

Supplementary Text: Calculating the maximal contribution of ancestral polymorphism to the faster X effect seen in *D. pseudoobscura* - *D. persimilis*

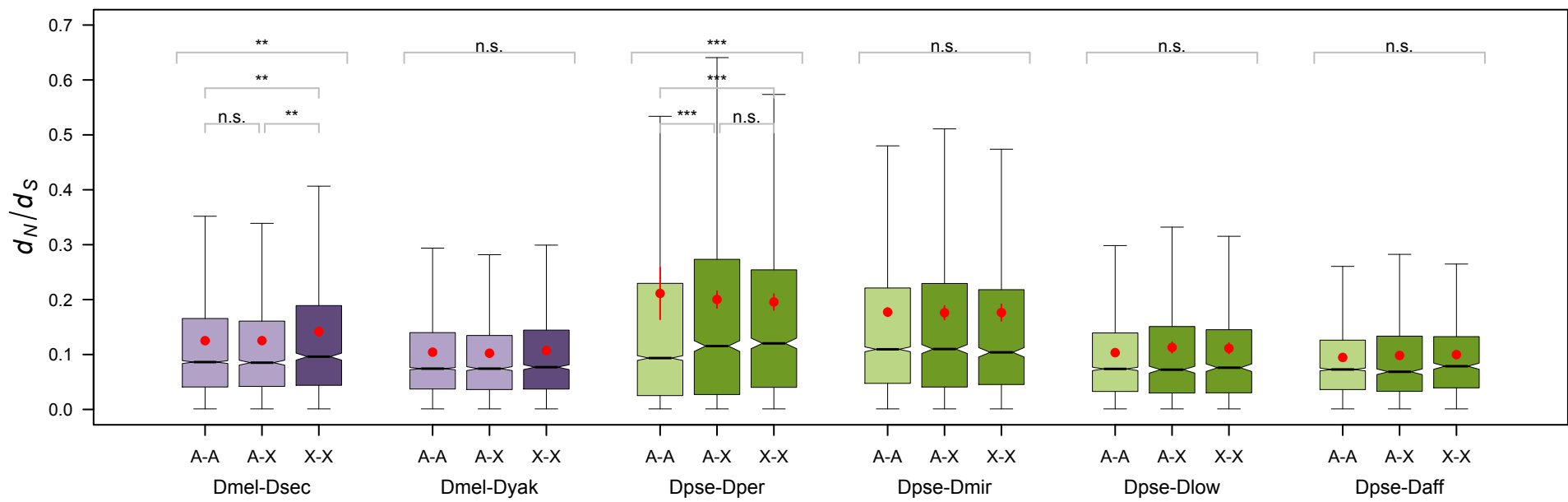
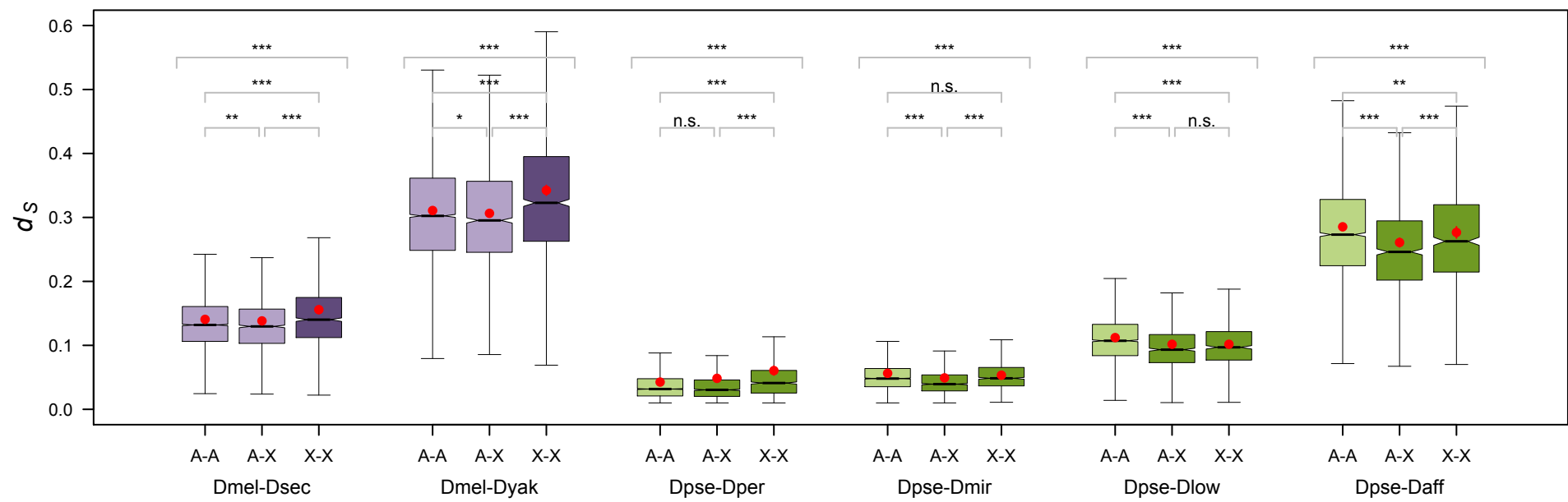
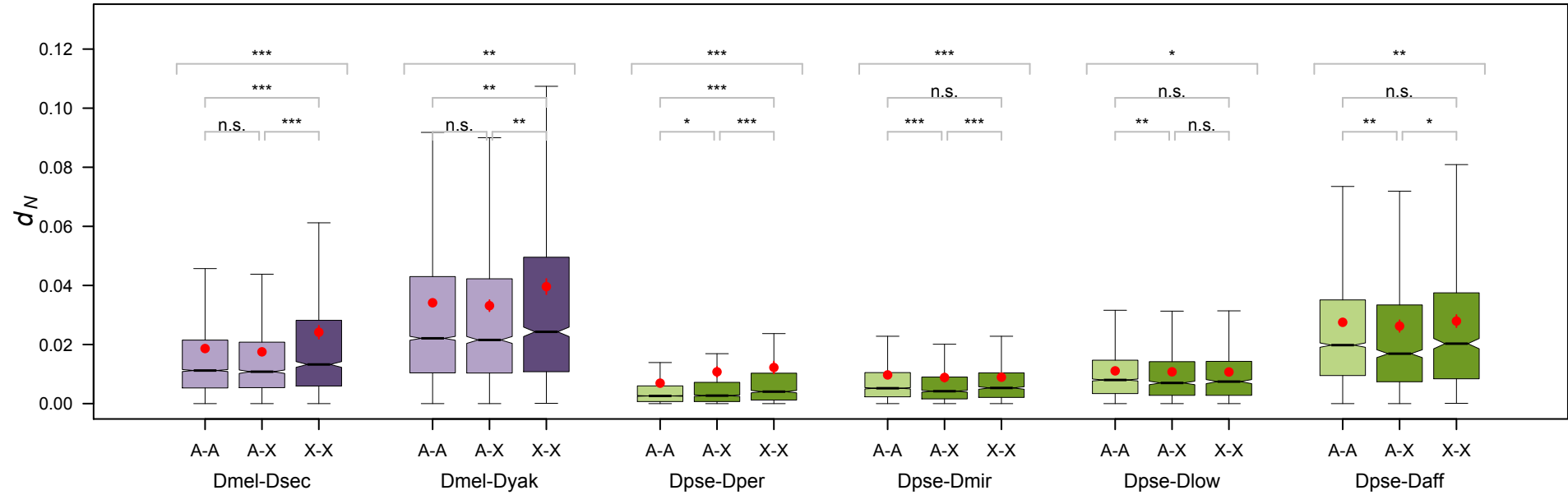
First, we find a rough correction for the between species divergence for within-species diversity by reducing the divergence values according to the mean diversity levels. For the *D. pseudoobscura* - *D. persimilis* divergence (abbreviated to *pse* - *per*), the mean K_A for the AA, XX, and XA genes are 0.0080, 0.012 and 0.014, respectively (for consistency with the polymorphism data below, we use means rather than medians). The corresponding mean K_S values are 0.038, 0.042 and 0.051. To estimate the contribution of polymorphism to the *pse* values, we can combine the polymorphism data from the current study with that of Haddrill *et al.* (2010), giving mean π_S values of 0.0221 and 0.0165 for AA and XX synonymous sites. The contribution of polymorphism from *D. persimilis* is probably lower than this: from (Machado *et al.* 2002), we find that the autosomal silent diversity in *D. persimilis* averages about 0.648 times that for *D. pseudoobscura* (although these values come from only 9 autosomal introns, the genomic data of (McGaugh and Noor 2012) for XL and chromosome 2 also support a substantially reduced level of diversity in *D. persimilis* compared with *D. pseudoobscura*). Using the estimate of 0.648 for the relative diversity level in *per*, the correction for the within-species diversity contribution to K_S is $0.5 \times (1 + 0.648) = 0.824$ times the *pse* diversity value, *i.e.* 0.0186 for AA and 0.0147 for XX and XA combined (hereafter X). Averaging the K_S values for XX and AX, we obtain corrected K_S values of 0.0194 and 0.0318 for A and X, respectively.

