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## Supplemental Information

# Mate Selection in Self-Compatible Wild Tobacco Results from Coordinated Variation in Homologous Self-Incompatibility Genes

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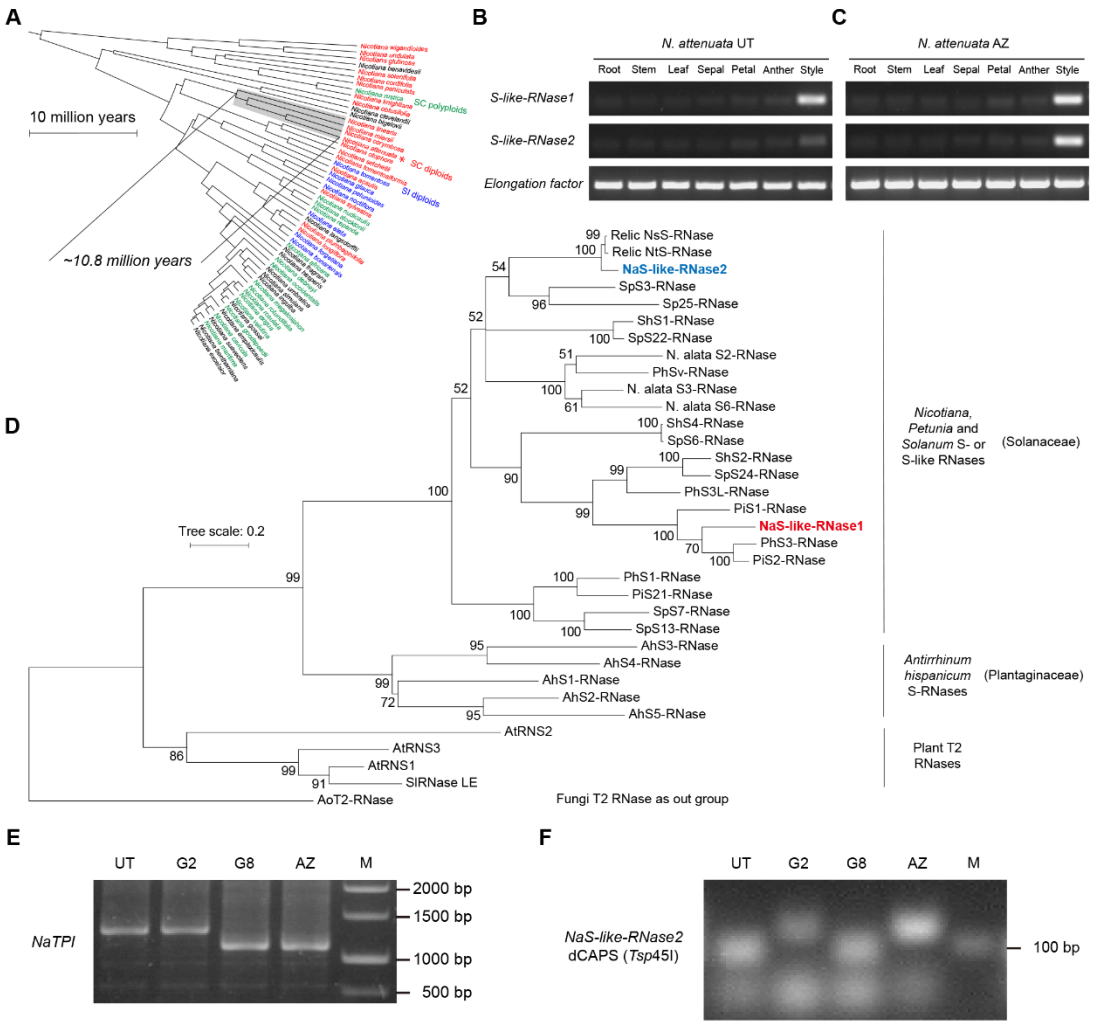


