

SUPPLEMENTARY MATERIAL

Genomic DNA hybridizations

Methods:

To correct for possible differences in hybridization efficiencies of individual oligos between *Mus musculus* and *Mus spretus* we performed Comparative Genomic Hybridizations (CGH) for all 3 *Mus spretus* samples using C57BL/6 as the reference. For each *Mus spretus* animal 1.5 μ g of genomic DNA was labelled using the BioPrime Plus Array CGH Indirect Genomic Labeling Systems (Invitrogen) according to the manufacturer's instructions. The C57BL/6 reference was labelled three times independently each time using 1.5 μ g of genomic DNA. Labelled genomic DNA of both species were combined in equal amounts and hybridized competitively to a microarray under a coverslip (Implen LifterSlip 24x60l) for 16 hours at 52°C in an Advalytix SlideBooster. As before, slides were washed in graded SSC/SDS and spun dry. The arrays were normalized using block-wise LOWESS and SD regularization (Yang et al. 2002) as described in (Quackenbush 2002).

Result:

To ascertain binding of the oligos to genomic DNA we first blasted all oligos that are represented on the microarray against the full genome sequence of *Mus musculus*. 80 % of the oligos showed genomic BLAST hits covering at least 60 bp of the 65mer oligo

(75% of the oligos covered the full 65mer sequence). Thus, the vast majority of the oligo sequences are suitable for genomic DNA hybridizations.

Different hybridization characteristics of oligos between both species can visually be inspected in an RI plot, where the $\log_2(R_i/G_i)$ for each oligo on the array is plotted as a function of the $\log_{10}(R_i * G_i)$ product intensities (G_i is the intensity of the hybridized genomic DNA in *musculus*, R_i is the intensity of the hybridized genomic DNA of *spretus*).

As shown in Supplementary Figure 4 for the three individuals of *Mus spretus* hybridized against a common *musculus* reference there are outliers present in both species. To test whether the same outliers can be found in each of the three *spretus* individuals (which would indicate a systematic effect of the oligo sequence on hybridization intensity) we performed a one-class analysis in SAM on the \log_2 -transformed ratios (R_i/G_i). At a False Discovery Rate of 5% we identified 1,098 genes, which are statistically significant. We tested whether these genes could be found among the list of differentially expressed genes between *musculus* and *spretus* in the different tissues. For all tissues we found the same proportion of genes with a potential systematic effect of sequence changes on the measured expression level (i.e., ~5%).

Quackenbush, J. 2002. Microarray data normalization and transformation. *Nature*

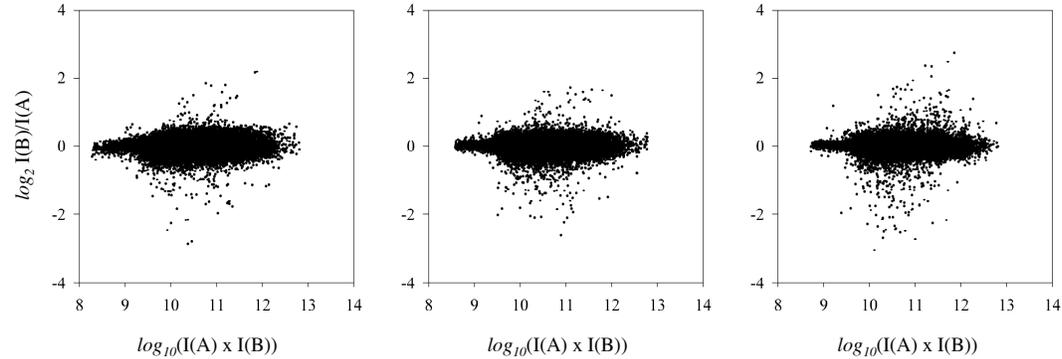
Genetics **32 Suppl:** 496-501.

Yang, Y.H., S. Dudoit, P. Luu, D.M. Lin, V. Peng, J. Ngai, and T.P. Speed. 2002.

Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res* **30**: e15.

Supplementary Figure 4

RI (“ratio to intensity”) plot from hybridizations of genomic DNA from *Mus musculus* and *Mus spretus*. Each panel shows one of the three *Mus spretus* individuals used in the study (*SP1*, *SP2*, *SP3* from left to right).



Supplementary Table 1

Chromosomal distribution of differentially expressed genes between species and subspecies of *Mus* in different tissues