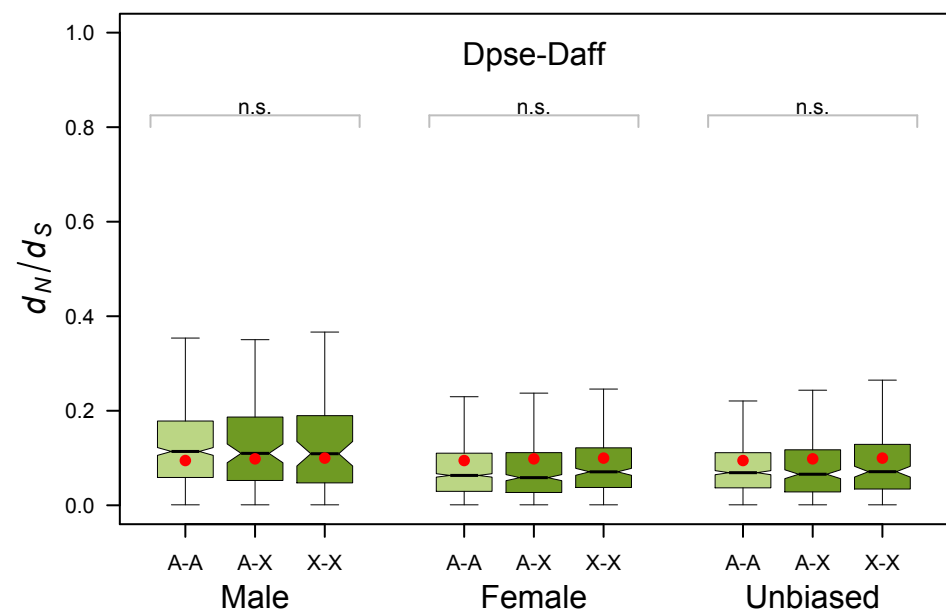
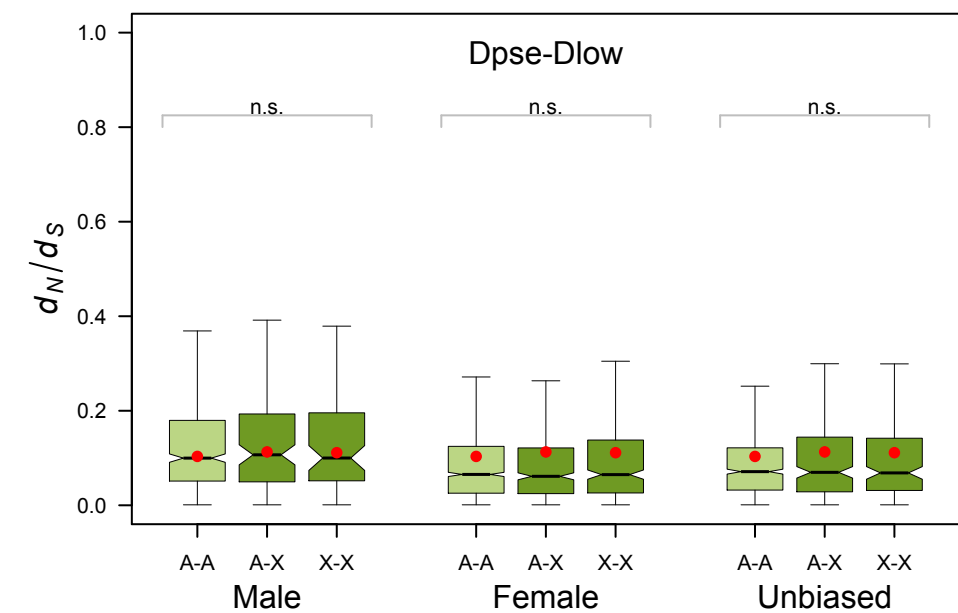
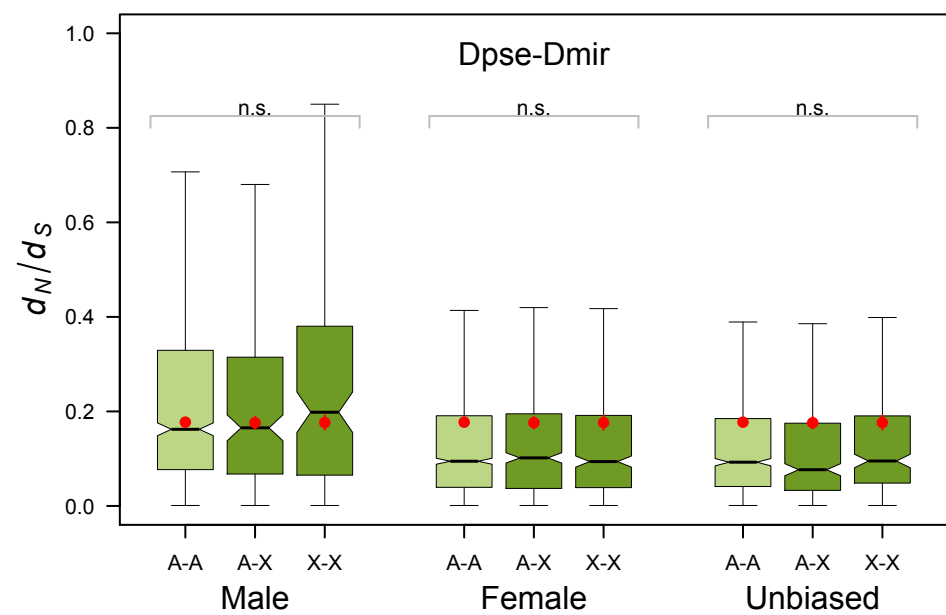
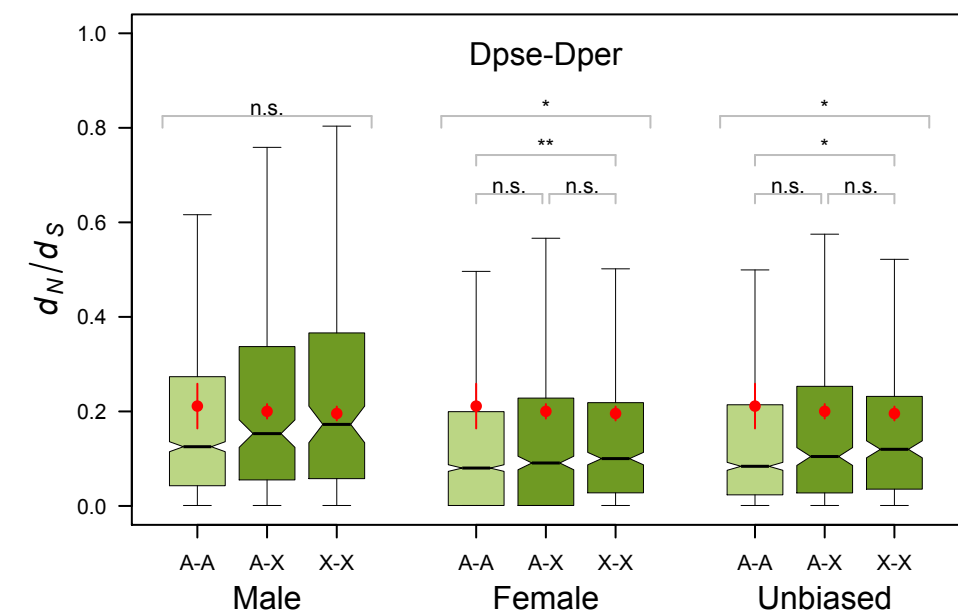
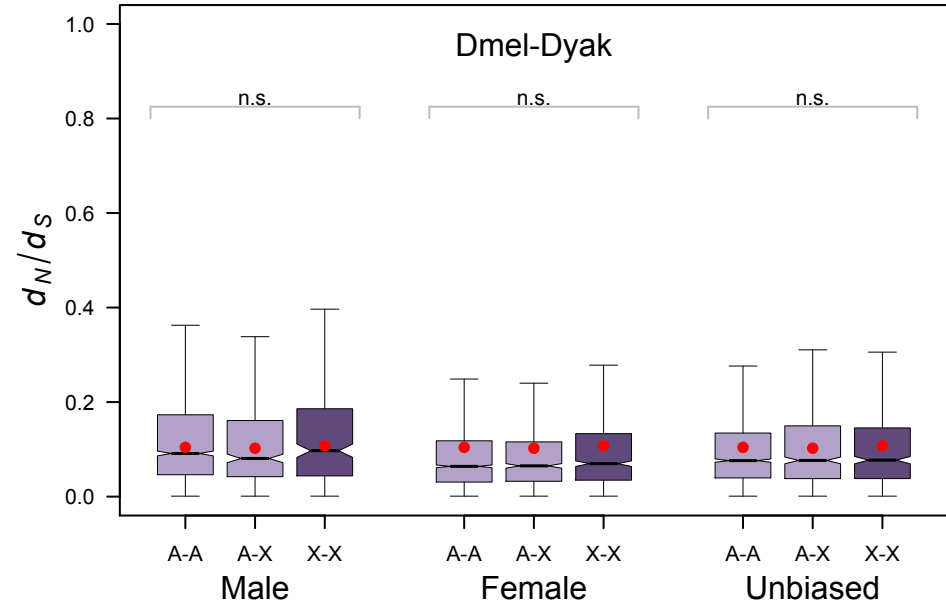
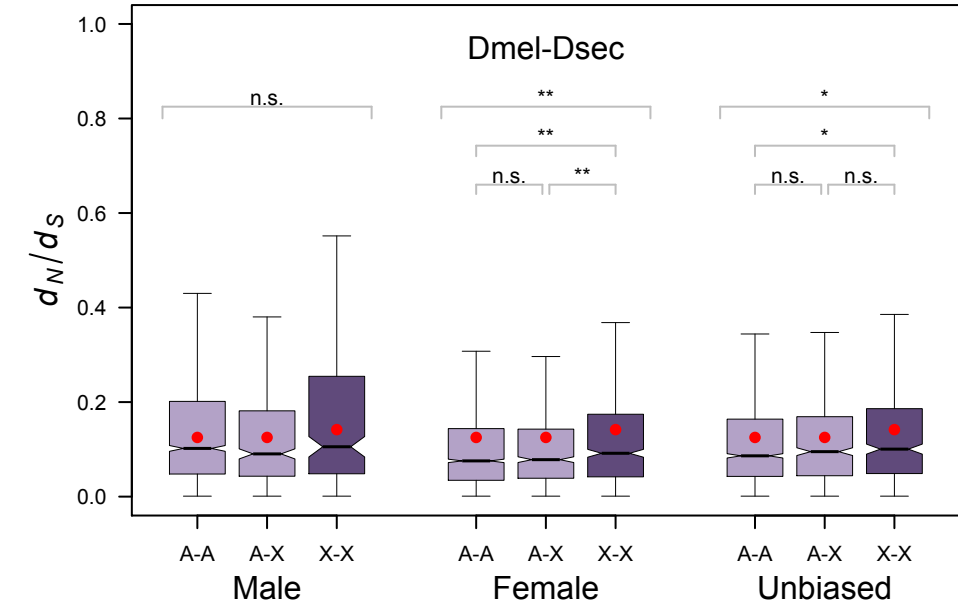
The ratio of the corrected value of K_S to π_S provides an estimate of the time separating the species in units of $2N_e$ generations (as net divergence is an estimate of $2u \times$ the speciation time, and π_S of $4N_e u$). Using mean π_S values for *pse*, this is about 0.88 for A and 1.80 for X, with the higher value for X-linked loci due to scaling by its lower N_e . These values of the divergence time are shorter than the average time to neutral fixation ($4N_e$ generations), and well within the range where a high fraction of neutral fixations involve ancestral polymorphisms. For example, a divergence time of 1 yields around 98% of fixations within a single lineage are due to such events; for a value of 2, it is 80% (Charlesworth *et al.* 2005, equations 14 and 15).

