

1 **Sexual conflict over remating interval is modulated by**
2 **the sex peptide pathway**

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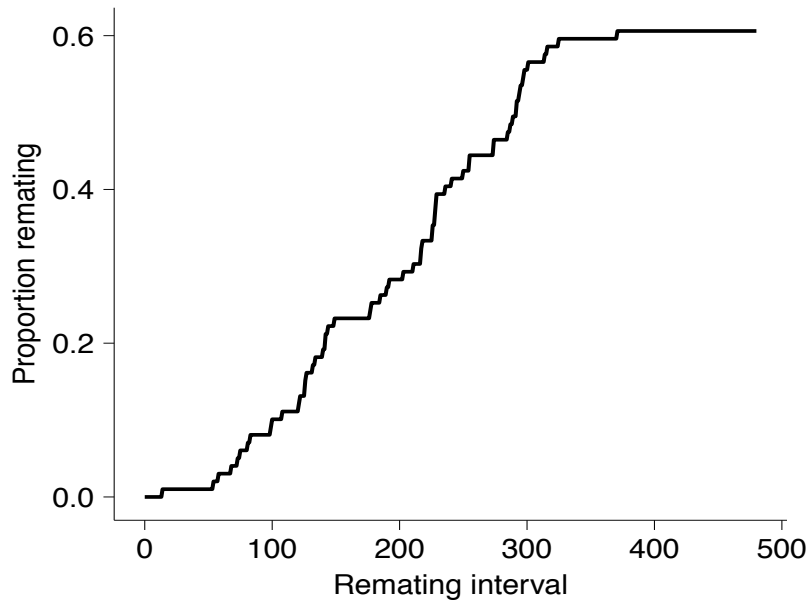
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14 **Electronic Supplementary Information**

15 Figures S1-S6.

16 Tables S1-S8.

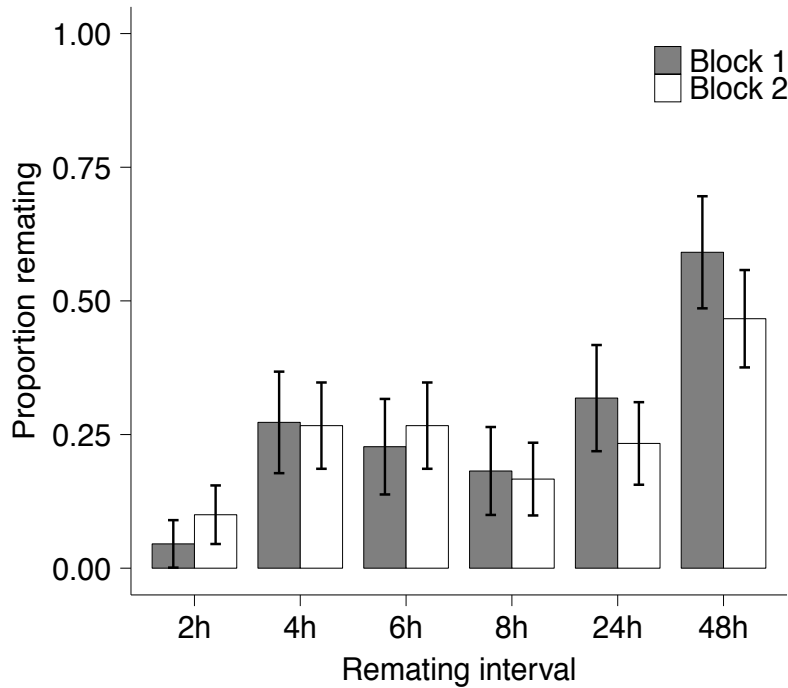
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19 **Figure S1.** Cumulative remating frequency of wild type females under continuous male
20 exposure. Shown is the cumulative proportion of wild type females remating, against
21 time in minutes, when continuously exposed to wild type males following first matings
22 with wild type males. The data show that ~60% of females remated within 6 hours (360
23 minutes).

