

SUPPLEMENTARY MATERIAL

Table S1. Primer sequences, gene IDs, annealing temperatures, and sequence sizes of genes studied in *D. willistoni* and *D. insularis*. The same primers were used for both species with the exception of *toy* for which a different forward primer was needed for *insularis*. Primers were determined from complete genome sequences (Drosophila 12 Genomes Consortium 2007).

Gene (FlyBase ID number)	Primer sequence (5'→3')	Annealing temperature	Total number of bases
<i>Adh</i> (0000055)	<i>AdhF</i> : ATTGGTCTGGACACCAGTCC <i>AdhR</i> : AAATTTAAATGCCCGAATCC	57°C	612
<i>ci</i> (0004859)	<i>CiF</i> : AAGGTTGCTACAAGGCGTATTC <i>CiR</i> : GGCTCCATTTTTAGTTCGGTAA	58°C	836
		58°C	836
<i>Ank</i> (0011747)	<i>AnkF</i> : TGCTCACAATTTGGGATATG <i>AnkR</i> : CAGATGAACGGGTGGTCTAA	56°C	451
		56°C	474
<i>ey</i> (0005558)	<i>EyF</i> : CTGAGGCTAGGATTCAGGTTTG <i>EyR</i> : ACAGCGGAGGGGTTAATAGAAC		1318
<i>Toy</i> (0019650)	<i>ToyF</i> : AAAACTGCGTACCCAACGTC <i>ToyR</i> : CCCGCTAAGTCCGATACATT	53°C	854
<i>insularis</i>	<i>ToyF2</i> : TCTCCAATCGACGTGCAA	60°C	862
<i>Sav</i> (GK13578)	<i>GK13578F1</i> : CGCCACCTGAGGTTTGTATT <i>GK13578R2</i> : TCGACGTCTGTAAGCGACTG	52°C	1164
		52°C	1063

Table S2. Distribution of polymorphic sites in alignments of nuclear DNA allele sequences. The prefix of each haplotype name denotes the species and population(s) in which that sequence was found (*Di*, *D. insularis* from St. Lucia; *DwS*, *D. willistoni* from St. Lucia; *DwE*, *D. willistoni* from Ecuador; *DwSE*, same sequence found in both *D. willistoni* populations). Haplotype numbers are arbitrary. Note that some allele haplotypes were inferred using PHASE rather than directly observed as homozygotes or resolved by cloning. “Site number” is the relative to the first base in the sequence alignment. “Count” is the number of times a given haplotype was sampled from each species.

Alcohol dehydrogenase (Adh)

Haplotype	Count	Site number								
		160	163	242	271	358	370	470	541	608
<i>Di</i> -Hap03	59	C	C	G	C	A	G	C	G	T
<i>Di</i> -Hap02	37	T	.	.
<i>Di</i> -Hap04	23	A	T	.	.
<i>Di</i> -Hap01	6	T	C	.
<i>Di</i> -Hap05	2	.	.	A
<i>Di</i> -Hap06	1	A
<i>Di</i> -Hap07	1	A	T	.	A
<i>Di</i> -Hap08	1	T	G	.	.	T	.	T	.	.
<i>Di</i> -Hap09	1	.	.	.	A
<i>Di</i> -Hap10	1	.	.	.	A	.	A	T	.	.

Table S2 *continued.*

Alcohol dehydrogenase (Adh)

