Table S2: Genes which differ significantly in abdomen expression between fertile and sterile introgression lines for delta =1.6

id	FlyBase	arm	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(False Discovery Rate)	Mean Fertile Expression(log2)	Mean Sterile Expression(log2)	ttest_p-value
145795_at	FBgn0031653	2L	7.93144	1.85068	0.23333	3.59862	0	8.30304	10.15372	0.00021
150588_at	FBgn0039330	3R	6.53679	2.43518	0.37253	5.54970	0	6.63453	9.06971	0.00475
150423_at	FBgn0039051	3R	4.47766	1.02520	0.22896	2.02826	0	5.49839	6.52358	0.00272
147126_i_at	FBgn0033788	2R	4.17456	2.37773	0.56958	5.44454	0	6.39876	8.77649	0.01008
150193_at	FBgn0038702	3R	4.09594	1.13771	0.27776	2.18287	0	6.09118	7.22889	0.00727
148255_at	FBgn0035667	3L	4.08922	0.98579	0.24107	1.97220	0	10.61048	11.59626	0.00373
143603_i_at	FBgn0010358	2R	-7.50971	-1.93881	0.25817	0.26292	0	10.33400	8.39519	0.00206
142132_at	FBgn0034716	2R	-5.40902	-1.25857	0.23268	0.41826	0	7.19704	5.93848	0.00062
150703_at	FBgn0039475	3R	-4.72915	-1.08421	0.22926	0.47474	0	10.97886	9.89465	0.00873
151094_at	FBgn0040609	3R	-4.72172	-0.66483	0.14080	0.63095	0	8.29680	7.63197	0.00021
150702_at	FBgn0039474	3R	-4.66850	-1.00373	0.21500	0.49915	0	10.88099	9.87726	0.00087
143470_at	FBgn0004431	3L	-3.90948	-0.69692	0.17826	0.61741	0	7.54621	6.84929	0.00107
141418_at	FBgn0036024	3L	-3.63178	-0.76872	0.21166	0.58816	0	9.40462	8.63591	0.00335

Table S3: Expression differences on autosomal arms in the testes the fertile (F) and sterile (S) introgression lines

Autosomal Arm	# of genes detected[1]	Cutoff criteria for S≠ F	# genes with significant expression difference between S and F males			
			S < F	S > F	S ≠ F	% (S ≠ F)[2]
2L	2052	Δ = 1.6	179	112	291	(14.18%)
2R	2231	Δ = 1.6	200	163	363	(16.27%)
3L	2163	Δ = 1.6	191	101	292	(13.50%)
3R	2803	Δ = 1.6	259	134	393	(14.02%)

[1] Genes with Log_2(signal intensity) >6.2

[2] Percentage of genes that show expression difference among those detected.

Table S4 The number of under-expressed genes vs the number of over-expressed genes in F vs. S male testes

Platform (# of genes detected)	Method of analysis	# genes with significant expression difference between S and F males		
		S<F	S>F	(S<F)/ (S>F)
X				
Microarray (1683)	t-test	193	100	1.93
	SAM ($\Delta = 1.6$)	103	26	3.96
RNA-seq (1410)	Fisher's exact ($p<0.005$)	63	42	1.50
	Fisher's exact ($p<0.001$)	52	32	1.63
Autosomes				
Microarray (9289)	t-test	1374	1201	1.14
	SAM ($\Delta = 1.6$)	838	510	1.64
RNA-seq (7628)	Fisher's exact ($p<0.005$)	454	467	0.98
	Fisher's exact ($p<0.001$)	365	358	1.02

Table S5 Gene Ontology classification of genes underexpressed in testis of sterile males. Nested categories are shown indented, below their parent.Only genes for which functional annotation exists are included. Note that categories may contain overlapping sets of genes, so nested categories will not sum to the totals of their parents.

