Supporting Information

Wu et al. 10.1073/pnas.1421397112

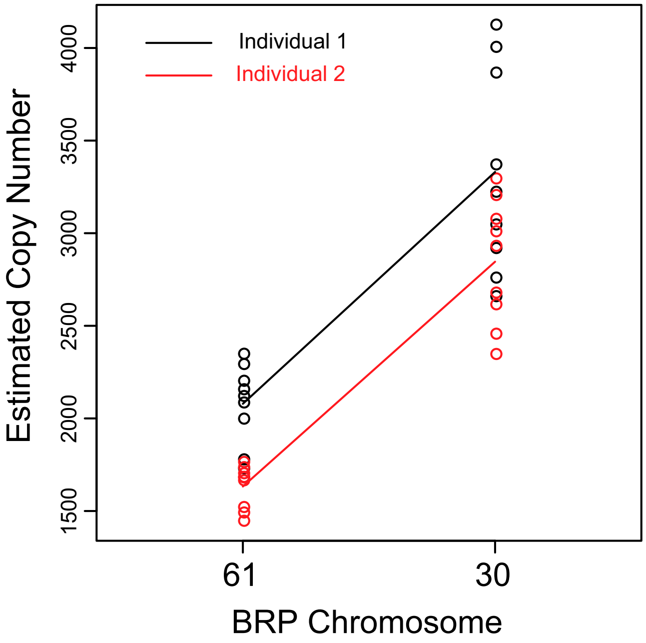

Fig. S1. Absolute qPCR analysis of copy number variation between *Silene noctiflora* BRP mitochondrial chromosomes 30 and 61.

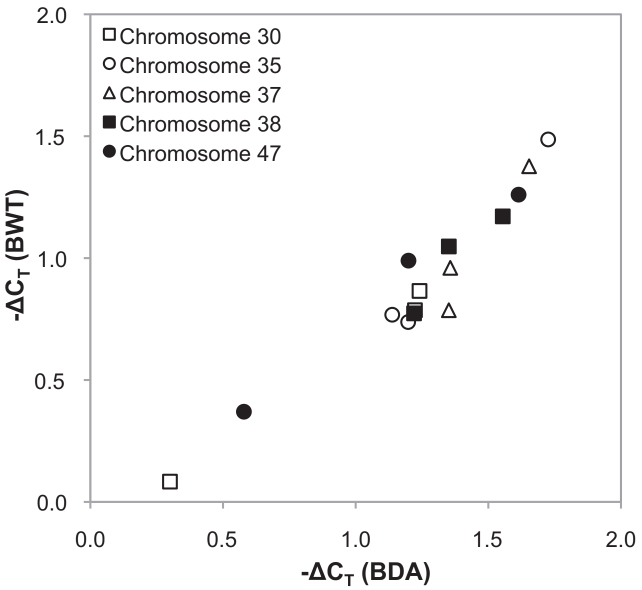

Fig. S2. Relative qPCR analysis of copy number variation among five *S. noctiflora* mitochondrial chromosomes in the BDA and BWT families. The ΔCt values were calculated relative to a nuclear reference gene (*XY4*), and each point represents an average of three biological replicates.

Table S1. Chromosome summary for *S. noctiflora* BRP and OSR mitochondrial genomes

