SUPPORTING INFORMATION

Natural variation and genetic determinants of *Caenorhabditis elegans* sperm size by Gimond *et al.*

CONTENTS

Supporting Files

File S1. Raw data in Excel format. Numbers below refer to different worksheets in Excel file

1. Male sperm size of 97 *C. elegans* strains (measurements in microns) (Figure 1)
2. Strain variation in male mating ability and fertility (Figures 3A and 3C)
3. Strain variation in ejaculate size (sperm number) (Figure 3E)
4. Strain variation in male fertilization success when competing with hermaphrodite self-sperm (Figure 4A)
5. Strain variation in male-male competitive ability (Figures 4C and 4E)
6. Strain variation in hermaphrodite sperm size (Figure 5A)
7. Strain variation in hermaphrodite sperm production (Figure 5C)
8. Strain variation in body size (Figure 6)
9. Male and hermaphrodite sperm size of strains N2, LSJ1, LSJ2 and CC1 (Figure 7)
10. Male and hermaphrodite sperm size of strains CX12311, CX13248, N2, and *nurf-1(n4295)* (Figure 8A-D)
11. Male sperm size measurements resulting from quantitative complementation tests using strains N2, LSJ1 and *nurf-1(n4295)* (Figure 8E)
12. Male sperm size of NURF complex mutants (Figure 9B)
13. Hermaphrodite sperm size of *isw-1(n3297)* versus N2 (Figure S2)
14. Male primary spermatocyte size of *isw-1(n3297)* versus N2 (Figure 9E)
15. Variation in body size of N2, LSJ1, *nurf-1(n4295)* and *isw-1(n3294)* (Figure 10)
16. Effect of *nurf-1* RNAi on male sperm size of *C. elegans* (Figure S1)
17. Effect of *nurf-1* RNAi on male sperm size of *C. plicata* (Figure S3)