Haplotype	Count	Site number																											
		013	068	091	109	178	208	211	253	277	334	346	352	370	382	394	406	412	415	424	454	475	478	490	517	526	541	559	604
DwS-Hap02	60	C	A	C	C	T	T	G	C	C	C	C	C	T	T	C	G	C	G	T	C	C	C	T	G	C	T	C	
DwSE-Hap0501	17	C	.	.	T
DwS-Hap01	15	T	T
DwS-Hap04	11	C
DwE-Hap04	5	C	.	.	T	T	.	C	.	.	C	.	
DwE-Hap03	3	C	.	.	T	C	.	.	C	.
DwSE-Hap0323	3	T
DwS-Hap07	3	T	T	.	C	.	.	C	.
DwSE-Hap1212	2	T	C	T	.	C	.	.	C	.
DwE-Hap10	2	C	.	.	C	.
DwE-Hap02	1	C	.	.	T	T
DwE-Hap05	1	C	.	.	T	T	.	.	T	.	.	.	A	T
DwE-Hap06	1	T	.	.	T	C	.	.	.	T	A	T
DwE-Hap07	1	C	.	.	T	T	T	C	.	.	C	T
DwE-Hap08	1	T	C	A	T	.	C	.	.	C	.
DwE-Hap09	1	.	.	A	.	.	C	.	.	T
DwE-Hap11	1	C	.	.	T	C	.	T	T	.	C	.	.	C
DwE-Hap13	1	C	.	.	T	T	T
DwE-Hap14	1	.	G	.	.	.	C	.	T	T
DwE-Hap15	1	C	.	.	T	C	T	.	C	.	.	C
DwE-Hap16	1	C	T
DwE-Hap17	1	C	.	.	T	C
DwE-Hap18	1	C	.	.	T	A	T	.	C	.	.	C	.
DwE-Hap19	1	C	.	.	T	A	.	.	.
DwE-Hap20	1	C	.	.	T	C
DwE-Hap21	1	C	T	.	C	.	.	.	C
DwE-Hap22	1	C	.	.	T	C	G	.	.
DwS-Hap06	1	C	A	.	T	C	.	.	C	.
DwS-Hap08	1	A
DwS-Hap09	1	T	.	.	G	C
DwS-Hap10	1	T
DwS-Hap11	1	T	T	C
DwS-Hap13	1	T	.	C	.	.	C	.

Table S2 *continued.*

Ankyrin (Ank)

Haplotype	Count	Site number										
		004	106	234	309	321	370	383	419	462	463	468
<i>Di</i> -Hap03	38	C	A	A	A	T	A	C	G	C	A	T
<i>Di</i> -Hap01	24	A	C	T	C	T	.	.
<i>Di</i> -Hap02	11	A	.	.	C	.	.	.
<i>Di</i> -Hap06	7	.	.	.	G
<i>Di</i> -Hap04	6	G	.
<i>Di</i> -Hap05	2	.	.	G
<i>Di</i> -Hap07	2	.	C	.	.	A	C	T	C	T	.	.
<i>Di</i> -Hap08	1	T	.	.	G
<i>Di</i> -Hap09	1	T	.	.	G	G	.
<i>Di</i> -Hap10	1	A
<i>Di</i> -Hap11	1	C	.	.	.
<i>Di</i> -Hap12	1	A	.	.	C	.	.	G
<i>Di</i> -Hap13	1	G

Haplotype	Count	Site number					
		004	058	110	153	298	477
<i>Dw</i> S-Hap01	56	C	C	G	T	C	—
<i>Dw</i> E-Hap01	40	C
<i>Dw</i> S-Hap02	32	A	—
<i>Dw</i> E-Hap02	1	.	.	C	.	.	C
<i>Dw</i> E-Hap03	1	G
<i>Dw</i> E-Hap04	1	T	G
<i>Dw</i> E-Hap05	1	A	C
<i>Dw</i> S-Hap03	1	.	.	.	C	A	—
<i>Dw</i> S-Hap04	1	.	T	.	.	.	—

Table S2 *continued.*

cubitus interruptus (ci)

Haplotype	Count	Site number								
		076	136	171	245	254	461	482	639	740
<i>Di</i> -Hap03	60	T	G	A	C	A	A	G	C	G
<i>Di</i> -Hap02	50	A	.	.
<i>Di</i> -Hap04	22	.	.	.	T	G
<i>Di</i> -Hap07	17	C	C	.	T	G
<i>Di</i> -Hap05	10	T	T
<i>Di</i> -Hap01	5	C	C	G
<i>Di</i> -Hap06	1	C
<i>Di</i> -Hap08	1	C	C	.	T	G	G	.	.	.

Haplotype	Count	Site number			
		145	158	192	605
<i>Dw</i> SE-Hap0104	106	G	G	A	C
<i>Dw</i> E-Hap01	24	.	.	.	A
<i>Dw</i> E-Hap02	16	.	.	G	A
<i>Dw</i> E-Hap03	1	.	A	.	A
<i>Dw</i> S-Hap02	1	A	.	.	.

