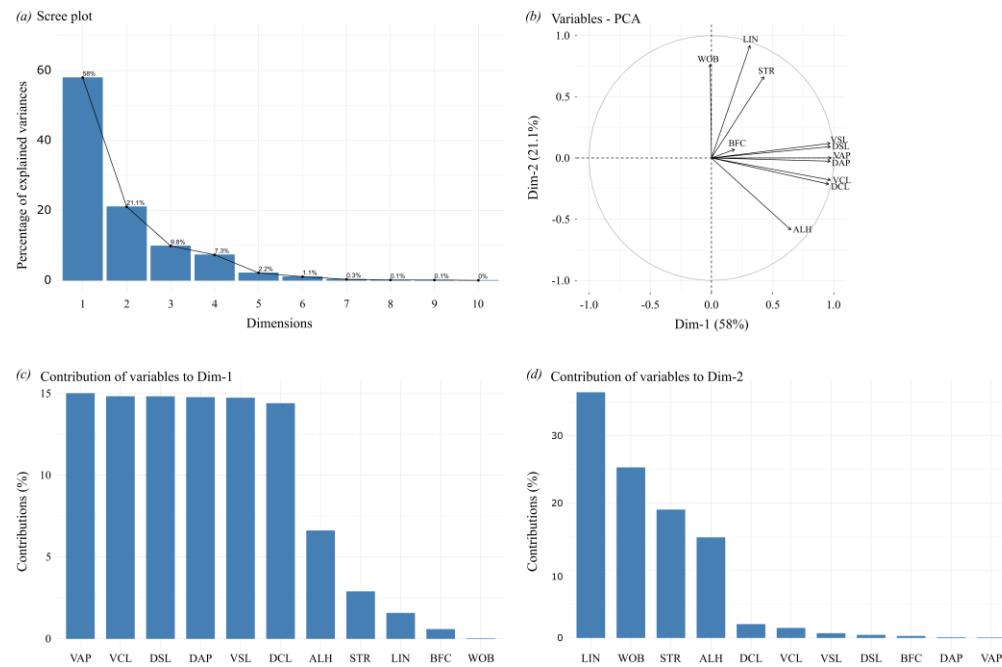


## Electronic supplementary material.

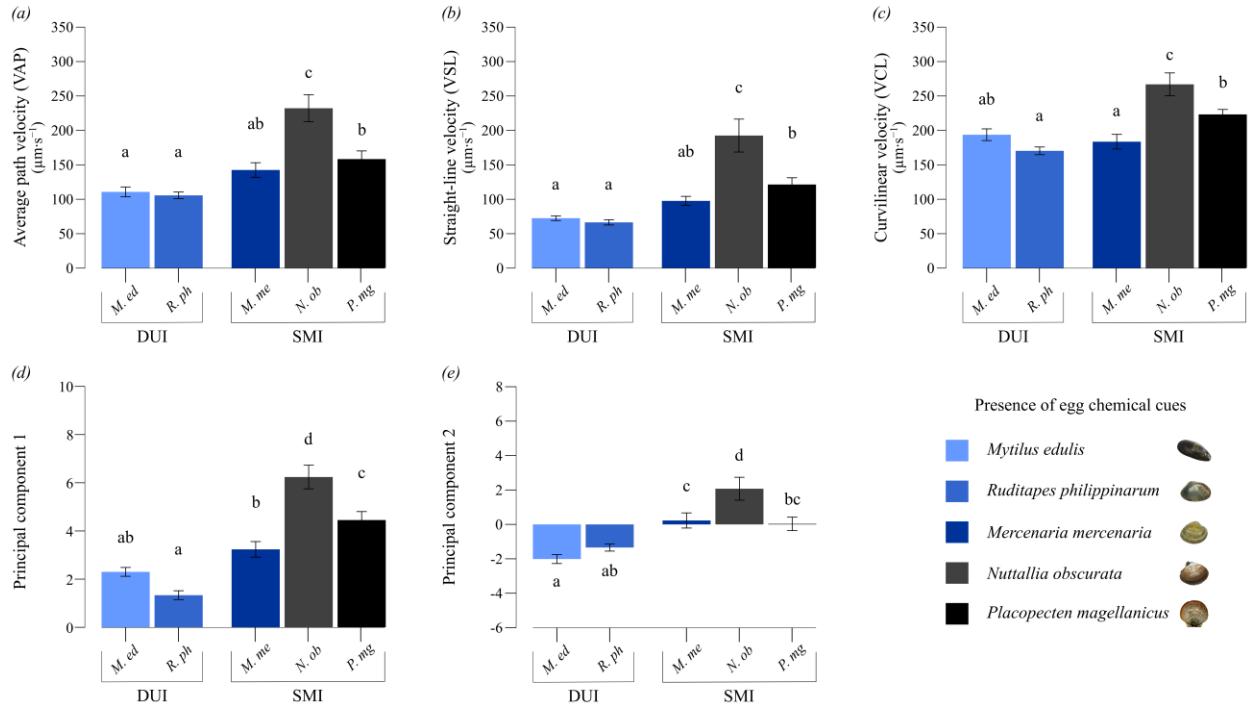
# LINKING PATERNALLY-INHERITED mtDNA VARIANTS AND SPERM PERFORMANCE

Stefano Bettinazzi, Sugahendni Nadarajah, Andréanne Dalpé, Liliana Milani, Pierre U Blier and Sophie Breton

