

SUPPLEMENTARY MATERIAL

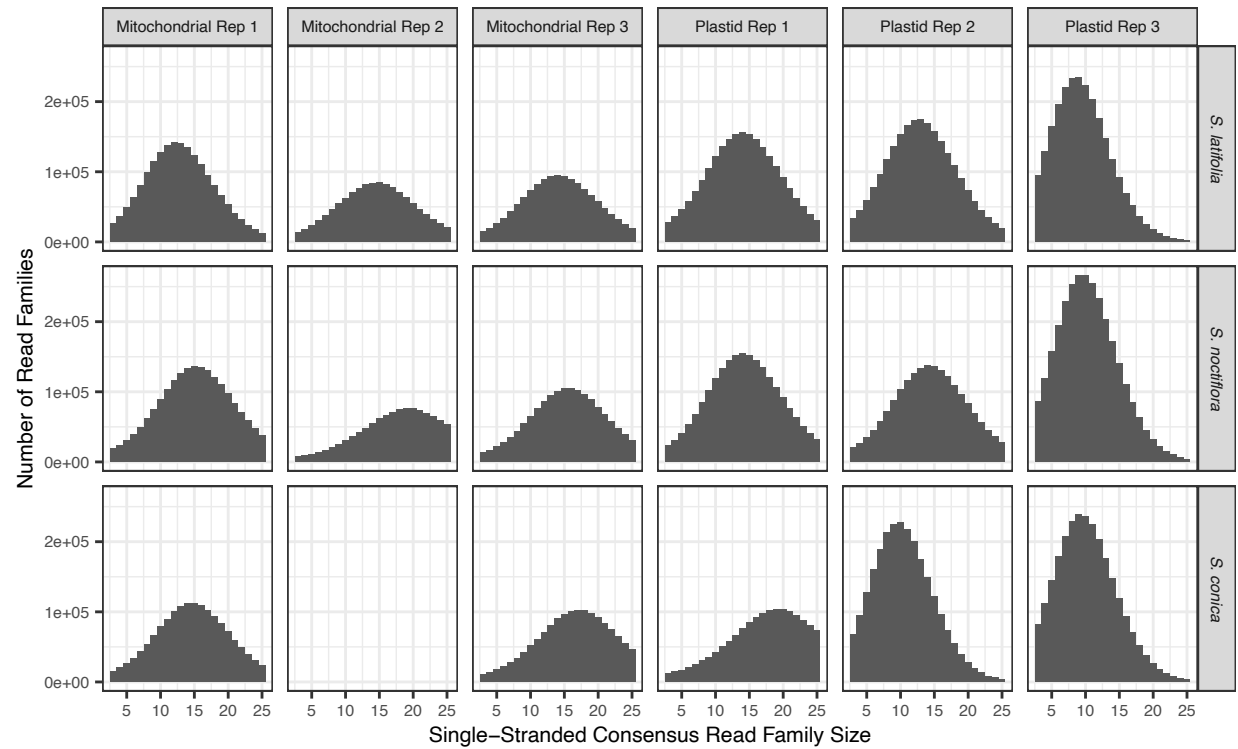


Figure S1. Summary of family sizes for single-stranded consensus sequences used for generation of double-stranded consensus sequences for each Duplex Sequencing library. The analysis pipeline required a minimum family size of 3 reads for each of the two complementary single-stranded families. The mitochondrial DNA library for the second biological replicate from *S. conica* was not sequenced because of an apparent library loading error.

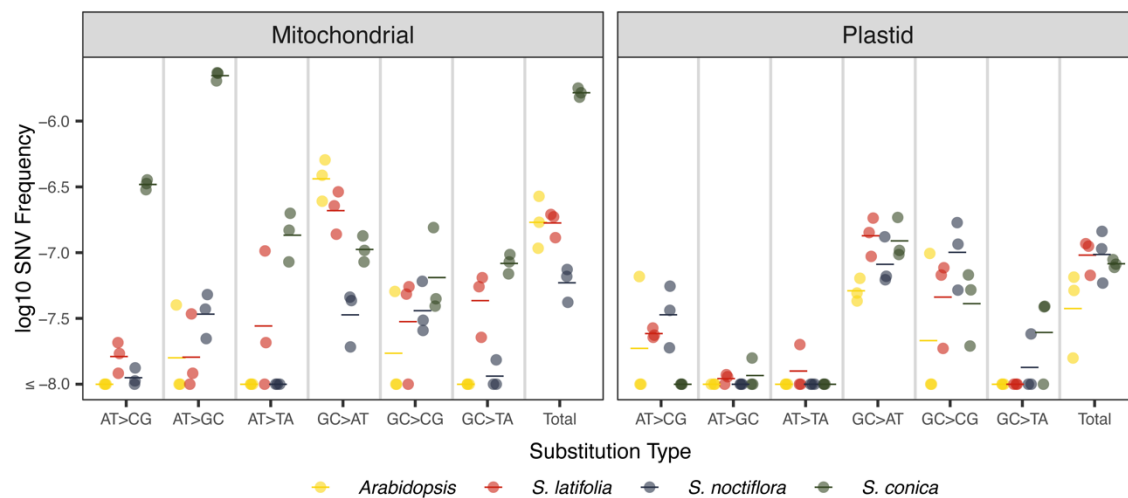


Figure S2. Variation in mitochondrial and plastid SNV frequencies and spectra across *Arabidopsis* and *Silene* species. The same data shown in Figure 2 are plotted on a log scale here.

