

Supplementary Information

Abnormal pairing of X and Y sex chromosomes during meiosis I in interspecific hybrids of *Phodopus campbelli* and *P. sungorus*

Satoshi Ishishita^{1†}, Kazuma Tsuboi^{1†}, Namiko Ohishi², Kimiyuki Tsuchiya³, Yoichi Matsuda^{1*}

¹ Laboratory of Animal Genetics, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan, ² Laboratory of Animal Cytogenetics, Graduate School of Science, Hokkaido University, North 10 West 8, Kita-ku, Sapporo, Hokkaido 060-0810, Japan, ³ OOYO SEIBUTSU Inc., Minato-ku, Tokyo 107-0062, Japan

† These authors contributed equally to this work

* Corresponding author.

Table S1. Body weight, testis weight, and relative testis weight in *Phodopus campbelli*, *P. sungorus*, and their F₁ hybrids

Animal	Number of individuals	Body weight ^a (g)	Testis weight ^a (mg)	Relative testis weight ^{a,b}
<i>P. campbelli</i>	3	33.6 ± 5.8	1109.3 ± 145.3	3.33 ± 0.38
<i>P. sungorus</i>	3	32.9 ± 5.6	957.0 ± 67.5	2.95 ± 0.43
F ₁	29	19.8 ± 2.2	176.8 ± 143.5	0.89 ± 0.72
F ₁ (Type A)	9	19.8 ± 3.0	27.2 ± 8.7	0.14 ± 0.05
F ₁ (Type B)	9	19.5 ± 2.1	142.6 ± 77.9	0.72 ± 0.35
F ₁ (Type C)	11	20.1 ± 1.7	327.2 ± 81.9	1.65 ± 0.41

^a Values are given as mean ± standard deviation.

^b Testis weight (g)/body weight (g) × 100.

Table S2. Quantification of asynapsis of X and Y chromosomes, association of X or Y chromosomes and autosomes, and association of autosome pairs in the pachytene spermatocytes of *Phodopus campbelli* and *P. sungorus* and pachytene-like spermatocytes of F₁ hybrids

Animal	ID	Number of observed cells	X-Y ^a	X//Y ^b	n.d. ^c	XY-Autosomes ^d	Autosomes-Autosomes ^e
<i>P. campbelli</i>	110315	55	54 98.18%	0 0%	1 1.82%	0 0%	0 0%
	110324	51	51 100%	0 0%	0 0%	0 0%	0 0%
	total	106	105 99.09 ± 1.29%	0 0%	1 0.91 ± 1.29%	0 0%	0 0%
<i>P. sungorus</i>	110307	45	45 100%	0 0%	0 0%	0 0%	0 0%
	110310	102	102 100%	0 0%	0 0%	0 0%	0 0%
	total	147	147 100%	0 0%	0 0%	0 0%	0 0%
F ₁ (Type B)	1-1	44	30 68.18%	14 31.82%	0 0%	4 9.09%	0 0%
	2-2n	56	35 62.50%	20 35.71%	1 1.79%	0 0%	0 0%
	3-1n	66	53 80.30%	13 19.70%	0 0%	0 0%	1 1.52%
	6-1	90	73 81.11%	13 14.44%	4 4.44%	2 2.22%	0 0%
	14-1n	40	27 67.50%	12 30.00%	1 2.50%	1 2.50%	0 0%
	20-3	67	44 65.67%	20 29.85%	3 4.48%	0 0%	2 2.99%
	total	363	262 70.88 ± 7.87%	92 26.92 ± 8.09%	9 2.20 ± 2.01%	7 2.30 ± 3.52%	3 0.75 ± 1.25%
F ₁ (Type C)	3-2	92	63 68.48%	28 30.43%	1 1.09%	0 0%	0 0%
	3-3n	44	35 79.55%	3 6.82%	6 13.64%	0 0%	0 0%
	8-1	76	46 60.53%	14 18.42%	16 21.05%	0 0%	0 0%
	6-2	61	36 59.02%	13 21.31%	12 19.67%	0 0%	0 0%
	18-1n	37	26 70.27%	9 24.32%	2 5.41%	4 10.81%	1 2.70%
	total	310	206 67.57 ± 8.28%	67 20.26 ± 8.74%	37 12.17 ± 8.75%	4 2.16 ± 4.83%	1 0.54 ± 1.21%

^a Number of cells with synaptic X and Y chromosomes.