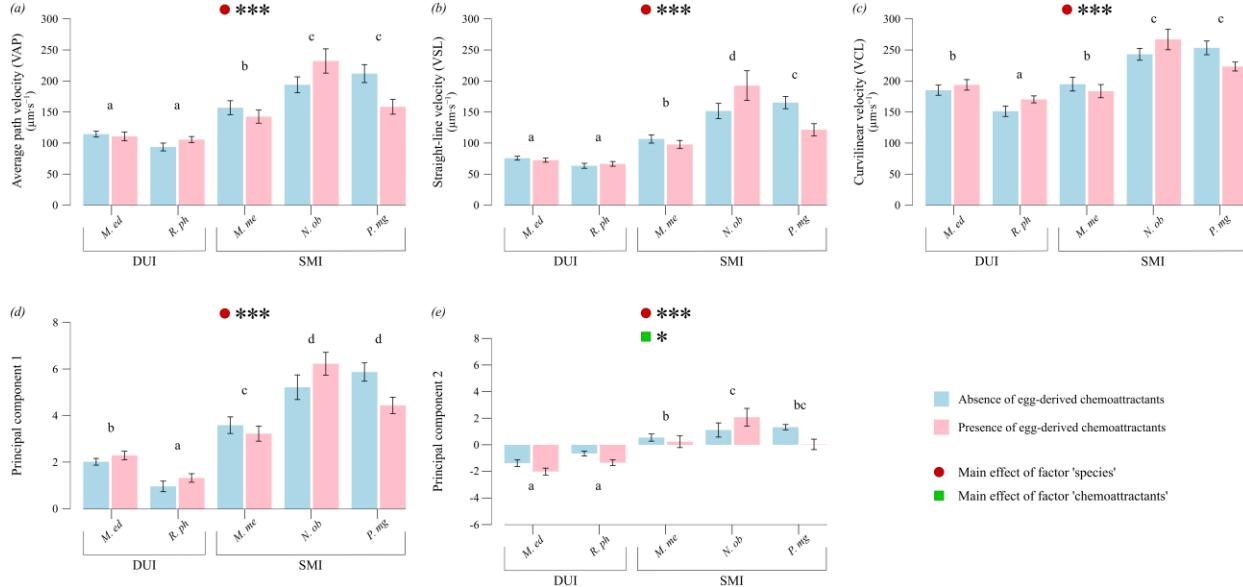
## SUPPORTING FIGURES AND TABLES



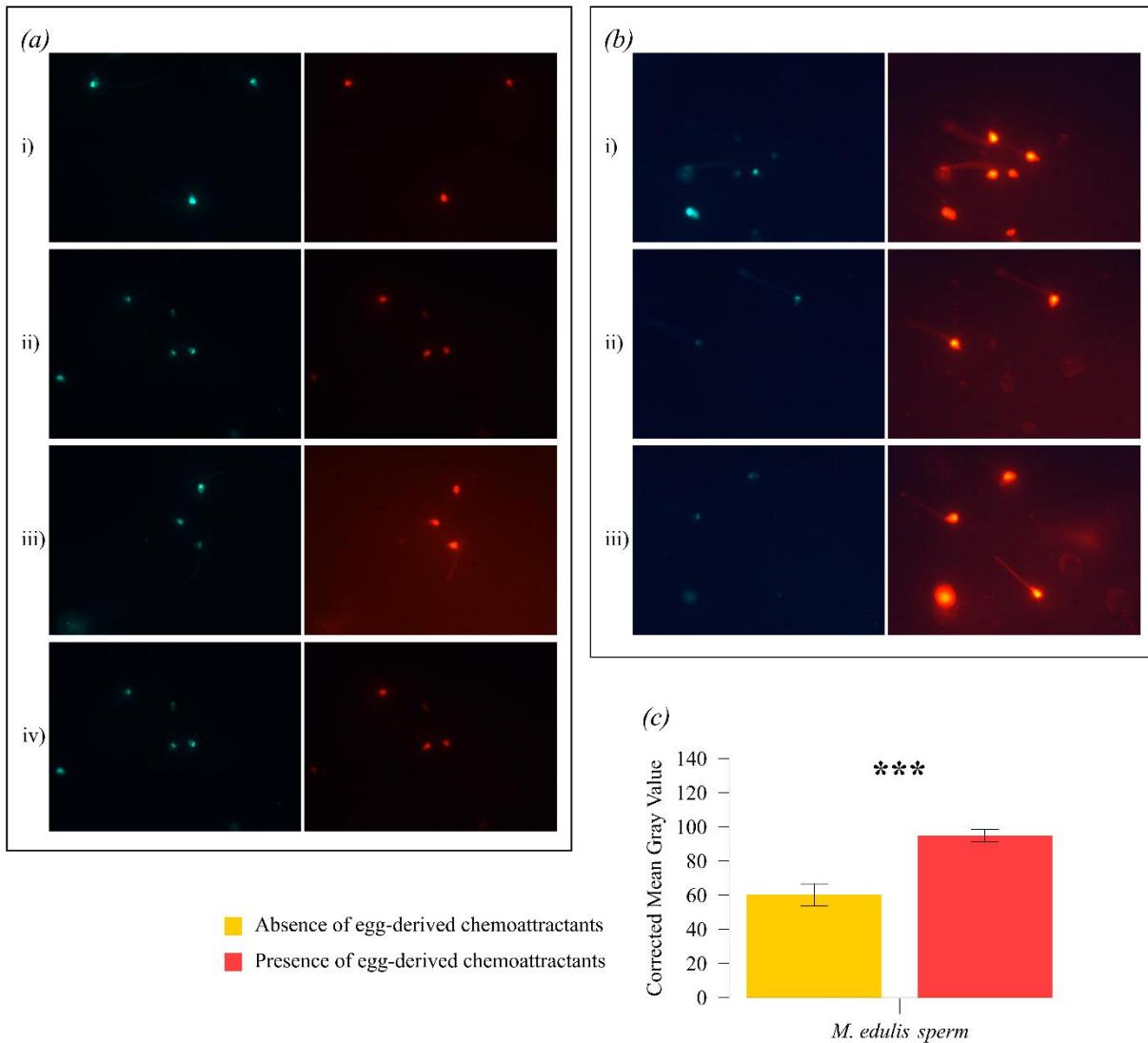
**Figure s1. PCA summary.** (a) Percentage of explained variance of each principal component. (b) Variable correlation plots. (c) Contribution of variables to the first principal component (PC1). (d) Contribution of variables to the second principal component (PC2). Sperm motility parameters: DAP, average path distance ( $\mu\text{m}$ ); DSL, straight-line distance ( $\mu\text{m}$ ); DCL, curvilinear distance ( $\mu\text{m}$ ); VAP, average path velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ); VSL, straight-line velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ); VCL, curvilinear velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ); STR, straightness (VSL/VAP); LIN, linearity (VSL/VCL); ALH, amplitude of lateral head displacement ( $\mu\text{m}$ ); BFC, beat-cross frequency (Hz); WOB, wobble coefficient (VAP/VCL). Additional information in table s1.