Using this estimate of the divergence time, we can now ask whether the higher K_A values for the X-linked vs. autosomal loci can be explained solely by genetic drift fixing ancestral polymorphisms. The expected divergence between two independently evolving lineages resulting from fixation of ancestral polymorphism is simply equal to the ancestral pairwise diversity, π -anc, for neutral mutations, and lower for slightly deleterious mutations (Charlesworth *et al.* 2005). The upper limit to the expected contribution to the K_A values from ancestral polymorphisms for the X-linked loci is thus provided by the relevant π_A values. We can take the *pse* values as a probable overestimate of these, since *pse* shows signs of a recent population expansion (Machado *et al.* 2002). Unfortunately, the results for the slowly-evolving genes obtained by Haddrill *et al.* (Haddrill *et al.* 2010), and for the fast-evolving genes in Table 2, are rather different: a mean 0.00066 for slowly-evolving XL genes, and a mean of 0.00136 for fast-evolving XR genes, respectively. However, if we are again conservative and use the higher value, we obtain a maximum contribution of ancestral polymorphism to K_A of 0.00136; this is only about 10% of the estimate for K_A for the X-linked loci combined. It therefore seems impossible to account for the high X values of K_A by fixations of ancestral polymorphisms. The magnitude of this discrepancy is so large that it has a very low probability of arising by chance— even ignoring the contribution of ancestral polymorphism to K_S , the adjusted K_A values still result in significantly higher K_A/K_S for X-linked loci (mean adjusted K_A to unadjusted K_S values for X-linked loci is 0.168, autosomal is 0.121; Mann-Whitney U test, $p = 2e-13$).

References

- Haddrill PR, Loewe L, Charlesworth B. 2010. Estimating the parameters of selection on nonsynonymous mutations in *Drosophila pseudoobscura* and *D. miranda*. *Genetics*. 185:1381-1396.
- Machado CA, Kliman RM, Markert JA, Hey J. 2002. Inferring the history of speciation from multilocus DNA sequence data: the case of *Drosophila pseudoobscura* and close relatives. *Molecular biology and evolution*. 19:472-488.
- McGaugh SE, Noor MAF. 2012. Genomic impacts of chromosomal inversions in parapatric *Drosophila* species. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 367:422-429.
- Charlesworth B, Bartolomé C, Noël V. 2005. Estimating the incidence of ancestral polymorphisms. *Genet Res*. 86:149-157.





Supplementary Table S1. X-autosome comparison of rates of evolution, using pairwise Ka, Ks, and Ka/Ks rates as calculated by Gestimator. Medians and 95% confidence intervals are given for each species pair. Significance from Kruskal-Wallis and Mann-Whitney U tests is indicated by *** P < 0.001; ** P < 0.01; * P < 0.05; '-' indicates that the Kruskal-Wallis test was not significant and Mann-Whitney U tests were thus not performed. X-autosome comparisons are highlighted in gray.

