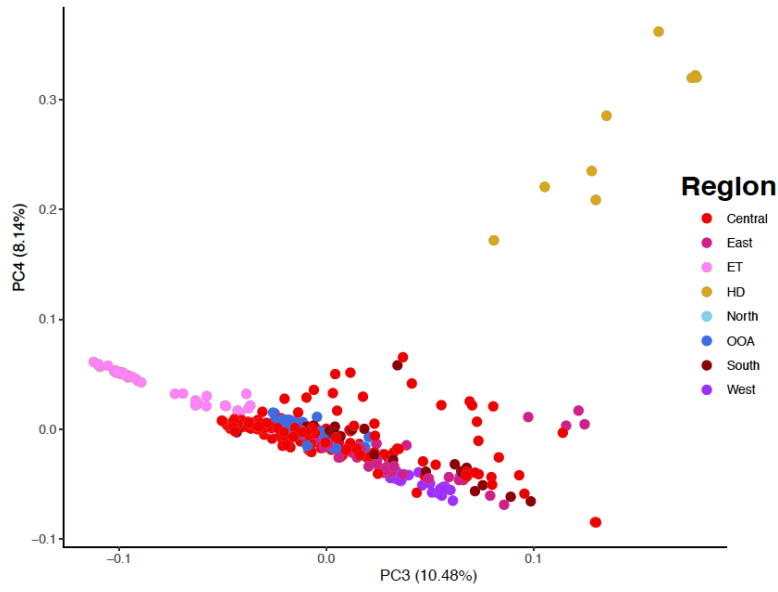
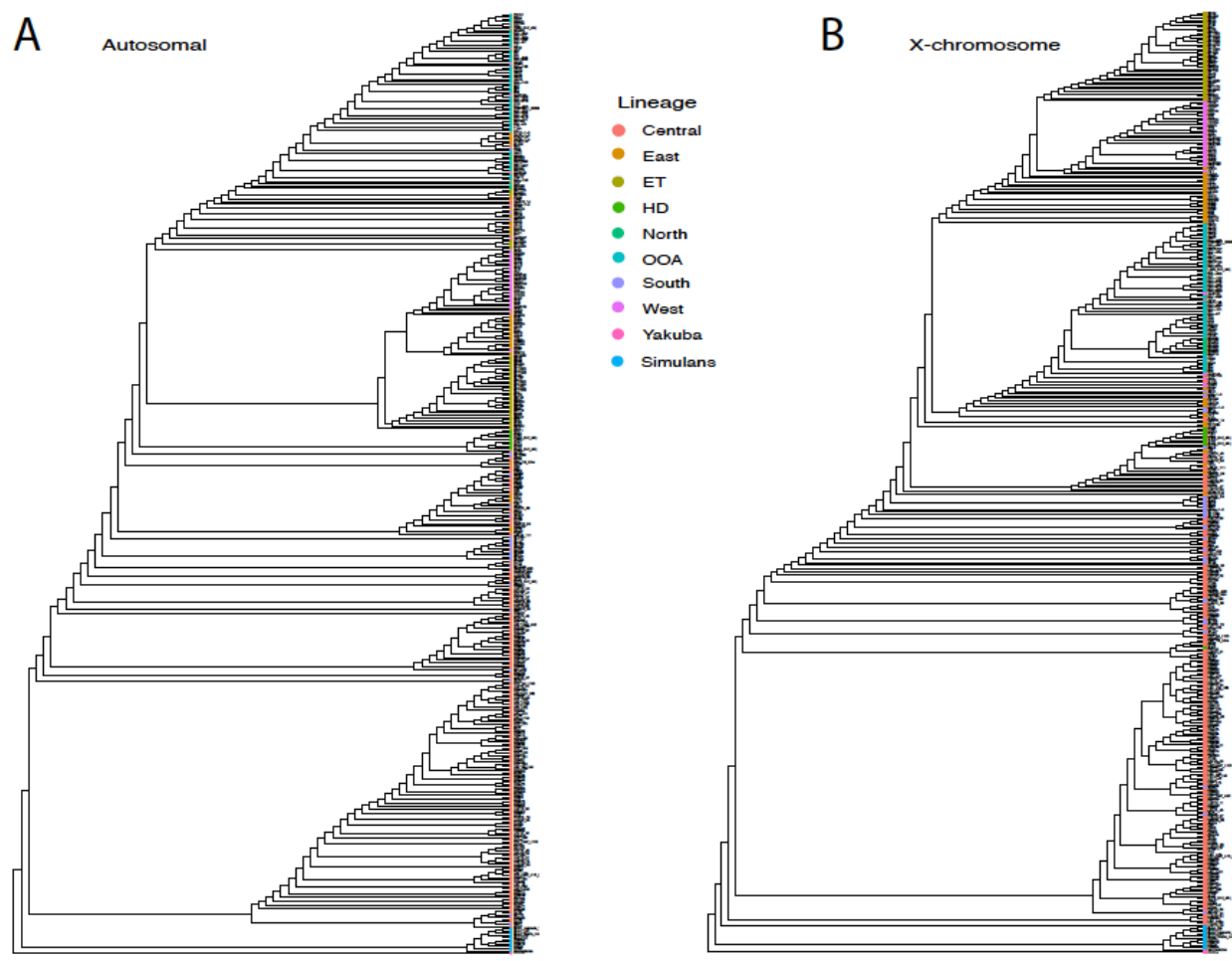