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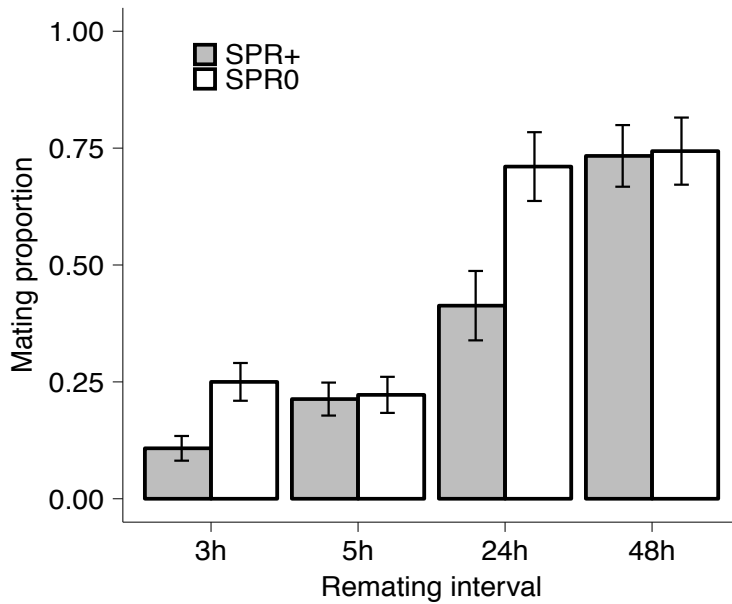


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27 **Figure S2.** Proportion of wild type females remating at different remating intervals in
 28 remating tests. Shown are two experimental blocks (block 1,2) of the proportion of wild
 29 type females that remated with wild type males at the remating intervals shown, after
 30 first mating with wild type males. ~30% of females remated as early as 4h after their
 31 first mating.

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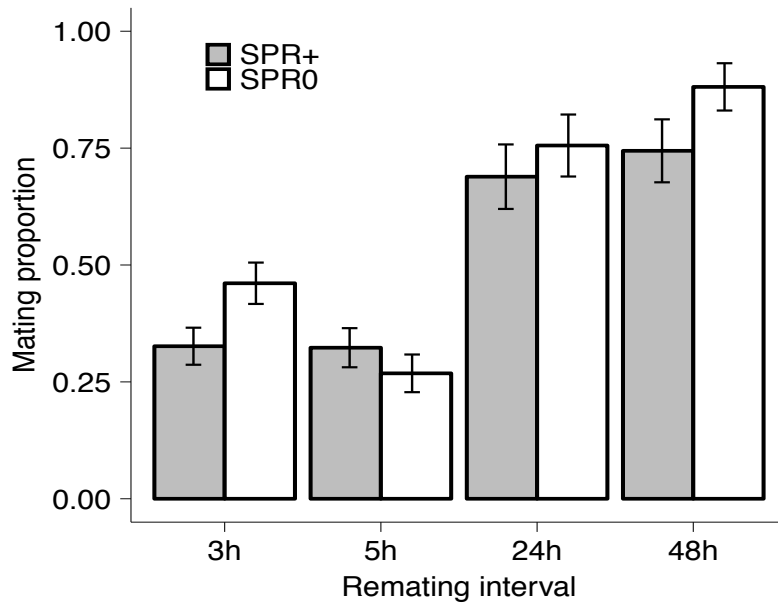


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35 **Figure S3.** Proportion of individuals that remated at different remating intervals in the
36 main fitness experiment. The proportion of control (SPR^+ , grey bars) or SPR^0 (white bars)
37 females in the main fitness experiment that remated with dsRed-sperm males at the
38 remating intervals shown, following first matings with GFP-sperm males. Consistent with
39 the data from the wild type and $SPR^{+/0}$ experiments (figures S1, S2, S4, S5) a high
40 proportion of females remated at the early time points tested, even in the control
41 females.