Supporting Figures

FigureS1

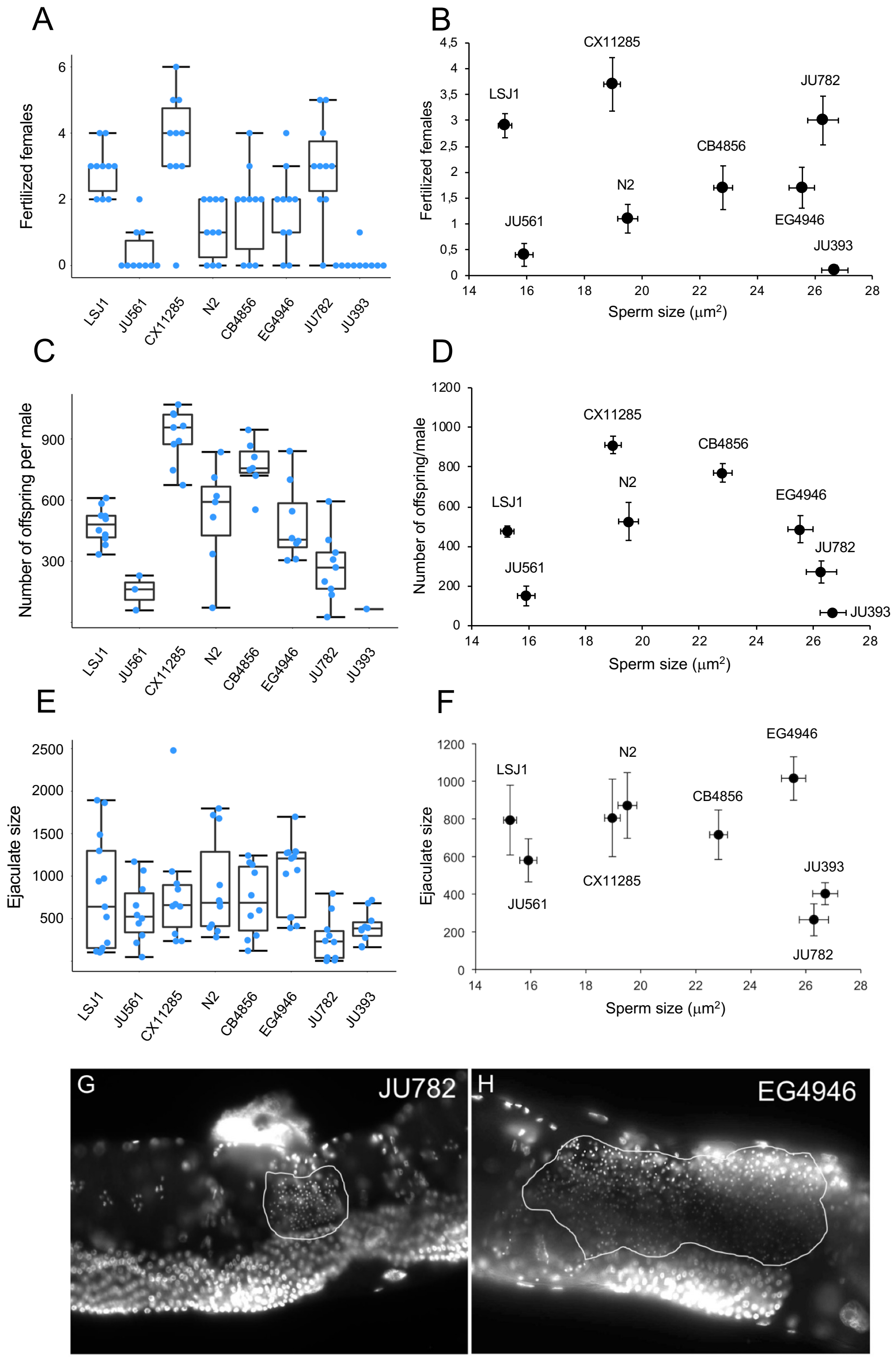

Figure S1. Differences in male mating ability and fertility between strains with variable male sperm size

Male mating ability, fertility, and ejaculate size in eight *C. elegans* strains with different average male sperm size. Strains with smallest (LSJ1) to largest (JU393) male sperm are arranged from left to right. (A) Significant strain variation in the number of *fog-2* females fertilized by a single male during an eight-hour window (Kruskal-Wallis, χ^2=45.12, df=7, P<0.0001) and (B) absence of correlation with average male sperm size ($\rho_{Spearman}$=-0.23, P=0.59). (C) Significant strain variation in the number of offspring sired by a single male during eight hours of mating with up to 10 females (Kruskal-Wallis, χ^2=37.78, df=7, P<0.0001) and (D) absence of correlation with average male sperm size ($\rho_{Spearman}$=-0.29, P=0.49). (E) Significant strain variation in ejaculate size, as measured by the number of sperm deposited by one male in a single mating (ANOVA, $F_{7, 86}$=3.77, P=0.0014) and (F) no correlation between average ejaculate size and male sperm size ($\rho_{Spearman}$=-0.33, P=0.42, log-transformed data). (G, H) Images of DAPI-stained *fog-2* females after single male mating event of strains (G) JU782 and (H) EG4946; The white lines delineate the mass of inseminated sperm. Samples sizes were between 10 and 13 animals per strain per experiment. (Note: Figures S1C and S1D are also included in main Figure 2 of the manuscript as Figures 2A and 2B)