Category	GO	Autosome			X			X^2 P	
		# of genes	# changed	Fraction underexpressed	# of genes	# changed	Fraction underexpressed		
Biological process	GO:0008150								
behavior	GO:0007610	90	11	0.122	35	2	0.057	0.955	0.3284
cell communication	GO:0007154	1027	114	0.111 *	216	18	0.083 *	1.183	0.2768
cellular process	GO:0009987	4313	424	0.098 *	790	43	0.054	13.239	0.0003
cell differentiation	GO:0030154	207	25	0.121	42	3	0.071	0.700	0.4027
regulation of cellular process	GO:0050794	224	32	0.143 **	49	3	0.061	1.944	0.1632 (includes regulation of cellular physiological process)
cellular physiological process	GO:0050875	3921	387	0.099 *	721	39	0.054	12.449	0.0004
cell cycle	GO:0007049	320	21	0.066	79	3	0.038	0.772	0.3796
cell death	GO:0008219	185	30	0.162 **	26	1	0.038	2.256	0.1331
cell division	GO:0051301	77	8	0.104	22	2	0.091	0.026	0.8716
cell motility	GO:0006928	154	21	0.136 *	32	2	0.063	1.088	0.2969
cell organization and biogenesis	GO:0016043	557	58	0.104	119	10	0.084	0.362	0.5474
cell proliferation	GO:0008283	211	22	0.104	37	5	0.135 *	0.244	0.6215
cellular metabolism	GO:0044237	2846	274	0.096	506	26	0.051	9.144	0.0025
chromosome segregation	GO:0007059	82	5	0.061	16	0	0.000	0.966	0.3256
regulation of cellular physiological process	GO:0051244	160	22	0.138 *	36	3	0.083	0.619	0.4316 (significance due to cell death)
transport	GO:0006810	895	89	0.099	163	9	0.055	2.742	0.0977

development	GO:0007275	1077 77	118 2	0.110 * 0.026	222 8	21 1	0.095 ** 0.125	0.351	0.55340.1790
male gamete generation	GO:0048232							1.806	
physiological process	GO:0007582	4458	435	0.098 *	801	42	0.052	14.405	0.0001 (includes cellular physiological process)
regulation of biological process	GO:0050789	792	84	0.106	143	9	0.063	2.118	0.1456
Cellular component	GO:0005575								
cell	GO:0005623	2339	197	0.084	451	22	0.049	5.739	0.0166
extracellular region	GO:0005576	163	26	0.160 **	30	4	0.133	0.098	0.7540
organelle	GO:0043226	1560	114	0.073	293	8	0.027	7.590	0.0059
protein complex	GO:0043234	877	53	0.060	160	4	0.025	3.000	0.0833
Molecular function	GO:0003674								
binding	GO:0005488	1623	145	0.089	340	23	0.068	1.443	0.2297
catalytic activity	GO:0003824	2544	265	0.104 **	461	26	0.056	8.647	0.0033 (significant due to proteases)
enzyme regulator activity	GO:0030234	254	23	0.091	42	2	0.048	0.747	0.3876
signal transducer activity	GO:0004871	684	64	0.094	141	10	0.071	0.622	0.4304
structural molecule activity	GO:0005198	505	29	0.057	101	6	0.059	0.005	0.9414
transcription regulator activity	GO:0030528	579	48	0.083	116	4	0.034	2.905	0.0883
translation regulator activity	GO:0045182	54	1	0.019	6	1	0.167	3.092	0.0787
transporter activity	GO:0005215	720	95	0.132 **	129	8	0.062	4.113	0.0426

*P < 0.05 **P < 0.01

Table S6 Summary of the number of reads mapped to *Drosophila simulans* genome according to the annotation of dmRefseq from UCSC

	Sterile		Fertile	
	# of reads	Percentage	# of reads	Percentage
Mapped reads	15150489	/	25621701	/
Uniquely mapped reads	14308455	/	24383254	/
Genomic region				
Exon	9461970	66.13%	16380909	67.18%
Intron	2259120	15.79%	3681018	15.10%
Exon-Intron junction	742180	5.18%	1319187	5.41%
intergenic/unannotated	1845185	12.90%	3002140	12.31%