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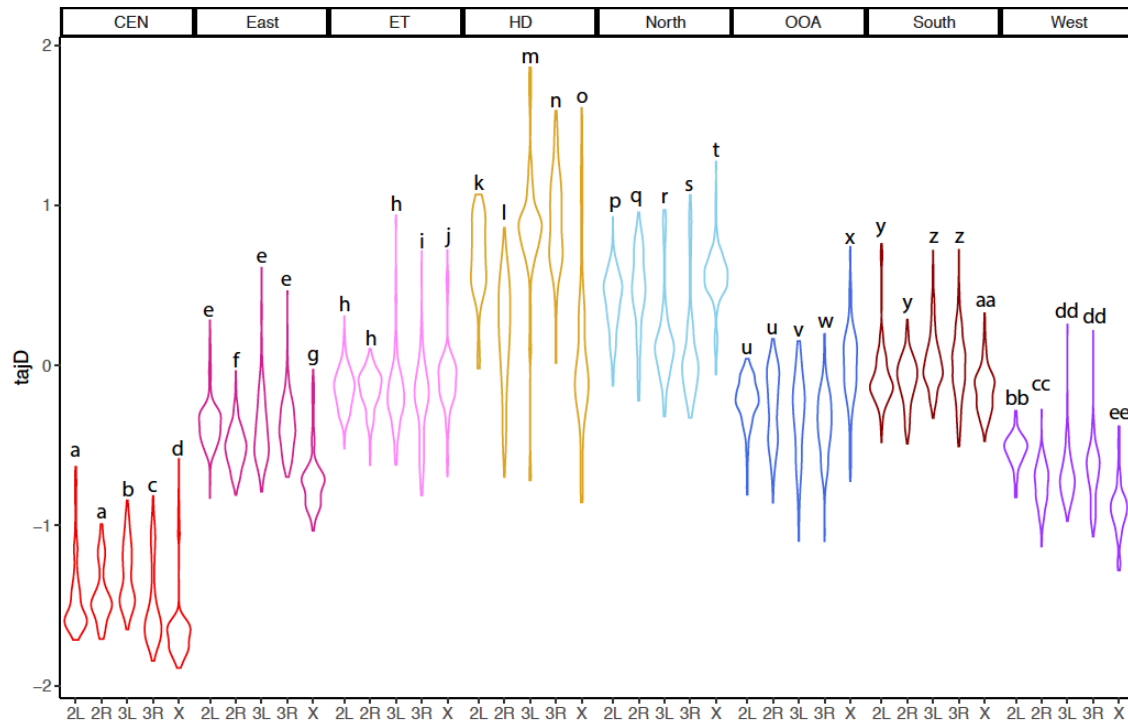
3 **Figure S1: PCs 3 and 4 of a genome-wide PCA.** Points are colored based on the geo-
4 genetic lineage. Percent of variance explained by each PC is indicated in brackets.



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6 **Figure S2: Full ML phylogeny of all samples** based on (A) autosomes and (B) the X
 7 chromosome only. Tip colors correspond with the geo-genetic lineages defined herein,
 8 plus *D. simulans* and *D. yakuba* as outgroups.

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11 **Figure S3: Tajima's D by chromosome for each geo-genetic lineage.** Letters denote
 12 significantly different groups.

