

Table ESM1: A compilation of published empirical studies addressing sexual selection against hybrids in parapatric and sympatric taxa displaying or not homogamy (F1, F2: first and second generation hybrids; BC: backcross hybrids).

Taxa1	Taxa2	Mating cue	Hybrid phenotype	Evidence for natural selection against hybrids	Mate preferences tested in	Type of hybrid tested	Evidence for SS against hybrids	Niche overlap between parental populations	Reference
<u>Wolf spiders</u>									
<i>Schizocosa ocreata</i>	<i>Schizocosa royneri</i>	Courtship behavior	A mix of courtship sequences of the two parental species (dysfunctional)	Not found	Taxa 1, 2 & F1	Laboratory : F1, F2, BC from forced copulations	Yes	No	[1]
<u>Fruit fly*</u>									
<i>Drosophila pseudoobscura</i>	<i>Drosophila persimilis</i>	Courtship behavior, cuticular hydrocarbon	Anomalously low courtship intensity (transgressive,dysfunctional)	Not tested	Taxa 1 & 2	Laboratory F1, BC	Yes	Important	[2]
<u>Threespine stickleback</u>									
Benthic <i>Gasterosteus aculeatus</i>	Limnetic <i>Gasterosteus aculeatus</i>	Nesting site, microhabitat, body size	Intermediate morphology and behavior, nest in taxa 2 habitat	Lower foraging and growth ability in nature	Taxa 2	Laboratory raised F1	In the lab → No In nature → Yes	No	[3, 4]
Lake* <i>Gasterosteus aculeatus</i>	Stream* <i>Gasterosteus aculeatus</i>	Nest, courtship behavior, aggressiveness	Intermediate, lower display, & subdominant	Genetic incompatibilities checked but not found	Taxa 1, 2, F1	Laboratory raised F1	No	No	[5]
<u>Butterfly</u>									
<i>Heliconius sydnio</i>	<i>Heliconius melpomene</i>	Wing color patterns, courtship behavior	Intermediate pattern, lower courtship probability when a male encounters a female with a wing pattern different from its own	Increased predation due to non adapted mimetic wing colors, F1 female sterile	Taxa 1, 2, F1	Laboratory F1	Yes	No	[6]
<u>Green tree frog</u>									
<i>Hyla cinerea</i>	<i>Hyla gratiosa</i>	Song	Intermediate male song frequency	Maybe ecological (habitat use and desiccation tolerance), impossible BC between females F1 and <i>cinerea</i> males	Taxa 1	Synthetic signal mimicking natural hybrids	Yes	Important	[7]

*no homogamy between the parental populations

Table ESM1 (continued)