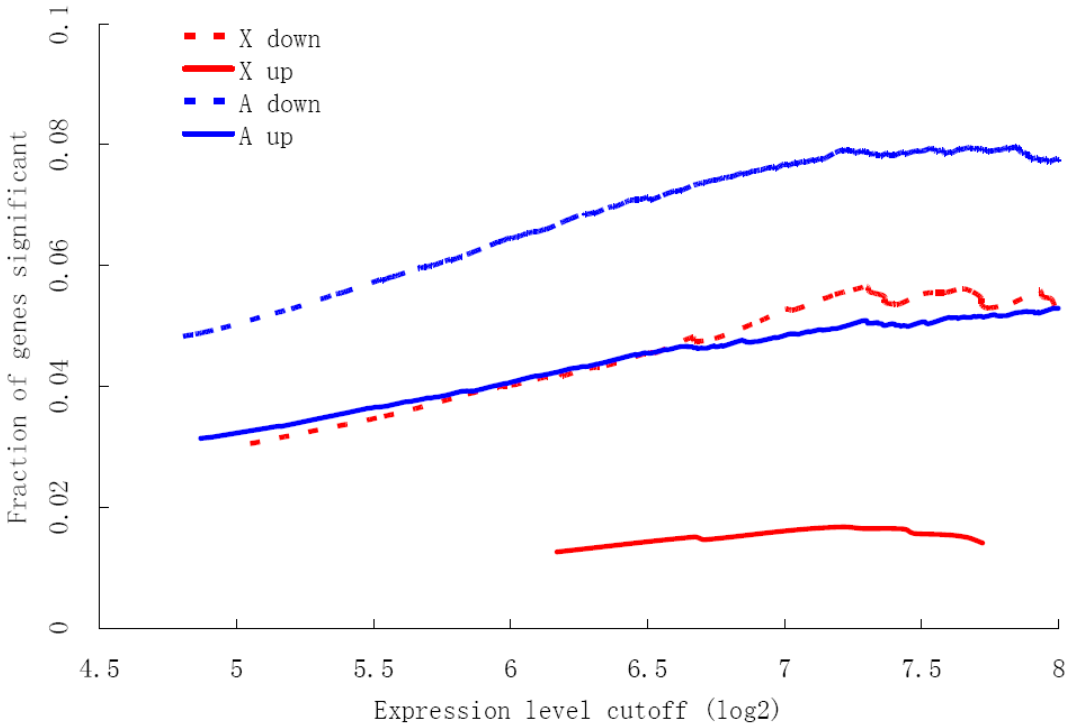

Figure S1: Proportion of genes that are significantly different in expression between testis of fertile and sterile males as called by SAM (Δ = 1.6). The proportion is based on all genes with expression levels greater than a given cutoff. This figure includes the total number of genes in the array.