Figure S2

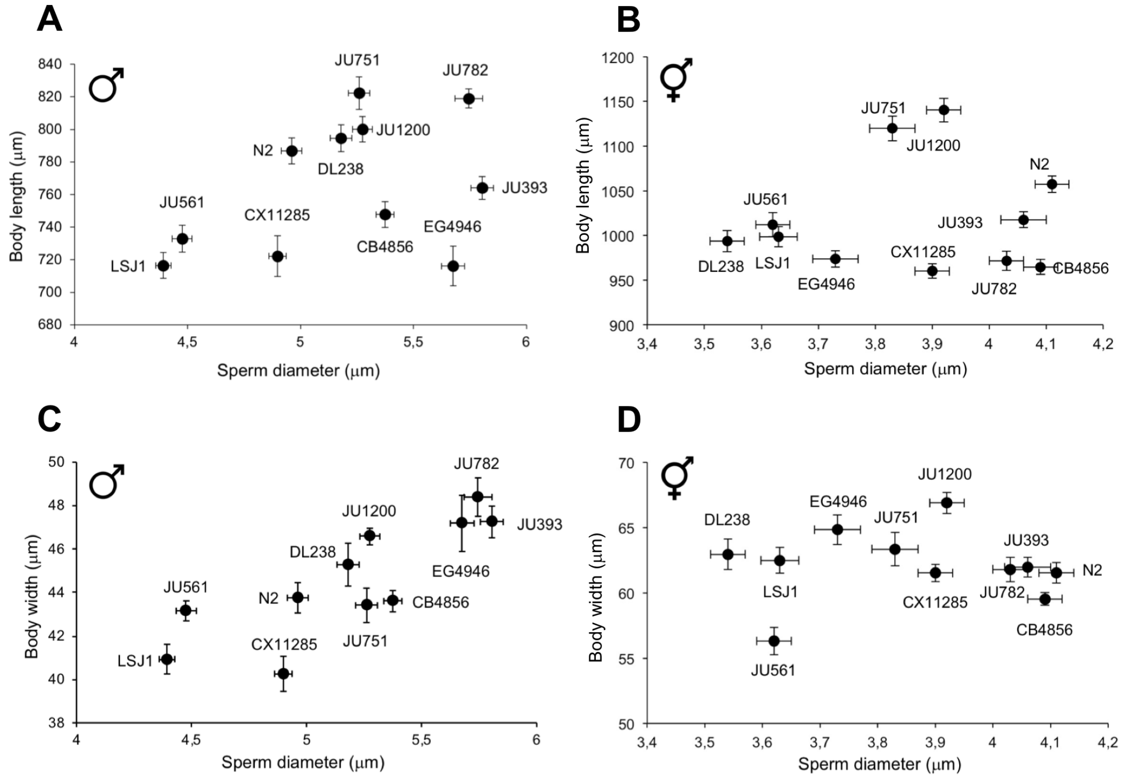

Figure S2. Allometric relationships between sperm and body size

Absence of scaling relationships between sperm size (mean diameter) and body length in (A) males ($F_{1,10}$=1.88, R^2=0.17, P=0.20) and (B) hermaphrodites ($F_{1,10}$=0.005, R^2=0.0005, P=0.94). Significant positive association between body width and sperm size in (C) males ($F_{1,10}$=16.61, R^2=0.65, P=0.0028) but not (D) hermaphrodites ($F_{1,10}$=0.43, R^2=0.05, P=0.53). Sperm size measures based on least-squares regression of strain mean (LSM); all data log-transformed for statistical analyses. Body length and width was measured in 10-29 individuals per strain and per sex (Table S6).

Figure S3

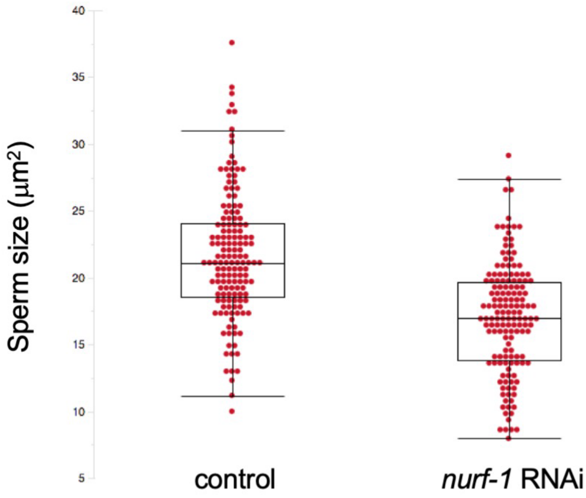

Figure S3. Effect of *nurf-1* RNAi on male sperm size of *C. elegans*. *nurf-1* RNAi significantly reduces male sperm size of the reference strain N2 (ANOVA, effect *treatment*: $F_{1, 280} = 90.91$, $P<0.0001$; effect *individual(treatment)*: $F_{18, 280} = 3.07$, $P<0.0001$). Fifteen spermatids from each of 10 individuals were measured per treatment (N=150).