<u>Pecos pupfish and Sheepshead minnow</u>									
<i>Cyprinodon pecosensis</i> *	<i>Cyprinodon variegatus</i>	Visual cue, territory (linked to dominance), courtship behavior	Display more male aggression and female pursuing than both taxa, Equals taxa. 1 in dominance over taxa 1	No (and even heterosis)	Taxa 1 & 2	Laboratory F1	No	Important	[8]
<u>Quail</u>									
<i>Coturnix coturnix coturnix</i>	<i>Coturnix japonica</i>	Male call	Most call parameters intermediate, some similar to taxa 1 or 2, some new. Greater intra and inter individual variation than parents	Not found	Taxa 1 and 2	Recorded laboratory F1 calls	Taxa 1 → No Taxa 2 → Yes	Important	[9]
<u>Grasshopper</u>									
<i>Chorthippus brunneus</i>	<i>Chorthippus jacobsi</i>	Male call and courtship song	Intermediate male song	No intrinsic viability or fertility decrease	Taxa 1, 2, F1, F2, BC	Laboratory F1	Yes	Important	[10]
<i>Chorthippus albomarginatus</i>	<i>Chorthippus osciei</i>	Male call and courtship song	Courtship song parameters mostly resembling Taxa 1	Embryon and nymph mortality higher in hybrids with a Taxa 2 mother	Taxa 1, 2, F1, BC	Laboratory F1	Taxa 1, F1, F2, & BC → No Taxa 2 → Yes	No	[11]
<u>Cichlid fish</u>									
<i>Pundamilia pundamilia</i>	<i>Pundamilia nyererei</i>	Male nuptial coloration and courtship	Intermediate color and courtship frequency	Not found	Taxa 1, 2, F1, F2	Laboratory F1 and F2	Yes	Important	[12 , 13]
<i>Pseudotropheus emmiltos</i>	<i>Pseudotropheus fainzilberi</i>	Undetermined, probably a mix of olfactory and visual signals, plus male courting sounds	F1 and most F2 intermediate colors and patterns, some F2 similar to one or the other parent	Not found	Taxa 1 and 2	Laboratory raised F2 and F1	F1 → No F2 → Variable	Important	[14 , 15]
<u>Flycatcher</u>									
<i>Ficedula albicollis</i> (collared)	<i>Ficedula hypoleuca</i> (pied)	Plumage color, song, courtship behavior, nest site	Intermediate male plumage color (overlapping with parental extremes)	Lower survival rate, F1 females sterile, F1 males sperm inviable	Taxa 1 , F1	Natural F1	Yes	Important	[16]

*no homogamy between the parental populations

Table ESM1 (continued)

<u>Green lacewing</u>									
<i>Chrysoperla adamsi</i>	<i>Chrysoperla plorabunda</i>	Duetting vibrational signal	Intermediate acoustic signal	Not tested	Taxa 1, 2, F1	Laboratory F1 and synthetic signal	Yes	Complete	[17]
<u>Chorus frog</u>									
<i>Pseudocaris feriarum</i>	<i>Pseudocaris nigrita</i>	Male song	Intermediate male song	Low fertilization success of males	Taxa 1	Synthetic signal mimicking laboratory raised F1 calls.	Yes	Important	[18]
<u>Swordtail fish</u>									
<i>Xiphophorus birchmanni</i>	<i>Xiphophorus malinche</i>	Male olfactory signals	Not analysed	Not tested	Taxa 1 and 2	Natural hybrids (F2 & BC)	No	Important	[19]

*no homogamy between the parental populations

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ESM2: GEOGRAPHICAL ORIGIN OF MICE INVOLVED IN THIS STUDY.



Trapping sites in Jutland (Denmark). The bold black line represents the genetic center of the hybrid zone as defined in Raufaste et al 2005.

Hybrid sites that yielded mice are represented by empty diamonds. Sites at the borders of the hybrid zone from where our "border populations" originate are represented by circles, white for *musculus* and grey for *domesticus*. White squares north of the *musculus* border are sites that yielded close allopatric *musculus* mice.

Biological material

Mice breeding scheme and housing conditions

Mice were crossed in the laboratory following an outbreeding scheme, in which the population distinctiveness was kept. Laboratory born mice were weaned at 24 days and sibling females were separated from their brothers 7 days later. Pairs were formed between non-sibling, and a given mouse was involved in an experiment only when it had spent at least 3 weeks with a potential mate.

All mice were kept in the animal facilities of the University of Montpellier 2. They were housed under controlled conditions with a 12:12 photoperiod (lights on between 5 AM and 5 PM), and food and water were available *ad libitum*.

Genetic markers and hybrid index estimation.

