

FIGURES S1-S5

To the article:

Sexual selection drives weak positive selection in protamine genes and high promoter divergence, enhancing sperm competitiveness.

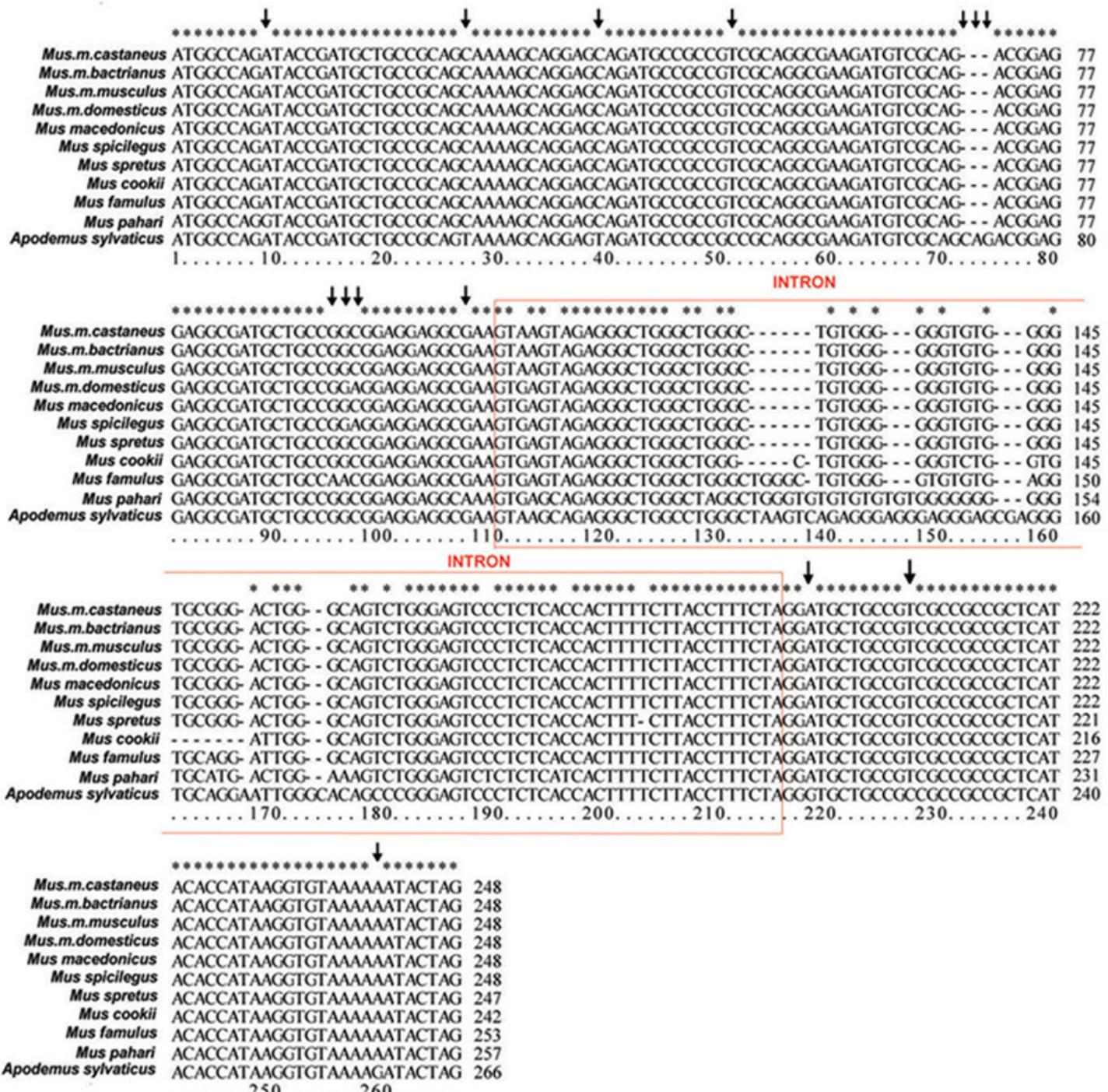
by

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Figure S1. Multiple alignment of protamine 1 sequences. (A) DNA and (B) protein sequences of 10 rodent species. Note that most of the coding variation in protamine 1 sequence corresponds with synonymous substitutions in the protein. Only 3 residues (at position 32, 36 and 51) differentiates *Apodemus sylvaticus* (outgroup) with the species of the ingroup