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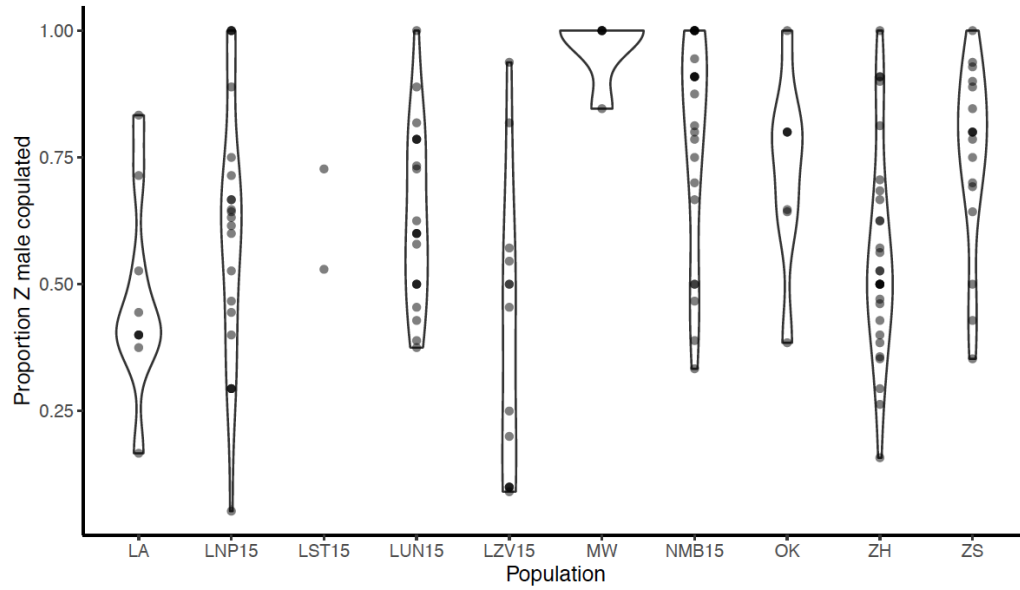
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20 **Figure S4: Strength of female mate preference differs by population.** Proportion of
 21 Z males copulated in a two-way choice trial between Z and M males and female flies
 22 from 10 sampling locales in Subtropical Africa. Points represent individual trials for
 23 individual isolines.