Figure S4

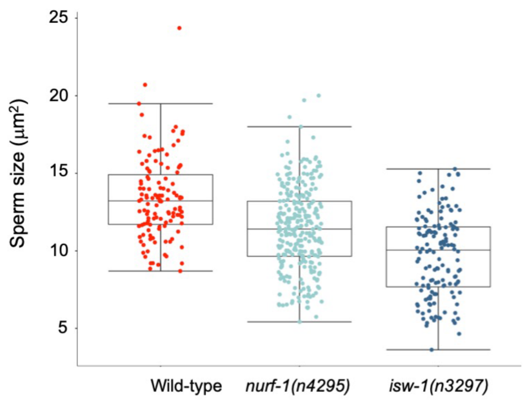

Figure S4. Hermaphrodite sperm size of *isw-1(n3297)* compared to N2 wild-type and *nurf-1(n4295).* Hermaphrodite sperm size of *isw-1(n3297)* is significantly reduced compared to the N2 wild-type (ANOVA, effect *strain*: $F_{1, 280}$ =90.91, P<0.0001; effect *individual(strain)*: $F_{18, 280}$ =3.07, P<0.0001).

Figure S5

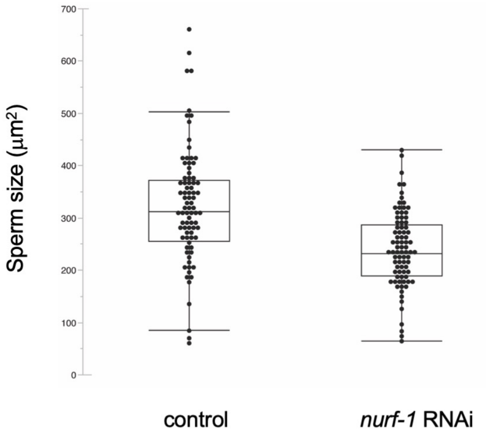

Figure S5. Effect of *nurf-1* RNAi on male sperm size of the gonochoristic species, *C. plicata.* *nurf-1 (C. elegans)* RNAi significantly reduces male sperm size of the strain SB355 (ANOVA, effect *treatment*: $F_{1,\ 163}$ =27.56, P<0.0001; effect *individual(treatment)*: $F_{10,163}$ =8.10, P<0.0001). 8-15 spermatids from each of 7 individuals were measured per treatment.

Supporting Tables

Table S1. List of *Caenorhabditis* strains used in this study. For additional strain information, see CeNDR: https://www.elegansvariation.org)

STRAIN	ORIGIN	DATE OF ISOLATION
AB1	Adelaide, Australia	1983
AB4	Adelaide, Australia	1983
CB4851	Bergerac, France	pre-1949
CB4852	Unknown	pre-1966
CB4853	Altadena, USA	1974/05
CB4854	Altadena, USA	1974/05
CB4856	Oahu, Hawaii	1972/08
CB4857	Claremont, USA	1972/11
CB4858	Pasadena, USA	1973
CB4932	Taunton, United Kingdom	pre-1991
CX11262	Los Angeles, USA	2003/09
CX11264	Los Angeles, USA	2003/09
CX11271	Pasadena, USA	2003/09
CX11276	Los Angeles, USA	2003/09
CX11285	Los Angeles, USA	2003/09
CX11292	Los Angeles, USA	2004/02
CX11307	Los Angeles, USA	2003/09
CX11314	Los Angeles, USA	2003/09
CX11315	Los Angeles, USA	2003/09
DL200	Addis Ababa, Ethiopia	2007/12
DL226	Corvallis, USA	2007
DL238	Manuka, Hawaii	2008/07/15
ED3005	Edinburgh, United Kingdom	2004/10/25
ED3011	Edinburgh, United Kingdom	2004/11/26
ED3012	Edinburgh, United Kingdom	2004/11/26
ED3017	Edinburgh, United Kingdom	2004/12/03
ED3040	Johannesburg, South Africa	2006/03
ED3046	Ceres, South Africa	2006/03
ED3048	Ceres, South Africa	2006/03
ED3049	Ceres, South Africa	2006/03
ED3052	Ceres, South Africa	2006/03
ED3073	Limuru, Kenya	2006/03
ED3077	Nairobi, Kenya	2006/03
EG4347	Eugene, USA	2006/10
EG4349	Salt Lake City, USA	2006/10
EG4724	Amares, Portugal	2007/03
EG4725	Amares, Portugal	2007/03
EG4946	Salt Lake City, USA	2007/09/27
JT11398	Lake Forest Park, USA	2003/12
JU1088	Kakegawa, Japan	2007/03/14
JU1172	Concepcion, Chile	2007/04
JU1200	Dundonald, United Kingdom	2007/08/01
JU1212	Primel Trégastel, France	2007/09/24
JU1213	Primel Trégastel, France	2007/09/24
JU1242	Santeuil, France	2007/10/14
JU1246	Santeuil, France	2007/10/14
JU1395	Saut-aux-Loups, France	2008/03/01
JU1400	Sevilla, Spain	2008/03
JU1409	Carmona, Spain	2008/03/31
JU1440	Barcelona, Spain	2008/06/09
JU1491	Le Blanc, France	2008/08/17
JU1530	Orsay, France	2008/09/09
JU1568	Ivry-sur-Seine, France	2008/10/05
JU1580	Orsay, France	2008/10/06
JU1581	Orsay, France	2008/10/23
JU1586	Le Blanc, France	2008/11/03
JU1652	Montevideo, Uruguay	2009
JU1896	Athens, Greece	2010/01/02
JU258	Ribeiro Frio, Madeira	2001/10