		A-A	X-X	A-X	Kruskal-Wallis tests	Mann-Whitney U tests			
						A-A vs. X-X	A-X vs. X-X	A-X vs. A-A	
All genes	<i>Dmel-Dsec</i>	N	5800	1318	1826				
		Ka	0.01166 (0.01126 - 0.01202)	0.01365 (0.01286 - 0.01474)	0.01102 (0.01044 - 0.01179)	6.77e-09***	3.78e-08***	3.78e-08***	0.2827
		Ks	0.11520 (0.11410 - 0.11630)	0.11335 (0.11100 - 0.11620)	0.11405 (0.11230 - 0.11600)	0.5117	--	--	--
		Ka/Ks	0.10295 (0.10030 - 0.10595)	0.12175 (0.11520 - 0.13280)	0.10045 (0.09420 - 0.10640)	6.96e-10***	2.24e-09***	1.50e-08***	0.4607
	<i>Dmel-Dyak</i>	N	6081	1487	1900				
		Ka	0.02315 (0.02230 - 0.02378)	0.02571 (0.02380 - 0.02735)	0.02181 (0.02049 - 0.02332)	0.0002***	0.0005***	0.0003***	0.2269
		Ks	0.25950 (0.25750 - 0.26170)	0.25330 (0.25020 - 0.25780)	0.25490 (0.25105 - 0.25880)	0.3514	--	--	--
		Ka/Ks	0.09283 (0.08971 - 0.09471)	0.10650 (0.10070 - 0.11110)	0.08883 (0.08514 - 0.09465)	5.01e-06***	1.22e-05***	1.22e-05***	0.2657
	<i>Dpse-Dper</i>	N	4791	1127	1368				
		Ka	0.00300 (0.00287 - 0.00313)	0.00488 (0.00432 - 0.00531)	0.00312 (0.00290 - 0.00352)	2.89e-19***	4.48e-20***	2.34e-07***	0.0087**
		Ks	0.02815 (0.02767 - 0.02867)	0.03425 (0.03310 - 0.03568)	0.02585 (0.02509 - 0.02680)	8.79e-20***	1.63e-15***	1.42e-17***	0.0006***
		Ka/Ks	0.10830 (0.10380 - 0.11320)	0.15130 (0.13890 - 0.16640)	0.13080 (0.12395 - 0.14205)	1.11e-14***	8.49e-13***	0.0344*	2.45e-06***
	<i>Dpse-Dmir</i>	N	4756	1300	1616				
		Ka	0.00554 (0.00535 - 0.00573)	0.00573 (0.00538 - 0.00626)	0.00451 (0.00425 - 0.00484)	6.50e-09***	0.5891	1.13e-06***	1.72e-08***
		Ks	0.04219 (0.04161 - 0.04272)	0.04016 (0.03910 - 0.04113)	0.03402 (0.03310 - 0.03478)	4.57e-52***	0.0021**	2.01e-21***	2.44e-52***
		Ka/Ks	0.13645 (0.13185 - 0.14180)	0.14515 (0.13255 - 0.15415)	0.13575 (0.12830 - 0.14480)	0.2987	--	--	--
	<i>Dpse-Dlow</i>	N	3012	703	969				
		Ka	0.00830 (0.00789 - 0.00868)	0.00770 (0.00723 - 0.00857)	0.00726 (0.00673 - 0.00772)	0.0209*	0.3129	0.3129	0.0197*
Ks		0.09119 (0.09018 - 0.09237)	0.07855 (0.07616 - 0.08100)	0.07840 (0.07680 - 0.08065)	2.17e-43***	4.88e-25***	0.9791	8.49e-30***	
Ka/Ks		0.09048 (0.08688 - 0.09424)	0.10300 (0.09239 - 0.11630)	0.09247 (0.08566 - 0.09928)	0.1046	--	--	--	
<i>Dpse-Daff</i>	N	3022	706	973					
	Ka	0.02051 (0.01969 - 0.02136)	0.02129 (0.01964 - 0.02336)	0.01790 (0.01661 - 0.01903)	0.0044**	0.7436	0.0197*	0.0045**	
	Ks	0.23035 (0.22795 - 0.23310)	0.20820 (0.20400 - 0.21536)	0.20500 (0.20020 - 0.20910)	3.48e-30***	8.64e-13***	0.0545	3.54e-25***	
	Ka/Ks	0.09162 (0.08799 - 0.09539)	0.10480 (0.09593 - 0.11330)	0.09110 (0.08366 - 0.09730)	0.0393*	0.0386*	0.0581	0.8062	
Common genes	<i>Dmel-Dsec</i>	N	1960	464	623				
		Ka	0.00956 (0.00912 - 0.01005)	0.01120 (0.01007 - 0.01283)	0.01033 (0.00968 - 0.01137)	0.0002***	0.0002***	0.0637	0.0524
		Ks	0.11570 (0.11370 - 0.11735)	0.11030 (0.10690 - 0.11320)	0.11560 (0.11290 - 0.11880)	0.054	--	--	--
		Ka/Ks	0.08430 (0.08030 - 0.08877)	0.10430 (0.09274 - 0.11290)	0.09123 (0.08435 - 0.09945)	1.06e-05***	1.31e-05***	0.0263*	0.0263*
	<i>Dmel-Dyak</i>	N	1960	464	623				
		Ka	0.01903 (0.01825 - 0.02018)	0.02243 (0.01978 - 0.02463)	0.01940 (0.01729 - 0.02169)	0.0153*	0.0129*	0.1303	0.3237
		Ks	0.25860 (0.25495 - 0.26240)	0.25170 (0.24520 - 0.25830)	0.25550 (0.25080 - 0.26182)	0.3832	--	--	--
		Ka/Ks	0.07858 (0.07500 - 0.08222)	0.09136 (0.08108 - 0.10045)	0.07881 (0.07039 - 0.08658)	0.0010***	0.0006***	0.0273*	0.2916
	<i>Dpse-Dper</i>	N	1960	464	623				
		Ka	0.00209 (0.00195 - 0.00225)	0.00335 (0.00273 - 0.00409)	0.00244 (0.00205 - 0.00270)	5.52e-09***	5.35e-09***	0.0060**	0.0060**
		Ks	0.02699 (0.02624 - 0.02770)	0.03114 (0.02803 - 0.03307)	0.02507 (0.02350 - 0.02620)	9.69e-06***	0.0002***	1.61e-05***	0.0365*
		Ka/Ks	0.07930 (0.07317 - 0.08530)	0.11240 (0.09617 - 0.12721)	0.10150 (0.08841 - 0.11610)	5.94e-08***	2.80e-06***	0.2902	8.41e-05***
	<i>Dpse-Dmir</i>	N	1960	464	623				
		Ka	0.00451 (0.00427 - 0.00476)	0.00438 (0.00387 - 0.00509)	0.00381 (0.00333 - 0.00410)	0.0095**	0.7606	0.0232*	0.0111*
		Ks	0.04276 (0.04198 - 0.04364)	0.03977 (0.03817 - 0.04125)	0.03490 (0.03383 - 0.03619)	1.31e-22***	0.0077**	1.14e-07***	4.44e-23***
		Ka/Ks	0.10585 (0.10030 - 0.11310)	0.10885 (0.09747 - 0.12630)	0.11110 (0.09884 - 0.12320)	0.7181	--	--	--
	<i>Dpse-Dlow</i>	N	1960	464	623				
		Ka	0.00902 (0.00842 - 0.00935)	0.00804 (0.00730 - 0.00965)	0.00778 (0.00724 - 0.00882)	0.2988	--	--	--
Ks		0.09436 (0.09269 - 0.09607)	0.08013 (0.07782 - 0.08274)	0.08267 (0.08073 - 0.08448)	1.94e-32***	2.26e-22***	0.0974	4.99e-19***	
Ka/Ks		0.09241 (0.08718 - 0.09615)	0.10255 (0.08970 - 0.11740)	0.09836 (0.09126 - 0.10790)	0.1416	--	--	--	
<i>Dpse-Daff</i>	N	1960	464	623					
	Ka	0.02187 (0.02084 - 0.02314)	0.02218 (0.02000 - 0.02520)	0.01973 (0.01849 - 0.02199)	0.2291	--	--	--	
	Ks	0.23710 (0.23469 - 0.24000)	0.21035 (0.20610 - 0.21830)	0.21380 (0.20900 - 0.21870)	1.14e-23***	1.02e-14***	0.6401	6.02e-16***	
	Ka/Ks	0.09390 (0.08895 - 0.09812)	0.10915 (0.09914 - 0.12075)	0.09904 (0.08884 - 0.10680)	0.0537	--	--	--	

Supplementary Table S2. X-autosome comparison of rates of evolution, using pairwise dN, dS and dN/dS rates as calculated by PAML. Medians and 95% confidence intervals are given for each species pair. Significance from Kruskal-Wallis and Mann-Whitney U tests is indicated by *** P < 0.001; ** P < 0.01; * P < 0.05; '-' indicates that the Kruskal-Wallis test was not significant and Mann-Whitney U tests were thus not performed. X-autosome comparisons are highlighted in gray.