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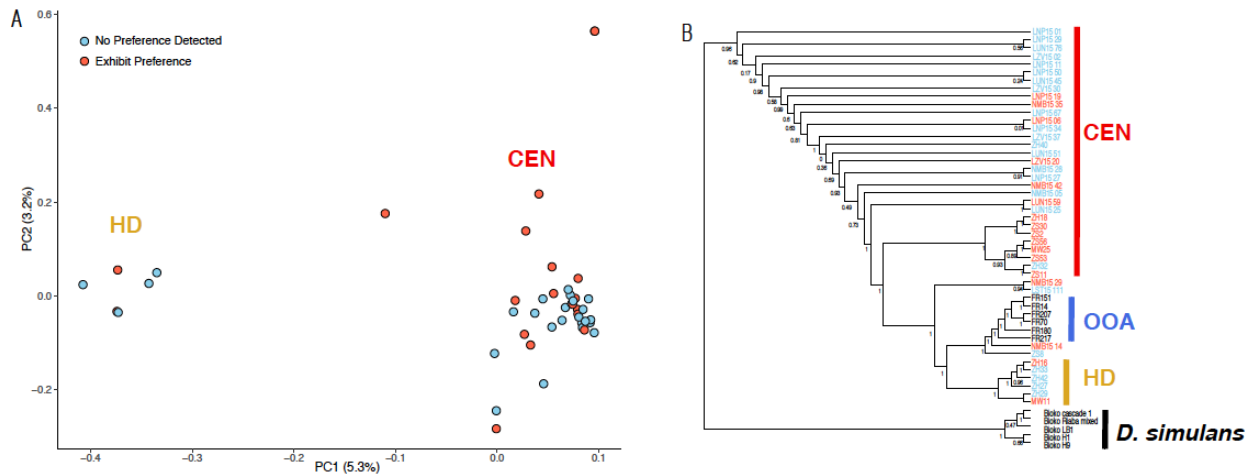
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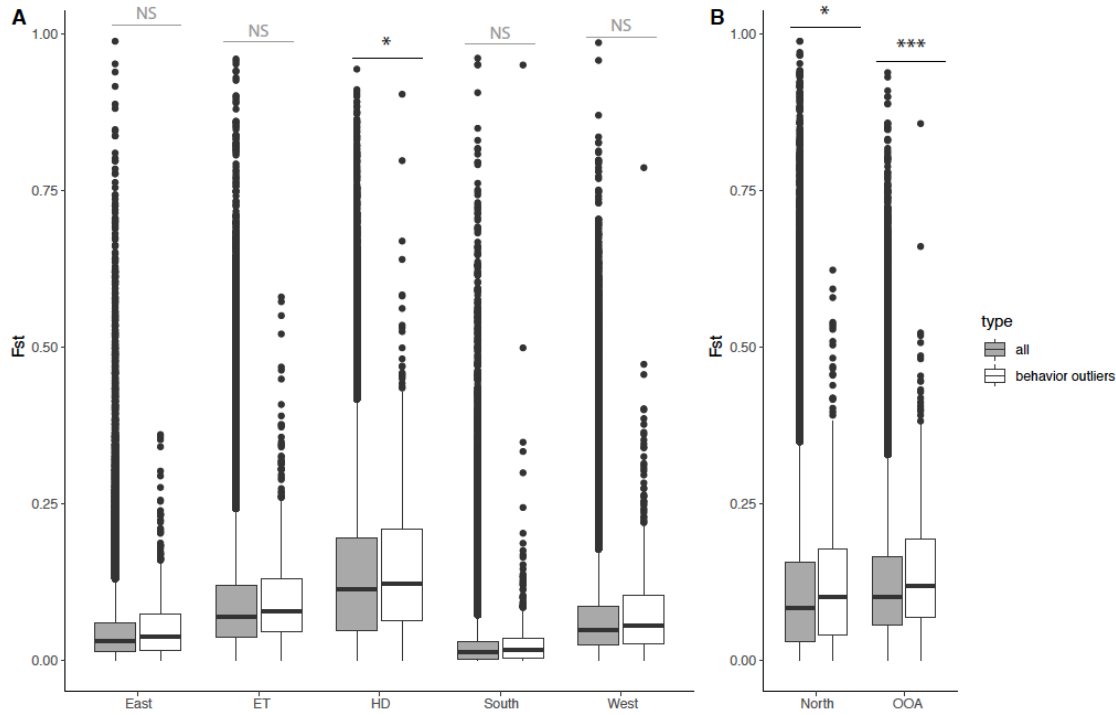
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33 **Figure S5: No genetic structure between lines that do/do not exhibit significant**
 34 **mate preference**; denoted in red and blue respectively. (A) PCA of all Central African
 35 lines that were phenotype for mate preference. Substantial genetic structure between
 36 HD and Central clades, but no structuring between lines that do/do not exhibit
 37 significant female mate preference. Moreover, there is no genetic structure between
 38 behavioral types across the first 8 PCs. Numbers in brackets denote the percent of
 39 variance explained by PC1 and PC2, respectively. (B) ML consensus phylogeny built
 40 from non-overlapping regions of 100KB using the rapid-hill climbing algorithm in
 41 RAxML, with 50 bootstraps per tree. Consensus phylogeny was built using these trees
 42 as input to ASTRAL. Each genetic clade is outlined with a thick vertical bar, with colors
 43 replicating the represented clades in Figure 1. We also include six lines from France
 44 (denoted by the blue OOA section) for context. We note that only 17 of 19 individuals
 45 that exhibit preference and have sequenced genomes are included in the phylogeny as
 46 2 genomes did not pass quality thresholds for our phylogenetic analyses (LA66 and
 47 LA69).