**Figure s2.** Basal sperm motility parameters in five bivalve species, DUI and SMI, with presence of chemoattractants. (a) Average path velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ). (b) Straight-line velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ). (c) Curvilinear velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ). (d) First principal component of the PCA combining sperm velocity parameters. (e) Second principal component of the PCA. Data are presented as means  $\pm$  s.e.m. Differences ( $p \leq 0.05$ ) in a *post hoc* Tukey's test are indicated by different letters. DUI species: *M. edulis* (*M. ed*,  $n = 11$ ), *R. philippinarum* (*R. ph*,  $n = 9$ ). SMI species: *M. mercenaria* (*M. me*,  $n = 9$ ), *N. obscurata* (*N. ob*,  $n = 5$ ), *P. magellanicus* (*P. mg*,  $n = 11$ ). Detailed summary is reported in electronic supplementary material, tables s2 and s3.



**Figure s3.** Sperm motility parameters comparison among five bivalve species, DUI and SMI, with and without chemoattractants. (a) Average path velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ). (b) Straight-line velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ). (c) Curvilinear velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ). (d) First principal component of the PCA combining sperm velocity parameters. (e) Second principal component of the PCA. Values are presented as means  $\pm$  s.e.m. A linear mixed model was implemented for each parameter separately. The main effect of the two fixed factors ‘species’ and ‘chemoattractants’ are indicated with a circle and square respectively.  $*p \leq 0.05$ ,  $**p \leq 0.01$ ,  $***p \leq 0.001$ . Differences among species are indicated by letters in subscript. DUI, doubly uniparental inheritance; SMI, strict maternal inheritance. Species: *M. edulis* (*M. ed*,  $n = 11$ ); *R. philippinarum* (*R. ph*,  $n = 9$ ); *M. mercenaria* (*M. me*,  $n = 9$ ); *N. obscurata* (*N. ob*,  $n = 5$ ); *P. magellanicus* (*P. mg*,  $n = 11$ ). Detailed summary is reported in electronic supplementary material, tables s2 and s4.



**Figure s4.** Membrane potential of *Mytilus edulis* (DUI) sperm mitochondria following the addition of egg-derived chemoattractants. The fluorescent dyes MitoSpy™ Green FM (400 nM; excitation/emission 490/516 nm) and MitoSpy™ Red CMXRos (500 nM; excitation/emission 577/598 nm) (BioLegend Inc, San Diego, California) were used to localize sperm mitochondria (green stain) and quantify their membrane potential (red stain), respectively. (a) Absence of egg-derived chemoattractants. (b) Presence of egg-derived chemoattractants. (c) Quantification and comparison of sperm mitochondria membrane potential without ( $n = 15$  spermatozoa) and with egg-derived chemoattractants ( $n = 15$  spermatozoa). Fluorescence intensity has been quantified as mean grey value per pixel and corrected for the relative background fluorescence. Values are presented as means  $\pm$  s.e.m. The effect of oocytes detection has been tested through a paired *t* test. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ .

**Table s1.** PCA summary. Contribution and correlation of the variables with principal components. The contributions of variables in accounting for the variability in a given principal component are expressed in percentage. Significant correlation coefficients ( $p \leq 0.05$ ) are shown in bold.

Table s1	PC1		PC2		
	Variable	Contribution	Correlation	Contribution	Correlation
DAP	14.74	<b>0.97</b>		0.03	-0.03
DSL	14.80	<b>0.97</b>		0.37	<b>0.09</b>
DCL	14.37	<b>0.96</b>		1.97	<b>-0.21</b>
VAP	14.99	<b>0.98</b>		0.00	0.00
VSL	14.71	<b>0.97</b>		0.62	<b>0.12</b>
VCL	14.80	<b>0.97</b>		1.40	<b>-0.18</b>
STR	2.87	<b>0.43</b>		18.97	<b>0.66</b>
LIN	1.55	<b>0.31</b>		36.37	<b>0.92</b>
ALH	6.60	<b>0.65</b>		14.84	<b>-0.59</b>
BFC	0.56	<b>0.19</b>		0.21	0.07
WOB	0.00	-0.01		25.22	<b>0.76</b>

**Table s2.** Data summary table. Sperm motility parameters measured. Inheritance: DUI, doubly uniparental inheritance; SMI, strict maternal inheritance. Species: MyEd, *M. edulis* ( $n = 11$ ); RuPh, *R. philippinarum* ( $n = 9$ ); MeMe, *M. mercenaria* ( $n = 9$ ); NuOb, *N. obscurata* ( $n = 5$ ); PiMg, *P. magellanicus* ( $n = 11$ ). Sperm motility parameters: DAP, average path distance ( $\mu\text{m}$ ); DSL, straight-line distance ( $\mu\text{m}$ ); DCL, curvilinear distance ( $\mu\text{m}$ ); VAP, average path velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ); VSL, straight-line velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ); VCL, curvilinear velocity ( $\mu\text{m}\cdot\text{s}^{-1}$ ); STR, straightness (VSL/VAP); LIN, linearity (VSL/VCL); ALH, amplitude of lateral head displacement ( $\mu\text{m}$ ); BFC, beat-cross frequency (Hz); WOB, wobble coefficient (VAP/VCL); PC1, principal component 1; PC2, principal component 2.

