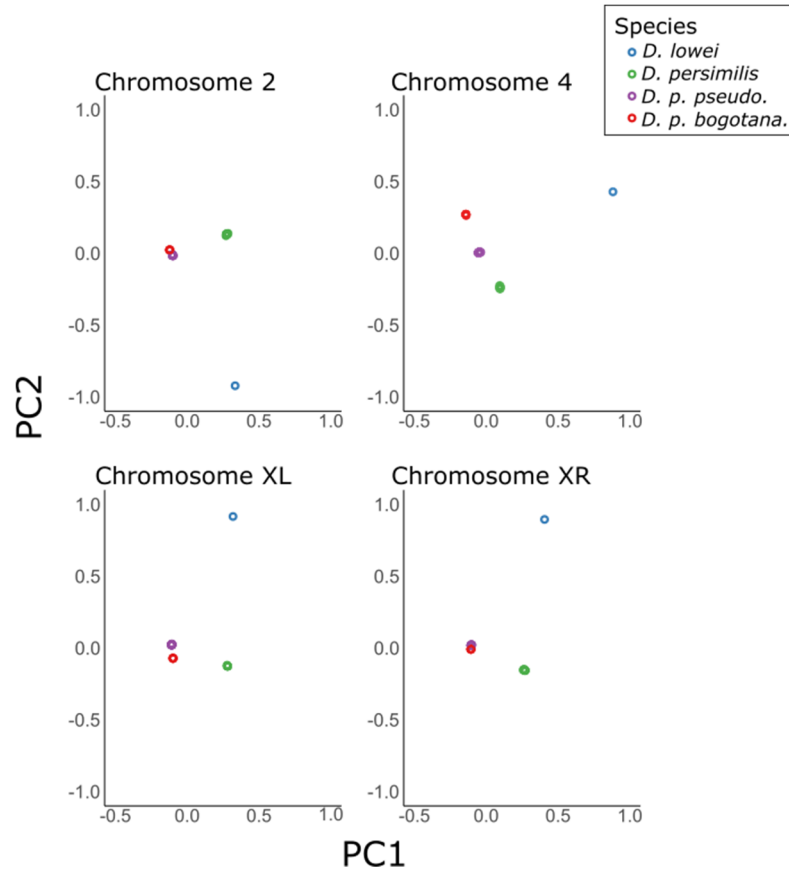
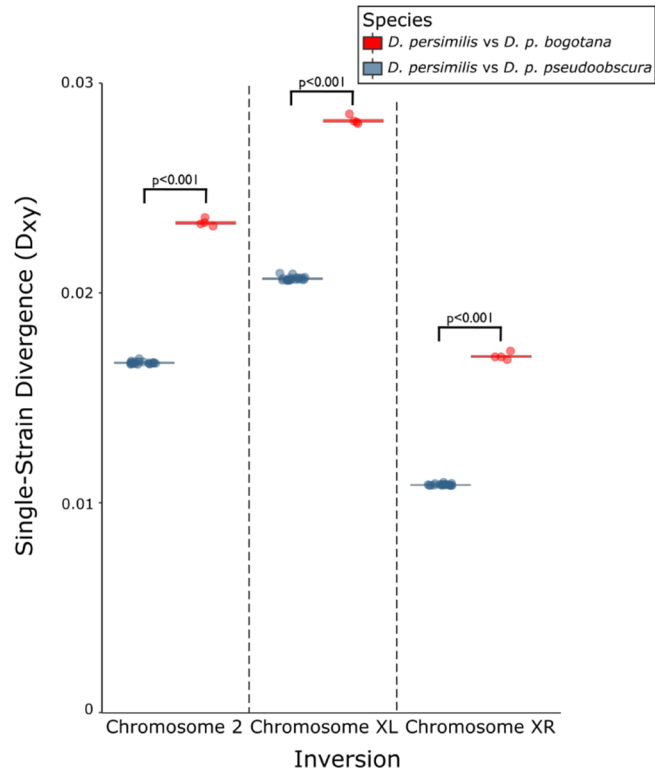


Supplementary Figures and Tables



Supplementary Figure 1 | Principal components analysis. The first two principal components are plotted for the four examined chromosome arms.



Supplementary Figure 2 | Divergence in inverted regions. For each individual *D. pseudoobscura* genome, divergence from *D. persimilis* is shown for inverted regions.

Supplementary Table 1 | Genomic data for introgression analysis.

Strains	Sequence Accessions
<i>Drosophila lowei</i>	
1 strain: Lab3Lowe	Raw sequences available under SRA Experiment SRX091467.
<i>Drosophila persimilis</i>	
8 strains: SCI, MSH7, MSH42, MatherG, Mather40, 111_51, 111_50, and 111_35	Raw sequences will be published to SRA by the time of publication.
<i>Drosophila pseudoobscura bogotana</i>	
4 strains: Toro4, Suta3, ER-White, SCinv	Raw sequences available under SRA Project PRJNA593268.
<i>Drosophila pseudoobscura pseudoobscura</i>	
19 strains: S1-A56, S10-A47, S11-A14, S12-M27, S13-A48, S14-A49, S15-A57, S16-A30, S17-M20, S18-M15, S19-A24, S20-M13, S21-M6, S22-A6, S3-M14, S4-A60, S5-M17, S6-A19, S9-A12	Raw sequences will be published to SRA by the time of publication.