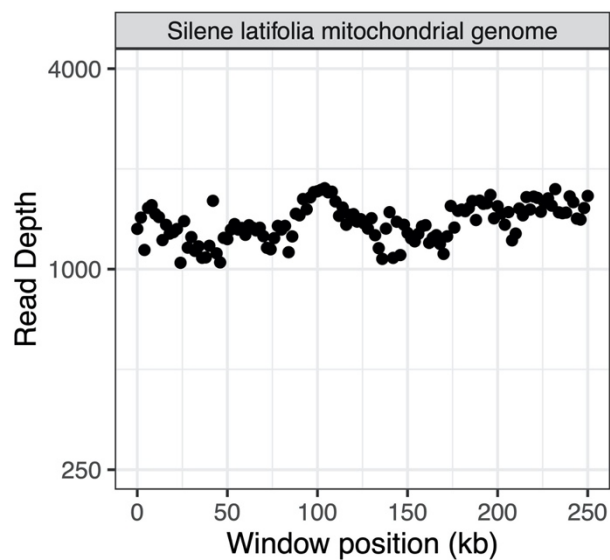


Figure S3. Summary of mitochondrial genome coverage in the *S. latifolia* UK2600 total-cellular sequencing library. Coverage is estimated based on reads mapping with a maximum of one mismatch and no indels in 2-kb windows.

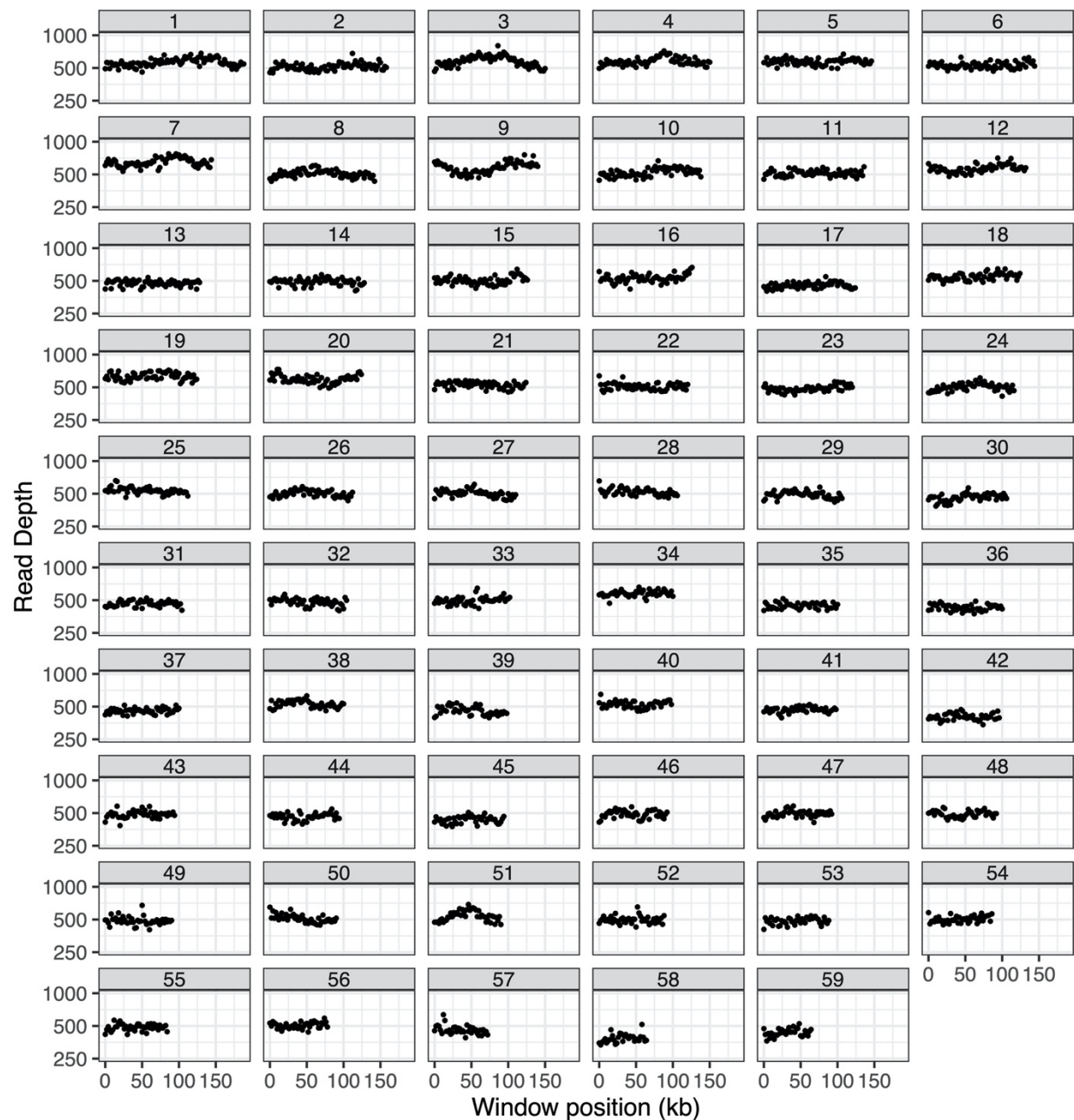


Figure S4. Summary of mitochondrial genome coverage in the *S. noctiflora* OSR total-cellular sequencing library. Coverage is estimated based on reads mapping with a maximum of one mismatch and no indels in 2-kb windows. Each panel represents a different chromosome within the multichromosomal genome.