**Figure S1. Phylogenetic Relationships, Ploidy, and Breeding System of 55 *Nicotiana* Species; Tissue-Specific Expression and Phylogenetic Analysis of NaS-like-RNases; *NaTPI* and *NaS-like-RNase2* dCAPS Markers that Distinguish Different Accessions. Related to Figures 1, 3, S3 and Table S3.** (A) The figure is modified from [S1] and [S2]. Branch lengths were obtained with penalized likelihood smoothing, implemented in *r8s* [1]. Red, self-compatible diploids; blue, self-incompatible diploids; green, self-compatible polyploids; black, unknown mating system and ploidy. *N. attenuata* and its closely related species are shaded in grey. (B and C) Transcript accumulation of *S-like-RNase1* and *2* in (B) UT and (C) AZ examined by RT-PCR. Total RNA was extracted from seven tissues as indicated. The synthesized cDNAs were used as templates in RT-PCR. *Nicotiana attenuata elongation factor* (*NaEF*) was used as a positive control. (D) Phylogenetic tree of S- and S-like-RNases. Numbers on branches indicate the bootstrap percentage values calculated from 1000 replicates, and only values greater than 50% are shown. Self-compatible species: *At*, *Arabidopsis thaliana*; *Ns*, *Nicotiana sylvestris*; *Nt*, *Nicotiana tabacum*; *Na*, *Nicotiana attenuata*; *Sl*, *Solanum lycopersicum*. Self-incompatible species: *Sp*, *Solanum peruvianum*; *Sh*, *Solanum habrochaites*; *Ph*, *Petunia x hybrida*; *Pi*, *Petunia inflata*; *Ah*, *Antirrhinum hispanicum*. Fungi *Aspergillus oryzae* T2 RNase was used as an out group for the phylogenetic analysis. (E) PCR products of the trypsin proteinase inhibitor (*NaTPI*) gene in four accessions. (F) PCR products of *S-like-RNase2* flanking sequences were digested by

*Tsp*45I. Genomic DNA was extracted from leaves of different accessions as template for PCR.