Supplementary Table 2 | Inversion breakpoints relative to the *D. miranda* reference genome. Breakpoints (Chr 2, XL, XR) were originally described in Machado *et al.* (2007) and provided here in coordinates of DroMir2.2.

Chromosome	Approximate Proximal Breakpoint	Approximate Distal Breakpoint
Chromosome 2	15,154,864	23,081,317
Chromosome XL	10,619,782	18,389,958
Chromosome XR	24,174,893	11,348,640

Supplementary Table 3 | Average divergence in inverted regions compared to collinear regions¹.

Chromosome	Average D_{xy} : Inverted	Average D_{xy} : Collinear	Average D_{xy} : Collinear _{FR}	p-value Inverted vs Collinear	p-value Inverted vs Collinear _{FR}
<i>D. persimilis</i> vs <i>D. p. pseudoobscura</i>					
Chromosome 2	0.0166	0.0125	0.0131	<0.001	<0.001
Chromosome XL	0.0207	0.0143	0.0130	<0.001	<0.001
Chromosome XR	0.0109	0.0094	0.0089	<0.001	<0.001
<i>D. persimilis</i> vs <i>D. p. bogotana</i>					
Chromosome 2	0.0233	0.0198	0.0203	<0.001	<0.001
Chromosome XL	0.0283	0.0235	0.0238	<0.001	<0.001
Chromosome XR	0.0171	0.0168	0.0153	0.202	<0.001

¹ Statistical comparisons of these estimates were made using Mann-Whitney U tests to compare the divergence estimates in each region, split into 50 kb windows.

Supplementary Table 4 | Using loci sampled from collinear_{FR} regions only, forward selection of the best model of *D. persimilis* - *D. p. bogotana* divergence using the maximized log-likelihood (LogL) under each model in likelihood-ratio tests.

H ₀	H ₁	Deg. of Freedom	LogL H ₀	LogL H ₁	LRT Statistic	P-value
Iso	IM	2	-25511.23	-25492.84	36.78	1.031e-08
IM	IIM1	1	-25492.84	-25492.84	0	-
IM	IIM2	3	-25492.84	-25442.42	100.84	1.025e-21
IIM1	IIM2	2	-25492.84	-25442.42	100.84	1.267e-22
IIM1	IIM3	1	-25492.84	-25447.73	90.22	2.131e-21
IIM1	IIM4	1	-25492.84	-25444.03	97.62	5.069e-23

Supplementary Table 5 | Maximum-likelihood estimates under models of *D. persimilis* - *D. pseudoobscura bogotana* divergence and values of the maximized log-likelihood (LogL).

Model	θ_1	θ_2	θ_A	θ_{C1}	θ_{C2}	V	T1	M1	M2	logL
<i>Loci sampled genome-wide</i>										
Divergence in Isolation										
ISO	2.756	1.992	3.468	-	-	3.626	-	-	-	-33702.73
Divergence in isolation with migration										
IM	2.599	1.721	3.391	-	-	4.083	-	0	0.251	-33659.39
Divergence in isolation with initial migration										
IIM1	2.599	1.721	3.391	-	-	4.082	1e-06	0	0.251	-33659.39
IIM2	2.631	1.950	1.825	9.587	1.830	3.380	1.089	0.187	0.164	-33538.92
IIM3	2.661	1.715	2.303	10.453	1.839	3.413	0.909	-	0.353	-33543.76
IIM4	2.654	2.288	1.543	9.735	1.807	3.215	1.158	0.300	-	-33542.89
<i>Loci sampled from collinear regions only</i>										
Divergence in Isolation										
ISO	2.857	2.026	4.120	-	-	3.162	-	-	-	-25511.23
Divergence in isolation with migration										
IM	2.724	1.738	4.082	-	-	3.538	-	0	0.368	-25492.84
Divergence in isolation with initial migration										
IIM1	2.724	1.739	4.082	-	-	3.533	0.005	0	0.369	-25492.84
IIM2	2.710	1.988	1.995	8.328	1.903	3.938	1.202	0.424	0.237	-25442.42
IIM3	2.751	1.586	2.835	8.878	1.935	2.930	0.936	-	0.694	-25447.73
IIM4	2.732	2.371	1.718	8.616	1.873	2.820	1.234	0.569	-	-25444.03

Supplementary Table 6 | Tajima's relative rate test. Genome-wide comparison of the substitution rates of each lineage compared to the outgroup *D. lowei* (including both inverted/collinear and intergenic/genic SNPs from chromosomes 2, 3, XR, and XL)