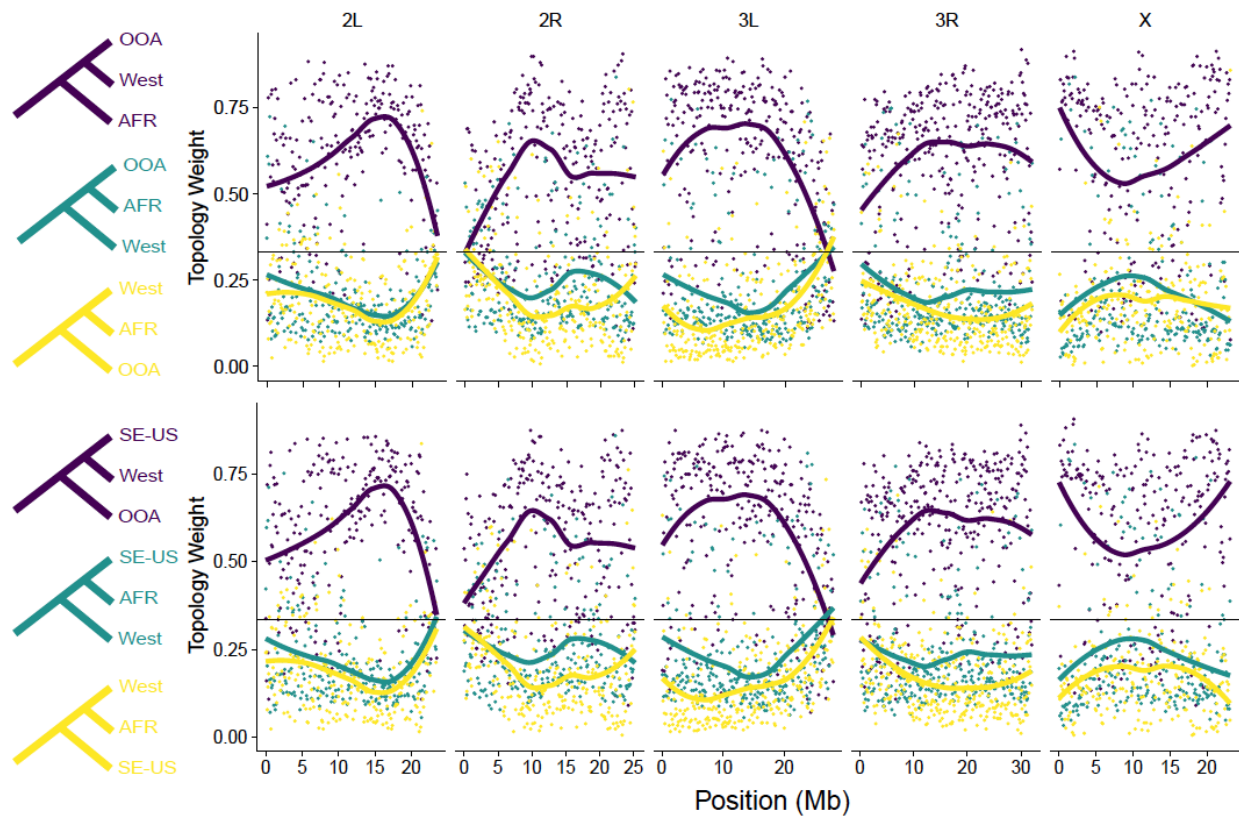
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50 **Figure S6: Behavioral outliers are highly differentiated between Central Africa and**
 51 **other geo-genetic lineages.** *PBE outliers for behavior show increased F_{st} relative to the*
 52 *rest of the genome for all comparisons (genome-wide versus outlier: $F=7.5732$,*
 53 *$df=1$, $p=0.0059$).* *However, when contrasting F_{st} between Central Africa and specific*
 54 *geo-genetic lineages, differences between F_{st} for genome-wide versus PBE behavior*
 55 *outliers is only significant for Central Africa versus OOA, North Africa, and HD.*

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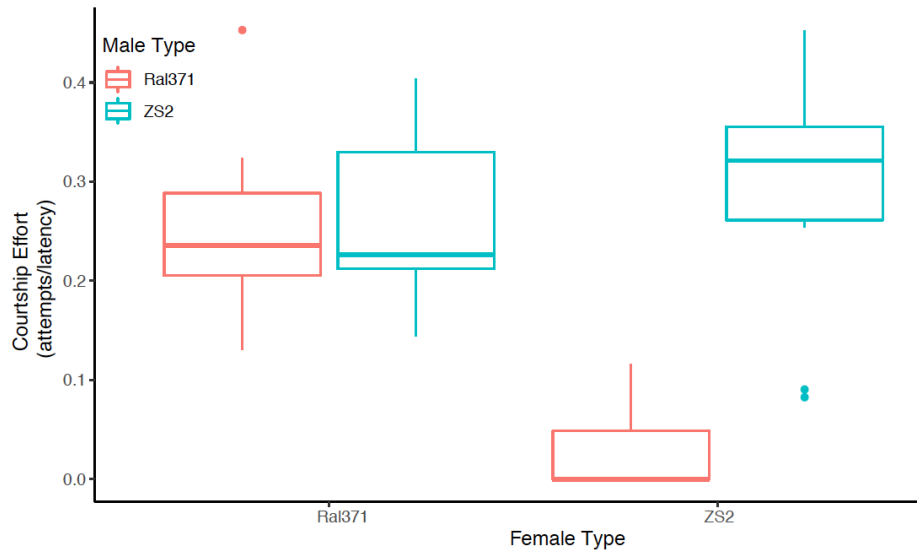
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58 **Figure S7: Weighted topologies between West Africa and OOA.** *Highly similar*
 59 *landscapes of weighted topologies between West Africa and each of all OOA lines and*
 60 *just those from the SE-United States (SE-US) suggest a largely shared landscape of*
 61 *introgression.*

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66 **Figure S8: Courtship effort differs between males of different genotypes.**
 67 Courtship effort (defined as the number of courtship attempts divided by latency) per
 68 behavioral type of male (M=Ral371, Z=ZS2) for each female in a two-way choice
 69 experiment. Z males attempt more courtings, particularly in the context of Z females.

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