JU310	Le Blanc, France	2002/08/25
JU311	Merlet, France	2002/09/08
JU323	Merlet, France	2002/09/08
JU346	Merlet, France	2002/09/08
JU360	Franconville, France	2002/09/02
JU363	Franconville, France	2002/09/16
JU367	Franconville, France	2002/09/16
JU393	Hermanville, France	2002/09
JU394	Hermanville, France	2002/09
JU397	Hermanville, France	2002/09
JU406	Hermanville, France	2002/12/30
JU440	Beauchêne, France	2003/09/12
JU561	Sainte-Barbe, France	2004/10/03
JU642	Le Perreux-sur-Marne, France	2004/12/14
JU751	Le Perreux-sur-Marne, France	2005/06/08
JU774	Carcavelos, Portugal	2005/07/10
JU775	Lisbon, Portugal	2005/07/10
JU778	Lisbon, Portugal	2005/07/10
JU782	Lisbon, Portugal	2005/07/10
JU792	Fréchendets, France	2005/08/31
JU830	Tübingen, Germany	2005/09/28
JU847	Obernai, France	2005/10/03
KR314	Vancouver, Canada	1984/05
LKC34	Unknown city, Madagascar	2005/06/17
LSJ1	Bristol, United Kingdom	1951
N2	Bristol, United Kingdom	1951
MY1	Lingen, Germany	2002/07
MY10	Roxel, Germany	2002/07
MY16	Mecklenbeck, Germany	2002/07
MY18	Roxel, Germany	2002/07
MY23	Roxel, Germany	2002/07
PB303	NA	1998/11/14
PB306	NA	1998/11/28
PS2025	Altadena, USA	Unknown
PX179	Eugene, USA	2001/10/02
QX1211	San Francisco, USA	2007/11/26
QX1233	Berkeley, USA	2007/11/24
RC301	Freiburg, Germany	1983
WN2002	Wageningen, Netherlands	2007/11/20

Table S2. Male sperm size variation (means of cross-sectional area, microns) and CV in 97 *C. elegans* strains.