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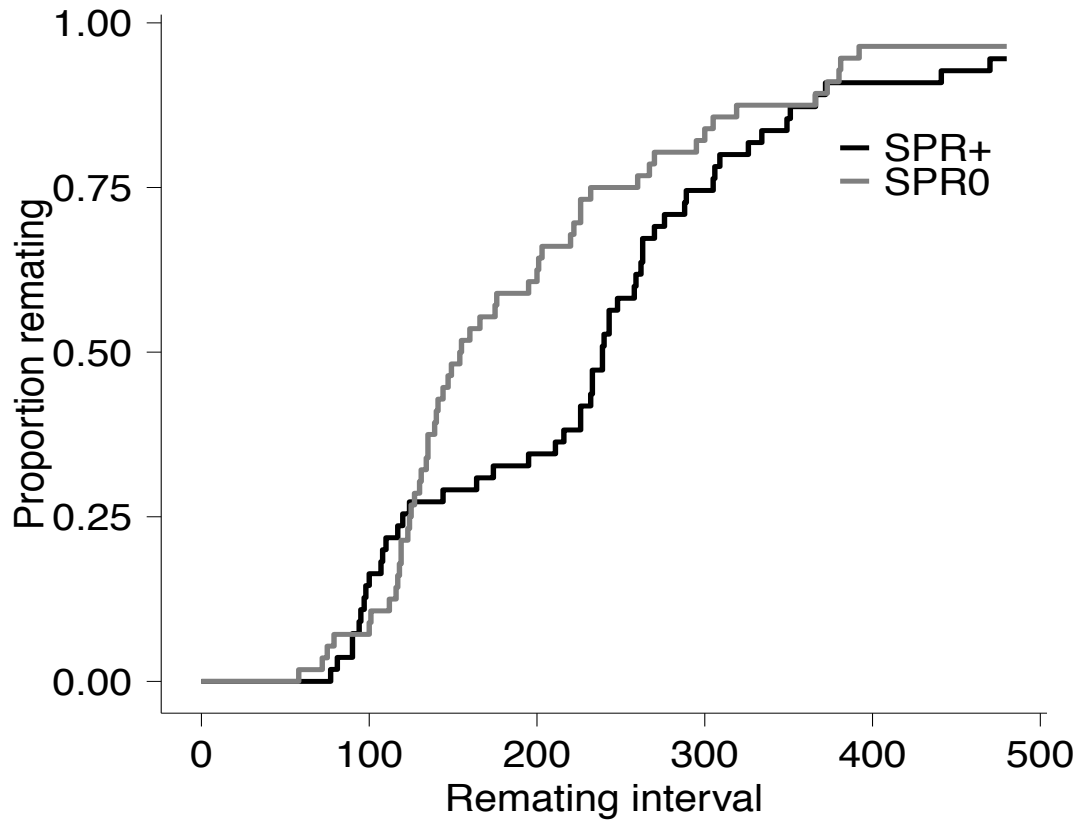
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45 **Figure S4.** Proportion of individuals that remated at different remating intervals for the
46 sperm count experiment. The proportion of control (SPR^+ , grey bars) or SPR^0 (white
47 bars) females in the sperm count experiment that remated with dsRed-sperm males at
48 the remating intervals shown, following first matings with GFP-sperm males. Consistent
49 with the data from the wild type and $SPR^{+/0}$ experiments (Figures S1, S2, S3, S5) a high
50 proportion of females remated at the early time points tested, even in the control
51 females.

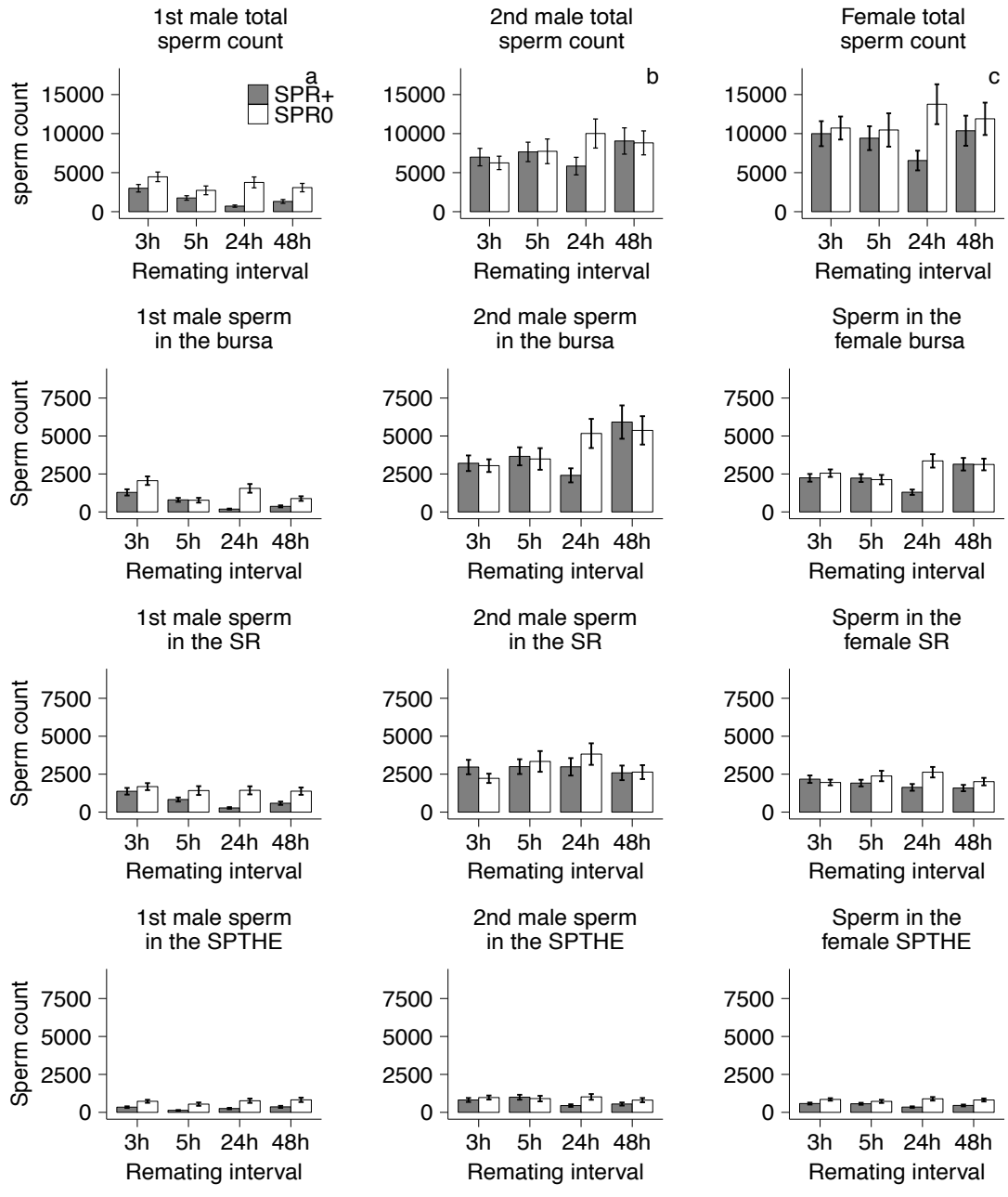
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54 **Figure S5.** Cumulative remating frequency of unmanipulated ("natural") rematings by