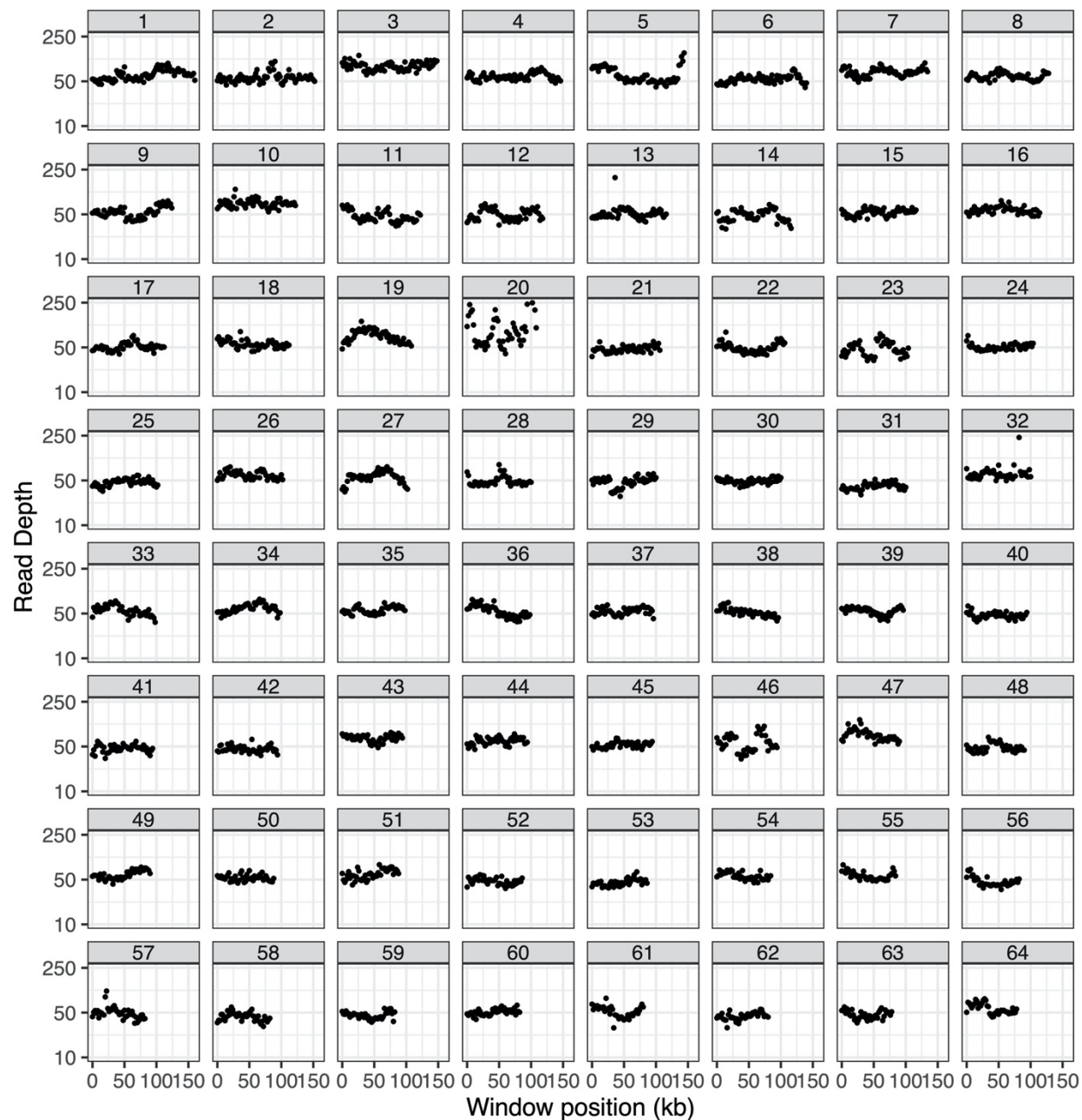


Figure S5a. Summary of coverage for mitochondrial chromosomes 1 through 64 in the *S. conica* ABR total-cellular sequencing library. Coverage is estimated based on reads mapping with a maximum of one mismatch and no indels in 2-kb windows. Note that four of the data points on chromosome 20 and two of the data points on chromosome 32 exceeded a coverage of 250 \times and are not shown to improve readability of the plots.