Table S2 *continued.*

twin of eyeless [&& is this correct? Not given in the main text &&] (*toy*)

Haplotype	Count	Site number															
		152	231	237	240	319	373	451	452	480	485	632	662	692	710	796	841
<i>Di</i> -Hap01	26	T	T	A	G	C	C	T	C	A	C	T	C	T	T	C	T
<i>Di</i> -Hap06	25	T	A
<i>Di</i> -Hap02	17	T	A	C
<i>Di</i> -Hap04	16	T	.	.	.	T	A	C	T	.	.	.	A
<i>Di</i> -Hap08	5	.	.	.	T	T	.	.	.	A	.	.	.
<i>Di</i> -Hap11	3	G	A	T	T	A	C	.	.	.	T	.
<i>Di</i> -Hap07	2	T	A	A
<i>Di</i> -Hap10	2	T
<i>Di</i> -Hap03	1	T	A	C	A
<i>Di</i> -Hap05	1	.	.	.	T	T	.	.	.	A	.	.	A
<i>Di</i> -Hap09	1	T	A	C	.	.	C	.	.
<i>Di</i> -Hap12	1	G
<i>Di</i> -Hap13	1	A
<i>Di</i> -Hap14	1	C	A	T	A	T	.
<i>Di</i> -Hap15	1	T	.	.	T	A
<i>Di</i> -Hap16	1	.	.	.	T	T	A	.	.	A	.	.	.

Haplotype	Count	Site number																												
		006	075	170	322	337	430	502	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	599	617	665	683	802	804
<i>DwSE</i> -Hap0102	80	G	T	G	G	A	A	G	T	G	—	—	—	—	—	—	—	—	—	—	—	—	—	—	C	T	G	A	G	G
<i>DwSE</i> -Hap0605	19	C	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwSE</i> -Hap0206	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	.	A	G	.	.
<i>DwS</i> -Hap07	6	.	.	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwE</i> -Hap04	4	.	.	.	T	—	—	—	—	—	—	—	—	—	—	—	—	—	—	.	A
<i>DwS</i> -Hap04	3	.	C	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwS</i> -Hap08	2	T	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwE</i> -Hap07	2	A	A	T	G	A	T	T	A	C	C	A	T	T	A	C	C	A	.	A	G	.	.
<i>DwE</i> -Hap01	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	C
<i>DwE</i> -Hap03	1	T	.	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwS</i> -Hap03	1	.	.	A	.	G	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwS</i> -Hap05	1	.	.	.	T	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DwS</i> -Hap09	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	.	A	G	A	.

Table S3. Neutrality tests based on the ratio of non-synonymous to synonymous substitutions (dN/dS).

Locus	Species	Population(s)	Exon(s)				
			SS	NSS	dN	dS	dN/dS
Adh	<i>D. insularis</i>	STL	148.77	460.23	0.0020	0.0123	0.1635
	<i>D. willistoni</i>	STL+ECU	147.67	461.33	0.0001	0.0289	0.0046
		STL	147.67	461.33	0.0000	0.0235	0.0000
		ECU	147.67	461.33	0.0002	0.0289	0.0066
Sav	<i>D. insularis</i>	STL	240.88	740.12	0.0014	0.0091	0.1506
	<i>D. willistoni</i>	STL+ECU	238.35	736.65	0.0003	0.0044	0.0789
		STL	238.43	736.57	0.0003	0.0049	0.0693
		ECU	238.28	736.72	0.0003	0.0035	0.0941
Ank	<i>D. insularis</i>	STL	82.14	283.86	0.0026	0.0054	0.4867
	<i>D. willistoni</i>	STL+ECU	81.83	284.17	0.0002	0.0002	0.8538
		STL	81.84	284.16	0.0001	0.0003	0.2846
		ECU	82.83	286.17	0.0003	0.0011	0.2937
Ci	<i>D. insularis</i>	STL	176.61	600.39	0.0000	0.0094	0.0000
	<i>D. willistoni</i>	STL+ECU	175.70	604.30	0.0010	0.0000	N/A
		STL	175.67	604.33	0.0000	0.0000	N/A
		ECU	175.79	604.21	0.0010	0.0000	N/A
Toy	<i>D. insularis</i>	STL	152.82	435.18	0.0002	0.0080	0.0213
	<i>D. willistoni</i>	STL+ECU	151.65	436.35	0.0002	0.0069	0.0234
		STL	151.62	436.38	0.0002	0.0074	0.0319
		ECU	151.70	436.30	0.0000	0.0057	0.0000
Ey	<i>D. willistoni</i>	STL+ECU	192.53	554.47	0.0014	0.0142	0.1018
		STL	192.76	554.24	0.0019	0.0129	0.1476
		ECU	192.76	554.24	0.0005	0.0120	0.0429

STL = St. Lucia, ECU = Ecuador

SS = number of synonymous sites

NSS = number of non-synonymous sites

dN = mean number of non-synonymous substitutions per non-synonymous site

dS = mean number of synonymous substitutions per synonymous site

N/A = cannot be calculated (dS = 0)

Table S4. Tests for linkage disequilibrium (LD) using a two-tailed Fisher's exact test, calculated in DnaSP. Only those pairs of polymorphic nucleotides that showed significant LD after Bonferroni correction are given.