Genome	Chromosome no.	Length, bp	GC content, %	Intact genes	Gene names	GenBank accession no.	Unique?
BRP	1	191,194	40.71	3	*ccmB-a*, *rpl5-1*, *cob*	KP053825	N
BRP	2	160,886	40.67	1	*ccmFc* (exon 1 2)	KP053826	N
BRP	3	153,950	41.18	1	*nad1* (exon 2, 3), ***Ψrps4*, *ΨtrnI(CAT)***	KP053827	N
BRP	4	152,677	41.25	1	*nad5* (exon 1, 2)	KP053828	N
BRP	5	148,955	41.25	3	*trnfM(CAT)*, *rrn18-a*, *rrn5-a*	KP053829	N
BRP	6	147,899	40.82	2	*atp9*, *nad1* (exon 5)	KP053830	N
BRP	7	147,089	41.12	1	*nad5* (exon 4, 5)	KP053831	N
BRP	8	140,355	40.69	2	*nad2* (exon 1, 2), *nad6*	KP053832	N
BRP	9	139,867	40.79	1	*ccmC*	KP053833	N
BRP	10	139,300	41.08	1	*nad9*	KP053834	N
BRP	11	134,804	41.16	1	*nad4* (exon 1, 2, 3)	KP053835	N
BRP	12	130,045	40.82	0	—	KP053836	N
BRP	13	129,952	40.27	0	***ΨtrnN(GTT), ΨtrnQ(TTG)***	KP053837	N
BRP	14	127,928	40.03	0	—	KP053838	N
BRP	15	127,208	40.41	0	—	KP053839	N
BRP	16	127,125	42.28	1	*cox1*, ***ΨmttB-2***	KP053840	N
BRP	17	126,908	40.34	1	*rrn26-a*	KP053841	N
BRP	18	126,794	40.85	0	—	KP053842	N
BRP	19	126,475	40.61	0	—	KP053843	N
BRP	20	126,261	40.66	1	*rrn26-b*	KP053844	N
BRP	21	122,562	40.36	1	*nad5* (exon 3)	KP053845	N
BRP	22	120,807	40.54	0	***ΨccmFn***	KP053846	Y
BRP	23	120,245	40.66	1	*mttB-1*	KP053847	N
BRP	24	118,597	40.73	0	***Ψrpl5-2***	KP053848	N
BRP	25	118,216	41.15	2	*cox3*, *atp8*, ***ΨtrnfM(CAT)***	KP053849	N
BRP	26	117,164	39.96	0	***ΨccmC***	KP053850	Y
BRP	27	116,776	39.89	0	—	KP053851	Y
BRP	28	115,687	40.99	1	*nad7* (exon 1, 2, 3, 4)	KP053852	N
BRP	29	115,049	40.41	0	—	KP053853	N
BRP	30	114,891	40.82	0	—	KP053854	N
BRP	31	114,765	40.72	0	—	KP053855	Y
BRP	32	112,465	42.12	2	*matR-a*, *nad1* (exon 4)	KP053856	N
BRP	33	108,907	40.50	0	—	KP053857	N
BRP	34	108,809	41.06	2	*rrn18-b*, *rrn5-b*	KP053858	N
BRP	35	108,105	41.26	1	*nad1* (exon 1)	KP053859	N
BRP	36	106,455	40.58	1	*nad3-a*	KP053860	N
BRP	37	103,632	40.67	0	—	KP053861	N
BRP	38	102,980	40.05	0	***Ψatp6***	KP053862	Y
BRP	39	102,297	40.15	2	*atp6*, *atp1*	KP053863	N
BRP	40	101,611	41.08	0	—	KP053864	Y
BRP	41	101,161	40.82	0	—	KP053865	N
BRP	42	101,035	40.47	1	*rps13*	KP053866	N
BRP	43	100,496	41.46	3	*trnfM(CAT)*, *rrn18-c*, *rrn5-c*	KP053867	N
BRP	44	99,709	41.10	1	*trnI-CAT*	KP053868	N
BRP	45	98,295	41.25	2	*rrn18-d*, *rrn5-d*, ***Ψnad2* (exon 3, 4, 5)**	KP053869	Y
BRP	46	98,155	40.59	1	*ccmFn*	KP053870	N
BRP	47	97,625	40.49	0	***ΨtrnfM(CAT), Ψrps3***	KP053871	N
BRP	48	96,769	40.87	0	***ΨtrnQ(TTG)***	KP053872	N
BRP	49	94,695	41.18	2	*nad2* (exon 3, 4, 5), *trnY-GTA*	KP053873	N
BRP	50	94,110	41.34	2	*rrn18-e*, *rrn5-e*	KP053874	Y
BRP	51	93,608	39.91	0	—	KP053875	N
BRP	52	92,884	41.04	1	*rrn26-c*	KP053876	N
BRP	53	92,345	40.92	0	—	KP053877	N
BRP	54	92,286	40.78	3	*trnI-CAT*, *rrn18-f*, *rrn5-f*, ***ΨtrnW(CCA)***	KP053878	N
BRP	55	91,549	40.82	2	*nad3-b*, *cox2*	KP053879	N
BRP	56	90,680	40.98	0	—	KP053880	N
BRP	57	89,546	40.58	0	***Ψatp6***	KP053881	Y
BRP	58	88,097	40.05	1	*rrn26-d*, ***Ψrps12***	KP053882	N
BRP	59	83,984	40.71	1	*trnI-CAT*	KP053883	N
BRP	60	81,391	40.98	3	*atp4-a*, *nad4L-a*, *ccmB-b*, ***ΨtrnI(CAT)***	KP053884	N
BRP	61	73,423	41.69	3	*matR-b*, *nad1* (exon 4), *nad5* (exon 3)	KP053885	Y
BRP	62	65,256	40.71	3	*atp4-b*, *nad4L-b*, *ccmB-c*	KP053886	N

Table S1. Cont.