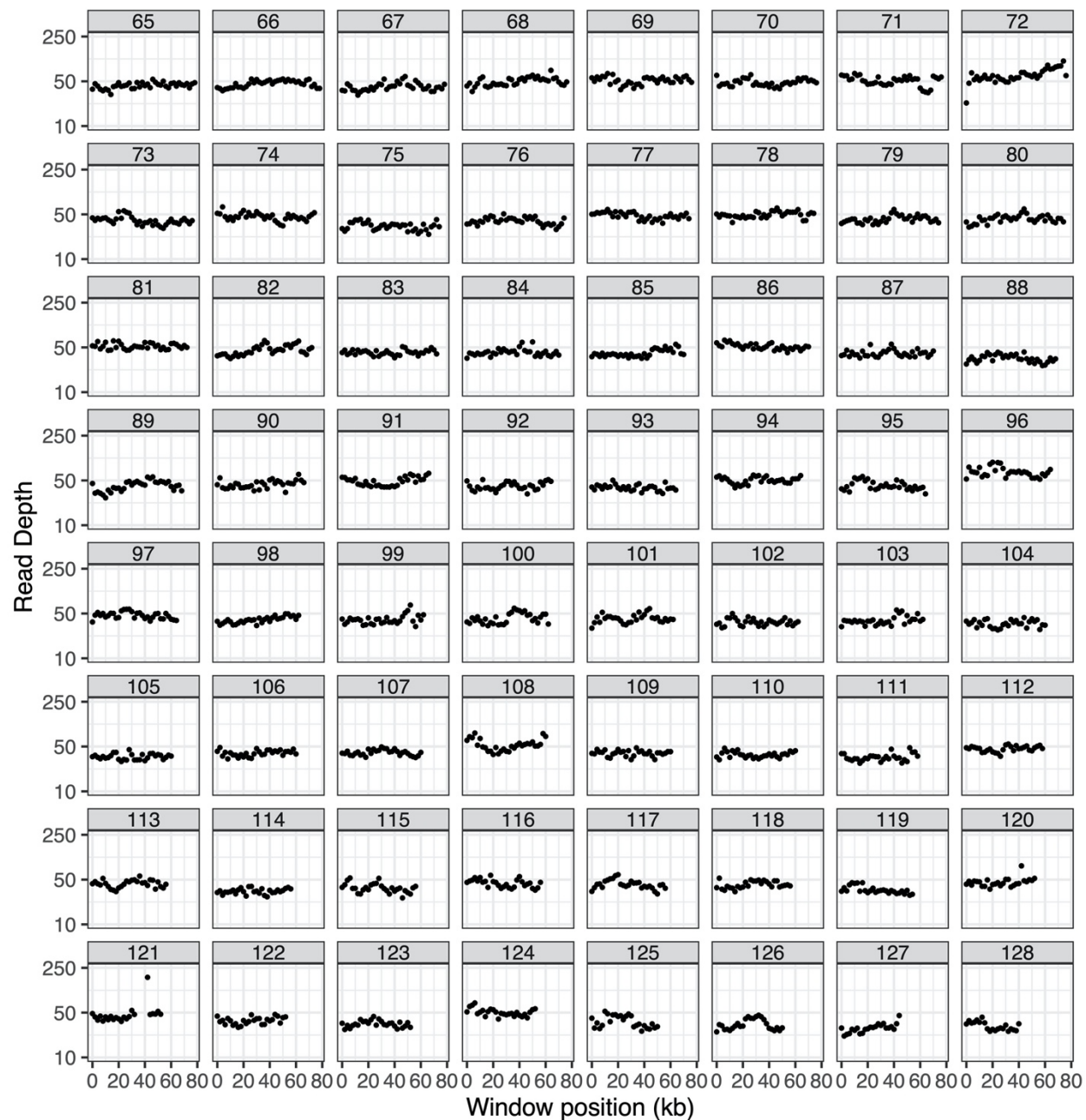


Figure S5b. Summary of coverage for mitochondrial chromosomes 65 through 128 in the *S. conica* ABR total-cellular sequencing library. Coverage is estimated based on reads mapping with a maximum of one mismatch and no indels in 2-kb windows. Note that four of the data points on chromosome 121 exceeded a coverage of 250 \times and are not shown to improve readability of the plots.

Table S1. Primers used in the *S. conica* ddPCR analysis. The two nuclear markers are referred to by the identifiers for the homologs in *Arabidopsis thaliana*.

| Gene | Genome | Forward Primer | Reverse Primer |
|------------------|---------------|------------------------|-------------------------|
| <i>nad9</i> | mitochondrial | CAAGAAGTGGGTCAAAGAATG | CGAGAATTAAAACGAGTACTCAG |
| <i>matR</i> | mitochondrial | GATCAGAATGGTACCCGAATC | CCTTGAAGATTGCTAGGAGTTG |
| <i>rpoB</i> | plastid | CGGGAGATGCAAGAACAAAC | CTGATCTTCCTCCCAATCTG |
| <i>petA</i> | plastid | CTATCCCATTTTTGCCCAGC | CCCCTTTCTTGCCGTTAGC |
| <i>AT1G06040</i> | nuclear | CCTTTATCTTCTGTGTGGAGG | GGCTGTGCACTTTGGTTGG |
| <i>AT1G07630</i> | nuclear | CAGAAAAAGTGGAATGATGCTC | CAATGAGATGAAGTGCTCTACC |