Alcohol dehydrogenase (Adh)

Species	Pop	S	LD				
			Site1	Site2			
<i>D. insularis</i>	STL	9	370	470			
<i>D. willistoni</i>	STL+ECU	28	208	277			
			277	334			
			277	478			
			277	517			
			277	559			
			382	478			
			382	517			
			382	559			
			478	517			
			478	559			
			517	559			
			<i>D. willistoni</i>	STL	11	277	334
						478	517
478	559						
517	559						
<i>D. willistoni</i>	ECU	24	517	559			

Table S4 *continued.*

salvador (sav)

Species	Pop	S	LD	
			Site1	Site2
<i>D. insularis</i>	STL	12	81	153
			81	271
			153	271
			271	292
			271	650
			282	292
			282	348
			292	348
			667	683
			667	683
<i>D. willistoni</i>	STL+ECU	13	662	810
			662	928
			810	928
<i>D. willistoni</i>	STL	9	525	1168
			662	810
			662	928
			810	928

Table S4 *continued.*

Ankyrin (Ank)

Species	Pop	S	LD	
			Site1	Site2
<i>D. insularis</i>	STL	11	321	370
			321	383
			321	419
			321	462
			370	383
			370	419
			370	462
			383	419
			383	462
			419	462

cubitus interruptus (ci)

Species	Pop	S	LD	
			Site1	Site2
<i>D. insularis</i>	STL	9	076	136
			076	171
			076	245
			076	254
			076	482
			136	171
			136	245
			136	254
			136	482
			245	254
			245	482
			254	482
			639	740
<i>D. willistoni</i>	STL+ECU	4	192	605

Table S4 *continued.*

twin of eyeless [&& is this correct? Not given in the main text &&] (*toy*)

Species	Pop	S	LD	
			Site1	Site2
<i>D. insularis</i>	STL	16	152	231
			152	237
			152	796
			231	237
			231	796
			237	796
			240	692
			319	632
			319	662
			319	841
			485	632
			632	662
			632	841
			662	841
<i>D. willistoni</i>	STL+ECU	15	322	617
			505	506
			599	665
			599	683
			665	683
<i>D. willistoni</i>	STL	10	599	665
			599	683
			665	683
<i>D. willistoni</i>	ECU	10	322	617
			505	506
			599	665
			599	683
			665	683

Table S4 *continued.*

eyeless (ey)

Species	Pop	S	LD		Species	Pop	S	LD		Species	Pop	S	LD									
			Site1	Site2				Site1	Site2				Site1	Site2								
<i>D. willistoni</i>	STL+ECU	19	36	173	<i>D. willistor</i>	STL+ECU	19	613	1208	<i>D. willistor</i>	STL	17	613	1208								
			36	512				640	1063				640	1063								
			36	516				822	828				822	828								
			36	613				822	933				822	933								
			36	822				822	1208				822	1208								
			36	828				828	933				828	933								
			36	933				828	1208				828	1208								
			36	1208				933	1208				933	1208								
			173	512				<i>D. willistor</i>	STL				17	ECU	14	36	512	<i>D. willistor</i>	ECU	14	36	822
			173	516												36	516				173	512
			173	613												36	613				173	516
			173	640												36	822				173	613
			173	822												36	828				173	828
			173	828												36	933				173	933
			173	933												36	1208				173	1208
			173	1063												173	640				512	516
			173	1208												173	1063				512	613
			512	516												512	516				512	828
			512	613												512	613				512	933
			512	822												512	822				512	1208
512	828	512	828	516	613																	
512	933	512	933	516	828																	
512	1208	512	1208	516	933																	
516	613	516	613	516	1208																	
516	822	516	822	613	828																	
516	828	516	828	613	933																	
516	933	516	933	613	1208																	
516	1208	516	1208	828	933																	
613	822	613	822	828	1208																	
613	828	613	828	933	1208																	
613	933	613	933																			