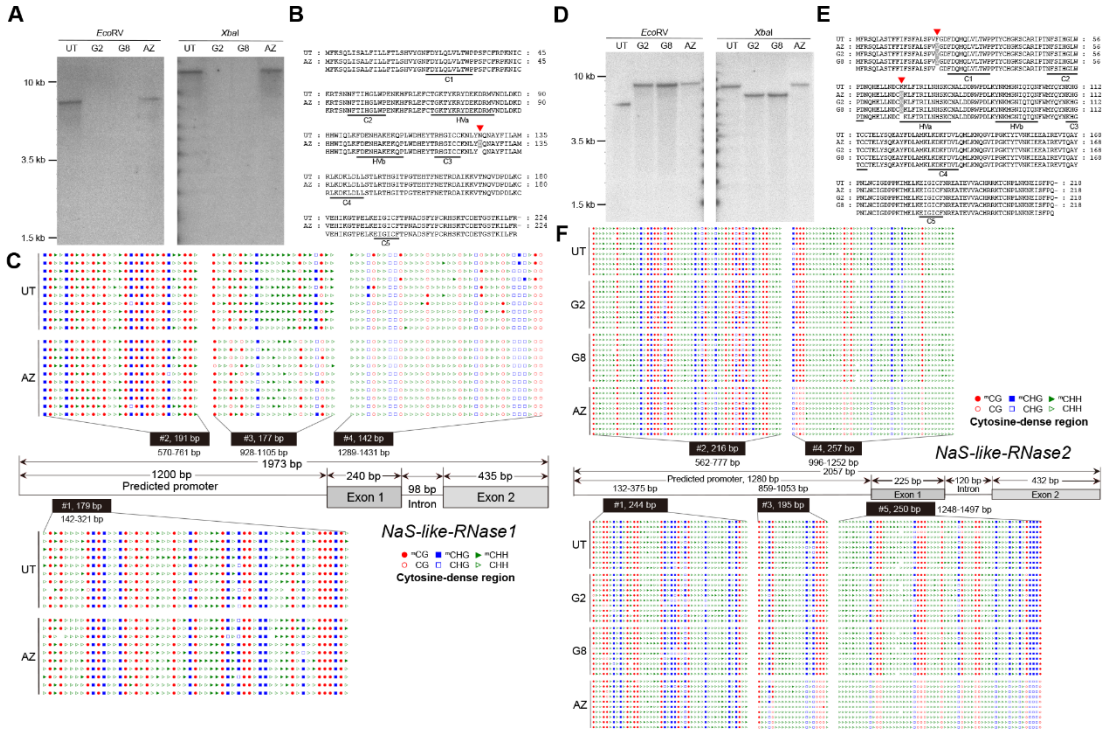


**Figure S2. Comparison of Copy Number, Amino Acid Sequence and Cytosine Methylation of *NaS-like-RNase1* and *2* in Four Natural Accessions. Related to Figure 2 and Table S5.** (A and D) Southern blot analysis to identify *NaS-like-RNase1* (A) and *2* (D) copy number. Genomic DNA isolated from leaf tissue was separately digested by *Eco*RV or *Xba*I and probed by the 240 bp (A) or 245 bp (D) fragment, which is used for both VIGS constructs and the quantification of gene silencing. The primers amplifying the fragments are listed in Table S2. Molecular weights in base pairs (bp) are shown on the left side of the blots. (B and E) Amino acid sequence alignment of NaS-like-RNase1 (B) and 2 (E) alleles. Different residues are highlighted by red triangles. The conserved regions C1 through C5 and hypervariable regions HVa and HVb are underlined. (C and F) Cytosine methylation frequencies in the predicted promoter and first exon of *S-like-RNase1* (C) and *2* (F) are shown. Four (C) or five (F) black boxes represent CpG-dense regions for methylation analysis. The middle panel illustrates the spatial distribution of the CpG-dense regions within the predicted promoter and first exon. DNA for bisulfite assays was isolated from styles. The top and bottom panels are graphical representations of cytosine methylation corresponding to the CpG-dense regions. Filled symbols indicate cytosine methylation; empty symbols indicate a lack of methylation. Red circles represent CG sites, blue squares represent CHG sites and green triangles represent CHH sites, H = A, T or C. Analysis was performed with CyMATE. The DNA elements shown are not to scale.