Table S2. Summary of library sequencing and mapping. Reported coverage values and mapping percentages are for duplex consensus sequences.

| Library | Library Type | SRA Accession | Raw Read Pairs | Mito Coverage (bp) | Plastid Coverage (bp) | Mito Mapping (%) | Plastid Mapping (%) | Unmapped (%) |
|---------------------------------|------------------------|---------------|----------------|--------------------|-----------------------|------------------|---------------------|--------------|
| Silene conica chloroplast 1 | Chloroplast duplex | SAMN17011097 | 1.15E+08 | 9.25E+07 | 2.03E+08 | 29.49% | 64.88% | 5.63% |
| Silene conica chloroplast 2 | Chloroplast duplex | SAMN17011098 | 1.15E+08 | 1.33E+08 | 2.72E+08 | 31.48% | 64.13% | 4.39% |
| Silene conica chloroplast 3 | Chloroplast duplex | SAMN17011099 | 1.42E+08 | 1.37E+08 | 2.70E+08 | 31.72% | 62.55% | 5.73% |
| Silene conica mito 1 | Mitochondrial duplex | SAMN17011100 | 1.01E+08 | 2.18E+08 | 1.77E+07 | 80.87% | 6.58% | 12.55% |
| Silene conica mito 3 | Mitochondrial duplex | SAMN17011101 | 1.14E+08 | 2.16E+08 | 2.01E+07 | 77.21% | 7.16% | 15.63% |
| Silene latifolia chloroplast 1 | Chloroplast duplex | SAMN17011085 | 1.33E+08 | 2.89E+07 | 2.83E+08 | 7.39% | 72.35% | 20.26% |
| Silene latifolia chloroplast 2 | Chloroplast duplex | SAMN17011086 | 1.28E+08 | 3.54E+07 | 2.41E+08 | 8.82% | 60.10% | 31.08% |
| Silene latifolia chloroplast 3 | Chloroplast duplex | SAMN17011087 | 1.01E+08 | 3.68E+07 | 2.73E+08 | 9.13% | 67.73% | 23.14% |
| Silene latifolia mito 1 | Mitochondrial duplex | SAMN17011088 | 1.13E+08 | 1.16E+08 | 2.67E+07 | 38.26% | 8.83% | 52.91% |
| Silene latifolia mito 2 | Mitochondrial duplex | SAMN17011089 | 9.79E+07 | 4.93E+07 | 1.63E+07 | 24.87% | 8.21% | 66.92% |
| Silene latifolia mito 3 | Mitochondrial duplex | SAMN17011090 | 8.83E+07 | 6.56E+07 | 2.23E+07 | 27.94% | 9.48% | 62.58% |
| Silene noctiflora chloroplast 1 | Chloroplast duplex | SAMN17011091 | 1.37E+08 | 1.06E+08 | 2.55E+08 | 27.63% | 66.35% | 6.02% |
| Silene noctiflora chloroplast 2 | Chloroplast duplex | SAMN17011092 | 1.25E+08 | 9.39E+07 | 2.20E+08 | 28.18% | 66.12% | 5.70% |
| Silene noctiflora chloroplast 3 | Chloroplast duplex | SAMN17011093 | 1.37E+08 | 1.25E+08 | 3.19E+08 | 25.69% | 65.36% | 8.95% |
| Silene noctiflora mito 1 | Mitochondrial duplex | SAMN17011094 | 1.32E+08 | 2.76E+08 | 1.56E+07 | 83.03% | 4.68% | 12.29% |
| Silene noctiflora mito 2 | Mitochondrial duplex | SAMN17011095 | 1.06E+08 | 1.88E+08 | 1.22E+07 | 81.54% | 5.28% | 13.18% |
| Silene noctiflora mito 3 | Mitochondrial duplex | SAMN17011096 | 1.03E+08 | 1.93E+08 | 2.39E+07 | 71.53% | 8.84% | 19.63% |
| Silene conica ABR | Total cellular shotgun | SAMN17011105 | 4.98E+08 | 3.53E+08 | 1.68E+09 | 0.49% | 2.31% | 97.20% |
| Silene noctiflora OSR | Total cellular shotgun | SAMN17011104 | 5.76E+08 | 3.45E+09 | 2.10E+09 | 2.51% | 1.53% | 95.96% |
| Silene latifolia UK2600 | Total cellular shotgun | SAMN17011102 | 2.81E+08 | 5.95E+08 | 5.51E+09 | 0.50% | 4.64% | 94.86% |
| Silene latifolia Kew 32982 | Total cellular shotgun | SAMN17011103 | 3.04E+08 | | | | | |