Table S5. Neutrality tests based on Fu's F_S and Tajima's D

Locus	Species	Population(s)	Exon(s)		Intron(s)	
			F_S	D	F_S	D
Adh	<i>D. insularis</i>	STL	-3.380	-0.893	-	-
		STL+ECU	-24.682	-1.529	-	-
	<i>D. willistoni</i>	STL	-5.370	-0.940	-	-
		ECU	-16.248	-1.531	-	-
Sav	<i>D. insularis</i>	STL	-3.551	0.118	1.300	0.148
		STL+ECU	-2.230	-0.971	-3.873	-1.491
	<i>D. willistoni</i>	STL	-1.196	-0.746	-1.407	-1.129
		ECU	-0.162	0.314	-1.548	-1.116
Ank	<i>D. insularis</i>	STL	-3.178	-0.302	2.792	1.741
		STL+ECU	-8.234	-1.734	1.839	1.178
	<i>D. willistoni</i>	STL	-3.874	-1.392	2.120	1.619
		ECU	-3.353	-1.575	-1.530	-1.115
Ci	<i>D. insularis</i>	STL	1.067	0.738	-0.104	-0.211
		STL+ECU	1.415	0.951	-4.629	-1.325
	<i>D. willistoni</i>	STL	-	-	-2.252	-1.023
		ECU	0.703	0.576	-1.530	-1.115
Toy	<i>D. insularis</i>	STL	-1.050	-0.382	-2.890	-0.111
		STL+ECU	-1.306	-0.766	-3.678	-1.457
	<i>D. willistoni</i>	STL	-0.611	-0.553	-2.007	-1.069
		ECU	1.514	-0.658	-1.148	-0.947
Ey	<i>D. willistoni</i>	STL+ECU	-0.922	0.950	0.642	0.502
		STL	-0.084	0.950	0.739	0.388
		ECU	-0.070	0.936	0.843	0.927

STL = St. Lucia, ECU = Ecuador

F_S = Fu's (1992) F_S ; significantly positive or negative values shown in bold italics

D = Tajima's (1989) D ; significantly positive or negative values shown in bold italics

Figure S1. In situ hybridization of a) *eyeless*, b) *cubitus interruptus*, and c) *Ankyrin*, on polytene chromosomes of *D. insularis*. Hybridization signals on sections 78B (*ci* and *Ank*) and 78D (*ey*) are arrowheaded.

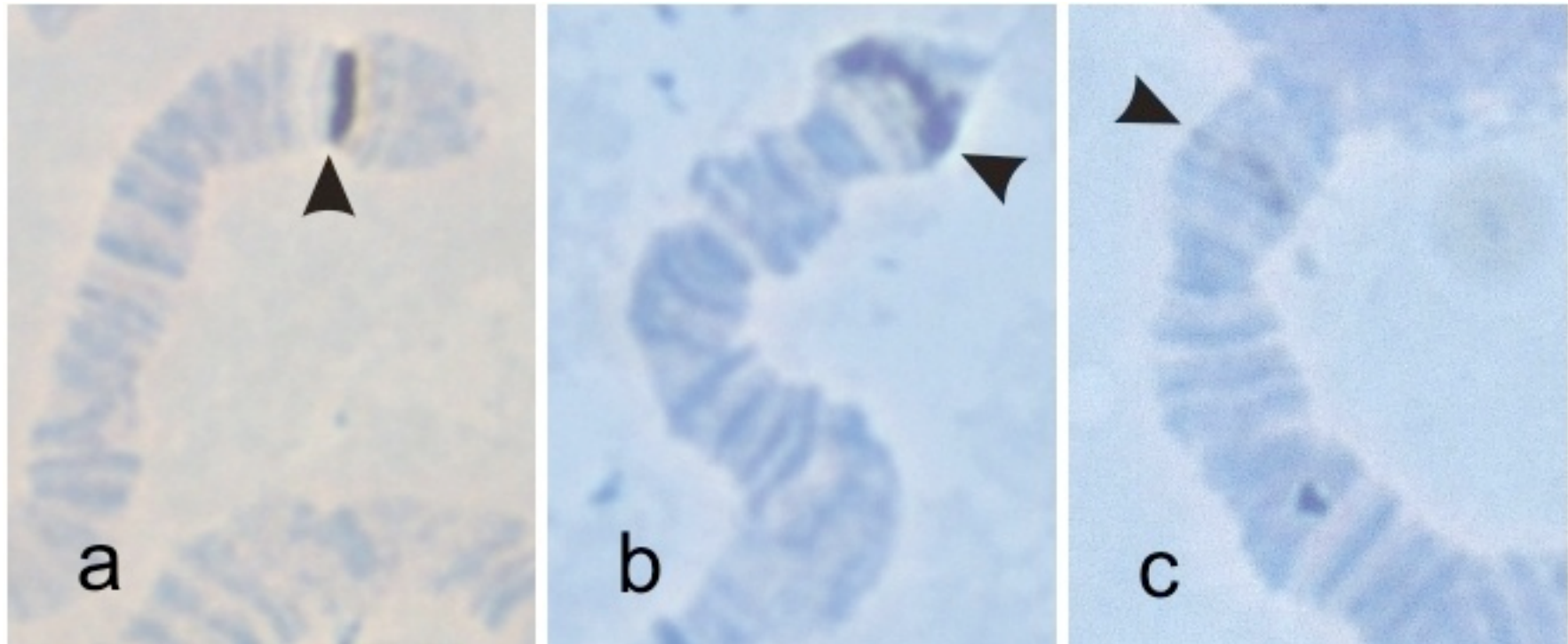
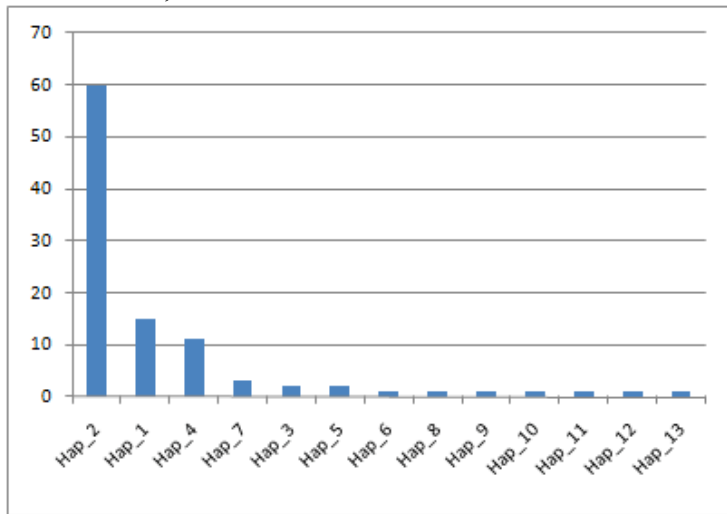