Comparison of the complete mouse genome sequence (laboratory strain C57/Bl6) to BAC-end sequences of the MSM mouse strain (*M. m. molossinus*, Abe et al. 2004) reveals numerous polymorphic indels of LINE (L1 family) and SINE (B1 and B2 families) transposable elements. We defined PCR primers flanking the insertion sites of these transposons and that thus allowed characterizing the presence/absence of the transposon according to the size(s) of the PCR product(s) on genomic DNA, after electrophoresis on agarose gels. Typing in this way a panel of wild mice covering the geographic ranges of *domesticus* and *musculus* allowed us to discover loci with very contrasted allele frequencies between the two subspecies (alternatively fixed or nearly so). In this study we used 18 such autosomal loci, and 2 X-linked loci, described in supplementary table S1. We also added a marker on the Y chromosome, an 18 bp deletion in the *Zfy2* gene that is characteristic of *musculus*, detected using the methods described in Boissinot and Boursot (1997).

We used these genotyping results to calculate a hybrid index (HI, defined throughout as the proportion of *musculus* ancestry) for the wild mouse samples used in this study, and for the wild parents of descendants participating in this study. Since some loci were not fully diagnostic between the two subspecies, we estimated HI by maximum likelihood (see e.g. Buerkle 2005 for the formula used to calculate likelihood), using

parental allele frequencies determined on wild samples covering the European ranges of the two subspecies (20 mice from different localities for each subspecies). These parental insertion frequencies are reported in supplementary table S4. Likelihoods were calculated for all possible values of HI to a precision of 10^{-3} . We thus found the maximum likelihood estimate and took the boundaries of the 2 Log Likelihood unit intervals around the maximum as support limits of the estimates of HI.

Behavioural tests protocols

Mate preference: the choice tests

Preference was assessed during two-way choice tests. The test apparatus consisted of a Y-shaped tubular Plexiglas device connected to three boxes, the start box and two peripheral boxes [1]. The apparatus was surrounded by a 20cm high cardboard in an attempt to partially homogenize the mouse visual field, and the experiment took place under low light intensity. The day before each test the mouse was left (~10min) to explore the entire empty apparatus, to reduce stress, neophobia and spatial investigation not directed towards the stimuli during the experiment. Urine stimuli (10µl each) were spread over 2cm² delimited areas at the extremity of each peripheral box. The urines were labelled so that the behaviour recording was blind. The left and right positions of the two stimuli were shifted between tests to avoid any effect of laterality. A given test mouse (the nose) was introduced in the start box, separated from the rest of the apparatus with a perforated transparent sliding door; the slide door was then opened and the test started as soon as the mouse entered the Y maze. A choice test lasted 5 minutes during which the time spent by the mouse in the different parts of the apparatus (centre, left and right) and time spent in contact sniffing or touching the stimuli were recorded with 'The Observer' 5.0.31 software [2].

Odour similarity: habituation-discrimination and habituation generalisation tests

The ability to perceive differences between two odorant stimuli was assessed via habituation-discrimination or habituation-generalisation tests [see review in 3]. The rationale of this test is that when a mouse is presented with a novel stimulus it investigates it spontaneously,

the intensity of this behaviour being proportional to the familiarity of the mouse with the stimulus: it will diminish as the mouse gains familiarity. Immediately following this phase (i.e. habituation) when the mouse is presented with one or two other stimuli it investigates less intensively the stimulus that is identical to (i.e. discrimination test) or resembles the most (i.e. generalisation test) the habituation stimulus, while it shows significantly more interest towards a stimulus different from the habituation one. Such experiments allow the assessment of odour similarity between 2 to 3 stimuli, and it was validated for the house mouse [e.g. 4, 5]. Here we used the same protocol as described by Smadja and Ganem (2008), consisting of a 10 minutes habituation phase to a 10 μ l stimulus followed by a 5 minutes phase where two 10 μ l stimuli are presented for discrimination (when only one of the stimulus is different from the habituation) or generalisation (when the two stimuli are new). The experimental apparatus comprised two transparent Plexiglas boxes separated by a 20cm long transparent tube.