**Table s2**

DAP	DSL	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2	Inheritance	Species	Treatment	Chem	ID
52.90	32.39	93.14	107.70	68.10	183.81	63.99	38.74	10.58	23.11	59.83	1.65	-1.77	DUI	MyEd	Ctrl	N	MyEd_1
9.70	6.73	14.42	21.49	14.99	32.81	73.17	51.80	2.56	29.28	69.60	-2.42	1.15	DUI	MyEd	Rot	N	MyEd_1
9.38	6.93	13.37	19.98	15.04	28.95	72.33	55.30	1.96	33.31	71.78	-2.45	1.51	DUI	MyEd	Ama	N	MyEd_1
7.15	3.92	14.26	15.54	9.66	31.20	55.47	32.68	2.17	45.75	52.27	-2.87	-1.08	DUI	MyEd	Omy	N	MyEd_1
58.69	37.96	100.53	129.96	87.97	214.06	68.65	44.32	9.41	30.10	63.30	2.46	-1.01	DUI	MyEd	Oxa	N	MyEd_1
104.38	40.19	140.71	165.54	76.38	225.83	45.19	32.86	8.64	28.01	74.01	3.19	-1.83	DUI	MyEd	Ctrl	ch	MyEd_1
10.77	8.31	14.75	14.65	11.30	20.05	79.85	64.59	1.51	25.37	78.67	-2.46	2.57	DUI	MyEd	Rot	ch	MyEd_1
9.47	7.79	11.82	13.69	11.30	17.05	87.03	68.06	0.79	29.55	78.53	-2.44	3.08	DUI	MyEd	Ama	ch	MyEd_1
8.67	7.32	10.49	13.33	11.37	15.96	87.64	72.22	2.01	28.02	82.14	-2.33	3.32	DUI	MyEd	Omy	ch	MyEd_1
54.19	30.52	95.23	106.95	64.99	187.67	66.61	40.11	11.54	23.75	59.46	1.78	-1.79	DUI	MyEd	Oxa	ch	MyEd_1
65.31	46.66	92.66	108.46	79.79	152.52	77.07	53.05	8.68	30.48	69.16	2.18	0.21	DUI	MyEd	Ctrl	N	MyEd_2
8.11	4.39	15.09	22.54	13.76	42.30	55.90	38.17	3.57	40.30	59.17	-2.57	-0.68	DUI	MyEd	Rot	N	MyEd_2
8.25	6.19	13.35	27.14	21.25	43.69	72.35	51.22	3.16	34.04	65.37	-2.22	0.82	DUI	MyEd	Ama	N	MyEd_2
8.95	5.66	18.22	24.88	16.88	52.88	61.77	36.46	4.86	46.40	52.16	-2.17	-1.10	DUI	MyEd	Omy	N	MyEd_2
52.59	32.82	77.62	109.51	71.61	155.20	70.46	47.02	10.35	26.12	67.04	1.55	-0.64	DUI	MyEd	Oxa	N	MyEd_2
67.14	45.80	116.62	116.47	80.54	200.60	71.20	39.96	12.00	26.51	57.23	2.81	-1.85	DUI	MyEd	Ctrl	ch	MyEd_2
9.10	6.55	12.32	12.38	8.91	16.76	76.84	60.43	1.51	27.07	76.71	-2.65	2.19	DUI	MyEd	Rot	ch	MyEd_2
6.39	5.75	8.10	8.97	8.08	11.38	89.50	69.87	0.72	27.32	77.70	-2.62	3.21	DUI	MyEd	Ama	ch	MyEd_2
6.60	6.02	9.10	8.98	8.19	12.37	91.22	66.39	0.61	31.31	72.72	-2.58	2.87	DUI	MyEd	Omy	ch	MyEd_2
41.76	31.67	68.75	105.76	82.55	167.53	80.01	53.34	8.82	29.64	65.61	1.52	0.14	DUI	MyEd	Oxa	ch	MyEd_2
50.41	37.41	94.46	113.40	83.41	195.51	73.92	40.84	9.32	26.42	55.22	2.01	-1.39	DUI	MyEd	Ctrl	N	MyEd_3
14.55	6.85	21.96	20.82	9.53	32.31	63.24	49.35	2.68	33.05	72.90	-2.44	0.86	DUI	MyEd	Rot	N	MyEd_3
9.27	7.50	11.27	12.61	10.20	15.33	83.76	68.27	0.84	23.69	81.90	-2.57	3.13	DUI	MyEd	Ama	N	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	N	MyEd_3
44.29	31.90	81.30	85.67	63.58	153.32	74.41	42.96	8.75	30.71	57.30	1.13	-1.00	DUI	MyEd	Oxa	N	MyEd_3
49.63	31.93	91.12	96.06	66.14	169.67	74.04	37.78	11.05	21.54	53.58	1.54	-1.87	DUI	MyEd	Ctrl	ch	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Rot	ch	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	ch	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	ch	MyEd_3
49.18	32.12	92.18	89.81	59.87	162.63	70.49	38.73	9.90	22.42	55.43	1.28	-1.69	DUI	MyEd	Oxa	ch	MyEd_3
68.25	39.71	100.57	125.83	79.64	182.85	64.65	43.54	8.55	29.31	68.01	2.20	-0.78	DUI	MyEd	Ctrl	N	MyEd_4
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Rot	N	MyEd_4
7.21	6.57	8.83	12.08	11.14	14.43	90.96	73.76	1.11	30.06	80.85	-2.41	3.57	DUI	MyEd	Ama	N	MyEd_4
4.87	4.18	6.77	6.62	5.69	9.20	85.85	61.79	0.49	28.49	71.98	-2.86	2.45	DUI	MyEd	Omy	N	MyEd_4
52.41	36.49	111.77	82.47	56.41	173.07	68.42	32.86	11.45	18.38	48.18	1.58	-2.72	DUI	MyEd	Oxa	N	MyEd_4
59.00	39.34	115.72	94.26	63.18	182.44	68.19	34.77	10.43	29.28	51.29	1.98	-2.29	DUI	MyEd	Ctrl	ch	MyEd_4
10.25	8.05	13.59	19.58	14.42	28.75	81.59	64.70	1.85	22.44	78.32	-2.35	2.54	DUI	MyEd	Rot	ch	MyEd_4
12.90	6.41	16.15	27.65	18.69	33.34	74.81	60.04	2.85	43.11	80.13	-2.03	2.18	DUI	MyEd	Ama	ch	MyEd_4
6.13	4.95	8.44	15.60	10.60	24.80	79.00	62.68	2.13	23.42	74.94	-2.60	2.17	DUI	MyEd	Omy	ch	MyEd_4
47.13	32.01	93.51	85.84	58.22	164.12	68.89	36.18	9.47	29.59	52.27	1.22	-1.93	DUI	MyEd	Oxa	ch	MyEd_4
50.74	29.47	91.10	117.82	72.55	206.85	63.87	37.37	11.04	25.88	58.24	1.84	-2.00	DUI	MyEd	Ctrl	N	MyEd_5
21.86	9.21	41.87	44.09	22.98	78.03	49.83	34.07	5.52	26.58	61.87	-1.70	-1.39	DUI	MyEd	Rot	N	MyEd_5
19.03	5.39	26.48	26.04	7.38	36.33	37.47	29.61	3.07	26.59	76.33	-2.86	-0.82	DUI	MyEd	Ama	N	MyEd_5
15.06	14.88	15.99	20.48	20.24	21.75	98.82	93.07	0.78	25.05	94.18	-1.71	5.43	DUI	MyEd	Omy	N	MyEd_5
52.16	34.31	89.69	116.32	78.52	194.98	68.12	40.26	10.33	28.35	59.31	1.94	-1.51	DUI	MyEd	Oxa	N	MyEd_5
49.05	26.85	104.62	97.32	55.98	200.47	57.56	29.86	11.75	28.58	49.65	1.52	-3.15	DUI	MyEd	Ctrl	ch	MyEd_5