A



B

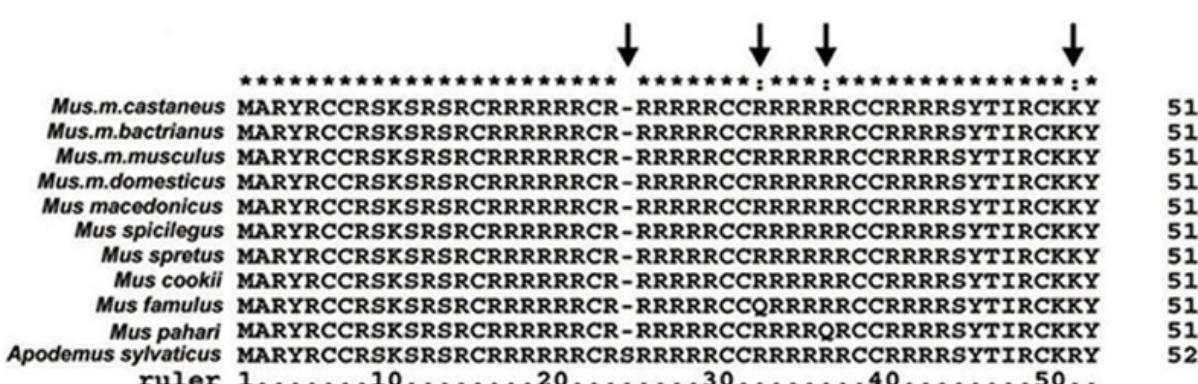


Figure S2. Multiple alignment of protamine 2 sequences. (A) DNA and (B) protein sequences of the 10 rodent species. Sequence alignments show non-synonymous variation at residues 22, 80 and 106. Non-synonymous variation at codon 98 differentiates *Apodemus sylvaticus* (outgroup) from all the (ingroup) species of *Mus*.

A

<i>Mus.m.castaneus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus.m.bactrianus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus.m.musculus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus.m.domesticus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus macedonicus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus spicilegus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus spretus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus famulus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus pahari</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Mus cookii</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
<i>Apodemus sylvaticus</i>	ATGGTTCGCTACCGAATGAGGGAGCCCCAGTGAGGGTCCCACCCAGGGGCTGGACAAGACCATTGACCGGAGGAGCAGGGCAAGGGCTGAGCC	100
	1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100	
	*****	*****
<i>Mus.m.castaneus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTACATAGGATCCACAAGAGGGC	200
<i>Mus.m.bactrianus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTACATAGGATCCACAAGAGGGC	200
<i>Mus.m.musculus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTACATAGGATCCACAAGAGGGC	200
<i>Mus.m.domesticus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Mus macedonicus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Mus spicilegus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Mus spretus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Mus famulus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Mus pahari</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Mus cookii</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
<i>Apodemus sylvaticus</i>	CAGAGCCGTAGAGGACTATGGGAGCACACAGGGGCCACACACCACAGACACAGGGCTGCTCTCGTAAGAGGCTGATAGGATCCACAAGAGGGC	200
	110.....120.....130.....140.....150.....160.....170.....180.....190.....200	
	*****	*****
	INTRON	
<i>Mus.m.castaneus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus.m.bactrianus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus.m.musculus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus.m.domesticus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus macedonicus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus spicilegus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus spretus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus famulus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus pahari</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Mus cookii</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
<i>Apodemus sylvaticus</i>	TCGGTCATGCAGAAGGCCGGAGGAGACACTCTGCCGCACAGGGGGCATCGAGAGTAAGCACCCCACAGCCGAGCCCCCTGGCACCTGTGCTGCT	300
	210.....220.....230.....240.....250.....260.....270.....280.....290.....300	
	*****	*****
	INTRON	
<i>Mus.m.castaneus</i>	GCTGCCGTCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus.m.bactrianus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus.m.musculus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus.m.domesticus</i>	GCTGCCGTCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus macedonicus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus spicilegus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus spretus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus famulus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus pahari</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Mus cookii</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
<i>Apodemus sylvaticus</i>	GCTGCCCATCTAACCCCTGCTGCCCTCTAGGAGCTAGCAAACCTCGACTTTCTACAGGCTGAGAAGATCCCAGGGAGGAGGAGATCGAGGT	400
	310.....320.....330.....340.....350.....360.....370.....380.....390.....400	
	*****	*****
	INTRON	
<i>Mus.m.castaneus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus.m.bactrianus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus.m.musculus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus.m.domesticus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus macedonicus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus spicilegus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus spretus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus famulus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus pahari</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Mus cookii</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
<i>Apodemus sylvaticus</i>	GCAGGAATGTAGGAGGCACCATCACTAA 429	
	410.....420	

B

Figure S3. Multiple alignment of protamine 1 promoter sequences from 10 species of *Mus*.

Figure S4. Multiple alignment of protamine 2 promoter from 10 species of *Mus*

Figure S5. Divergence of promoter sequences. The probability density distribution of branch lengths from 10,000 replicates of protamine 1 promoter (pP1) and protamine 2 promoter (pP2) is shown together with that obtained from the intron sequences. The intron sequence was used as proxy of neutral divergence. *M. spicilegus*, *M. spretus* and *M. macedonicus*, which are the species with highest levels of sperm competition, show the highest lengths in pP2. *M. cookii* and *M. pahari* were excluded from the analysis since their branch lengths remain undefined in the unrooted intron tree built without the information of *Apodemus sylvaticus*.