		A-A	X-X	A-X	Kruskal-Wallis tests	Mann-Whitney U tests		
						A-A vs. X-X	A-X vs. X-X	A-X vs. A-A
All_genes	<i>Dmel-Dsec</i>	N 5710	1300	1778				
		dN 0.01120 (0.01090 - 0.01160)	0.01325 (0.01220 - 0.01420)	0.01080 (0.01030 - 0.01140)	9.08e-08***	3.22e-07***	3.22e-07***	0.2905
		dS 0.13190 (0.13055 - 0.13340)	0.14015 (0.13750 - 0.14230)	0.12960 (0.12740 - 0.13230)	9.12e-13***	4.54e-10***	2.36e-12***	0.0088**
		dN/dS 0.08625 (0.08410 - 0.08890)	0.09615 (0.08990 - 0.10275)	0.08515 (0.08130 - 0.09135)	0.0041**	0.0042**	0.0067**	0.8613
	<i>Dmel-Dyak</i>	N 5977	1463	1848				
		dN 0.02210 (0.02140 - 0.02300)	0.02430 (0.02250 - 0.02590)	0.02155 (0.02000 - 0.02265)	0.0038**	0.0064**	0.0042**	0.2763
		dS 0.30240 (0.29940 - 0.30480)	0.32280 (0.31590 - 0.33050)	0.29530 (0.29005 - 0.29920)	1.43e-17***	9.00e-15***	1.06e-15***	0.0138*
		dN/dS 0.07430 (0.07190 - 0.07640)	0.07700 (0.07210 - 0.08170)	0.07430 (0.06935 - 0.07750)	0.5668	--	--	--
	<i>Dpse-Dper</i>	N 5438	1233	1552				
		dN 0.00260 (0.00250 - 0.00270)	0.00405 (0.00370 - 0.00460)	0.00270 (0.00250 - 0.00290)	5.15e-20***	5.85e-21***	6.15e-09***	0.0342*
		dS 0.03170 (0.03110 - 0.03250)	0.04110 (0.03930 - 0.04265)	0.03040 (0.02890 - 0.03130)	8.84e-27***	2.79e-25***	4.52e-20***	0.1802
		dN/dS 0.09355 (0.08980 - 0.09780)	0.12030 (0.10940 - 0.13380)	0.11545 (0.10390 - 0.12550)	8.04e-07***	9.44e-06***	0.3164	0.0008***
	<i>Dpse-Dmir</i>	N 3831	999	1336				
		dN 0.00520 (0.00500 - 0.00530)	0.00530 (0.00470 - 0.00570)	0.00420 (0.00390 - 0.00440)	4.43e-07***	0.6884	0.0004***	2.61e-07***
		dS 0.04810 (0.04710 - 0.04880)	0.04850 (0.04640 - 0.05040)	0.03935 (0.03825 - 0.04085)	2.09e-35***	0.2362	6.41e-24***	1.82e-32***
	dN/dS 0.10950 (0.10470 - 0.11460)	0.10400 (0.09509 - 0.11490)	0.10995 (0.10048 - 0.11740)	0.6006	--	--	--	
<i>Dpse-Dlow</i>	N 2968	694	949					
	dN 0.00800 (0.00750 - 0.00830)	0.00745 (0.00690 - 0.00810)	0.00700 (0.00650 - 0.00750)	0.0209*	0.2716	0.3848	0.0229*	
	dS 0.10715 (0.10580 - 0.10875)	0.09695 (0.09460 - 0.10025)	0.09325 (0.09160 - 0.09590)	1.05e-24***	2.26e-10***	0.0735	6.04e-21***	
	dN/dS 0.07380 (0.07045 - 0.07655)	0.07600 (0.06785 - 0.08615)	0.07240 (0.06620 - 0.07980)	0.7701	--	--	--	
<i>Dpse-Daff</i>	N 2969	694	949					
	dN 0.01980 (0.01880 - 0.02040)	0.02030 (0.01870 - 0.02215)	0.01690 (0.01590 - 0.01850)	0.0042**	0.7361	0.0187*	0.0042**	
	dS 0.27310 (0.26999 - 0.27630)	0.26270 (0.25510 - 0.26926)	0.24610 (0.24060 - 0.25121)	4.14e-17***	0.0056**	9.11e-05***	1.48e-17***	
	dN/dS 0.07280 (0.06960 - 0.07630)	0.07870 (0.07120 - 0.08440)	0.06860 (0.06240 - 0.07440)	0.3901	--	--	--	
Common_genes	<i>Dmel-Dsec</i>	N 1714	390	560				
		dN 0.00910 (0.00850 - 0.00990)	0.01020 (0.00920 - 0.01180)	0.00980 (0.00890 - 0.01070)	0.0351*	0.0393*	0.2275	0.2275
		dS 0.13650 (0.13440 - 0.13915)	0.14100 (0.13755 - 0.14650)	0.13390 (0.12870 - 0.13726)	0.0023**	6.88E-02	0.0025**	0.0161*
		dN/dS 0.06705 (0.06310 - 0.07160)	0.07390 (0.06430 - 0.07915)	0.07685 (0.06810 - 0.08150)	0.0771	--	--	--
	<i>Dmel-Dyak</i>	N 1714	390	560				
		dN 0.01820 (0.01735 - 0.01900)	0.01885 (0.01670 - 0.02200)	0.01840 (0.01655 - 0.02080)	0.4887	--	--	--
		dS 0.30860 (0.30455 - 0.31190)	0.33015 (0.31850 - 0.34100)	0.29970 (0.29195 - 0.30890)	7.29e-06***	4.41e-05***	1.67e-05***	0.0853
		dN/dS 0.06000 (0.05720 - 0.06360)	0.05915 (0.05105 - 0.06630)	0.06185 (0.05765 - 0.06721)	0.4025	--	--	--
	<i>Dpse-Dper</i>	N 1714	390	560				
		dN 0.00200 (0.00180 - 0.00210)	0.00260 (0.00210 - 0.00310)	0.00220 (0.00190 - 0.00250)	0.0001***	0.0001***	0.0884	0.0418*
		dS 0.03015 (0.02935 - 0.03120)	0.03690 (0.03390 - 0.03940)	0.02965 (0.02765 - 0.03125)	4.06e-07***	8.08e-07***	2.83e-06***	0.4746
		dN/dS 0.06530 (0.06050 - 0.07170)	0.07625 (0.06590 - 0.08842)	0.07910 (0.07014 - 0.09161)	0.0028**	0.0374*	0.7277	0.0077**
	<i>Dpse-Dmir</i>	N 1714	390	560				
		dN 0.00420 (0.00400 - 0.00450)	0.00365 (0.00310 - 0.00430)	0.00340 (0.00305 - 0.00385)	0.0137*	0.2570	0.2570	0.0122*
		dS 0.04865 (0.04750 - 0.05020)	0.04850 (0.04465 - 0.05100)	0.04105 (0.03935 - 0.04210)	1.71e-17***	0.9794	2.54e-10***	3.19e-17***
	dN/dS 0.08440 (0.07835 - 0.09145)	0.07715 (0.06730 - 0.08480)	0.08400 (0.07340 - 0.09736)	0.4546	--	--	--	
<i>Dpse-Dlow</i>	N 1714	390	560					
	dN 0.00820 (0.00780 - 0.00870)	0.00710 (0.00610 - 0.00780)	0.00740 (0.00680 - 0.00835)	0.0337*	0.0276*	0.1738	0.4254	
	dS 0.11165 (0.10920 - 0.11350)	0.09760 (0.09465 - 0.10145)	0.09650 (0.09370 - 0.10010)	2.55e-18***	7.32e-10***	0.6729	4.72e-14***	
	dN/dS 0.07115 (0.06835 - 0.07540)	0.06980 (0.06015 - 0.08090)	0.07530 (0.06800 - 0.08580)	0.2607	--	--	--	
<i>Dpse-Daff</i>	N 1714	390	560					
	dN 0.02070 (0.01955 - 0.02220)	0.01970 (0.01650 - 0.02190)	0.01880 (0.01675 - 0.02070)	0.2053	--	--	--	
	dS 0.28270 (0.27860 - 0.28760)	0.26640 (0.25909 - 0.27535)	0.25520 (0.24740 - 0.26270)	8.07e-13***	0.0002***	0.0662	4.23e-12***	
	dN/dS 0.07175 (0.06805 - 0.07640)	0.07430 (0.06535 - 0.08140)	0.07420 (0.06310 - 0.08050)	0.9303	--	--	--	