40.16	28.12	68.62	70.62	50.25	114.53	62.94	37.62	8.67	17.81	59.00	0.13	-1.51	SMI	PIMg	Rot	N	PIMg_10
9.32	8.09	11.85	12.68	11.01	16.11	86.25	65.85	0.89	25.62	76.21	-2.51	2.80	SMI	PIMg	Ama	N	PIMg_10
23.90	10.68	33.43	49.42	26.84	67.81	52.95	41.03	5.04	20.02	75.81	-1.72	-0.16	SMI	PIMg	Omy	N	PIMg_10
71.51	57.83	100.62	123.70	102.99	169.72	78.63	53.54	5.70	33.22	65.49	2.72	0.52	SMI	PIMg	Oxa	N	PIMg_10
95.64	73.67	125.96	176.86	142.70	229.45	81.56	59.32	7.02	35.41	72.84	4.83	1.01	SMI	PIMg	Ctrl	ch	PIMg_10
28.10	17.36	48.06	45.43	28.74	76.09	59.15	38.10	7.11	16.99	63.92	-1.17	-1.07	SMI	PIMg	Rot	ch	PIMg_10
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PIMg	Ama	ch	PIMg_10
17.74	7.43	25.63	33.45	16.30	50.19	47.81	36.12	3.86	21.71	74.77	-2.43	-0.40	SMI	PIMg	Omy	ch	PIMg_10
58.51	46.91	101.83	90.42	72.93	154.14	76.61	44.20	8.93	24.07	56.44	1.88	-1.01	SMI	PIMg	Oxa	ch	PIMg_10
124.74	80.57	144.74	242.53	174.01	280.26	73.11	61.33	8.56	26.93	84.22	6.48	1.09	SMI	PIMg	Ctrl	N	PIMg_11
53.31	34.58	70.51	105.07	76.93	135.02	64.05	45.33	4.91	34.41	69.93	0.93	0.08	SMI	PIMg	Rot	N	PIMg_11
8.54	5.81	10.25	14.51	10.60	17.08	74.95	62.85	1.05	18.65	83.63	-2.78	2.63	SMI	PIMg	Ama	N	PIMg_11
20.71	10.15	32.13	28.84	14.34	44.89	54.60	39.69	4.24	21.27	72.59	-2.21	-0.20	SMI	PIMg	Omy	N	PIMg_11
101.86	70.47	126.72	154.39	108.23	192.43	73.00	55.92	5.81	35.97	76.09	3.96	0.91	SMI	PIMg	Oxa	N	PIMg_11
112.66	75.24	148.67	184.08	130.03	239.86	73.49	52.16	7.91	32.88	71.72	5.17	0.10	SMI	PIMg	Ctrl	ch	PIMg_11
48.84	36.62	71.00	83.47	66.04	116.59	63.19	43.11	4.30	33.01	67.15	0.47	-0.10	SMI	PIMg	Rot	ch	PIMg_11
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PIMg	Ama	ch	PIMg_11
18.44	7.79	26.96	25.48	10.98	37.09	48.57	35.33	3.13	23.19	73.84	-2.62	-0.35	SMI	PIMg	Omy	ch	PIMg_11
83.46	65.37	114.99	134.98	108.34	183.11	81.02	57.02	6.10	35.20	70.04	3.46	0.90	SMI	PIMg	Oxa	ch	PIMg_11

**Table s3.** Sperm motility parameters comparison between five bivalve species, both DUI and SMI, with and without chemoattractants. Values are presented as means  $\pm$  s.e.m. The effect of the factor 'species' ( $F$  and  $p$ ) was assessed separately for each parameter and presence/absence of egg chemical cues by means of a one way ANOV A, followed by a Tukey's multi comparison test (result indicates by letters in subscript). Significant differences ( $p \leq 0.05$ ) are shown in bold. Inheritance: For abbreviations refer to table s2.

