

## Supplementary Data

Supplementary Table S1. Descriptions of the sequenced regions.

	Cytology	Characteristics	Amplification Primers	Temp
<i>Adh</i>	49B	846 bp aligned 181 synonymous 124 5' UTR+intron	5' ccgactagaaagcatcac 5' atttgaatggttagatatgc	54°
v1-71.20 (CG17549)	49A	530 bp aligned 47 synonymous 342 intron	5' ttgtagccaccacctcca 5' aYactgtgggcggtattc	56°
v14-60.15 (CG5682)	48F	594 bp aligned 143 synonymous	5' ttgaggctcggagaacag 5' catcgaggctcggatctt	58°
<i>bib</i>	48E	953 bp aligned 164 synonymous 179 intron	5' tacgatttcggacttgcgaa 5' atgctctctgtacgctgttg	58°
<i>Gpdh</i>	47B	1141 bp aligned 174 synonymous 361 intron	5' ttgccagggtccagttg 5' gggaatctgcaagtatagt	61°
<i>tim</i>	42E	502 bp aligned 112 synonymous	5' gatccgaagagcaccaagag 5' gtggcatcgggtgccctca	60°

Supplementary Table S2. Sequence-based estimates of the recombination parameter.

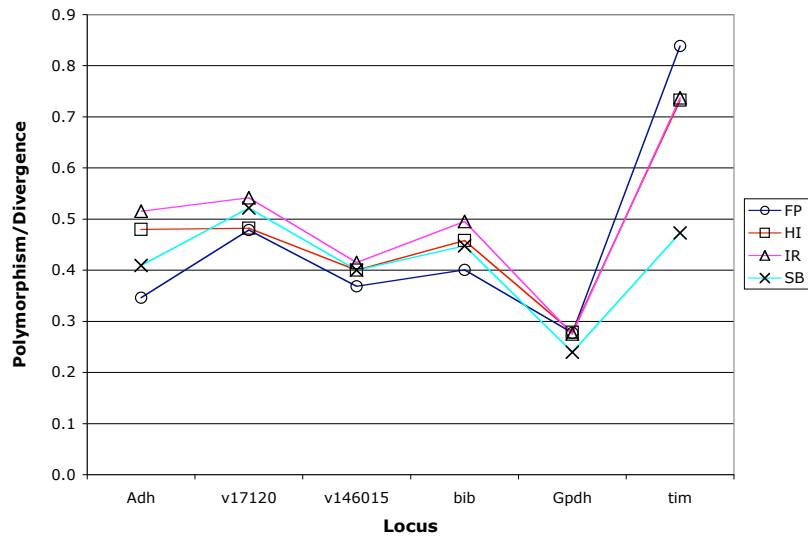
	FP	HI	IR	SB	Total
<i>Adh</i>					
$R_H$	747	>10000	521	293	786
$r$ per bp	0.89	-	0.62	0.35	0.94
v1-71.20					
$R_H$	596	300	>10000	32.9	130
$r$ per bp	1.13	0.57	-	0.06	0.25
v14-60.15					
$R_H$	319	144	90.5	35.1	95.3
$r$ per bp	0.54	0.24	0.15	0.06	0.16
<i>bib</i>					
$R_H$	106	436	153	111	123
$r$ per bp	0.11	0.47	0.16	0.12	0.13
<i>Gpdh</i>					
$R_H$	2304	4896	442	326	621
$r$ per bp	2.02	4.29	0.39	0.29	0.55
<i>tim</i>					
$R_H$	>10000	134	>10000	46.8	156
$r$ per bp	-	0.27	-	0.09	0.31

$R_H$  corresponds to the Hudson [1] estimator of  $4Nr$  for the entire region, and when informative, the corresponding rate between adjacent sites is presented.