Genome	Chromosome no.	Length, bp	GC content, %	Intact genes	Gene names	GenBank accession no.	Unique?
BRP	63	64,875	42.50	0	—	KP053887	Y
OSR	1	191,963	40.70	3	*ccmB-a, rpl5-1, cob*	NC_016393	N
OSR	2	161,299	40.66	1	*ccmFc* (exon 1, 2)	NC_016396	N
OSR	3	153,996	41.17	1	*nad1* (exon 2, 3), ***Ψrps4, ΨtrnI(CAT)***	NC_016348	N
OSR	4	152,707	41.25	1	*nad5* (exon 1, 2)	NC_016397	N
OSR	5	148,612	41.22	3	*trnfM(CAT), rrn18-b, rrn5-b*	NC_016372	N
OSR	6	147,958	40.80	2	*atp9, nad1* (exon 5)	NC_016355	N
OSR	7	146,587	41.03	2	*cox3, atp8,* ***ΨtrnfM(CAT)***	NC_016381	N
OSR	8	145,239	41.10	1	*nad5* (exon 4, 5)	NC_016390	N
OSR	9	142,006	40.70	2	*nad2* (exon 1, 2), *nad6*	NC_016382	N
OSR	10	140,341	40.78	1	*ccmC*	NC_016349	N
OSR	11	138,824	41.08	1	*nad9*	NC_016377	N
OSR	12	134,806	41.14	1	*nad4* (exon 1, 2, 3)	NC_016345	N
OSR	13	130,152	40.81	0	—	NC_016363	N
OSR	14	130,008	40.25	0	***ΨtrnN(GTT), ΨtrnQ(TTG)***	NC_016171	N
OSR	15	129,012	40.81	0	—	NC_016384	N
OSR	16	128,843	40.33	1	*rrn26-a*	NC_016365	N
OSR	17	127,930	40.03	0	—	NC_016346	N
OSR	18	127,245	40.40	0	—	NC_016357	N
OSR	19	127,193	42.28	1	*cox1,* ***ΨmttB-2***	NC_016398	N
OSR	20	126,609	40.64	1	*rrn26-d*	NC_016368	N
OSR	21	126,452	40.58	0	—	NC_016389	N
OSR	22	122,890	40.35	1	*nad5* (exon 3)	NC_016352	N
OSR	23	122,485	40.60	1	*mttB-1*	NC_016364	N
OSR	24	119,408	40.69	0	***Ψrpl5-2***	NC_016360	N
OSR	25	115,341	40.98	1	*nad7* (exon 1, 2, 3, 4)	NC_016359	N
OSR	26	114,914	40.81	0	—	NC_016383	N
OSR	27	113,308	42.15	2	*matR, nad1* (exon 4)	NC_016391	N
OSR	28	108,290	41.02	2	*rrn18-d, rrn5-d*	NC_016374	N
OSR	29	108,152	41.25	1	*nad1* (exon 1)	NC_016362	N
OSR	30	108,040	40.58	0	—	NC_016354	Y
OSR	31	107,738	40.08	0	—	NC_016361	Y
OSR	32	106,477	40.57	1	*nad3-a*	NC_016378	N
OSR	33	104,288	40.96	1	*rrn26-b,* ***ΨtrnQ(TTG)***	NC_016366	N
OSR	34	103,926	40.70	1	*trnI-CAT*	NC_016401	N
OSR	35	103,557	40.05	0	—	NC_016344	Y
OSR	36	103,548	40.22	1	*rps13-1*	NC_016347	Y
OSR	37	103,320	40.70	0	—	NC_016370	N
OSR	38	102,347	40.14	2	*atp6, atp1*	NC_016356	N
OSR	39	100,876	40.46	1	*rps13-2*	NC_016379	N
OSR	40	100,579	41.45	3	*trnfM(CAT), rrn18-a, rrn5-a*	NC_016371	N
OSR	41	100,078	40.47	0	—	NC_016350	N
OSR	42	98,550	40.59	1	*ccmFn*	NC_016376	N
OSR	43	97,627	40.50	0	***Ψrps3, ΨtrnfM(CAT)***	NC_016351	N
OSR	44	96,564	41.12	1	*trnI-CAT*	NC_016387	N
OSR	45	96,233	40.71	0	—	NC_016392	N
OSR	46	95,084	40.92	0	—	NC_016353	Y
OSR	47	94,621	41.15	2	*nad2* (exon 3, 4, 5), *trnY-GTA*	NC_016358	N
OSR	48	94,201	39.91	0	—	NC_016399	N
OSR	49	92,946	41.03	1	*rrn26-e*	NC_016369	N
OSR	50	92,480	40.75	3	*trnI-CAT, rrn18-e, rrn5-e,* ***ΨtrnW(CCA)***	NC_016375	N
OSR	51	92,366	40.92	0	—	NC_016388	N
OSR	52	91,804	41.15	1	*rrn26-c*	NC_016367	Y
OSR	53	91,595	40.79	2	*nad3-c, cox2*	NC_016386	N
OSR	54	89,951	40.93	0	—	NC_016400	N
OSR	55	86,782	39.77	1	*nad3-b,* ***Ψrps12***	NC_016385	N
OSR	56	81,416	40.97	3	*atp4-b, nad4L-b, ccmB-c,* ***ΨtrnI(CAT)***	NC_016395	N
OSR	57	74,922	40.57	2	*rrn18-c, rrn5-c*	NC_016373	Y
OSR	58	67,018	40.71	0	***ΨtrnfM(CAT)***	NC_016380	Y
OSR	59	66,365	40.66	3	*atp4-a, nad4L-a, ccmB-b*	NC_016394	N