Table S3. *Silene* mitochondrial SNV count and frequency data with and without *k*-mer filtering

| Species | Rep | k-mer Filt | SNV Counts | | | | | | | SNV Frequencies | | | | | | |
|--------------------------|-----|------------|------------|-----|-----|-----|-----|-----|-------|-----------------|---------|---------|---------|---------|---------|---------|
| | | | A>C | A>G | A>T | C>A | C>G | C>T | Total | A>C | A>G | A>T | C>A | C>G | C>T | Total |
| <i>Silene conica</i> | 1 | 10 | 53 | 356 | 26 | 13 | 6 | 18 | 472 | 3.0E-07 | 2.0E-06 | 1.5E-07 | 9.7E-08 | 4.5E-08 | 1.3E-07 | 1.5E-06 |
| <i>Silene conica</i> | 2 | 10 | 27 | 176 | 15 | 4 | 9 | 6 | 237 | 3.6E-07 | 2.3E-06 | 2.0E-07 | 6.9E-08 | 1.6E-07 | 1.0E-07 | 1.8E-06 |
| <i>Silene conica</i> | 3 | 10 | 67 | 462 | 17 | 13 | 6 | 13 | 578 | 3.3E-07 | 2.3E-06 | 8.5E-08 | 8.5E-08 | 3.9E-08 | 8.5E-08 | 1.6E-06 |
| <i>Silene conica</i> | 1 | None | 150 | 483 | 58 | 49 | 12 | 217 | 969 | 8.5E-07 | 2.7E-06 | 3.3E-07 | 3.6E-07 | 8.9E-08 | 1.6E-06 | 3.1E-06 |
| <i>Silene conica</i> | 2 | None | 64 | 238 | 24 | 20 | 12 | 98 | 456 | 8.5E-07 | 3.2E-06 | 3.2E-07 | 3.5E-07 | 2.1E-07 | 1.7E-06 | 3.4E-06 |
| <i>Silene conica</i> | 3 | None | 158 | 610 | 50 | 59 | 17 | 231 | 1125 | 7.9E-07 | 3.0E-06 | 2.5E-07 | 3.9E-07 | 1.1E-07 | 1.5E-06 | 3.2E-06 |
| <i>Silene latifolia</i> | 1 | 10 | 1 | 1 | 0 | 4 | 3 | 18 | 27 | 5.7E-09 | 5.7E-09 | 0.0E+00 | 3.0E-08 | 2.2E-08 | 1.3E-07 | 8.7E-08 |
| <i>Silene latifolia</i> | 2 | 10 | 1 | 0 | 1 | 2 | 2 | 5 | 11 | 1.3E-08 | 0.0E+00 | 1.3E-08 | 3.5E-08 | 3.5E-08 | 8.6E-08 | 8.2E-08 |
| <i>Silene latifolia</i> | 3 | 10 | 1 | 2 | 6 | 1 | 0 | 10 | 20 | 5.0E-09 | 1.0E-08 | 3.0E-08 | 6.5E-09 | 0.0E+00 | 6.5E-08 | 5.7E-08 |
| <i>Silene latifolia</i> | 1 | None | 9 | 8 | 6 | 17 | 5 | 42 | 87 | 1.1E-07 | 9.7E-08 | 7.3E-08 | 2.7E-07 | 8.1E-08 | 6.8E-07 | 6.0E-07 |
| <i>Silene latifolia</i> | 2 | None | 5 | 8 | 14 | 17 | 6 | 58 | 108 | 1.0E-07 | 1.7E-07 | 2.9E-07 | 4.7E-07 | 1.7E-07 | 1.6E-06 | 1.3E-06 |
| <i>Silene latifolia</i> | 3 | None | 9 | 16 | 18 | 19 | 10 | 52 | 124 | 1.5E-07 | 2.7E-07 | 3.1E-07 | 4.3E-07 | 2.3E-07 | 1.2E-06 | 1.2E-06 |
| <i>Silene noctiflora</i> | 1 | 10 | 3 | 5 | 1 | 0 | 4 | 3 | 16 | 1.3E-08 | 2.2E-08 | 4.4E-09 | 0.0E+00 | 2.6E-08 | 1.9E-08 | 4.2E-08 |
| <i>Silene noctiflora</i> | 2 | 10 | 1 | 8 | 0 | 0 | 7 | 5 | 21 | 6.0E-09 | 4.8E-08 | 0.0E+00 | 0.0E+00 | 6.1E-08 | 4.3E-08 | 7.4E-08 |
| <i>Silene noctiflora</i> | 3 | 10 | 2 | 7 | 0 | 2 | 4 | 6 | 21 | 1.1E-08 | 3.7E-08 | 0.0E+00 | 1.5E-08 | 3.1E-08 | 4.6E-08 | 6.6E-08 |
| <i>Silene noctiflora</i> | 1 | None | 3 | 8 | 3 | 4 | 6 | 12 | 36 | 1.3E-08 | 3.5E-08 | 1.3E-08 | 2.6E-08 | 3.8E-08 | 7.7E-08 | 9.4E-08 |
| <i>Silene noctiflora</i> | 2 | None | 2 | 11 | 1 | 2 | 13 | 9 | 38 | 1.2E-08 | 6.6E-08 | 6.0E-09 | 1.7E-08 | 1.1E-07 | 7.8E-08 | 1.3E-07 |
| <i>Silene noctiflora</i> | 3 | None | 5 | 13 | 2 | 11 | 9 | 11 | 51 | 2.7E-08 | 6.9E-08 | 1.1E-08 | 8.4E-08 | 6.9E-08 | 8.4E-08 | 1.6E-07 |