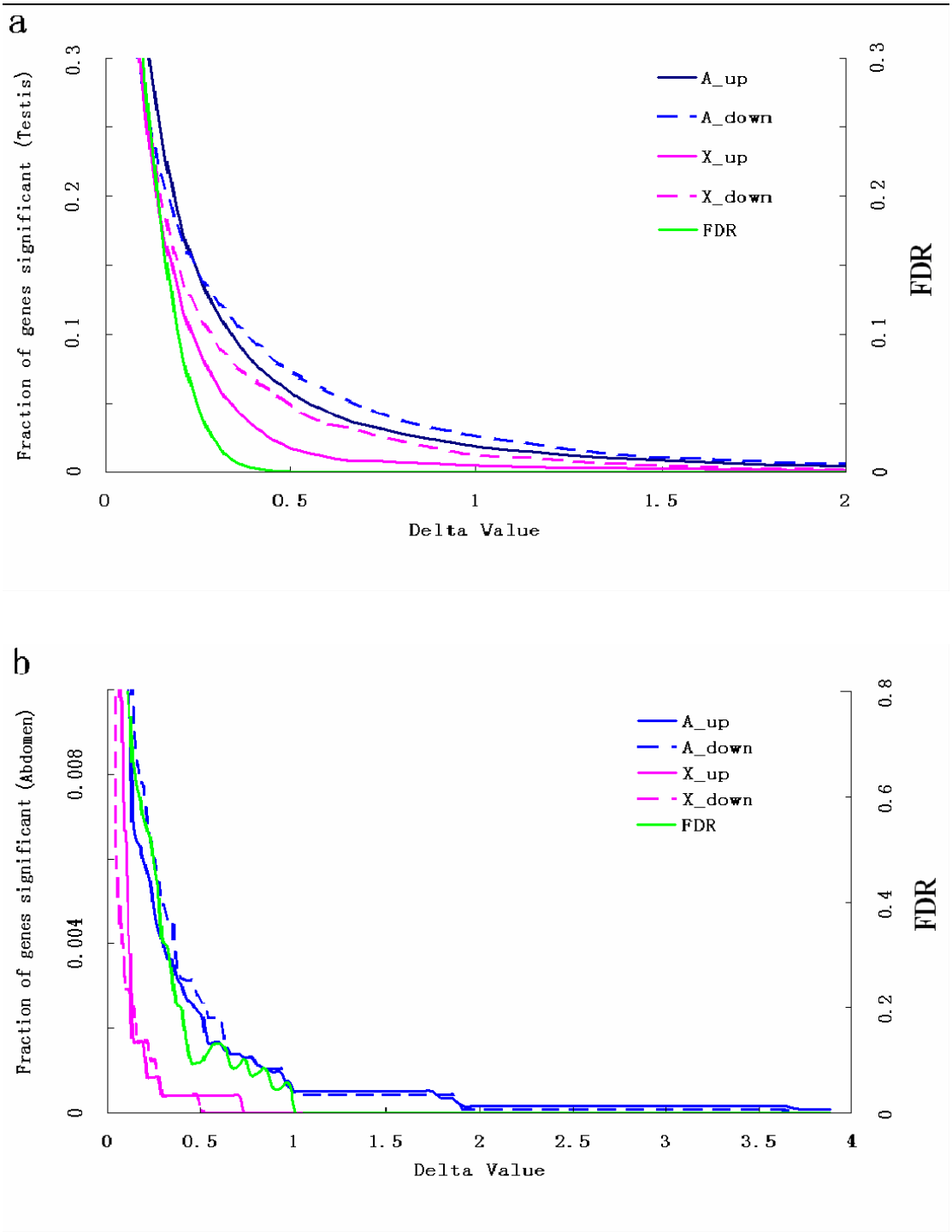

Figure S2: Proportion of genes that are significantly different in expression between fertile and sterile males and calculated false discovery rates for a) testis and b) abdomens as the delta value used to call significance varies. These proportions are calculated for all genes, regardless of expression level. False discovery rates are assessed by permutation.