^b Number of cells with asynaptic X and Y chromosomes.

^c Number of cells in which X and Y chromosomes synapsis could not be determined.

^d Number of cells that showed association between X or Y chromosomes and autosomes.

^e Number of cells that showed association between autosome pairs.

Frequencies are given as mean ± standard deviation.

Table S3. Morphologies of XY bodies and quantification of γ -H2AFX staining in autosomes and association between the XY bodies and autosomes in the pachytene spermatocytes of *Phodopus campbelli* and *P. sungorus* and pachytene-like spermatocytes of F₁ hybrids

Animal	ID	Number of observed cells	X-Y ^a	X/Y			γ -H2AFX in autosomes ^e	XY-autosomes association ^f
				(Normal) ^b	(Broad) ^c	(Separated) ^d		
<i>P. campbelli</i>	110324	84	83 98.81%	1 1.19%	0 0%	0 0%	0 0%	0 0%
	110315	88	88 100%	0 0%	0 0%	0 0%	0 0%	0 0%
	total	172	171 99.40 ± 3.54%	1 0.60 ± 0.71%	0 0%	0 0%	0 0%	0 0%
<i>P. sungorus</i>	110307	70	70 100%	0 0%	0 0%	0 0%	0 0%	0 0%
	110310	104	104 100%	0 0%	0 0%	0 0%	0 0%	1 0.96%
	total	174	174 100%	0 0%	0 0%	0 0%	0 0%	1 0.48 ± 0.68%
F ₁ (Type B)	4-a-1	56	32 57.14%	20 35.71%	1 1.79%	3 5.36%	3 5.36%	2 3.57%
	6-1	87	54 62.07%	22 25.29%	6 6.90%	5 5.75%	8 9.20%	3 3.45%
	20-3	56	50 89.29%	6 10.71%	0 0%	0 0%	1 1.79%	0 0%
	total	199	136 69.50 ± 17.31%	48 23.91 ± 12.56%	7 2.89 ± 3.58%	8 3.70 ± 3.21%	12 5.45 ± 3.71%	5 2.34 ± 2.03%
F ₁ (Type C)	1-a-2	86	61 70.93%	23 26.74%	0 0%	2 2.33%	2 2.33%	2 2.33%
	18-1	54	42 77.78%	11 20.37%	1 1.85%	0 0%	2 3.70%	2 3.70%
	a-1	81	72 88.89%	8 9.88%	1 1.23%	0 0%	3 3.70%	0 0%
	total	221	175 79.20 ± 9.06%	42 19.00 ± 8.52%	2 1.03 ± 0.94%	2 0.78 ± 1.34%	7 3.24 ± 0.80%	4 2.01 ± 1.87%

^a Number of cells with normal XY bodies and synapsed X and Y chromosomes.

^b Number of cells with normal XY bodies and unsynapsed X and Y chromosomes.

^c Number of cells with broad XY bodies comprising unsynapsed X and Y chromosomes.

^d Number of cells with separated XY bodies and unsynapsed X and Y chromosomes.

^e Number of cells containing autosomal regions stained with γ -H2AFX antibody. Staining signals on autosomes associated with the XY body were excluded.

^f Number of cells that showed association between the XY bodies and autosomes.

n, number of animals; frequencies are given as mean ± standard deviation.

Table S4. Morphological abnormalities of spermatozoa in *Phodopus campbelli*, *P. sungorus*, and their F₁ hybrids.

Morphology of spermatozoa	<i>P. campbelli</i>	<i>P. sungorus</i>	F ₁ (Type C)
Normal	176	180	14
Shelving curve hook	2	2	176
Bent hook	12	5	135
Thick hook	9	11	93
Fallen head with short hook	0	2	38
Short hook	0	0	10
Other	1	0	34
Total (%) ^a	200 (12.0)	200 (10.0)	500 (97.2)

^aFrequencies of abnormal sperm heads.