Figure S2. Frequency distribution of alleles (introns plus exons) at each locus, for each population and species. Counts are shown on the ordinate and haplotypes arbitrarily numbered along the abscissa.

Alcohol dehydrogenase (Adh)

D. willistoni, St. Lucia



D. willistoni, Ecuador

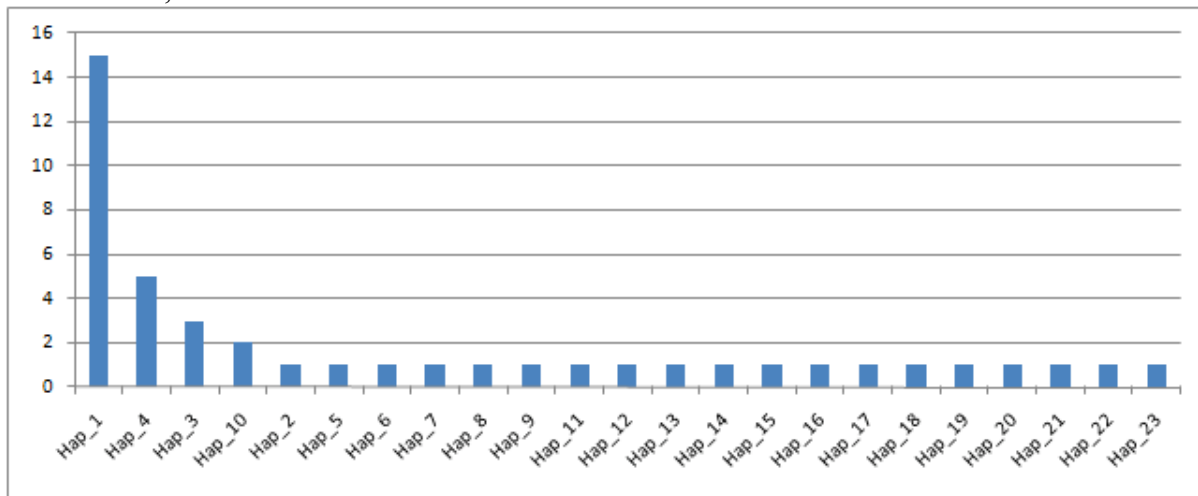
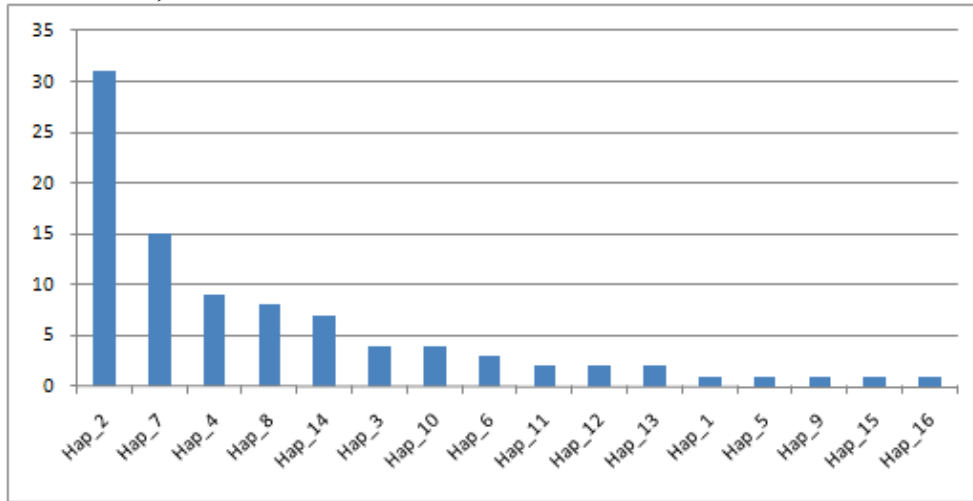