Gene names listed in bold text are apparent pseudogenes and are not included in reported counts of intact genes.

Table S2. Summary of genes with duplicate copies

Gene	BRP	OSR
atp4	2	2
atp6	**3**	1
ccmB	3	3
ccmC	2	1
ccmFn	**2**	1
matR	2	1
mttB	**2**	**2**
nad1-exon4	2	1
nad2-exon3-4–5	**2**	1
nad3	2	3
nad4L	2	2
nad5-exon3	2	1
rpl5	**2**	**2**
rps13	1	**2**
rrn18	6	5
rrn26	4	5
rrn5	6	5
trnfM	4(**2**)	5(**3**)
trnI	5(**2**)	5(**2**)

Bold values indicate duplicate copies that are not identical in sequence.

Table S3. Summary of SNPs and indels between *S. noctiflora* BRP and OSR mitochondrial genomes by chromosome

BRP chromosome	OSR chromosome	Alignment length, bp	SNPs	Pairwise divergence, %	Indels
1	1	191,178 (183,146)	53 (35)	0.0277 (0.0191)	69 (2)
2	2	160,871 (154,660)	47 (38)	0.0292 (0.0246)	56 (7)
3	3	153,941 (148,001)	38 (29)	0.0247 (0.0196)	51 (1)
4	4	152,629 (146,526)	40 (26)	0.0262 (0.0177)	64 (3)
5	5	148,528 (142,851)	60 (45)	0.0404 (0.0315)	56 (13)
6	6	147,876 (141,262)	44 (34)	0.0298 (0.0241)	77 (4)
7	8	145,173 (139,261)	38 (30)	0.0262 (0.0215)	55 (6)
8	9	140,321 (134,921)	60 (46)	0.0428 (0.0341)	35 (7)
9	10	139,856 (134,655)	65 (48)	0.0465 (0.0356)	55 (7)
10	11	138,770 (133,274)	14 (9)	0.0101 (0.0068)	54 (6)
11	12	134,706 (129,147)	44 (41)	0.0327 (0.0317)	54 (5)
12	13	129,997 (124,609)	157 (136)	0.1208 (0.1091)	90 (25)
13	14	129,916 (124,166)	17 (12)	0.0131 (0.0097)	73 (2)
18	15	126,779 (121,856)	24 (21)	0.0189 (0.0172)	30 (6)
17	16	126,896 (121,874)	24 (15)	0.0189 (0.0123)	72 (13)
14	17	127,898 (123,055)	65 (45)	0.0508 (0.0366)	14 (8)
15	18	127,139 (121,774)	178 (137)	0.14 (0.1125)	40 (3)
16	19	127,108 (123,761)	55 (45)	0.0433 (0.0364)	27 (3)
20	20	126,249 (120,618)	50 (35)	0.0396 (0.029)	67 (22)
19	21	126,331 (121,004)	72 (63)	0.057 (0.0521)	48 (5)
21	22	122,543 (117,773)	39 (31)	0.0318 (0.0264)	52 (7)
23	23	120,232 (115,404)	57 (40)	0.0474 (0.0347)	55 (3)
24	24	118,551 (113,467)	38 (31)	0.0321 (0.0273)	44 (7)
28	25	115,291 (110,105)	41 (30)	0.0356 (0.0272)	41 (4)
29	41	99,913 (95,730)	132 (106)	0.1321 (0.1107)	52 (21)
30	26	114,881 (110,284)	40 (28)	0.0348 (0.0254)	32 (1)
32	27	111,964 (108,369)	43 (40)	0.0384 (0.0369)	25 (11)
34	28	107,867 (103,455)	67 (57)	0.0621 (0.0551)	38 (4)
35	29	108,091 (103,682)	50 (39)	0.0463 (0.0376)	45 (9)
36	32	106,444 (101,975)	13 (9)	0.0122 (0.0088)	33 (2)
48	33	96,751 (93,336)	50 (37)	0.0517 (0.0396)	31 (5)
37	37	102,938 (98,933)	57 (46)	0.0554 (0.0465)	31 (1)
39	38	102,294 (98,371)	19 (15)	0.0186 (0.0152)	32 (5)
41	45	96,194 (91,940)	96 (78)	0.0998 (0.0848)	32 (1)
42	39	100,840 (96,744)	16 (13)	0.0159 (0.0134)	26 (3)
43	40	100,494 (96,472)	16 (14)	0.0159 (0.0145)	40 (6)
44	44	95,964 (91,991)	58 (52)	0.0604 (0.0565)	36 (8)
46	42	98,060 (93,901)	42 (36)	0.0428 (0.0383)	30 (7)
47	43	97,575 (93,677)	57 (36)	0.0584 (0.0384)	26 (7)
49	47	94,510 (90,422)	43 (33)	0.0455 (0.0365)	53 (6)
51	48	91,098 (86,923)	53 (45)	0.057 (0.0507)	43 (15)
52	49	92,884 (89,132)	37 (32)	0.0398 (0.0359)	27 (3)
54	50	92,286 (88,468)	31 (27)	0.0336 (0.0305)	28 (3)
53	51	92,336 (88,599)	57 (45)	0.0617 (0.0508)	33 (3)
55	53	91,549 (87,685)	14 (10)	0.0153 (0.0114)	36 (0)
56	54	88,975 (85,757)	398 (354)	0.4473 (0.4128)	105 (46)
58	55	73,705 (69,875)	44 (33)	0.0531 (0.0418)	38 (5)
59	34	78,100 (74,903)	70 (55)	0.0896 (0.0734)	27 (6)
60	56	81,379 (78,014)	41 (30)	0.0504 (0.0385)	28 (7)
62	59	65,222 (62,352)	67 (52)	0.1027 (0.0834)	36 (9)
Total		5,761,093 (5,528,160)	2,931 (2,344)	0.0509 (0.0424)	2,242 (363)

Values in parentheses are calculated after removal of homopolymer regions (*Materials and Methods*).

Table S4. Summary of read depth for the single-copy regions of each chromosome in the *S. noctiflora* BRP mitochondrial genome