55 once-mated SPR^+ and SPR^0 females continuously exposed to wild type males.



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57 **Figure S6. Number of sperm from the first and second male in storage in the female.**

58 The number of sperm is in arbitrary units of fluorescence derived from the number of
 59 fluorescent pixels in each of the captured images (mean \pm se). Shown in the LH column in
 60 each case is the sperm count for the first male (green fluorescence), in the middle
 61 column the count for the second male (red fluorescence) and in the RH column the total
 62 sperm count (total fluorescence). The top row shows the sperm counts for all the sperm

63 storage organs plus the bursa, the second row the sperm counts for the bursa only, the
64 third for the seminal receptacle (SR) only and the bottom row for the two spermathecae
65 combined (SPTHE). The results show that there was little correlation between the
66 pattern of sperm storage and offspring paternity / numbers shown in figure 1 in the
67 main text.

68 **Table S1.** The number of flies that were initially setup, that mated, that did not mate
 69 (unmated), that were lost during the setup and the mating success, for each remating
 70 timepoint ('time') and female *SPR* status in the main fitness experiment.

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time	<i>SPR</i> ^{+ / 0}	setup	mated	unmated	lost	mating success	se
0h	<i>SPR</i> ⁺	400	336	22	12	0.84	0.02
0h	<i>SPR</i> ⁰	400	310	88	2	0.78	0.02
3h	<i>SPR</i> ⁺	139	15	123	1	0.11	0.03
3h	<i>SPR</i> ⁰	116	29	86	1	0.25	0.04
5h	<i>SPR</i> ⁺	136	29	106	1	0.21	0.04
5h	<i>SPR</i> ⁰	117	26	90	1	0.22	0.04
24h	<i>SPR</i> ⁺	46	19	25	2	0.41	0.07
24h	<i>SPR</i> ⁰	38	27	11	0	0.71	0.07
48h	<i>SPR</i> ⁺	45	33	12	0	0.73	0.07
48h	<i>SPR</i> ⁰	39	29	8	2	0.74	0.07

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74 **Table S2.** The number of flies that were initially setup, that mated, that did not mate
 75 (unmated), that were lost during the setup and the mating success, for each remating
 76 timepoint ('time') and female *SPR* status in the main sperm storage experiment.