Tables s3	Inheritance	Species	DAP	DSL	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2
	DUI	MyEd	57.96 $\pm$ 2.75 <sup>a</sup>	36.88 $\pm$ 1.71 <sup>a</sup>	96.86 $\pm$ 4.05 <sup>ab</sup>	114.06 $\pm$ 4.66 <sup>a</sup>	75.41 $\pm$ 3.16 <sup>a</sup>	184.87 $\pm$ 8.19 <sup>ab</sup>	67.57 $\pm$ 1.74 <sup>a</sup>	41.82 $\pm$ 1.57 <sup>a</sup>	10.44 $\pm$ 0.49 <sup>a</sup>	25.59 $\pm$ 0.99 <sup>a</sup>	61.6 $\pm$ 1.59 <sup>a</sup>	2.02 $\pm$ 0.14 <sup>a</sup>	-1.38 $\pm$ 0.26 <sup>a</sup>
	DUI	RuPh	47.83 $\pm$ 3.29 <sup>a</sup>	30.06 $\pm$ 1.87 <sup>a</sup>	79.35 $\pm$ 4.24 <sup>a</sup>	93.42 $\pm$ 6.28 <sup>a</sup>	63.16 $\pm$ 4.04 <sup>a</sup>	150.95 $\pm$ 8.34 <sup>a</sup>	70.51 $\pm$ 1.43 <sup>a</sup>	43.56 $\pm$ 1.31 <sup>a</sup>	7.34 $\pm$ 0.4 <sup>b</sup>	27.76 $\pm$ 1.22 <sup>ab</sup>	62.31 $\pm$ 2.05 <sup>a</sup>	0.97 $\pm$ 0.23 <sup>a</sup>	-0.66 $\pm$ 0.19 <sup>a</sup>
	SMI	MeMe	92.15 $\pm$ 6.57 <sup>b</sup>	58.32 $\pm$ 3.47 <sup>b</sup>	115.31 $\pm$ 6.13 <sup>bc</sup>	156.69 $\pm$ 11.27 <sup>b</sup>	106.29 $\pm$ 6.65 <sup>b</sup>	194.51 $\pm$ 11.01 <sup>b</sup>	71.08 $\pm$ 2.41 <sup>a</sup>	54.88 $\pm$ 2.1 <sup>b</sup>	7.97 $\pm$ 0.49 <sup>b</sup>	27.17 $\pm$ 1.07 <sup>a</sup>	77.66 $\pm$ 1.76 <sup>b</sup>	3.59 $\pm$ 0.36 <sup>b</sup>	0.54 $\pm$ 0.27 <sup>b</sup>
	SMI	NuOb	102.65 $\pm$ 8.88 <sup>b</sup>	76.51 $\pm$ 6.39 <sup>c</sup>	129.03 $\pm$ 7.79 <sup>c</sup>	193.58 $\pm$ 12.73 <sup>bc</sup>	151.32 $\pm$ 12.26 <sup>b</sup>	242.62 $\pm$ 9.6 <sup>b</sup>	76.68 $\pm$ 3.59 <sup>ab</sup>	60.87 $\pm$ 4.38 <sup>bc</sup>	7.26 $\pm$ 0.46 <sup>b</sup>	33.89 $\pm$ 1.74 <sup>b</sup>	77.28 $\pm$ 2.9 <sup>b</sup>	5.22 $\pm$ 0.53 <sup>c</sup>	1.11 $\pm$ 0.53 <sup>b</sup>
	SMI	PlMg	112.4 $\pm$ 5.89 <sup>b</sup>	82.25 $\pm$ 3.92 <sup>c</sup>	136.9 $\pm$ 4.46 <sup>c</sup>	211.56 $\pm$ 14.47 <sup>c</sup>	164.82 $\pm$ 9.82 <sup>c</sup>	252.95 $\pm$ 11.04 <sup>c</sup>	78.84 $\pm$ 1.59 <sup>b</sup>	63.03 $\pm$ 1.79 <sup>c</sup>	7.76 $\pm$ 0.33 <sup>b</sup>	29.93 $\pm$ 1.74 <sup>ab</sup>	80 $\pm$ 2.82 <sup>b</sup>	5.87 $\pm$ 0.39 <sup>c</sup>	1.32 $\pm$ 0.2 <sup>b</sup>
	F	(4,40)	29.69	(4,40)	50.13	(4,40)	22.11	(4,40)	23.27	(4,40)	39.13	(4,40)	17.58	(4,40)	5.93
	P	<b>1.69E-11</b>	<b>4.62E-15</b>	<b>1.08E-09</b>	<b>5.42E-10</b>	<b>2.51E-13</b>	<b>2.11E-10</b>	<b>7.50E-08</b>	<b>2.11E-08</b>	<b>7.50E-04</b>	<b>2.26E-10</b>	<b>1.44E-05</b>	<b>7.20E-03</b>	<b>3.23E-08</b>	<b>8.45E-14</b>