Chromosome	Length, bp	Mean read depth	Median read depth	SD read depth
1	188,337	24.48	24	5.90
2	160,272	20.68	20	5.43
3	153,950	23.00	23	5.51
4	152,063	22.43	22	6.03
5	145,718	23.77	23	6.21
6	147,899	22.07	22	5.93
7	146,465	20.08	20	5.21
8	139,545	24.18	24	5.95
9	139,867	21.09	21	5.55
10	138,847	20.91	21	5.55
11	134,804	23.03	23	5.71
12	130,045	20.07	20	5.78
13	129,952	19.53	19	5.30
14	127,559	18.12	18	4.89
15	127,208	25.05	25	5.85
16	122,637	24.83	24	6.67
17	123,324	23.08	23	5.57
18	126,053	18.07	18	5.08
19	124,904	21.39	21	5.47
20	121,966	24.10	24	6.39
21	119,469	22.45	22	5.65
22	120,428	19.08	19	5.22
23	120,245	21.99	22	5.63
24	110,327	21.91	21	5.65
25	117,701	24.52	24	5.98
26	117,164	20.88	20	5.53
27	116,776	19.88	20	5.30
28	115,687	21.87	22	5.65
29	113,654	20.29	20	5.48
30	114,891	25.26	25	6.10
31	106,657	19.30	19	4.99
32	89,361	16.26	16	4.88
33	108,169	21.36	21	5.62
34	105,645	20.59	20	5.83
35	108,105	18.48	18	5.24
36	104,704	22.23	22	5.64
37	103,264	19.66	19	5.30
38	99,401	16.49	16	4.62
39	101,906	23.22	23	6.14
40	101,096	21.25	21	5.33
41	98,560	18.73	18	5.17
42	101,035	17.38	17	5.02
43	97,426	21.56	21	5.56
44	97,250	16.99	17	5.05
45	93,322	20.80	20	5.45
46	97,701	15.22	15	4.18
47	97,126	17.81	18	4.75
48	96,769	14.97	15	4.73
49	94,316	20.47	20	5.56
50	90,874	24.38	24	6.13
51	89,277	21.35	21	5.70
52	86,046	21.35	21	5.16
53	90,208	20.16	20	5.45
54	87,668	20.66	20	5.74
55	88,886	19.97	20	5.19
56	85,565	23.82	24	5.39
57	85,966	15.75	15	4.69
58	82,018	21.79	21	5.64
59	81,689	23.63	23	5.34
60	73,153	17.60	17	4.85
61	51,443	12.22	12	3.93
62	58,932	14.46	14	4.91
63	63,513	13.99	14	4.33

All values are calculated after excluding repeated sequences in each chromosome (*Materials and Methods*).

Table S5. ANOVA table from comparison of estimated copy numbers from absolute qPCR assays of *S. noctiflora* BRP mitochondrial chromosomes 30 and 61

Effect	df	SS	MS	F	*P*
Biological replicate	1	1,950,730	1,950,730	31.44	<0.001
Chromosome	1	13,674,126	13,674,126	220.41	<0.001
Marker (chromosome)	4	2,042,877	510,719	8.23	<0.001