Supplementary Table 3. Comparisons of Ka/Ks between X-linked and autosomal genes, divided by sex-biased expression.

		A-A	X-X	A-X	Kruskal-Wallis tests	Mann-Whitney U tests		
						A-A vs. X-X	A-X vs. X-X	A-X vs. A-A
Male-biased genes	<i>Dmel-Dsec</i>	<i>N</i> 1591 <i>Ka/Ks</i> 0.1208 <i>CI</i> (0.1143-0.1287)	431 0.1091 (0.0906-0.1236)	228 0.14155 (0.1165-0.1703)	0.0068**	0.0245*	0.0065**	0.0903
	<i>Dmel-Dyak</i>	<i>N</i> 1657 <i>Ka/Ks</i> 0.113 <i>CI</i> (0.1066-0.121)	446 0.10405 (0.0906-0.1184)	251 0.1357 (0.118-0.1515)	0.0060**	0.0403*	0.0060**	0.0499*
	<i>Dpse-Dper</i>	<i>N</i> 1055 <i>Ka/Ks</i> 0.1508 <i>CI</i> (0.139-0.163)	213 0.1821 (0.1521-0.2081)	142 0.2242 (0.1675-0.2834)	0.0074**	0.0181*	0.2648	0.0946
	<i>Dpse-Dmir</i>	<i>N</i> 1060 <i>Ka/Ks</i> 0.21565 <i>CI</i> (0.2023-0.2288)	247 0.2149 (0.1966-0.2513)	173 0.2501 (0.2112-0.304)	0.5515	--	--	--
	<i>Dpse-Dlow</i>	<i>N</i> 534 <i>Ka/Ks</i> 0.13055 <i>CI</i> (0.1185-0.1371)	118 0.14225 (0.114-0.18)	73 0.1487 (0.1091-0.1774)	0.5157	--	--	--
	<i>Dpse-Daff</i>	<i>N</i> 535 <i>Ka/Ks</i> 0.1378 <i>CI</i> (0.1276-0.1549)	119 0.152 (0.1173-0.1854)	75 0.148 (0.1253-0.1878)	0.6617	--	--	--
Female-biased genes	<i>Dmel-Dsec</i>	<i>N</i> 2295 <i>Ka/Ks</i> 0.09248 <i>CI</i> (0.0878-0.0967)	751 0.0906 (0.0844-0.095)	646 0.11755 (0.1075-0.1255)	3.14e-08***	6.68e-08***	7.79e-07***	0.7343
	<i>Dmel-Dyak</i>	<i>N</i> 2404 <i>Ka/Ks</i> 0.07911 <i>CI</i> (0.0758-0.082)	773 0.07873 (0.0717-0.0851)	737 0.09791 (0.0916-0.1058)	2.63e-07***	2.86e-07***	1.70e-05***	0.8728
	<i>Dpse-Dper</i>	<i>N</i> 1706 <i>Ka/Ks</i> 0.08904 <i>CI</i> (0.0813-0.097)	538 0.10465 (0.0949-0.1203)	477 0.1191 (0.1057-0.1364)	1.17e-05***	1.47e-05***	0.1211	0.0198*
	<i>Dpse-Dmir</i>	<i>N</i> 1747 <i>Ka/Ks</i> 0.1129 <i>CI</i> (0.1071-0.1214)	621 0.1256 (0.1118-0.1397)	516 0.1252 (0.109-0.1405)	0.1517	--	--	--
	<i>Dpse-Dlow</i>	<i>N</i> 1265 <i>Ka/Ks</i> 0.07623 <i>CI</i> (0.0726-0.0814)	438 0.0745 (0.0647-0.0841)	330 0.08551 (0.0677-0.098)	0.1667	--	--	--
	<i>Dpse-Daff</i>	<i>N</i> 1270 <i>Ka/Ks</i> 0.07546 <i>CI</i> (0.0708-0.0795)	439 0.07262 (0.0662-0.0804)	331 0.08976 (0.0811-0.1027)	0.0133*	0.0153*	0.0176*	0.7365
Unbiased genes	<i>Dmel-Dsec</i>	<i>N</i> 1769 <i>Ka/Ks</i> 0.1032 <i>CI</i> (0.0988-0.1084)	583 0.1098 (0.0985-0.1177)	420 0.1313 (0.1138-0.1459)	0.0005***	0.0003***	0.0070**	0.5611
	<i>Dmel-Dyak</i>	<i>N</i> 1871 <i>Ka/Ks</i> 0.09415 <i>CI</i> (0.0898-0.0977)	616 0.094335 (0.0857-0.1022)	473 0.1064 (0.097-0.1149)	0.102	--	--	--
	<i>Dpse-Dper</i>	<i>N</i> 1125 <i>Ka/Ks</i> 0.09595 <i>CI</i> (0.0891-0.1045)	317 0.1249 (0.1036-0.1541)	265 0.1517 (0.1243-0.1843)	2.14e-05***	0.0001***	0.48	0.0041**
	<i>Dpse-Dmir</i>	<i>N</i> 1090 <i>Ka/Ks</i> 0.11815 <i>CI</i> (0.1111-0.1267)	372 0.1043 (0.0877-0.1196)	309 0.1358 (0.1207-0.1566)	0.0073**	0.0518	0.0064**	0.0709
	<i>Dpse-Dlow</i>	<i>N</i> 745 <i>Ka/Ks</i> 0.08777 <i>CI</i> (0.0808-0.0959)	232 0.08791 (0.0776-0.108)	175 0.1021 (0.0835-0.1197)	0.3134	--	--	--
	<i>Dpse-Daff</i>	<i>N</i> 747 <i>Ka/Ks</i> 0.09078 <i>CI</i> (0.0845-0.0968)	232 0.08599 (0.0694-0.0967)	175 0.09632 (0.077-0.1179)	0.1827	--	--	--

Supplementary Table 4. Comparisons of dN/dS between X-linked and autosomal genes, divided by sex-biased expression.