Table S4. *Silene* plastid SNV count and frequency data with and without *k*-mer filtering

| Species | Rep | k-mer Filt | SNV Counts | | | | | | | SNV Frequencies | | | | | | |
|--------------------------|-----|------------|------------|-----|-----|-----|-----|-----|-------|-----------------|---------|---------|---------|---------|---------|---------|
| | | | A>C | A>G | A>T | C>A | C>G | C>T | Total | A>C | A>G | A>T | C>A | C>G | C>T | Total |
| <i>Silene conica</i> | 1 | 10 | 0 | 2 | 1 | 3 | 4 | 8 | 18 | 0.0E+00 | 1.6E-08 | 7.9E-09 | 3.9E-08 | 5.2E-08 | 1.0E-07 | 8.8E-08 |
| <i>Silene conica</i> | 2 | 10 | 0 | 0 | 0 | 4 | 7 | 10 | 21 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 3.9E-08 | 6.8E-08 | 9.7E-08 | 7.7E-08 |
| <i>Silene conica</i> | 3 | 10 | 0 | 1 | 0 | 0 | 2 | 19 | 22 | 0.0E+00 | 6.0E-09 | 0.0E+00 | 0.0E+00 | 2.0E-08 | 1.9E-07 | 8.2E-08 |
| <i>Silene conica</i> | 1 | None | 1 | 4 | 1 | 3 | 4 | 12 | 25 | 7.9E-09 | 3.2E-08 | 7.9E-09 | 3.9E-08 | 5.2E-08 | 1.6E-07 | 1.2E-07 |
| <i>Silene conica</i> | 2 | None | 0 | 0 | 0 | 6 | 8 | 20 | 34 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 5.8E-08 | 7.7E-08 | 1.9E-07 | 1.3E-07 |
| <i>Silene conica</i> | 3 | None | 0 | 1 | 1 | 2 | 4 | 26 | 34 | 0.0E+00 | 6.0E-09 | 6.0E-09 | 2.0E-08 | 3.9E-08 | 2.5E-07 | 1.3E-07 |
| <i>Silene latifolia</i> | 1 | 10 | 4 | 2 | 0 | 1 | 2 | 10 | 19 | 2.3E-08 | 1.1E-08 | 0.0E+00 | 9.4E-09 | 1.9E-08 | 9.4E-08 | 6.7E-08 |
| <i>Silene latifolia</i> | 2 | 10 | 4 | 0 | 3 | 0 | 7 | 13 | 27 | 2.7E-08 | 0.0E+00 | 2.0E-08 | 0.0E+00 | 7.7E-08 | 1.4E-07 | 1.1E-07 |
| <i>Silene latifolia</i> | 3 | 10 | 4 | 2 | 0 | 0 | 7 | 19 | 32 | 2.4E-08 | 1.2E-08 | 0.0E+00 | 0.0E+00 | 6.7E-08 | 1.8E-07 | 1.2E-07 |
| <i>Silene latifolia</i> | 1 | None | 8 | 6 | 4 | 2 | 3 | 31 | 54 | 4.5E-08 | 3.4E-08 | 2.3E-08 | 1.9E-08 | 2.8E-08 | 2.9E-07 | 1.9E-07 |
| <i>Silene latifolia</i> | 2 | None | 10 | 4 | 11 | 10 | 10 | 38 | 83 | 6.7E-08 | 2.7E-08 | 7.3E-08 | 1.1E-07 | 1.1E-07 | 4.2E-07 | 3.4E-07 |
| <i>Silene latifolia</i> | 3 | None | 7 | 12 | 6 | 6 | 9 | 30 | 70 | 4.1E-08 | 7.1E-08 | 3.5E-08 | 5.8E-08 | 8.7E-08 | 2.9E-07 | 2.6E-07 |
| <i>Silene noctiflora</i> | 1 | 10 | 3 | 0 | 1 | 0 | 5 | 6 | 15 | 1.9E-08 | 0.0E+00 | 6.3E-09 | 0.0E+00 | 5.2E-08 | 6.2E-08 | 5.9E-08 |
| <i>Silene noctiflora</i> | 2 | 10 | 5 | 0 | 0 | 2 | 14 | 11 | 32 | 3.6E-08 | 0.0E+00 | 0.0E+00 | 2.4E-08 | 1.7E-07 | 1.3E-07 | 1.5E-07 |
| <i>Silene noctiflora</i> | 3 | 10 | 11 | 0 | 1 | 0 | 14 | 8 | 34 | 5.6E-08 | 0.0E+00 | 5.1E-09 | 0.0E+00 | 1.2E-07 | 6.6E-08 | 1.1E-07 |
| <i>Silene noctiflora</i> | 1 | None | 5 | 1 | 1 | 1 | 5 | 9 | 22 | 3.1E-08 | 6.3E-09 | 6.3E-09 | 1.0E-08 | 5.2E-08 | 9.3E-08 | 8.6E-08 |
| <i>Silene noctiflora</i> | 2 | None | 6 | 1 | 0 | 2 | 14 | 19 | 42 | 4.4E-08 | 7.3E-09 | 0.0E+00 | 2.4E-08 | 1.7E-07 | 2.3E-07 | 1.9E-07 |
| <i>Silene noctiflora</i> | 3 | None | 12 | 0 | 2 | 2 | 14 | 17 | 47 | 6.1E-08 | 0.0E+00 | 1.0E-08 | 1.7E-08 | 1.2E-07 | 1.4E-07 | 1.5E-07 |