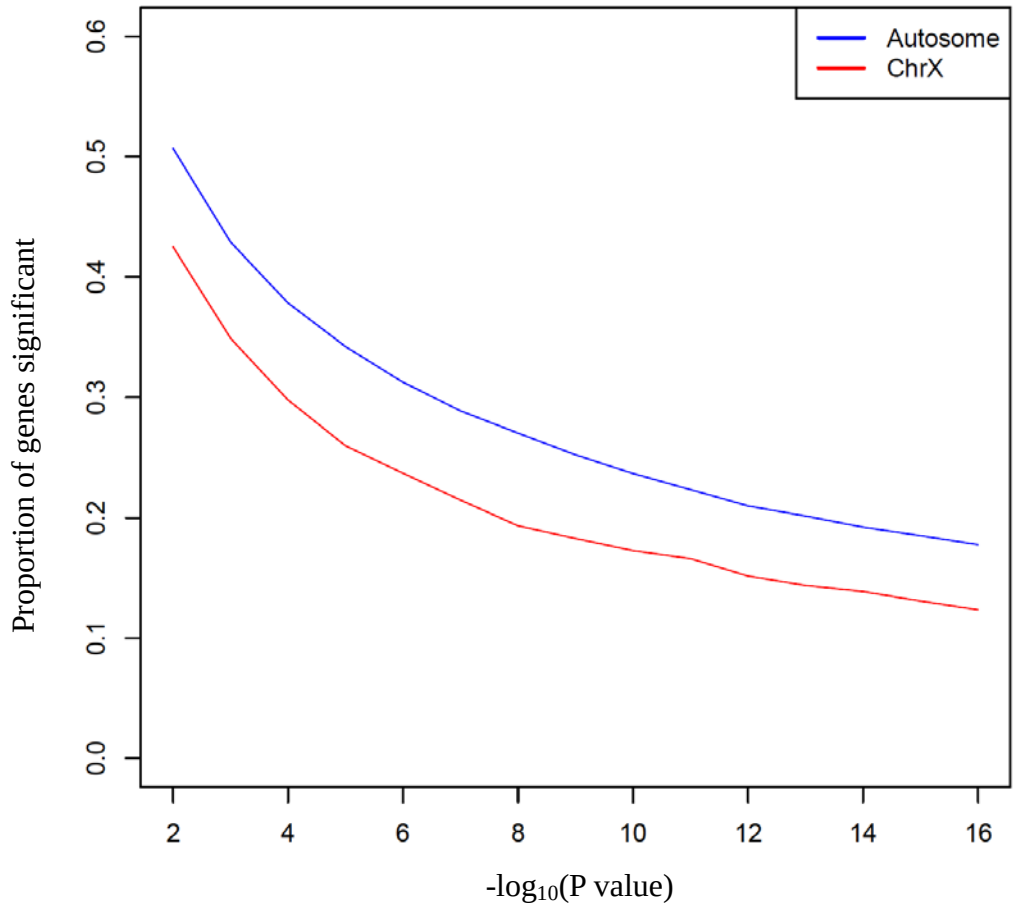

Figure S3: Proportion of genes that are significantly different in expression between testes of fertile and sterile males as a function of -log10(P). The P value is called by Fisher's exact test in RNA-seq analysis. Note that the proportion is lower for X-linked than for autosomal genes at all P values.

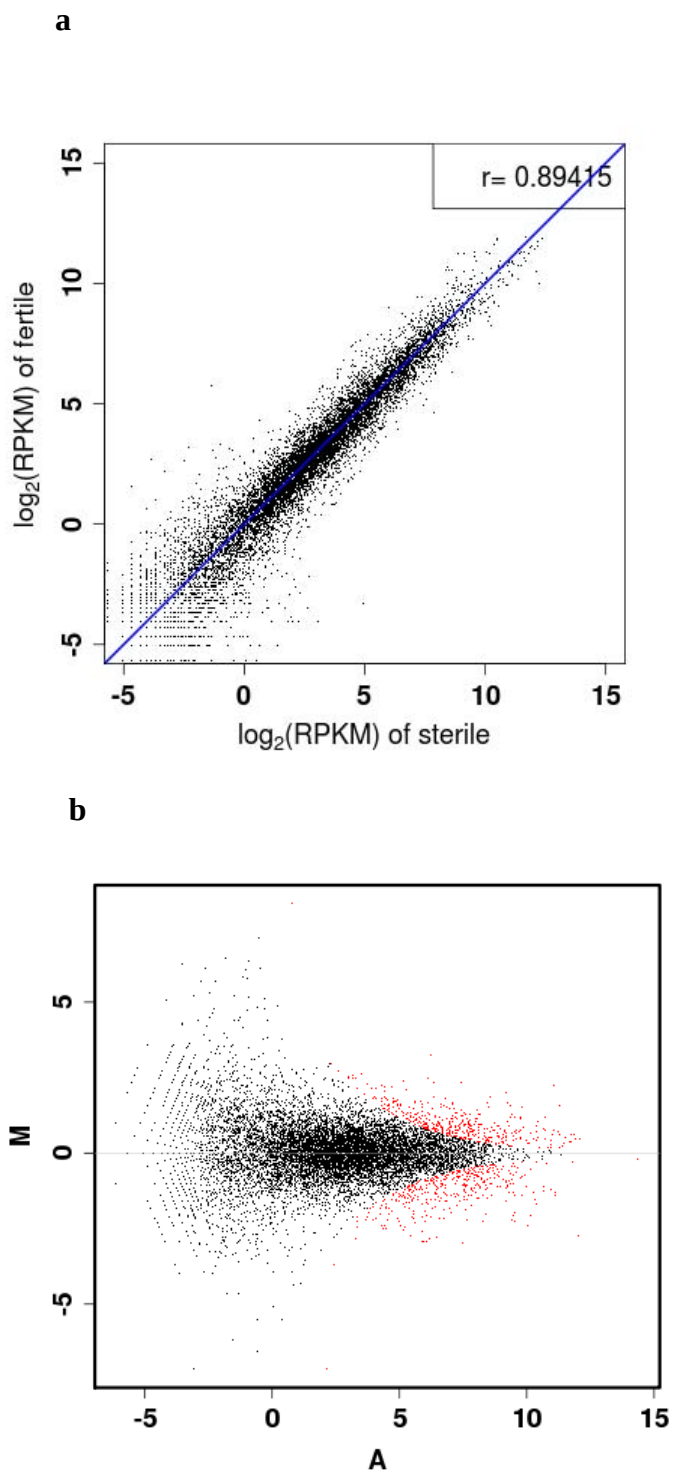

Figure S4 **Scatterplot (a) and MA plot (b) comparing gene expression profiles in sterile and fertile lines**. Each point in the graph presents an individual gene. The red points in the MA-plot are the genes identified as differentially expressed at p-value < 0.001 by Fisher's exact test. In the MA plot, A=[$\log_2$(RPKM of sterile)+$\log_2$(RPKM of fertile)]/2; M=$\log_2$(RPKM of sterile/RPKM of fertile).