					Kruskal-Wallis tests	Mann-Whitney U tests			
		A-A	X-X	A-X		A-A vs. X-X	A-X vs. X-X	A-X vs. A-A	
Male-biased genes	<i>Dmel-Dsec</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1562 0.102 (0.0947-0.1094)	418 0.0904 (0.0787-0.1017)	227 0.1055 (0.0865-0.1329)	0.0714	--	--	--
	<i>Dmel-Dyak</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1623 0.0913 (0.0854-0.0968)	433 0.0808 (0.0745-0.0936)	249 0.0971 (0.0829-0.1139)	0.1432	--	--	--
	<i>Dpse-Dper</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1184 0.12535 (0.1152-0.138)	234 0.15305 (0.131-0.1818)	158 0.17255 (0.125-0.2151)	0.0523	--	--	--
	<i>Dpse-Dmir</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	865 0.1622 (0.1535-0.1803)	203 0.1653 (0.1442-0.2045)	135 0.1983 (0.1599-0.2547)	0.7818	--	--	--
	<i>Dpse-Dlow</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	523 0.0998 (0.0914-0.1109)	114 0.1068 (0.0868-0.127)	75 0.1 (0.0766-0.1383)	0.8937	--	--	--
	<i>Dpse-Daff</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	523 0.1139 (0.1017-0.124)	114 0.10985 (0.0908-0.1293)	75 0.1091 (0.0832-0.1323)	0.9545	--	--	--
Female-biased genes	<i>Dmel-Dsec</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	2263 0.0756 (0.0717-0.0801)	731 0.0782 (0.0711-0.0833)	632 0.0917 (0.0815-0.0993)	0.0030**	0.0030**	0.0078**	0.9409
	<i>Dmel-Dyak</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	2368 0.06405 (0.0611-0.0676)	752 0.06505 (0.0603-0.0719)	722 0.06975 (0.0642-0.0768)	0.0667	--	--	--
	<i>Dpse-Dper</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1993 0.0802 (0.073-0.0881)	625 0.0909 (0.0798-0.1052)	523 0.1002 (0.0884-0.1129)	0.0124*	0.0098**	0.2359	0.2484
	<i>Dpse-Dmir</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1432 0.0946 (0.088-0.1008)	515 0.1017 (0.0846-0.1124)	413 0.0939 (0.084-0.1098)	0.9881	--	--	--
	<i>Dpse-Dlow</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1243 0.0653 (0.0604-0.0693)	427 0.0613 (0.0498-0.0678)	324 0.0649 (0.0558-0.0789)	0.3678	--	--	--
	<i>Dpse-Daff</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1244 0.0632 (0.0602-0.067)	427 0.0585 (0.0525-0.0639)	324 0.0708 (0.0633-0.0839)	0.0729	--	--	--
Unbiased genes	<i>Dmel-Dsec</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1751 0.0863 (0.0814-0.09)	578 0.09515 (0.0861-0.1027)	417 0.1005 (0.0884-0.1133)	0.0230*	0.0240*	0.2005	0.2005
	<i>Dmel-Dyak</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1850 0.0762 (0.071-0.0802)	609 0.0766 (0.0692-0.0872)	467 0.0772 (0.0702-0.0845)	0.7527	--	--	--
	<i>Dpse-Dper</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	1271 0.0839 (0.0773-0.0905)	355 0.1046 (0.0842-0.125)	296 0.11985 (0.1003-0.1449)	0.0140*	0.0175*	0.3914	0.1721
	<i>Dpse-Dmir</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	863 0.0926 (0.0842-0.1009)	309 0.0767 (0.0617-0.0948)	247 0.0951 (0.0794-0.1071)	0.1303	--	--	--
	<i>Dpse-Dlow</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	739 0.0712 (0.0668-0.0762)	226 0.0697 (0.0595-0.0845)	171 0.0685 (0.0568-0.0853)	0.9225	--	--	--
	<i>Dpse-Daff</i>	<i>N</i> <i>dN/dS</i> <i>CI</i>	739 0.069 (0.0635-0.0745)	226 0.0657 (0.0565-0.0733)	171 0.0711 (0.0539-0.0824)	0.5885	--	--	--

Supplementary Table S5. To assess the quantitative effect of sex bias on the faster-X effect in both clades simultaneously, we took only the genes that could be aligned in all species ($n = 2974$), and estimated a standardized rate of protein evolution, ω , for each clade, using PAML's codeml (using the M0 codon model, with the transition-transversion rate estimated from the data and the codon frequencies estimated from the base frequencies, and using the tree with the best likelihood under the codon model for each gene). We then fit a linear model to the log-transformation of this ω estimate, with the clade, X- or autosomal linkage, and the level of sex bias as factors. Sex bias is quantified as the log of the male to female expression ratio, with the source of the expression values as given in the main text. Note that the factors with negative coefficients are inferred to lower the rate of evolution in this model.

	coefficients	std error	t-value	Pr(> t)	
	-3.24335	0.02921	-111.052	< 2.00E-16	***
Pseudo clade	0.41061	0.04464	9.198	< 2.00E-16	***
X-linkage	-0.03127	0.08538	-0.366	0.7142	
sex bias	0.16071	0.03635	4.422	9.97E-06	***
Pseudo clade: X-linkage	-0.02884	0.10461	-0.276	0.7828	
Pseudo clade:sex bias	0.57728	0.09223	6.259	4.15E-10	***
X-linkage:sex bias	-0.27485	0.10489	-2.62	0.0088	**
Pseudo clade: X-linkage:sex bias	-0.28443	0.18652	-1.525	0.1273	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 1.449 on 5940 degrees of freedom
Multiple R-squared: 0.03052, Adjusted R-squared: 0.02938
F-statistic: 26.71 on 7 and 5940 DF, p-value: < 2.20E-16

Supplementary Table S6. Polymorphism and divergence statistics for alleles from a Mesa Verda population of *D. pseudoobscura*.