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time	<i>SPR</i> ^{+/-0}	setup	mated	unmated	lost	mating success	se
0h	<i>SPR</i> ⁺	400	357	22	21	0.89	0.02
0h	<i>SPR</i> ⁰	400	338	37	25	0.84	0.02
3h	<i>SPR</i> ⁺	141	46	94	1	0.33	0.04
3h	<i>SPR</i> ⁰	128	59	68	1	0.46	0.04
5h	<i>SPR</i> ⁺	130	42	83	5	0.32	0.04
5h	<i>SPR</i> ⁰	123	33	88	2	0.27	0.04
24h	<i>SPR</i> ⁺	45	31	14	0	0.69	0.07
24h	<i>SPR</i> ⁰	45	34	8	3	0.76	0.07
48h	<i>SPR</i> ⁺	43	32	10	1	0.74	0.07
48h	<i>SPR</i> ⁰	42	37	4	1	0.88	0.05

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80 **Table S3.** ANOVA table for first male offspring production with remating interval ('time')
 81 and female *SPR* status ('SPR') and their interactions as explanatory factors.

			Resid.	Resid.		
	Df	Deviance	Df	Dev	F	Pr(>F)
NULL	NA	NA	121	1484.360	NA	NA
time	3	272.767	118	1211.593	8.885	0.000
SPR	1	6.100	117	1205.493	0.596	0.442
time:SPR	3	118.700	114	1086.794	3.867	0.011

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83 **Table S4.** ANOVA table for second male offspring production with remating interval
 84 ('time') and female *SPR* status ('SPR') and their interactions as explanatory factors.

			Resid.	Resid.		
	Df	Deviance	Df	Dev	F	Pr(>F)
NULL	NA	NA	208	2807.138	NA	NA
time	3	464.178	205	2342.960	17.746	0.000
SPR	1	368.103	204	1974.857	42.219	0.000
time:SPR	3	114.450	201	1860.407	4.376	0.005

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86 **Table S5.** ANOVA table for female offspring production with remating interval ('time')
 87 and female *SPR* status ('SPR') and their interactions as explanatory factors.

			Resid.	Resid.		
	Df	Deviance	Df	Dev	F	Pr(>F)
NULL	NA	NA	208	99190.47	NA	NA
time	3	6704.451	205	92486.02	6.258	0.000
SPR	1	14634.439	204	77851.58	40.983	0.000
time:SPR	3	6076.582	201	71775.00	5.672	0.001

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89 **Table S6.** ANOVA table showing the analysis of first male sperm numbers (arbitrary units
90 of fluorescence) stored in the female reproductive tract with sperm storage organ
91 ('tissue'), remating interval ('time') and female *SPR* status ('SPR') and their interactions
92 as explanatory factors.

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL	NA	NA	815	1830577	NA	NA
time	3	55356.625	812	1775220	6.466	0.000
SPR	1	76245.565	811	1698975	26.717	0.000
tissue	2	88035.587	809	1610939	15.424	0.000
time:SPR	3	28283.131	806	1582656	3.304	0.020
time:tissue	6	20957.835	800	1561698	1.224	0.292
SPR:tissue	2	1391.093	798	1560307	0.244	0.784
time:SPR:tissue	6	10357.879	792	1549949	0.605	0.727

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94 **Table S7.** ANOVA table for second male sperm numbers (arbitrary units of fluorescence)
 95 stored in the female reproductive tract with sperm storage organ ('tissue'), remating
 96 interval ('time') and female SPR status ('SPR') and their interactions as explanatory
 97 factors.

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	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL	NA	NA	815	2998814	NA	NA
time	3	28323.921	812	2970490	3.492	0.015
SPR	1	2992.911	811	2967497	1.107	0.293
tissue	2	613738.427	809	2353759	113.501	0.000
time:SPR	3	29962.817	806	2323796	3.694	0.012
time:tissue	6	46521.726	800	2277274	2.868	0.009
SPR:tissue	2	3964.935	798	2273309	0.733	0.481
time:SPR:tissue	6	9772.519	792	2263537	0.602	0.729

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101 **Table S8.** ANOVA table for total sperm numbers (arbitrary units of fluorescence) stored
 102 in the female reproductive tract with sperm storage organ ('tissue'), remating interval
 103 ('time') and female SPR status and their interactions as explanatory factors.

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL	NA	NA	815	3280204	NA	NA
time	3	5640.558	812	3274563	0.598	0.616
SPR	1	35197.246	811	3239366	11.194	0.001
tissue	2	660016.339	809	2579350	104.950	0.000
time:SPR	3	43634.068	806	2535715	4.626	0.003
time:tissue	6	24901.855	800	2510814	1.320	0.246
SPR:tissue	2	7993.723	798	2502820	1.271	0.281
time:SPR:tissue	6	13777.527	792	2489042	0.730	0.625

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