Strain	Sperm Size (mean)	SEM	CV (within-individual)	CV (betwee-individual)	N individuals	N sperm
AB1	23.94	1.68	16.49	5.22	7	140
AB4	22.13	0.83	18.75	6.46	7	140
CB4852	21.44	1.91	17.55	5.68	7	140
CB4853	21.98	2.36	22.13	4.66	7	140
CB4854	25.07	1.73	21.09	4.05	7	140
CB4856	22.83	0.70	16.42	5.60	7	140
CB4857	22.89	2.05	20.67	4.35	7	140
CB4858	22.08	1.53	17.63	7.01	7	140
CB4932	21.06	0.88	15.65	7.69	7	140
CX11262	21.81	1.65	16.93	8.79	7	140
CX11264	20.85	1.29	16.33	4.91	7	140
CX11271	25.03	1.55	18.86	7.04	7	140
CX11276	21.56	1.24	18.82	6.23	7	140
CX11285	18.97	1.60	17.16	6.81	7	140
CX11292	21.40	1.51	19.59	7.48	7	140
CX11307	23.95	1.82	17.03	5.90	7	140
CX11314	20.76	1.83	18.55	6.06	7	140
CX11315	20.82	1.36	15.20	7.69	7	140
DL200	20.62	0.62	16.97	3.31	7	140
DL226	24.36	2.09	18.99	5.82	7	140
DL238	21.29	1.52	19.70	8.92	7	140
ED3005	24.36	1.47	24.36	5.44	7	140
ED3011	21.17	1.39	25.29	13.02	7	140
ED3012	22.72	1.05	16.88	6.27	7	140
ED3017	23.23	1.70	20.66	4.92	7	140
ED3040	22.45	1.14	20.66	9.96	7	140
ED3046	24.32	1.22	18.63	4.75	7	140
ED3048	20.73	2.29	19.68	6.12	7	140
ED3049	21.68	0.72	13.74	4.87	7	140
ED3052	21.97	1.91	16.82	7.09	7	140
ED3073	22.98	1.45	16.42	5.25	7	140
ED3077	22.63	0.79	20.15	6.86	7	140
EG4347	24.05	1.28	19.25	7.10	7	140
EG4349	21.49	2.00	18.71	5.43	7	140
EG4724	20.96	2.24	14.97	6.88	7	140
EG4725	21.08	1.24	18.07	8.55	7	140
EG4946	25.55	1.68	19.75	6.28	7	140
JT11398	22.17	1.80	15.50	5.90	7	140
JU258	20.79	1.46	15.15	5.98	7	140
JU310	24.36	1.23	19.54	5.79	7	140
JU311	24.06	2.07	20.87	13.30	7	140
JU323	23.80	2.55	22.45	6.77	7	140
JU346	23.35	1.66	14.92	5.96	7	140
JU360	23.91	1.34	17.15	7.36	7	140
JU363	24.90	1.38	19.22	5.31	7	140
JU367	23.37	0.94	14.16	5.77	7	140
JU393	26.70	0.92	18.15	10.12	7	140
JU394	24.16	1.39	16.32	4.12	7	140
JU397	23.12	0.95	12.10	6.74	6	120
JU406	21.36	1.96	17.72	7.32	7	140
JU440	23.22	1.01	17.77	6.27	7	140
JU561	15.92	2.06	19.17	12.52	7	140
JU642	21.21	1.55	13.97	11.23	7	140
JU751	21.94	2.41	18.75	9.02	7	140
JU774	22.10	1.68	21.79	10.10	7	140
JU775	22.48	1.89	21.71	10.49	7	140
JU778	20.37	1.46	22.84	8.98	7	140
JU782	26.29	2.68	23.26	3.23	7	140
JU792	20.59	1.40	14.52	7.78	7	140
JU830	24.16	1.06	15.54	3.07	7	140
JU847	22.06	1.73	17.73	5.16	7	140
JU1088	23.41	1.19	14.26	8.82	7	140
JU1172	22.57	2.48	20.17	5.61	7	140