	DUI	MyEd	63.07 $\pm$ 4.57 <sup>a</sup>	39.98 $\pm$ 2.02 <sup>a</sup>	111.17 $\pm$ 4.18 <sup>b</sup>	110.45 $\pm$ 7.16 <sup>a</sup>	72.27 $\pm$ 3.39 <sup>a</sup>	193.4 $\pm$ 8.51 <sup>ab</sup>	67.15 $\pm$ 2.99 <sup>ab</sup>	37.61 $\pm$ 1.72 <sup>a</sup>	11.14 $\pm$ 0.53 <sup>a</sup>	25.85 $\pm$ 1.58 <sup>a</sup>	56.57 $\pm$ 2.07 <sup>a</sup>	2.3 $\pm$ 0.18 <sup>ab</sup>	-2.01 $\pm$ 0.26 <sup>a</sup>
	DUI	RuPh	53.13 $\pm$ 2.61 <sup>a</sup>	31.03 $\pm$ 1.86 <sup>a</sup>	88.97 $\pm$ 3.57 <sup>a</sup>	105.4 $\pm$ 4.82 <sup>a</sup>	66.23 $\pm$ 3.8 <sup>a</sup>	170.04 $\pm$ 5.6 <sup>a</sup>	62.85 $\pm$ 1.78 <sup>a</sup>	39.17 $\pm$ 1.69 <sup>a</sup>	8.58 $\pm$ 0.32 <sup>ab</sup>	27.04 $\pm$ 2.07 <sup>ab</sup>	62.13 $\pm$ 1.81 <sup>ab</sup>	1.33 $\pm$ 0.18 <sup>a</sup>	-1.34 $\pm$ 0.21 <sup>ab</sup>
	SMI	MeMe	83.12 $\pm$ 5.97 <sup>ab</sup>	54.59 $\pm$ 3.42 <sup>b</sup>	108.18 $\pm$ 6.07 <sup>ab</sup>	142.24 $\pm$ 10.55 <sup>ab</sup>	97.4 $\pm$ 6.54 <sup>ab</sup>	183.29 $\pm$ 10.63 <sup>a</sup>	72.13 $\pm$ 2.87 <sup>bc</sup>	54.44 $\pm$ 2.48 <sup>b</sup>	9.55 $\pm$ 1.34 <sup>ab</sup>	24.61 $\pm$ 2.26 <sup>a</sup>	75.35 $\pm$ 1.66 <sup>cd</sup>	3.23 $\pm$ 0.32 <sup>b</sup>	0.23 $\pm$ 0.44 <sup>c</sup>
	SMI	NuOb	105.98 $\pm$ 9.69 <sup>b</sup>	81.28 $\pm$ 3.79 <sup>c</sup>	123.79 $\pm$ 12.47 <sup>bc</sup>	231.85 $\pm$ 19.55 <sup>c</sup>	192.2 $\pm$ 23.9 <sup>c</sup>	266.53 $\pm$ 16.58 <sup>c</sup>	82.69 $\pm$ 3.77 <sup>c</sup>	69.96 $\pm$ 5.68 <sup>c</sup>	7.65 $\pm$ 0.69 <sup>b</sup>	31.78 $\pm$ 1.74 <sup>ab</sup>	83.85 $\pm$ 3.09 <sup>d</sup>	6.23 $\pm$ 0.49 <sup>d</sup>	2.07 $\pm$ 0.66 <sup>d</sup>
	SMI	PlMg	95.2 $\pm$ 5.9 <sup>b</sup>	70.29 $\pm$ 4.23 <sup>c</sup>	136.98 $\pm$ 4.13 <sup>c</sup>	158.06 $\pm$ 11.88 <sup>b</sup>	121.15 $\pm$ 9.81 <sup>b</sup>	222.89 $\pm$ 7.2 <sup>b</sup>	76.78 $\pm$ 1.7 <sup>c</sup>	52.29 $\pm$ 2.87 <sup>b</sup>	7.96 $\pm$ 0.44 <sup>b</sup>	32.59 $\pm$ 1.39 <sup>b</sup>	67.45 $\pm$ 3.01 <sup>bc</sup>	4.44 $\pm$ 0.35 <sup>c</sup>	0.03 $\pm$ 0.39 <sup>bc</sup>
	F	(4,40)	13.93	(4,40)	35.65	(4,40)	11.75	(4,40)	24.14	(4,40)	13.11	(4,40)	7.53	(4,40)	3.77
	P	<b>3.32E-07</b>	<b>1.07E-12</b>	<b>2.09E-06</b>	<b>2.80E-08</b>	<b>3.26E-10</b>	<b>6.48E-07</b>	<b>1.20E-04</b>	<b>6.64E-09</b>	<b>1.00E-02</b>	<b>1.20E-04</b>	<b>4.62E-08</b>	<b>5.10E-12</b>	<b>2.20E-07</b>	