fbgn	location	category	n	div_sitesR	div_sitesS	gaps	raw_divR	raw_divS	kR	kS	kR_JC	kS_JC	pm_sites_R	pm_sites_S	S_R	S_S	pi_R	pi_S	theta_R	theta_S	TD_R	TD_S
FBgn0080425	XR	Unbiased	15	270.729	92.2708	99	5.26666	14.7333	0.01945	0.15967	0.01071	0.17954	270.777	92.2222	8	9	0.0059	0.01962	0.00908	0.03011	-1.2877	-1.2983
FBgn0080442	XR	Unbiased	15	358.197	118.802	683	2	22.6	0.00558	0.19023	0.0056	0.21941	358.211	118.788	0	11	0	0.02228	0	0.02847	-	-0.8378
FBgn0080491	XR	Unbiased	15	216.52	74.4791	200	0	15.0666	0	0.20229	0	0.23574	216.5	74.5	0	1	0	0.00178	0	0.00412	-	-1.1594
FBgn0080502	XR	Unbiased	15	381.666	113.333	264	7	24.3333	0.01834	0.2147	0.01856	0.25294	393.666	116.333	1	2	0.00033	0.00507	0.00078	0.00528	-1.1594	-0.1052
FBgn0080686	XR	Unbiased	15	276.062	89.9375	356	12.3333	33	0.04467	0.36692	0.04606	0.50387	289.055	94.9444	12	36	0.01159	0.10071	0.01276	0.11661	-0.3569	-0.5792
FBgn0080703	XR	Unbiased	12	257.5	81.5	43	3	23.3333	0.01165	0.28629	0.01174	0.36062	257.5	81.5	0	4	0	0.00985	0	0.01625	-	-1.3847
FBgn0080821	XR	Unbiased	15	365.197	120.802	1073	0	13.2	0	0.10926	0	0.11809	365.088	120.911	0	6	0	0.00756	0	0.01526	-	-1.7662
FBgn0080923	XR	Unbiased	15	313.52	97.4791	439	0	19.5333	0	0.20038	0	0.23314	313.444	97.5555	0	11	0	0.03241	0	0.03467	-	-0.2518
FBgn0080991	XR	Unbiased	15	386.187	123.812	645	0	26.1333	0	0.21107	0	0.24786	386.166	123.833	0	2	0	0.00215	0	0.00496	-	-1.4905
FBgn0081203	XR	Unbiased	13	216.404	80.5952	892	0.07692	10	0.00035	0.12407	0.00035	0.13563	222.358	83.641	2	6	0.00196	0.0141	0.00289	0.02311	-0.9092	-1.44
FBgn0081662	XR	Unbiased	15	392.062	114.937	255	4.26666	20	0.01088	0.174	0.01096	0.19798	411.1	119.9	3	11	0.00148	0.02446	0.00224	0.02821	-1.0094	-0.5123
FBgn0081676	A	Unbiased	14	64.7333	19.2666	181	5.71428	3.64285	0.08827	0.18907	0.09391	0.21786	64.7142	19.2857	2	4	0.00781	0.04957	0.00971	0.06521	-0.5324	-0.7903
FBgn0081984	XR	Unbiased	11	289.916	103.083	572	5	15.5454	0.01724	0.1508	0.01744	0.16836	289.924	103.075	0	5	0	0.01023	0	0.01656	-	-1.4646
FBgn0082012	A	Unbiased	11	83.1666	33.8333	439	0	2.0909	0	0.1618	0	0.06449	83.1666	33.8333	0	1	0	0.00537	0	0.01009	-	-1.1285
FBgn0082034	A	Female-biased	15	144.833	44.1666	1082	4.06666	6.13333	0.02807	0.13886	0.02861	0.15356	144.766	44.2333	1	2	0.00092	0.00602	0.00212	0.0139	-1.1594	-1.4905
FBgn0082078	A	Unbiased	14	381.188	131.811	221	43.6428	51.8571	0.14449	0.39342	0.12423	0.55763	380.809	132.19	7	23	0.00429	0.03674	0.00578	0.05471	-0.9451	-1.3867
FBgn0082108	A	Female-biased	15	420.562	131.437	755	57.8666	39	0.13759	0.29671	0.152	0.37766	420.522	131.477	13	6	0.00543	0.01216	0.0095	0.01403	-1.6841	-0.4653
FBgn0244147	XL	Unbiased	15	396.885	116.114	286	46.6	31.4	0.11741	0.27042	0.12769	0.33537	403.522	118.477	10	9	0.00457	0.01543	0.00762	0.02336	-1.5195	-1.2725
FBgn0244218	XL	Unbiased	15	412.583	127.416	138	27.8666	34.2	0.06754	0.26841	0.07077	0.33223	412.555	127.444	4	2	0.0018	0.00209	0.00298	0.00482	-1.2699	-1.4905
FBgn0244765	XR	Female-biased	15	183.27	50.7291	195	4.13333	16.1333	0.02255	0.31802	0.02289	0.41378	183.288	50.7111	1	13	0.00135	0.06084	0.00167	0.07884	-0.3988	-0.8973
FBgn0244843	XR	Unbiased	15	189.552	65.4479	809	0	14.3333	0	0.219	0	0.25898	189.477	65.5222	0	4	0	0.00988	0	0.01877	-	-1.5181
FBgn0244862	XR	Female-biased	14	355.344	109.655	783	64.4285	17.5714	0.18131	0.16024	0.20755	0.18027	393.892	122.107	28	4	0.01855	0.00557	0.02235	0.0103	-0.7267	-1.5097
FBgn0244919	XR	Female-biased	15	256.583	67.4166	646	13	8.73333	0.05066	0.12954	0.05245	0.14221	268	71	0	8	0	0.02441	0	0.03465	-	-1.0881
FBgn0245136	XR	Unbiased	15	299.406	105.593	136	4.2	21.3333	0.01402	0.20203	0.01416	0.23539	299.355	105.644	2	7	0.00127	0.01099	0.00205	0.02037	-1.0016	-1.6576
FBgn0245216	XR	Unbiased	15	302.697	96.302	324	1	21.6666	0.0033	0.22498	0.00331	0.26748	302.666	96.3333	0	6	0	0.01265	0	0.01915	-	-1.188
FBgn0246788	A	Unbiased	9	467.816	147.183	339	38	60	0.08122	0.40765	0.08597	0.58818	467.833	147.166	0	0	0	0	0	0	-	-
FBgn0246854	A	Unbiased	15	203.218	60.7812	339	4.8	18.3333	0.02361	0.30162	0.02399	0.38583	203.2	60.8	6	13	0.00712	0.04401	0.00908	0.06575	-0.7544	-1.3
FBgn0247266	A	Unbiased	7	398.937	126.062	648	38.5714	34.4285	0.09668	0.2731	0.10351	0.33958	402.595	128.404	10	5	0.00756	0.01557	0.01013	0.01589	-1.3593	-0.999
FBgn0247718	A	Unbiased	15	368.041	123.958	335	26.6666	30.4	0.07245	0.24524	0.07619	0.29699	368.2	123.8	6	15	0.00341	0.02907	0.00501	0.03726	-1.1157	-0.8767
FBgn0247974	A	Female-biased	14	361.077	100.922	1069	32.2857	26.4285	0.08941	0.26187	0.09521	0.32211	361.047	100.952	6	8	0.0027	0.01665	0.00522	0.02491	-1.7289	-1.2506
FBgn0248238	A	Unbiased	13	347.952	99.0476	245	13.1538	22.6153	0.0378	0.22832	0.03878	0.27227	376.128	109.871	2	5	0.00081	0.0098	0.00171	0.01466	-1.468	-1.1822
FBgn0248273	A	Female-biased	15	401.875	120.125	108	7	39.5333	0.01741	0.3291	0.01762	0.43325	401.833	120.166	0	6	0	0.00951	0	0.01535	-	-1.3325
FBgn0249640	XR	Unbiased	15	156.291	44.7083	669	0	11.0666	0	0.24753	0	0.3004	156.333	44.6666	0	1	0	0.00298	0	0.00688	-	-1.1594
FBgn0249878	XR	Female-biased	15	371.979	108.02	925	29.1333	29.2666	0.07831	0.27093	0.08271	0.33617	387.855	113.144	3	3	0.00103	0.0074	0.00237	0.00815	-1.685	-0.2725
FBgn0261117	A	Female-biased	15	361.927	82.0729	1926	27.9333	19.5333	0.07717	0.23799	0.08144	0.28631	363.833	83.1666	1	2	0.00036	0.01007	0.00084	0.00739	-1.1594	0.95399

Supplementary Table S7. Correlates of diversity and divergence

Variables	Covariates	Partial correlations (lower and upper one percentiles in brackets)			
		Fast-evolving		Female-biased	
		XR	A	X	A
π_S, K_S	K_A, Fop	0.005 (-0.305/0.246)	0.170 (-0.346/0.622)	0.401(-1.000/1.000)	-0.092 (-0.783/0.498)
π_S, Fop	K_A, K_S	0.099 (-0.307/0.401)	0.371* (-0.053/0.680)	0.370 (-1.000/1.000)	0.392* (-0.210/0.819)
π_S, K_A	K_S, Fop	0.094 (-0.285/0.426)	0.046 (-0.365/0.554)	-0.483(-1.000/1.000)	0.062 (-0.587/0.746)
K_A, K_S	π_S, Fop	0.296** (0.027/0.540)	0.417** (0.079/0.759)	0.057 (-1.000/1.000)	0.672** (0.189/0.905)
Fop, K_S	π_S, K_A	-0.080 (-0.353/0.232)	-0.132 (-0.614/0.759)	-0.322 (-1.000/1.000)	-0.087 (-0.665/0.589)
π_A, K_S	K_A, Fop	-0.024 (-0.218/0.781)	0.179 (-0.365/0.728)	-0.273 (-1.000/1.000)	-0.269 (-0.761/0.531)
π_A, Fop	K_A, K_S	0.093 (-0.198/0.381)	0.176 (-0.353/0.523)	0.326 (-1.000/1.000)	0.153 (-0.368/0.776)
π_A, K_A	K_S, Fop	0.517** (0.179/0.781)	0.351* (-0.033/0.726)	0.848 (-1.000/1.000)	0.372 (-0.023/0.777)
K_A, K_S	π_A, Fop	0.267** (0.003/0.530)	0.335* (-0.095/0.751)	0.145 (-1.000/1.000)	0.699** (0.001/0.902)
π_A, K_A	Fop, Exp	0.536** (0.201/0.782)	0.455** (0.040/0.688)	0.799 (-1.000/1.000)	0.183 (-0.164/0.702)
π_S, π_A	K_A, K_S	0.520** (0.204/0.663)	0.727** (0.301/0.877)	-0.278 (-1.000/1.000)	0.414*** (0.069/0.887)

* $p < 0.05$ (one-tailed test)

** $p < 0.01$ (one-tailed test)