Biological replicate*chromosome	1	3,226	3,226	0.05	0.82
Residuals	28	1,737,075	62,038		

MS, mean square; SS, sum of squares.

*Indicates an interaction between the two effects.

Table S6. Correlation in read depth for mitochondrial chromosome shared between *S. noctiflora* BRP and OSR

BRP				OSR			
Chromosome	Mean read depth	Median read depth	SD read depth	Chromosome	Mean read depth	Median read depth	SD read depth
1	24.48	24	5.90	1	25.92	26	7.36
2	20.68	20	5.43	2	23.51	23	6.86
3	23.00	23	5.51	3	26.47	26	7.27
4	22.43	22	6.03	4	26.36	26	7.06
5	23.77	23	6.21	5	26.64	26	6.95
6	22.07	22	5.93	6	24.88	24	7.00
7	20.08	20	5.21	8	22.81	22	6.70
8	24.18	24	5.95	9	25.74	26	7.03
9	21.09	21	5.55	10	25.58	25	7.14
10	20.91	21	5.55	11	24.01	23	7.02
11	23.03	23	5.71	12	25.80	25	7.13
12	20.07	20	5.78	13	22.90	22	6.71
13	19.53	19	5.30	14	22.62	22	6.45
14	18.12	18	4.89	17	22.41	22	6.40
15	25.05	25	5.85	18	26.07	26	6.97
16	24.83	24	6.67	19	28.38	28	7.06
17	23.08	23	5.57	16	25.21	25	6.83
18	18.07	18	5.08	15	23.96	24	6.96
19	21.39	21	5.47	21	24.21	24	6.82
20	24.10	24	6.39	20	27.72	27	7.52
21	22.45	22	5.65	22	24.59	24	6.57
23	21.99	22	5.63	23	22.96	23	6.23
24	21.91	21	5.65	24	23.88	24	6.63
28	21.87	22	5.65	25	26.04	26	7.14
29	20.29	20	5.48	41	23.52	23	7.03
30	25.26	25	6.10	26	25.23	25	6.67
32	16.26	16	4.88	27	23.78	23	6.41
34	20.59	20	5.83	28	24.68	24	6.85
35	18.48	18	5.24	29	22.28	22	6.57
36	22.23	22	5.64	32	23.37	23	6.94
37	19.66	19	5.30	37	22.01	22	6.31
39	23.22	23	6.14	38	26.53	26	7.61
41	18.73	18	5.17	45	21.70	21	6.49
42	17.38	17	5.02	39	22.13	22	6.72
43	21.56	21	5.56	40	24.95	25	6.76
44	16.99	17	5.05	44	21.15	21	6.18
46	15.22	15	4.18	42	18.82	19	5.57
47	17.81	18	4.75	43	22.86	22	7.58
48	14.97	15	4.73	33	22.82	22	6.48
49	20.47	20	5.56	47	23.45	23	6.43
51	21.35	21	5.70	48	24.05	24	6.64
52	21.35	21	5.16	49	23.83	23	6.30
53	20.16	20	5.45	51	25.93	25	7.30
54	20.66	20	5.74	50	23.63	23	6.77
55	19.97	20	5.19	53	24.15	24	7.12
56	23.82	24	5.39	54	24.49	24	7.26
58	21.79	21	5.64	55	25.50	25	7.11
59	23.63	23	5.34	34	27.16	27	7.25
60	17.60	17	4.85	56	26.03	25	6.83
62	14.46	14	4.91	59	22.39	22	7.00