**Table S4.** Sperm motility parameters comparison between five bivalve species, both DUI and SML, with and without chemoattractants. Values are presented as means  $\pm$  s.e.m. The main effect of the two fixed factors 'species' and 'chemoattractants' was assessed for each parameter separately through a linear mixed effect model, which took into account the by-subject variability. Differences among species and were determined through a post-hoc pairwise comparison, with  $p$ -values adjusted using Holm's correction for multiple testing. Significant differences ( $P \leq 0.05$ ) are shown in bold. Inheritance: DUI, doubly uniparental inheritance; SML, strict maternal inheritance. Species: Mytilus edulis ( $n = 11$ ); Ruditapes philippinarum ( $n = 9$ ); Nucula obsoleta ( $n = 9$ ); Mercenaria mercenaria ( $n = 9$ ); Macoma edulis ( $n = 11$ ). Control-N, basal sperm motility without chemoattractants. 'Control-N', basal sperm motility with chemoattractants. 'Control', main effect of factor 'chemoattractants'. For abbreviations refer to Table S2.

**Table S4**

Species	Inheritance	Treatment	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2
			DCL	VAP	VSL	STR	LIN	ALH	BFC	WOB	PC1	PC2	
Mytilus edulis	DUI	Control-N	57.96 $\pm$ 2.75*	36.88 $\pm$ 1.71*	9.686 $\pm$ 4.05*	114.06 $\pm$ 4.66*	75.41 $\pm$ 3.16*	184.87 $\pm$ 8.19*	67.57 $\pm$ 1.74*	10.44 $\pm$ 0.69*	25.59 $\pm$ 0.99*	61.6 $\pm$ 1.59*	-1.38 $\pm$ 0.26*
		Control	63.07 $\pm$ 4.57*	39.98 $\pm$ 0.02*	111.17 $\pm$ 4.14*	110.45 $\pm$ 7.16*	72.27 $\pm$ 3.39*	193.1 $\pm$ 8.51*	67.15 $\pm$ 2.99*	37.61 $\pm$ 1.72*	25.85 $\pm$ 1.58*	56.57 $\pm$ 2.07*	-2.01 $\pm$ 0.26*
Ruditapes philippinarum	DUI	Control-N	47.83 $\pm$ 3.39*	30.06 $\pm$ 1.87*	79.35 $\pm$ 4.24*	93.42 $\pm$ 6.38*	63.16 $\pm$ 4.04*	150.93 $\pm$ 3.4*	70.51 $\pm$ 1.43*	43.56 $\pm$ 1.31*	7.34 $\pm$ 0.48*	27.6 $\pm$ 1.22*	0.97 $\pm$ 0.23*
		Control	53.13 $\pm$ 2.41*	31.03 $\pm$ 1.86*	80.87 $\pm$ 3.56*	105.4 $\pm$ 4.23*	66.73 $\pm$ 3.87*	170.04 $\pm$ 5.61*	67.85 $\pm$ 1.70*	30.17 $\pm$ 1.69*	8.53 $\pm$ 0.32*	27.04 $\pm$ 2.07*	63.1 $\pm$ 1.81*
Nucula obsoleta	SML	Control-N	92.15 $\pm$ 6.37*	58.52 $\pm$ 3.47*	115.31 $\pm$ 6.13*	136.69 $\pm$ 1.27*	106.29 $\pm$ 6.65*	194.51 $\pm$ 1.00*	71.08 $\pm$ 2.41*	54.88 $\pm$ 2.18*	7.91 $\pm$ 0.89*	21.17 $\pm$ 1.07*	3.59 $\pm$ 0.26
		Control	83.12 $\pm$ 5.97*	54.59 $\pm$ 4.42*	108.18 $\pm$ 6.07*	142.24 $\pm$ 10.55*	97.4 $\pm$ 6.54*	183.29 $\pm$ 0.65*	72.15 $\pm$ 2.87*	54.44 $\pm$ 2.48*	9.55 $\pm$ 1.54*	24.61 $\pm$ 2.26*	75.3 $\pm$ 1.68*
Macoma edulis	SML	Control-N	102.65 $\pm$ 8.88*	76.51 $\pm$ 3.39*	120.03 $\pm$ 12.73*	193.58 $\pm$ 12.73*	151.32 $\pm$ 12.26*	242.62 $\pm$ 9.65*	76.68 $\pm$ 3.59*	60.87 $\pm$ 3.38*	7.26 $\pm$ 0.64*	33.89 $\pm$ 7.44*	5.22 $\pm$ 0.53*
		Control	105.98 $\pm$ 9.69*	81.28 $\pm$ 3.79*	121.79 $\pm$ 12.47*	192.2 $\pm$ 21.39*	136.9 $\pm$ 4.46*	266.53 $\pm$ 16.81*	83.09 $\pm$ 3.77*	69.96 $\pm$ 3.68*	7.63 $\pm$ 0.69*	31.78 $\pm$ 7.74*	6.23 $\pm$ 0.49*
Perna magallanica	SML	Control-N	112.4 $\pm$ 5.89*	82.25 $\pm$ 3.92*	121.56 $\pm$ 14.47*	164.82 $\pm$ 9.82*	21.15 $\pm$ 14.47*	252.95 $\pm$ 11.09*	78.84 $\pm$ 1.59*	63.03 $\pm$ 1.74*	7.76 $\pm$ 0.33*	29.93 $\pm$ 1.74*	5.87 $\pm$ 0.39*
		Control	95.2 $\pm$ 5.95*	82.25 $\pm$ 3.92*	158.06 $\pm$ 11.88*	121.15 $\pm$ 9.81*	136.89 $\pm$ 4.14*	212.75 $\pm$ 22.89*	76.78 $\pm$ 1.17*	52.29 $\pm$ 3.83*	7.96 $\pm$ 1.04*	32.59 $\pm$ 1.39*	4.44 $\pm$ 0.35*
Mercenaria mercenaria	SML	Control-N	F <sub>4,80</sub> =39.26, P<2.16e-06***	F <sub>4,80</sub> =60.23, P<2.16e-06***	F <sub>4,80</sub> =30.27, P<9.42e-06***	F <sub>4,80</sub> =20.37, P<1.63e-05***	F <sub>4,80</sub> =36.64, P<3e-13***	F <sub>4,80</sub> =21.75, P<1.45e-09***	F <sub>4,80</sub> =10.9, P<5.52e-08***	F <sub>4,80</sub> =6.6, P=5.60e-08***	F <sub>4,80</sub> =9.58, P=1.56e-08***	F <sub>4,80</sub> =34.4, P<2e-16***	F <sub>4,80</sub> =28.8, P=2.65e-11***
		Control	F <sub>4,80</sub> =30.26, P<2.16e-06***	F <sub>4,80</sub> =60.23, P<2.16e-06***	F <sub>4,80</sub> =30.27, P<9.42e-06***	F <sub>4,80</sub> =20.37, P<1.63e-05***	F <sub>4,80</sub> =36.64, P<3e-13***	F <sub>4,80</sub> =21.75, P<1.45e-09***	F <sub>4,80</sub> =10.9, P<5.52e-08***	F <sub>4,80</sub> =6.6, P=5.60e-08***	F <sub>4,80</sub> =9.58, P=1.56e-08***	F <sub>4,80</sub> =34.4, P<2e-16***	F <sub>4,80</sub> =28.8, P=2.65e-11***
Total		F <sub>1,47</sub> =0.59, P=0.34	F <sub>1,47</sub> =1.16, P=0.34	F <sub>1,47</sub> =1.16, P=0.34	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =0.76, P=0.38	F <sub>1,47</sub> =0.76, P=0.38	F <sub>1,47</sub> =0.76, P=0.38	F <sub>1,47</sub> =0.76, P=0.38	
		F <sub>1,47</sub> =0.59, P=0.34	F <sub>1,47</sub> =1.16, P=0.34	F <sub>1,47</sub> =1.16, P=0.34	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =2.36, P=0.13	F <sub>1,47</sub> =0.76, P=0.38	F <sub>1,47</sub> =0.76, P=0.38	F <sub>1,47</sub> =0.76, P=0.38	F <sub>1,47</sub> =0.76, P=0.38	