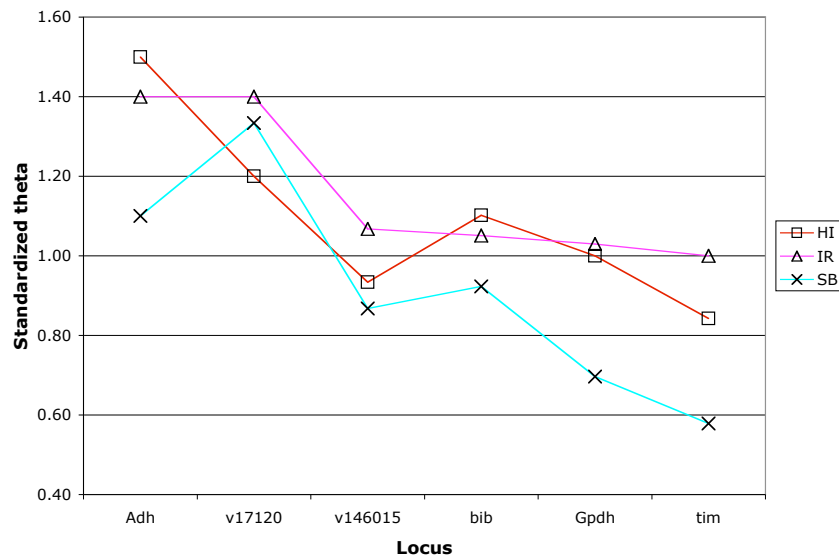
Supplementary Table S3. Assay methods for loci used to measure linkage relationships.

Locus	Cytology	Primers (5'-3')	Assay	Reference
<i>fu1</i>	18C	gccgagtgacattgagcag gctcggcggttctcgggtag	RFLP- <i>Clal</i>	Vieira et al. 2001 [2]
<i>Adh</i>	49B	ccgactagaaagcatcac attggaatggttagatatgc	RFLP- <i>HhaI</i>	McAllister 2002 [3]
v1-71.20	49A	ttgtagccaccacctcca ayactgtgggcggttattc	RFLP- <i>AluI</i>	This study
<i>bib</i>	48E	tacgatttcggacttgcgaa gggttacagattctggcag	RFLP- <i>BbrPI</i>	McAllister 2002 [3]
v68-86.1	48A	tcgcatctcaaaatthaattgac agcagcaaaacgaagcaaat	MS-(TG) <sub>n</sub>	Schlötterer 2000 [4]
<i>Gpdh</i>	47B	gttctattggccgcttatc tcggcattcttggcagcctc	MS-(CA) <sub>n</sub>	This study
v71-6	47B	cgaacagtttagccagaa acagataaacagttgcacag	MS-(GT) <sub>n</sub>	Schlötterer 2000 [4]
v68-62	44E	atgttgcaagctgtgttgc tcgggtgcattcgtagggtg	MS-(GTT) <sub>n</sub>	Schlötterer 2000 [4]
v3054	?	attcaatatctccaggctccc ttctgtgttatgctgcctgctgaac	MS-(GA) <sub>n</sub>	This study
v68-4	43A	aagacgggctgaggatgtgag aacaataaatgatgtgaaatc	MS-(CA) <sub>n</sub>	Schlötterer 2000 [4]
<i>tim</i>	42E	ttcaatatctccaggctccc gtttgccgtcaaagtgtagaac	MS-(GA) <sub>n</sub>	This study

Supplementary Figure S1. Pairwise sequence diversity in *D. americana* relative to fixed differences in comparison with *D. virilis*. Numbers of fixed differences at each locus were relative to the entire set of 40 sequences from *D. americana*. Loci are arranged according to relative position along Chromosome 4 with the centromere toward the left and telomere toward the right.



Supplementary Figure S2. Standardized estimate of heterozygosity ( $\hat{\pi}$ ) measured from the number of mutations. Observed  $\hat{\pi}$  in each northern sample is standardized using  $\hat{\pi}$  for the southern FP sample. A significant Spearman rank correlation is obtained relative to position on Chromosome 4 ( $r_s = -0.78$ , 95% CI: -0.91, -0.57).



## Supplementary References

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- [1] Hudson RR (1987) Estimating the recombination parameter of a finite population model without selection. *Genet Res* 50: 245-250.
- [2] Vieira J, McAllister BF, Charlesworth B (2001) Evidence for selection at the *fused1* locus of *Drosophila americana*. *Genetics* 158: 279-290.
- [3] McAllister BF (2002) Chromosomal and allelic variation in *Drosophila americana*: selective maintenance of a chromosomal cline. *Genome* 45: 13-21.
- [4] Schlötterer C (2000) Microsatellite analysis indicates genetic differentiation of the neo-sex chromosomes in *Drosophila americana americana*. *Heredity* 85: 610-616.