Supplemental materials on Haldane's rule in relation to the X:A imbalance hypothesis

All the issues discussed in this study, including the large X-effect on hybrid sterility, the X:autosome (X:A) imbalance hypothesis and the contrast between hybrid inviability and sterility, were raised to explain the phenomenon commonly referred to as Haldane's rule. According to Haldane's rule (Haldane 1922), in interspecific hybridization, the hemizygous sex (XY males or ZW females) is generally more severely affected than the homozygous sex (XX females or ZZ males) when the effect is asymmetric between sexes.

Muller (1942) proposed the X:A imbalance hypothesis to explain this rule. Hybrids of the homozygous sex have exactly one haploid genome from each parental species. Therefore, gene dosages are balanced between the two genomes. Hybrids of the hemizygous sex, in contrast, have its X chromosome from only one species. Its X and autosomes, hence, are not balanced. For an explicit model on the X:A imbalance in terms of gene expression, see Wu et al. (1996).

The X:A imbalance hypothesis had been commonly accepted until Coyne (1985) published an ingenious experiment that appeared to invalidate the explanation. He chose to study hybridization between *Drosophila simulans* and *D. mauritiana* which yields fertile hybrid females and sterile hybrid males. By using the attached-X technique, Coyne (1985) constructed hybrid females that have the X chromosome from one species and autosomes from both species. These hybrid females have as much X:A imbalance as the hemizygous hybrid males but are quite fertile. Coyne (1985) rejected the X:A imbalance hypothesis in favor of X-Y interaction.

By constructing a series of genotypes specifically to test X:Y interactions, Johnson et al. (1992) subsequently rejected X-Y interaction as the cause of hybrid male sterility between the two species. They suggested that X:autosome interaction should still be considered a possible cause of hybrid male sterility. With respect to Coyne's (1985) test, they reasoned that oogenesis and spermatogenesis are fundamentally very different

developmental processes. The test by Coyne (1985), elegant as it was, should not be applied to hybrid sterility. (Coyne's test should be good for hybrid inviability.)

The male-female difference in the rate of fertility evolution was analyzed in detail by Wu and Davis (1993). They showed that Haldane's rule in mammals and in *Drosophila* is largely an issue of hybrid sterility, with very few cases of hybrid inviability. This analysis has led to what was later referred to as the "fast male evolution" hypothesis (Turelli and Orr 2000). According to Wu and Davis (1993) and Wu and Palopoli (1994), hybrid male sterility has evolved 10-100 fold faster than hybrid inviability.

There have been a series of papers on Haldane's rule (Laurie 1997; Orr 1997; Presgraves and Orr 1998; Turelli 1998; Turelli and Orr 2000; Wu et al. 1996). Although these papers clarify many of the earlier confusions, two issues remain incompletely resolved. First, why has hybrid male sterility evolved so rapidly? Second, can the X:A imbalance hypothesis explain this rapid evolution? If it can, the hypothesis would then explain the majority of cases of Haldane's rule in mammals and in *Drosophila*. (The third and somewhat ancillary issue about Haldane's rule, i.e., the large X effect on hybrid male sterility, is discussed in the main text.)

With respect to the issue of fast evolution of hybrid male sterility, Wu and Davis (1993) suggested two explanations. An explanation is sexual selection driving the evolution of male reproduction. The second explanation (that is not mutually exclusive with the first) is a mechanistic one concerning gene expression regulation. This second explanation was not specific about the mechanism of X:A imbalance as the analysis of whole genome expression was not feasible then. By providing the empirical support for a mechanism whereby the X:A imbalance affects spermatogenesis in the hybrids, we also assume that the same mechanism does not apply to oogenesis or embryogenesis as female fertility and embryonic viability of these hybrids are apparently normal. If true, this current study may provide the missing piece in the explanation for Haldane's rule.

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