All values are calculated after excluding repeated sequences in each chromosome (*Materials and Methods*).

Table S7. ANOVA table from comparison of ΔCt values from relative qPCR assays of *S. noctiflora* mitochondrial chromosomes in the BDA and BWT families

Effect	df	SS	MS	F	*P*
Family	1	8.49	8.49	80.17	<0.001
Chromosome	4	8.20	2.05	19.36	<0.001
Biological replicate (family)	4	18.61	4.65	43.94	<0.001
Marker (chromosome)	10	24.38	2.44	23.02	<0.001
Family*chromosome	4	0.17	0.04	0.39	0.82
Residuals	242	25.63	0.11		

MS, mean square; SS, sum of squares.

*Indicates an interaction between the two effects.

Table S8. Summary of *S. noctiflora* populations

Population	Seed collection location/source
BRP	Nelson County, VA, United States
OSR	Giles County, VA, United States
BDA	Budapest, Hungary
BWT	Tübingen, Germany
NOU	Noutonice, Czech Republic
KEW 147	Unknown
KEW 1661	Unknown
KEW 1672	Unknown
KEW 12991	Cambridgeshire, England
KEW 22121	Bosnia-Herzegovina
KEW 36186	Ontario, Canada
KEW 120913	Oxfordshire, England

All populations starting with “KEW” are from the Kew Royal Botanic Gardens Millennium Seed Bank Collection, with the noted accession numbers.