Chrom ¹	between <i>spretus</i> and <i>musculus</i>						between <i>musculus</i> subspecies							
			brain		liver/kidney		testis		brain		liver/kidney		testis	
	No. of transcripts ²	% of total autosomal ³	No. of diff expr. genes ⁴	% of total autosomal ⁵	No. of diff expr. genes ⁴	% of total autosomal ⁵	No. of diff expr. genes ⁴	% of total autosomal ⁵	No. of diff expr. genes ⁴	% of total autosomal ⁵	No. of diff expr. genes ⁴	% of total autosomal ⁵	No. of diff expr. genes ⁴	% of total autosomal ⁵
1	1113	5.8	14	6.5	79	5.9	78	4.8	13	5.0	62	5.2	1	4.8
2	1398	7.2	15	7.0	86	6.5	119	7.3	26	9.9	75	6.3	3	14.3
3	911	4.7	6	2.8	59	4.4	82	5.1	14	5.3	62	5.2	1	4.8
4	1080	5.6	13	6.1	75	5.6	88	5.4	16	6.1	65	5.5	0	0.0
5	1130	5.8	8	3.7	79	5.9	107	6.6	20	7.6	72	6.0	2	9.5
6	1877	9.7	28	13.1	112	8.4	128	7.9	22	8.4	105	8.8	0	0.0
7	1380	7.1	18	8.4	110	8.3	116	7.2	18	6.9	90	7.6	0	0.0
8	906	4.7	7	3.3	67	5.0	84	5.2	18	6.9	65	5.5	1	4.8
9	1009	5.2	12	5.6	72	5.4	88	5.4	6	2.3	67	5.6	2	9.5
10	872	4.5	9	4.2	69	5.2	82	5.1	7	2.7	59	5.0	1	4.8
11	1353	7.0	21	9.8	89	6.7	120	7.4	17	6.5	90	7.6	2	9.5
12	1381	7.1	14	6.5	77	5.8	129	8.0	17	6.5	75	6.3	3	14.3
13	752	3.9	13	6.1	53	4.0	56	3.5	11	4.2	43	3.6	0	0.0
14	882	4.6	7	3.3	62	4.7	73	4.5	17	6.5	58	4.9	3	14.3
15	707	3.7	3	1.4	33	2.5	59	3.6	8	3.1	48	4.0	1	4.8
16	633	3.3	6	2.8	54	4.1	46	2.8	8	3.1	42	3.5	0	0.0
17	866	4.5	9	4.2	64	4.8	78	4.8	10	3.8	53	4.5	0	0.0
18	491	2.5	5	2.3	44	3.3	39	2.4	7	2.7	32	2.7	1	4.8
19	605	3.1	6	2.8	45	3.4	50	3.1	7	2.7	28	2.4	0	0.0
SUM (A)	19346	<u>% of total[#]</u>	214	<u>% of total[#]</u>	1329	<u>% of total[#]</u>	1622	<u>% of total[#]</u>	262	<u>% of total[#]</u>	1191	<u>% of total[#]</u>	21	<u>% of total[#]</u>

SUM (X)	629	3.1	6	2.7	38	2.8	42	2.5	17	6.1*	42	3.4	2	8.7
SUM (Y)	16		0		1		1		0		2		0	
SUM	19991		220		1368		1665		279		1235		23	

* significant (after Bonferroni correction) over-representation of X-chromosomal genes (P-value: 0.0078)

#percentage of X-chromosomal transcripts relative to all transcripts assayed

¹Chromosome number

²Number of transcripts assayed on microarray on each chromosome

³Percentage of total transcripts on particular autosome

⁴Number of differentially expressed genes on each chromosome

⁵Percentage of differentially expressed genes on the particular chromosome relative to the number of all differentially expressed genes on autosomes

Supplementary Table 3

Sample locations and number of generations mouse strains were kept in the lab

Subspecies	Generation	Geographic origin	Strain name
<i>M. m. musculus</i>	Collected in the wild	Czech Republic, Studenec	M2
<i>M. m. musculus</i>	Collected in the wild	Czech Republic, Tresov	M3
<i>M. m. musculus</i>	Collected in the wild	Czech Republic, Rousek	M4
<i>M. m. musculus</i>	Collected in the wild	Czech Republic, Pozdatin	M5
<i>M. m. musculus</i>	Collected in the wild	Czech Republic, Rejtar	M7
<i>M. m. musculus</i>	Collected in the wild	Czech Republic, Pozdatin	M8
<i>M. m. domesticus</i>	Collected in the wild	Germany, Niederbachem	D1
<i>M. m. domesticus</i>	Collected in the wild	Germany, Zuellighofen	D3
<i>M. m. domesticus</i>	Collected in the wild	Germany, Arzdorf	D5
<i>M. m. domesticus</i>	Collected in the wild	Germany, Kuerrighofen	D6
<i>M. m. domesticus</i>	Collected in the wild	Germany, Swisttal	D10
<i>M. m. domesticus</i>	Collected in the wild	Germany, Eimerzheim	D13
<i>M. m. ssp</i>	8	Iran, Teheran	THE
<i>M. m. ssp</i>	5	Iran, Birdjand	BID
<i>M. m. ssp</i>	5	Iran, Machad	MAC
<i>M. m. ssp</i>	5	Iran, Khak	KAK
<i>M. m. ssp</i>	10	India, Delhi	DHA
<i>M. m. ssp</i>	9	Pakistan, Rawalpindi	MPK
<i>M. m. castaneus</i>	8	India, Masinagudi	CIM
<i>M. m. castaneus</i>	2	Thailand, Pathumthani	CTP
<i>M. m. castaneus</i>	8	Taiwan, He-mei	CTA
<i>M. spretus</i>	1	Near Madrid	SP1
<i>M. spretus</i>	1	Near Madrid	SP2
<i>M. spretus</i>	1	Near Madrid	SP3