The mouse was introduced into one of the boxes, the starting box, which was separated from the test box by the 20cm long transparent tube and a perforated transparent sliding door. After one minute or so the door was removed to allow the mouse to enter the tube leading to the test box containing the stimuli. During the habituation phase, the stimulus was spread over a delimited 2cm² area at the extremity of the test box, on its floor. During the discrimination or generalisation phase two stimuli were spread in the middle of each of the two lateral sides of the box (10cm apart from each other). The discrimination/generalisation phase followed immediately the habituation phase, which involved isolating the mouse in the start box after the first phase and replacing the habituation stimulus box by a new one containing the two other stimuli. Here too the urine was labelled so that recording was blind, and left or right position of the two stimuli was shifted between tests. The measured variable was time spent in contact, sniffing or licking a given stimulus.

Statistical analysis

A two tailed Wilcoxon test for paired comparisons (stimulus “a” versus “b”) was applied for all tests except for the choice test with border *musculus* noses presented with stimuli of both subspecies where the test was one tailed based on earlier studies showing assortative preference in similar tests.

In order to compare preference between tests, we constructed an index “R” defined as the time spent in

contact with the homosubspecific stimulus divided by the total time in contact with both stimuli.

For comparisons between more than two conditions, e.g. testing the influence of geography or level of hybridisation on preference, we performed a mixed ANCOVA. Residuals distribution and homoscedasticity were checked post-hoc both with residual *versus* fitted values and Normal QQ plots, and with Shapiro’s and Bartlett’s tests. The distribution of residuals of variable R not conforming to a normal law, we applied an exponential transformation to our variable and checked that the residuals of “expR” conformed to the above-mentioned constraints, which was the case.

The maximal model included two factors, sex of chooser and category of the non-*musculus* stimulus, one covariate, “motivation” (i.e. willingness to participate to the test, defined as the sum of time spent in the right and left side of the apparatus), and their interactions as fixed effects. The chooser population of origin was included as a random factor. The covariate “motivation” was included in our model because preliminary analyses indicated that it was variable (mean=189.8, sd=28.4) and could slightly differ between the sexes (Wilcoxon test: W=4030, p=0.0599), and hence could have interfered with our measure of preference. Stimulus origin was considered as an ordinal factor, rather than a continuous variable, containing the coordinates of their origin on the transect or their HI, because uneven trapping success across the hybrid zone led to discontinuities along such continuum. Backward simplification of the model was performed following the procedure described in [6] to obtain the most parsimonious adequate model.

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Table ESM4: Description of the loci used to assign mice to the two subspecies and to calculate HI values of hybrids.