Figure S2 *continued.*

salvador (sav)

D. insularis, St. Lucia



D. willistoni, St. Lucia

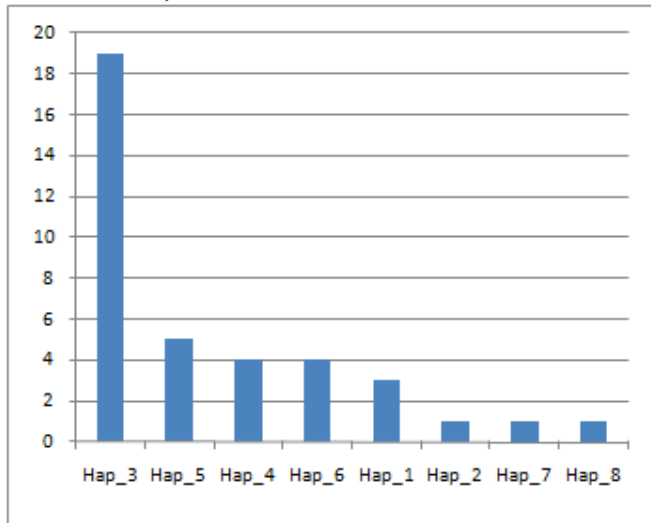
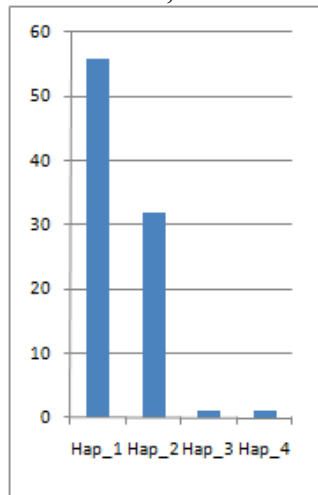


Figure S2 *continued.*

Ankyrin (Ank)

D. willistoni, St. Lucia



D. willistoni, Ecuador

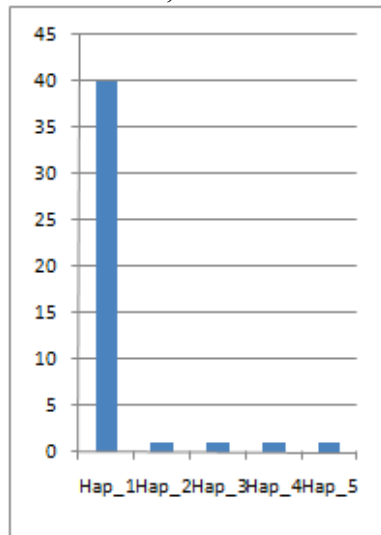
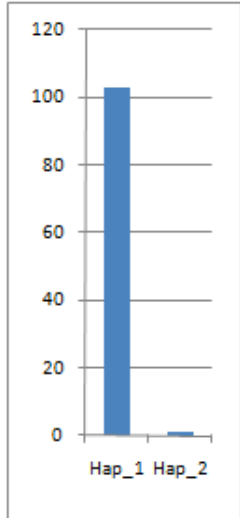