<u>Genomes Compared to Outgroup (<i>D. lowei</i>)</u>		<u>Substitutions</u>		<u>Test Statistic</u>
<u>Line1</u>	<u>Line2</u>	<u>m₁</u> (Line1,Outgroup)	<u>m₂</u> (Line2,Outgroup)	<u>X²</u>
<i>D. p. bogotana</i> Line SCinv	<i>D. p. bogotana</i> Line Suta3	135464	135233	0.20
<i>D. p. bogotana</i> Line SCinv	<i>D. p. bogotana</i> Line Toro4	133199	132939	0.25
<i>D. p. bogotana</i> Line SCinv	<i>D. p. bogotana</i> Line WhiteER	137670	137815	0.08
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S1-A56	362782	307843	4500.72**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S10-A47	363835	313950	3671.54**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S11-A14	361134	302789	5127.31**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S12-M27	355206	284279	7866.70**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S13-A48	355405	289593	6715.09**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S14-A49	360900	304058	4858.97**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S15-A57	363020	310913	4028.80**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S16-A30	353165	279822	8498.11**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S17-M20	315070	151071	57698.58**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S18-M15	353368	276725	9322.67**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S19-A24	354527	281630	8353.24**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S20-M13	348004	256521	13844.16**
<i>D. p. bogotana</i> Line SCinv	<i>D. pseudoobscura</i> Line S21-M6	354757	280638	8646.00**
<i>D. p. bogotana</i> Line Suta3	<i>D. p. bogotana</i> Line SCinv	135233	135464	0.20
<i>D. p. bogotana</i> Line Suta3	<i>D. p. bogotana</i> Line Toro4	134334	134335	0.00
<i>D. p. bogotana</i> Line Suta3	<i>D. p. bogotana</i> Line WhiteER	135565	135880	0.37
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S1-A56	360524	307364	4231.23**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S10-A47	361629	313153	3482.49**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S11-A14	358637	302069	4843.21**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S12-M27	352897	283684	7525.26**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S13-A48	353294	288965	6443.23**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S14-A49	358728	303642	4581.23**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S15-A57	360959	310232	3833.82**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S16-A30	350890	279369	8116.11**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S17-M20	312747	150658	56695.21**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S18-M15	351123	276035	8990.09**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S19-A24	351877	280727	8002.36**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S20-M13	345586	255997	13341.78**
<i>D. p. bogotana</i> Line Suta3	<i>D. pseudoobscura</i> Line S21-M6	352231	280059	8237.99**
<i>D. p. bogotana</i> Line Toro4	<i>D. p. bogotana</i> Line SCinv	132939	133199	0.25
<i>D. p. bogotana</i> Line Toro4	<i>D. p. bogotana</i> Line Suta3	134335	134334	0.00
<i>D. p. bogotana</i> Line Toro4	<i>D. p. bogotana</i> Line WhiteER	134961	135490	1.03
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S1-A56	360359	307187	4235.31**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S10-A47	361472	313088	3470.43**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S11-A14	358627	302044	4846.04**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S12-M27	353138	283941	7515.90**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S13-A48	353590	289344	6419.86**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S14-A49	358674	303731	4557.23**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S15-A57	360985	310337	3821.15**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S16-A30	350773	279509	8057.60**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S17-M20	313008	151078	56501.00**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S18-M15	351189	276235	8954.24**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S19-A24	352118	280950	8000.54**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S20-M13	345915	256036	13420.09**
<i>D. p. bogotana</i> Line Toro4	<i>D. pseudoobscura</i> Line S21-M6	352426	280109	8267.92**
<i>D. p. bogotana</i> Line WhiteER	<i>D. p. bogotana</i> Line SCinv	137815	137670	0.08

<i>D. p. bogotana</i> Line WhiteER	<i>D. p. bogotana</i> Line Suta3	135880	135565	0.37
<i>D. p. bogotana</i> Line WhiteER	<i>D. p. bogotana</i> Line Toro4	135490	134961	1.03
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S1-A56	362427	307752	4460.53**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S10-A47	363327	313437	3677.81**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S11-A14	360819	302644	5101.01**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S12-M27	354744	284331	7758.07**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S13-A48	355454	289729	6695.43**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S14-A49	360412	304036	4783.30**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S15-A57	362700	310561	4037.77**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S16-A30	352637	279820	8383.68**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S17-M20	314738	151530	57127.77**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S18-M15	353270	276742	9295.91**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S19-A24	354159	281495	8306.50**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S20-M13	347651	256585	13724.80**
<i>D. p. bogotana</i> Line WhiteER	<i>D. pseudoobscura</i> Line S21-M6	354241	280464	8575.71**

** Tajima's relative rate test significant at $p < 0.001$, X^2 test, 1 degree of freedom (Tajima 1993).