Locus name	Chromosome	Position (Mb)	Parental insertion frequency		Forward primer	Reverse primer
			<i>musculus</i>	<i>domesticus</i>		
11B1_143C	chr11	76.93	1.000	0.000	TTTGCAGCATCCAACAATTT	CACCCAGGTATGCTCCCTAA
18B1_432O	chr18	34.52	1.000	0.000	TATGCATGCTTGTGGGAGAG	CATCTTGATGTGGCCTACCA
4B2_416D	chr4	154.89	0.000	1.000	TCCTCAAAACGAGCAAAAGG	AATAATTTGGGGGTGGGATG
8B2_269N	chr8	4.74	1.000	0.000	TGGTGCCAAGGTATTGGTTT	TTTAAAGGCTTACCATTGAGAACA
11B2_080I	chr11	53.17	0.028	1.000	ATTTGGGAGGCCAAATTA	ATGGAAACTTCCCCCTTTT
2B2_499A	chr2	32.89	0.028	1.000	GCATTTCCACCTGACCGTAT	GGAAACTGGCCCACTGATAA
4B1_178D	chr4	34.84	0.028	1.000	CGTGCTGACTTTGGTTGAAA	GCAAGTTGGTCTGCTCCTTC
4B1_264O	chr4	94.13	0.028	1.000	TCGAAGACATTGAAAGGGAGA	CACACACACTGTAGCAAGGACA
5B2_323M	chr5	24.75	0.972	0.000	TTGGGTCAGTTAGACGACATTG	TTTCTCCATAATTTTTCAGGTTGA
8B2_368N	chr8	119.18	0.000	0.950	TCTGCAAACCTCAGAACGTG	AATGAGGCTCCTCCTCCAAT
9B1_433H	chr9	70.1	0.028	1.000	CAAATGGTGTTGCAAATGGA	CGGCAGAACCTCGAAAGTTA
11B2_155G	chr11	23	0.000	0.950	ATCCACCCTCCAGCCTAACT	GTGGGAGGCAGTAGGAGTCA
13B2_315P	chr13	54.73	0.000	0.900	AATGCCTTATGCCAACCAAG	ATGGGTTCAATTTGTGGGAAA
3B2_143N	chr3	127.43	0.111	1.000	TGACCAAGAAGATGCTCACG	TGGCAGAGGAAATCAAATCC
4B2_378L	chr4	126.28	0.000	0.950	TTTCAGCCGAATGTCCTACC	AGAGGGGAAAGCTTCCAGAG
11B2_189J	chr11	78.1	0.000	1.000	CACCCAGGTCCACAGAACTA	AGGGCTTGACCAGGAGTTCT
13B1_340G	chr13	56.86	0.028	0.850	ATGGTTTTGTGGGAGGTGTC	CTTCCTGGTCGCAGTTCTTC
3B2_373P	chr3	132.03	0.056	1.000	ATCTGTGTCCCACCAGCTCT	TGGGGATGGGAGATTTCATT
Syap1 [1]	chrX	159.3	0.000	1.000	TGGCTGAGTCACCACTTGTT	TGGGGAATGACATTTGAGGT
Btk [2]	chrX	131	0.000	1.000	AATGGGCTAGCGTAGTGCAG	AGGGGACGTACACTCAGCTTT

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Table ESM5 : Estimation of *musculus* ancestry (HI) for each trapped site calculated by maximum likelihood on the number of loci (n loci) obtained on sampled mice (n mice). Distance to the genetic center of the hybrid zone (Distance) was calculated as discribed in Raufaste et al. [1].