Table S5. Genome copy number estimates

| Species | Nuclear (=Unmapped) Coverage (bp) | Mito Coverage (bp) | Plastid Coverage (bp) | Nuclear Genome Size (bp) | Mito Genome Size (bp) | Plastid Genome Size (bp) | Nuclear Cov. | Mito Cov. | Plastid Cov. | Mito per Nuclear | Plastid per Nuclear |
|--------------------------|---|--------------------------|-----------------------------|--------------------------------|-----------------------------|--------------------------------|-----------------|--------------|-----------------|------------------------|---------------------------|
| <i>Silene latifolia</i> | 7.58E+10 | 3.53E+08 | 1.68E+09 | 2.67E+09 | 2.53E+05 | 1.52E+05 | 29.22 | 1394.70 | 11047.66 | 47.72 | 378.02 |
| <i>Silene noctiflora</i> | 1.41E+11 | 3.45E+09 | 2.10E+09 | 2.78E+09 | 6.73E+06 | 1.52E+05 | 52.70 | 512.25 | 13838.01 | 9.72 | 262.59 |
| <i>Silene conica</i> | 1.20E+11 | 5.95E+08 | 5.51E+09 | 9.30E+08 | 1.13E+07 | 1.47E+05 | 136.49 | 52.55 | 37486.69 | 0.38 | 274.64 |

Table S6. ddPCR droplet counts and copy number calculations

| Sample | Genome | Marker | Positive droplets | Negative droplets | Copies per reaction | Dilution Factor | Copies per nuclear copy |
|----------|---------|-----------|-------------------|-------------------|---------------------|-----------------|-------------------------|
| New1 | Mito | matR | 1244 | 6955 | 3967 | 1 | 0.98 |
| New2 | Mito | matR | 2413 | 10431 | 4900 | 1 | 1.09 |
| New3 | Mito | matR | 1987 | 11250 | 3820 | 1 | 0.91 |
| Original | Mito | matR | 2356 | 13893 | 3680 | 1 | 0.42 |
| New1 | Mito | nad9 | 1842 | 10249 | 3880 | 1 | 0.96 |
| New2 | Mito | nad9 | 1202 | 4781 | 5280 | 1 | 1.18 |
| New3 | Mito | nad9 | 990 | 5106 | 4160 | 1 | 1.00 |
| Original | Mito | nad9 | 2268 | 13951 | 3540 | 1 | 0.41 |
| New1 | Plastid | petA | 6502 | 7514 | 14660 | 200 | 727.54 |
| New2 | Plastid | petA | 6692 | 6199 | 17220 | 200 | 767.04 |
| New3 | Plastid | petA | 6392 | 8264 | 13480 | 200 | 644.98 |
| Original | Plastid | petA | 5601 | 1651 | 34820 | 200 | 804.16 |
| New1 | Plastid | rpoB | 5868 | 6456 | 15220 | 200 | 755.33 |
| New2 | Plastid | rpoB | 6803 | 6015 | 17800 | 200 | 792.87 |
| New3 | Plastid | rpoB | 6409 | 8078 | 13740 | 200 | 657.42 |
| Original | Plastid | rpoB | 10798 | 4448 | 28980 | 200 | 669.28 |
| New1 | Nuclear | AT1G06040 | 1524 | 7912 | 4140 | 1 | |
| New2 | Nuclear | AT1G06040 | 1751 | 8889 | 4240 | 1 | |
| New3 | Nuclear | AT1G06040 | 2070 | 10630 | 4180 | 1 | |
| Original | Nuclear | AT1G06040 | 3982 | 10165 | 7780 | 1 | |
| New1 | Nuclear | AT1G07630 | 1756 | 9820 | 3920 | 1 | |
| New2 | Nuclear | AT1G07630 | 2242 | 10039 | 4740 | 1 | |
| New3 | Nuclear | AT1G07630 | 2118 | 10890 | 4180 | 1 | |
| Original | Nuclear | AT1G07630 | 5315 | 10629 | 9540 | 1 | |

Table S7. Detection of mitochondrial SNVs in multiple biological replicates

| Species | SNV Detection | A>C | A>G | A>T | C>A | C>G | C>T | Total |
|--------------------------|---------------------------|-----|-----|-----|-----|-----|-----|-------|
| <i>Silene conica</i> | Shared among replicates | 17 | 100 | 5 | 3 | 1 | 2 | 128 |
| <i>Silene conica</i> | Unique to one replicate | 32 | 157 | 28 | 19 | 19 | 21 | 276 |
| <i>Silene latifolia</i> | Shared among replicates** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Silene latifolia</i> | Unique to one replicate | 3 | 3 | 7 | 7 | 5 | 33 | 58 |
| <i>Silene noctiflora</i> | Shared among replicates | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Silene noctiflora</i> | Unique to one replicate | 6 | 20 | 1 | 2 | 15 | 14 | 58 |

**The use of parentals for *k*-mer filtering may have biased against detection of shared SNVs in *S. latifolia* (see main text).