JU1200	22.00	1.37	18.35	3.85	7	140
JU1212	21.61	1.51	18.11	3.14	7	140
JU1213	23.80	1.44	24.69	21.07	7	140
JU1242	19.99	1.00	15.13	12.12	7	140
JU1246	21.40	2.59	19.01	7.33	7	140
JU1395	22.77	1.04	17.56	2.38	7	140
JU1400	19.56	1.91	18.74	5.19	7	140
JU1409	24.64	0.91	13.12	5.23	7	140
JU1440	20.49	2.44	19.04	4.92	7	140
JU1491	21.70	1.71	21.68	3.81	7	140
JU1530	25.71	1.63	17.35	20.12	7	140
JU1568	22.09	1.91	16.93	6.42	7	140
JU1580	22.00	0.76	15.02	7.87	7	140
JU1581	20.41	1.22	13.76	3.65	7	140
JU1586	20.16	1.47	19.50	9.14	7	140
JU1652	21.95	1.59	17.23	6.97	7	140
JU1896	23.21	0.81	18.26	8.87	7	140
KR314	22.11	3.11	21.29	14.20	6	120
LKC34	24.68	1.06	18.99	5.75	7	140
LSJ1	15.25	0.82	16.09	9.08	7	140
MY1	20.01	1.56	16.72	4.62	7	140
MY10	20.84	1.54	20.29	9.43	7	140
MY16	22.46	1.79	16.80	1.66	7	140
MY18	22.16	1.12	20.60	10.50	7	140
MY23	20.09	1.65	16.80	7.44	7	140
N2	19.51	2.17	20.68	3.70	7	140
PB303	23.01	0.83	18.10	4.90	7	140
PB306	22.15	1.47	18.60	5.82	7	140
PS2025	22.40	1.48	19.73	7.99	7	140
PX179	22.41	1.81	21.73	7.20	7	140
QX1211	22.11	1.02	18.49	6.97	7	140
QX1233	23.57	2.81	23.73	19.28	7	140
RC301	21.86	0.95	16.92	6.65	7	140
WN2002	22.37	2.06	20.86	7.31	7	140

Table S3. Mean sperm size and plugging phenotype of examined *C. elegans* strains. Plugging phenotype data from Andersen et al. (2012) and Palopoli et al. (2015).

Strain	Mean sperm size	Plugging =1, Non-plugging=0
AB1	23.5511	0
AB4	21.659	1
CB4852	21.0393	0
CB4853	21.356	1
CB4854	24.4207	1
CB4856	22.4802	1
CB4857	22.3462	0
CB4858	21.6617	1
CB4932	20.7466	0
CX11262	21.3944	1
CX11264	20.537	1
CX11271	24.5216	1
CX11276	21.1239	1
CX11285	18.6448	1
CX11292	20.9053	1
CX11307	23.5335	1
CX11314	20.3464	1
CX11315	20.5113	1
DL200	20.3174	1
DL226	23.8156	1
DL238	20.7834	1
ED3005	23.5608	1
ED3011	20.2955	1
ED3012	22.3289	0
ED3017	22.6823	1
ED3040	21.8398	1
ED3046	23.8324	1
ED3048	20.2543	1
ED3049	21.45	1
ED3052	21.5752	1
ED3073	22.6221	1
ED3077	22.0667	1
EG4347	23.5467	0
EG4349	21.0612	1
EG4724	20.6426	1
EG4725	20.631	1
EG4946	24.9506	0
JT11398	21.8455	0
JU1088	23.0792	1
JU1172	22.0359	1
JU1200	21.58	0
JU1212	21.2382	0
JU1213	22.6857	1
JU1242	19.6241	1
JU1246	20.9496	1
JU1395	22.3787	0
JU1400	19.1491	1
JU1409	24.3819	1
JU1440	20.0642	1
JU1491	21.1306	1
JU1530	24.8259	1
JU1568	21.6964	0
JU1580	21.6949	1
JU1581	20.1968	1
JU1586	19.6909	0
JU1652	21.5463	1
JU1896	22.7062	0
JU258	20.4938	1
JU310	23.8629	0
JU311	23.3055	0
JU323	23.1108	1
JU346	23.0378	1
JU360	23.4768	1
JU363	24.3861	1

JU367	23.1158	0
JU393	26.1593	1
JU394	23.8254	0
JU397	22.9068	1
JU406	20.9359	0
JU440	22.8045	0
JU561	15.4953	1
JU642	20.8737	1
JU751	21.4432	1
JU774	21.4306	1
JU775	21.8151	1
JU778	19.7247	1
JU782	25.4637	1
JU792	20.3049	1
JU830	23.8397	1
JU847	21.6807	1
KR314	21.366	1
LKC34	24.1826	1
LSJ1	15.0017	0
MY1	19.7027	1
MY10	20.3428	1
MY16	22.107	1
MY18	21.557	1
MY23	19.733	1
N2	19.0539	0
PB303	22.6011	1
PB306	21.7096	1
PS2025	21.8714	1
PX179	21.788	0
QX1211	21.6496	1
QX1233	22.4217	1
RC301	21.4845	1
WN2002	21.7914	0

Table S4. Strain variation in hermaphrodite sperm size (and comparison to male sperm size) (cross-sectional area in microns) (data shown in figure 5A).