Category	Site name	Distance (km)	n mice	n loci	HI	HI Low	HI high
close allopatic <i>musculus</i>	H3	113.99	6	118	0.988	0.967	0.998
close allopatic <i>musculus</i>	H7	113.01	1	20	1.000	0.918	1.000
close allopatic <i>musculus</i>	H1	112.9	9	171	0.987	0.971	0.996
close allopatic <i>musculus</i>	H2	111.07	4	81	1.000	0.988	1.000
close allopatic <i>musculus</i>	H10	110.17	5	99	1.000	0.990	1.000
close allopatic <i>musculus</i>	H12	110.12	7	140	0.970	0.944	0.987
close allopatic <i>musculus</i>	R1	100.83	1	20	1.000	0.924	1.000
close allopatic <i>musculus</i>	R6	100.71	7	141	1.000	0.991	1.000
close allopatic <i>musculus</i>	R14	99.4	5	99	1.000	0.990	1.000
close allopatic <i>musculus</i>	R17	98.39	1	19	1.000	0.948	1.000
close allopatic <i>musculus</i>	R12	97.39	5	99	0.999	0.963	1.000
close allopatic <i>musculus</i>	R16	95.6	4	81	1.000	0.980	1.000
border <i>musculus</i>	B7	72.82	5	95	0.987	0.958	0.999
border <i>musculus</i>	B6	68.86	2	42	1.000	0.975	1.000
border <i>musculus</i>	B4	65.74	1	20	1.000	0.951	1.000
border <i>musculus</i>	B12	62.79	6	119	0.991	0.973	0.998
border <i>musculus</i>	B3	62.79	6	116	0.995	0.979	0.999
border <i>musculus</i>	LA16	61.01	4	73	1.000	0.985	1.000
border <i>musculus</i>	B17	60.24	1	20	0.973	0.885	0.998
border <i>musculus</i>	B10	59.58	4	79	0.954	0.911	0.982
border <i>musculus</i>	LA19	58.31	1	20	0.935	0.808	1.000
border <i>musculus</i>	LA15	57.55	9	174	1.000	0.995	1.000
border <i>musculus</i>	LA3	55.81	4	69	1.000	0.965	1.000
border <i>musculus</i>	LA4	55.15	2	40	1.000	0.975	1.000
border <i>musculus</i>	LA17	54.55	7	134	1.000	0.986	1.000
border <i>musculus</i>	M5	50.65	4	80	1.000	0.980	1.000
border <i>musculus</i>	M7	50.04	1	20	1.000	0.951	1.000
border <i>musculus</i>	M4	49.38	7	142	1.000	0.993	1.000
border <i>musculus</i>	M1	49.05	6	121	1.000	0.984	1.000
border <i>musculus</i>	M3	47.65	1	21	1.000	0.949	1.000
H1	HZ65	20.77	10	184	0.859	0.817	0.894
H1	HZT1	19.71	7	143	0.935	0.896	0.965
H2	HZ59	15.95	4	82	0.891	0.826	0.942
H2	HZ60	15.71	14	285	0.889	0.857	0.918
H2	HZ49	14.75	1	20	0.959	0.858	0.997
H2	HZ44	14.57	1	18	0.726	0.559	0.861
H2	HZ48	14.55	3	61	0.941	0.886	0.975
H2	HZ54	14.118	1	21	0.700	0.540	0.834
H3	HZ38	12.97	10	204	0.789	0.744	0.831
H3	HZ50	12.95	2	40	0.812	0.709	0.892
H3	HZ27	10.81	6	123	0.913	0.867	0.949
H3	HZ37	10.2	6	123	0.861	0.809	0.905
H4	HZ62	9.63	5	102	0.688	0.620	0.752
H6	HZ35	6.45	10	206	0.829	0.787	0.866
-	HZ58	-5.26	3	60	0.057	0.024	0.112
H7	HZ12	-19.47	11	221	0.060	0.039	0.087
H7	HZ14	-20	6	119	0.071	0.041	0.112
H7	HZ9	-20.01	5	102	0.041	0.017	0.078
border <i>domesticus</i>	L7	-24.3	6	119	0.000	0.000	0.008
border <i>domesticus</i>	L6	-24.53	10	204	0.003	0.001	0.011
border <i>domesticus</i>	L3	-25.01	1	21	0.026	0.002	0.112
border <i>domesticus</i>	L10	-25.64	6	122	0.000	0.000	0.008
border <i>domesticus</i>	L14	-26.82	1	21	0.052	0.009	0.155
border <i>domesticus</i>	L8	-28.09	1	20	0.055	0.009	0.163
border <i>domesticus</i>	O4	-28.16	10	203	0.003	0.001	0.011
border <i>domesticus</i>	O5	-30.36	7	142	0.037	0.019	0.064
border <i>domesticus</i>	O1	-31.38	1	21	0.026	0.002	0.112
border <i>domesticus</i>	O3	-32.65	4	81	0.083	0.045	0.134
border <i>domesticus</i>	O7	-33.59	6	122	0.000	0.000	0.008
border <i>domesticus</i>	S11	-39.67	8	161	0.000	0.000	0.008
border <i>domesticus</i>	S10	-41.23	1	20	0.000	0.000	0.049
border <i>domesticus</i>	S7	-43.69	6	122	0.013	0.004	0.033
border <i>domesticus</i>	S8	-45.27	7	126	0.010	0.002	0.029

[1]. Raufaste N., Orth A., Belkhir K., Senet D., Smadja C., Baird S.J.E., Bonhomme F., Dod B., Boursot P. 2005 Inference of selection and migration in the danish house mouse hybrid zone. *Bio.l.l Linn. Soc.* 84, 593-616.