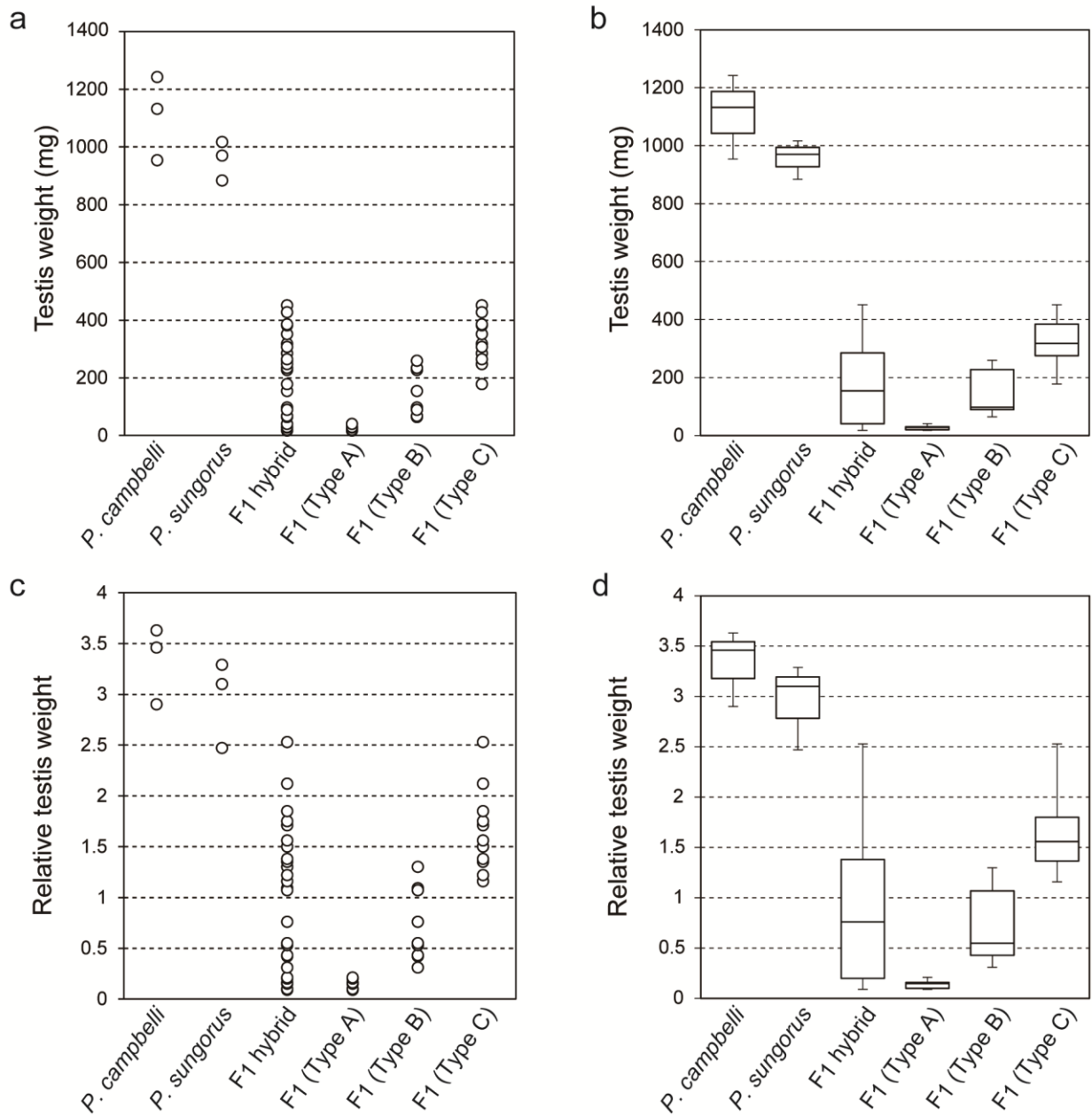


Figure S1. Testis weight and relative testis weight of *Phodopus campbelli*, *P. sungorus*, and their F₁ hybrids. a–d Testis weight and relative testis weight of *P. campbelli*, *P. sungorus*, and Type A, Type B, and Type C hybrids are shown by the dot blots (a, c) and box-and-whisker plots (b, d). The bottom and top of the box represent the first and third quartiles, the line inside the box represents the median, and the ends of the whiskers represent the minimum and maximum values.