Strain	Sex	Mean sperm size	SEM	N individuals	N spermatids
LSJ1	Male	15.25	0.23	7	140
LSJ1	Hermaphrodite	10.44	0.20	11	143
JU561	Male	15.91	0.31	7	140
JU561	Hermaphrodite	10.34	0.17	12	134
CX11285	Male	18.97	0.29	7	140
CX11285	Hermaphrodite	12.04	0.22	9	104
N2	Male	19.51	0.35	7	140
N2	Hermaphrodite	13.37	0.23	9	123
DL238	Male	21.29	0.39	7	140
DL238	Hermaphrodite	9.96	0.19	8	140
JU751	Male	21.94	0.38	7	140
JU751	Hermaphrodite	11.65	0.25	10	99
JU1200	Male	21.99	0.34	7	140
JU1200	Hermaphrodite	12.16	0.20	12	136
JU775	Male	22.48	0.46	7	140
JU775	Hermaphrodite	9.04	0.24	7	87
CB4856	Male	22.8	0.33	7	140
CB4856	Hermaphrodite	13.25	0.21	13	149
EG4946	Male	25.55	0.44	7	140
EG4946	Hermaphrodite	11.07	0.25	8	125
JU782	Male	26.29	0.53	7	140
JU782	Hermaphrodite	12.94	0.24	10	136
JU393	Male	26.70	0.45	7	140
JU393	Hermaphrodite	13.19	0.32	7	152

Table S5. Strain variation in hermaphrodite sperm production (data in Figure 5C).

Strain	Mean sperm number/spermatheca	SEM	N individuals
CB4856	137.00	3.28	31
CX11285	146.65	3.98	20
EG4946	151.83	7.22	23
JU393	150.15	5.48	20
JU561	136.38	5.20	29
JU782	152.74	4.00	27
LSJ1	175.96	7.81	23
N2	145.00	5.28	12

Table S6. Strain and sex differences in male and hermaphrodite body size (measures in microns). Strain and sex had significant effects on body length (ANOVA, effect *sex*: $F_{1,422}$=2325.80, P<0.0001; effect *strain:* $F_{10,422}$=12.78, P<0.0001; interaction *sex* x *strain*: $F_{10,422}$ =23.57, P<0.0001) and body width (ANOVA, effect *sex*: $F_{1,422}$=1549,46, P<0.0001; effect *strain:* $F_{10,422}$=15.25, P<0.0001; interaction *sex* x *strain*: $F_{10,422}$ =19.47, P<0.0001). (ANOVA analyses testing for the fixed effects of *strain* and *sex* on body length/width; all data log-transformed).

Strain	**Sex**	**Body length**	**SE length**	**Body width**	**SE width**	**N individuals**
LSJ1	M	716.5	7.9	40.9	0.6	24
LSJ1	H	998	11.2	62	0.9	23
JU561	M	732.9	8.1	43.1	0.4	19
JU561	H	1012	13.3	56	1.0	18
CX11285	M	722.2	12.5	40.2	0.8	19
CX11285	H	960	8.0	62	0.6	18
N2	M	786.8	8.1	43.7	0.6	23
N2	H	1057	9.2	62	0.7	20
DL238	M	794.7	8.2	45.2	0.9	21
DL238	H	994	12.0	63	1.1	17
JU751	M	822.3	10.0	43.4	0.7	15
JU751	H	1120	13.8	63	1.2	17
JU1200	M	800.1	7.8	46.6	0.3	21
JU1200	H	1140	13.2	67	0.8	21
CB4856	M	747.8	7.9	43.6	0.4	21
CB4856	H	965	8.3	60	0.4	19
EG4946	M	716.1	12.1	47.2	1.2	10
EG4946	H	974	9.1	65	1.1	16
JU782	M	819.0	5.8	48.4	0.8	24
JU782	H	972	10.7	62	0.9	15
JU393	M	764.0	6.9	47.2	0.7	17
JU393	H	1018	9.0	62	0.7	29