurev-genet-040620-022145

Yang JA, Lee SH, Goddard ME, Visscher PM. 2011. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* **88:** 76–82. doi:10.1016/j.ajhg.2010.11.011

Yang S, Wang L, Huang J, Zhang X, Yuan Y, Chen JQ, Hurst LD, Tian D. 2015. Parent–progeny sequencing indicates higher mutation rates in heterozygotes. *Nature* **523:** 463–467. doi:10.1038/nature14649

Yoder AD, Tiley GP. 2021. The challenge and promise of estimating the de novo mutation rate from whole-genome comparisons among closely related individuals. *Mol Ecol* **30:** 6087–6100. doi:10.1111/mec.16007

Received October 4, 2022; accepted in revised form March 28, 2023.