Table S9. PCR primer sequences

Primer name	Primer sequence
qPCR primers for screening 7 mitochondrial chromosomes	
OSR.37.3F	AGGGGAAGAAACCGAGTAAGAACT
OSR.37.3R	ACGTACTGGAATGAGTAGGCGATT
OSR.37.4F	ACTTAGTAATGAACCGTGCGTGAG
OSR.37.4R	GTTTGCATAACGGTGTTTATTCCA
OSR.37.5F	CAAGGGTAGCAAAGTGAGTGAAAG
OSR.37.5R	ATCTAAGCTCACAAGCAAATACCG
OSR.38.1F	TCTTTTCCGAGGACTCTTAATTGC
OSR.38.1R	AAGCGCGAAAGATTTTGTAGGTAG
OSR.38.2F	AATAGCATCCATACCCATTCGTCT
OSR.38.2R	CCAGTCGAGCATGAAGACAATTTA
OSR.38.3F	GCAACAGTTTCTGGACTAAGGACA
OSR.38.3R	AAGTCTTGGTGAATTCCTTTGCTT
OSR.47.1F	AAGAAAGCCTTCAACTAAGGAGCA
OSR.47.1R	CCCGCCTGTTTATCTTGTAACTTC
OSR.47.2F	TATGGTCATGGTTTCCTAATGCTG
OSR.47.2R	ATGATTGTGAGTCAACCGCTAAGA
OSR.47.3F	ATCTACTCGCCCCAATCAAAATTA
OSR.47.3R	ACGGGATGTTCACTAAGCCTCTAC
BRP.28.1F	TTATAGGCGCGCGTGATATAGTCT
BRP.28.1R	GGAAACTGTTGCTTACCCGTATTC
BRP.28.2F	AACGTTTGCATCATAAAGAGGAGA
BRP.28.2R	TGCTTTATAGTGGATCCTGAGTGC
BRP.28.3F	CAGGACTCTCTTGTCGGGGTTAAT
BRP.28.3R	AGATTGAGATAGAGCCTTGGGTGA
BRP.31.2F	ATTTTGTGCGGGAGTCTCTCAGT
BRP.31.2R	CTTCTTCTGATGCCTCAAGGTGT
BRP.31.3F	AGCCATCCCCTTAATGTAAACTCC
BRP.31.3R	GGCAATTCCCTTGCTAGCTTAGT
BRP.31.4F	AAAATACTGCGGGCTTACGTTTAG
BRP.31.4R	ATCTTGCGAGAGATGCAAGTGTAG
OSR.30.1F	AAAGGGTTCGTCTTGTTGAAAGAG
OSR.30.1R	AAAGGTGCTCTGGTGACACATATC
OSR.30.2F	TCTTACCTCCATGAAAGAAAGCAA
OSR.30.2R	TAAGACATAAGGCAGCTGTTCTGC
OSR.30.3F	GAGCAGTATGCTCTGATACCAACC
OSR.30.3R	AACTGGTAATGGTTGCAGGGTAAT
OSR.35.1F	GCCCAATCAAATTCACAAAATACA
OSR.35.1R	CTCTTGCTATCGAGCATCAAGGTA
OSR.35.2F	TCGCCTGCCTGTAACTTATACTTG
OSR.35.2R	ATCTTAGAGACCCGCCATATTCCT
OSR.35.3F	CAGCTTGTTCAAACAAAAGAATGG
OSR.35.3R	GATGCTTTTGGTATCAATGCAAAG
qPCR primers for nuclear reference gene	
SnX4_F	TTCCAGAGAAGCTTAGATCCGACT
SnX4_R	GACAGTTGTCGGTGTCTCTGAAGT
Conventional and qPCR primers for absolute quantification	
BRP.30_B_F24223	GCGGTCCGAAAATACCAATA
BRP.30_B_R24872	GTGCCATTTCCCTTCTGAAA
BRP.30_S_F24473	TATGACAGGCGCAAGTTCAG
BRP.30_S_R24594	TCCTGACCAGGCAAGCTACT
BRP.30_B_F48037	GATGTGAGCCTTTCCTTTCG
BRP.30_B_R48671	GGGCCATCTTTCCTTTAAGC
BRP.30_S_F48465	AGCGACACTTGGACTCTGCT
BRP.30_S_R48580	CGGGTCAATGAGTCTTCGTA
BRP.30_B_F80866	GAAGCAGCTGGAATCGAGAC
BRP.30_B_R81521	CGACCACTCTCCCTCAAGAA
BRP.30_S_F81038	TGAACGAAAGCAGGTCACAG
BRP.30_S_R81157	TCTTTGGGAAAAAGCGAGAA
BRP.61_B_F14252	AAGCCTGGCCACGTAGTTTA
BRP.61_B_R14901	TTCGCTGCTACTCGCTAGGT
BRP.61_S_F14666	ACCCGTTCCAATCCAACATA
BRP.61_S_R14787	GCCAGCGTCCATAAGAGAAG

Table S9. Cont.

Primer name	Primer sequence
BRP.61_B_F26132	CTCATGTACGCCCCATTCTT
BRP.61_B_R26779	CCACACACAACCAATCTCGT
BRP.61_S_F26302	CCTCCCCCTCGATTCTTTAG
BRP.61_S_R26423	ATGGGCAGTACGGTCTTTTG
BRP.61_B_F41911	GAAAGCCCGCTTGTAGGTTA
BRP.61_B_R42554	GAAATGCGTGGAATTCGTTT
BRP.61_S_F42000	CTTCCGCTTACGTCTTGGAC
BRP.61_S_R42120	GATTCTCGCTGGCTTTGAAC
Conventional PCR primers for genome assembly gap filling	
BRP.61_gap_F430	AAGCGAGCGAGACTCTCAAC
BRP.61_gap_R1062	AGGGGGTGGTTTCCACTAAG
BRP.61_gap_F544	TGAGGGTAGACTAATTGGCTTGTT
BRP.61_gap_R1202	AAGACTAGAAAGGGGTCTTTTGCT