Figure S2 *continued.*

cubitus interruptus (*ci*)

D. willistoni, St. Lucia



D. willistoni, Ecuador

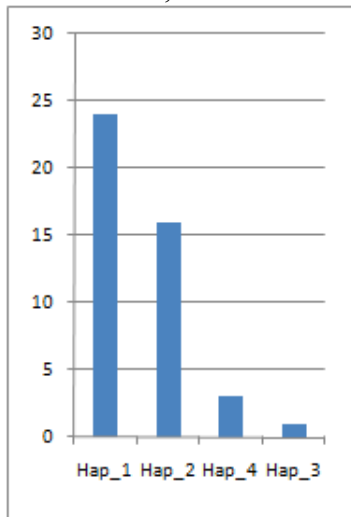
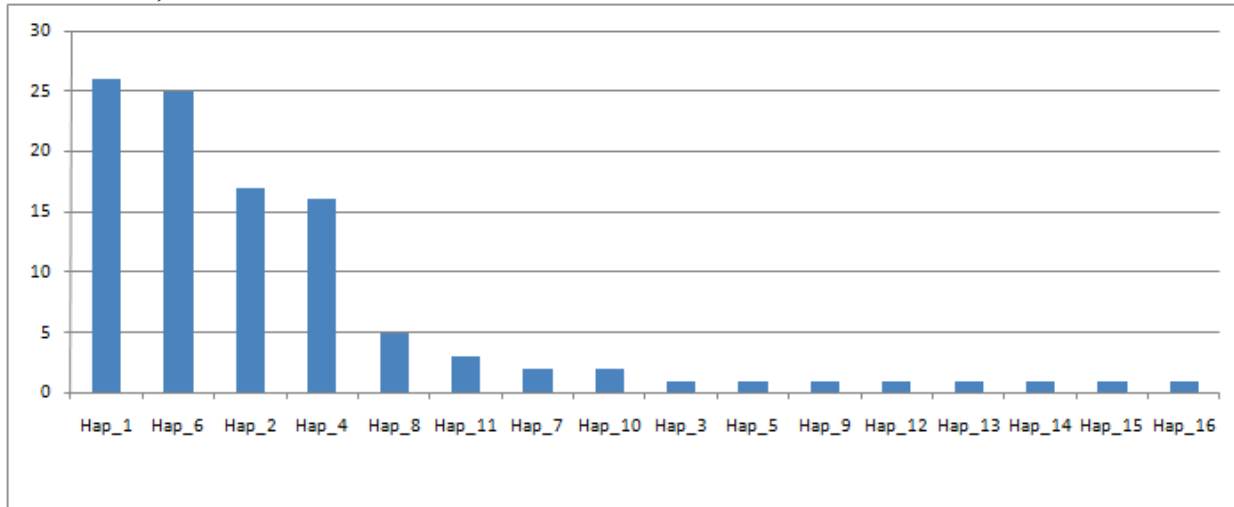


Figure S2 *continued.*

twin of eyeless [&& is this correct? Not given in the main text &&] (*toy*)

D. insularis, St. Lucia



D. willistoni, St. Lucia

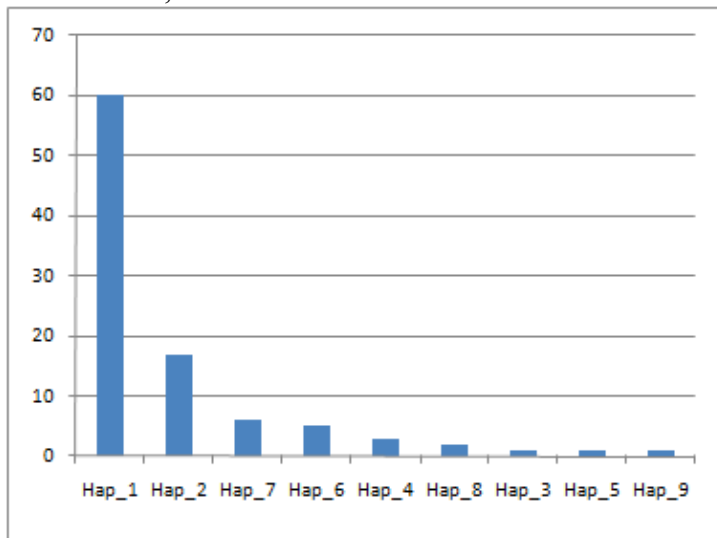


Figure S2 *continued.*

eyeless (ey)

D. willistoni, St. Lucia