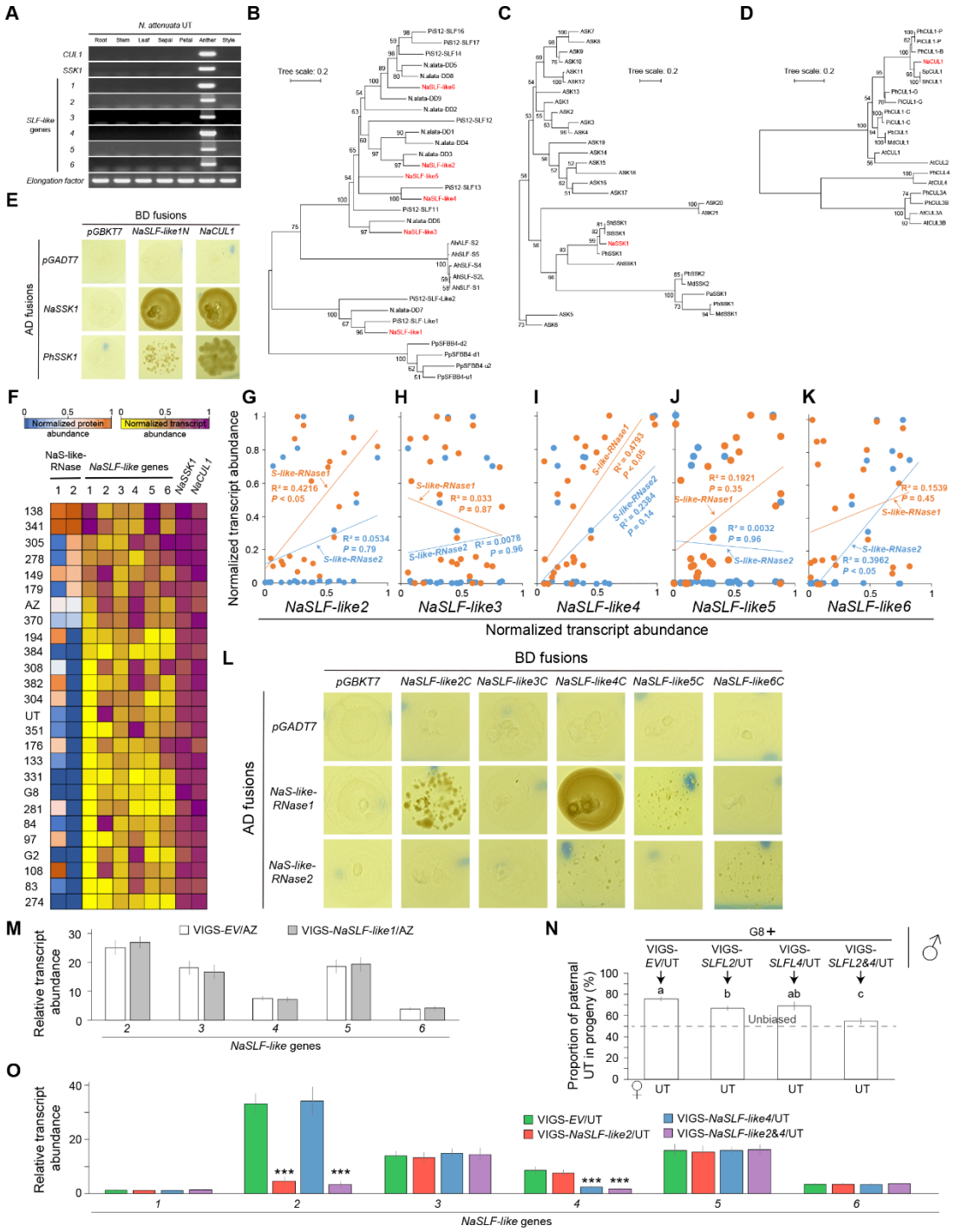


**Figure S3. Tissue-Specific Expression, Phylogeny and Protein-Protein Interaction Analysis of SCF$^{SLF\text{-}like1}$ Complexes; Correlation and Protein-Protein Interaction Analysis among *S-like-RNases* and *SLF-like2-6*; Silencing Specificity in VIGS-*NaSLF-like* Transgenic Lines and Redundancy of *NaSLF-like2* and *4* Function in Mate Selection. Related to Figure 3, Tables S1 and S3.** (A) Transcript accumulation pattern of *NaCUL1*, *SSK1* and *SLF-like1-6* in UT examined by RT-PCR. Total RNA was extracted from seven tissues as indicated. The synthesized cDNAs were used as templates in RT-PCR. The *NaEF* gene was used as a positive control. (B to D) Phylogenetic trees of (B) SLF or SLF-like proteins, (C) SKP1-like or SLF-interacting SKP1 proteins and (D) Cullin1 proteins are

presented. Numbers on branches indicate the bootstrap percentage values calculated from 1000 replicates: only values greater than 50% are shown. *Ah*, *Antirrhinum hispanicum*; *At*, *Arabidopsis thaliana*; *Md*, *Malus domestica*; *Na*, *Nicotiana attenuata*; *N. alata*, *Nicotiana alata*; *Pa*, *Prunus avium*; *Pb*, *Pyrus bretschneideri*; *Pi*, *Petunia inflata*; *Ph*, *Petunia hybrida*; *Pp*, *Pyrus pyrifolia*; *Sh*, *Solanum habrochaites*; *Sl*, *Solanum lycopersicum*; *Sp*, *Solanum peruvianum*. (E and L) Yeast cells containing different combinations of BD fusions and AD fusions (E: BD: *NaSLF-like1N*, encoding N-terminus of 1-60 amino acids, and *NaCUL1*, AD: *NaSSK1* and *PhSSK1*; L: *NaSLF-like2-6C*, encoding C-terminus without 1-60 amino acids, AD: *S-like-RNase1* and *2*) were tested for their growth on -Leu/-Trp/-His/-Ade dropout media. Empty vector *pGBKT7* and *pGADT7* were used as negative controls. (F) The normalized protein abundance (relative to NaEF, and normalized as $X' = X/X_{max}$) of NaS-like-RNase1 and 2, together with the normalized transcript abundance (relative to *NaEF* and normalized as $X' = X/X_{max}$) of *SLF-like1-6*, *SSK1* and *CUL1* were quantified in 26 *N. attenuata* natural accessions. (G-K) Correlation analyses among normalized transcript abundance of *NaS-like-RNases* and (G) *NaSLF-like2*; (H) *SLF-like3*; (I) *SLF-like4*; (J) *SLF-like5*; (K) *SLF-like6* are shown. *S-like-RNase1* and *2* data are displayed in orange or blue, respectively. (M) *NaSLF-like2* to *6* relative transcript abundance (mean ± SE, n=4, relative to *NaEF*) was quantified in pollen of empty vector (VIGS-*EV*/AZ) and *NaSLF-like1*-silenced (VIGS-*NaSLF-like1*/AZ) AZ transgenic lines. (N) The percentage of seeds sired by paternal UT genotype (mean ± SE, n=4) in progeny of mixed pollinations. Emasculated flowers of UT plants were pollinated with equal pollen mixtures from G8 and empty vector (VIGS-*EV*/UT), *NaSLF-like2*-silenced (VIGS-*SLFL2*/UT), *NaSLF-like4*-silenced (VIGS-*SLFL4*/UT), *NaSLF-like2* and *4*-co-silenced (VIGS-*SLFL2&4*/UT) UT transgenic lines, respectively. Each replicate represents a capsule from an independent pollination. Different letters indicate significant differences in a Tukey-corrected *post-hoc* test following a one-way ANOVA ($P < 0.05$). The dotted line indicates the unbiased seed set percentage for a 1:1 pollen mixture applied to the stigma. (O) *NaSLF-like1* to *6* relative transcript abundance (mean ± SE, n=4, relative to *NaEF*) was quantified in pollen of empty vector (VIGS-*EV*/UT), *NaSLF-like2*-silenced (VIGS-*NaSLF-like2*/UT), *NaSLF-like4*-silenced (VIGS-*NaSLF-like4*/UT), *NaSLF-like2* and *4*-co-silenced (VIGS-*NaSLF-like2&4*/UT) UT transgenic lines. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Student's *t*-test).

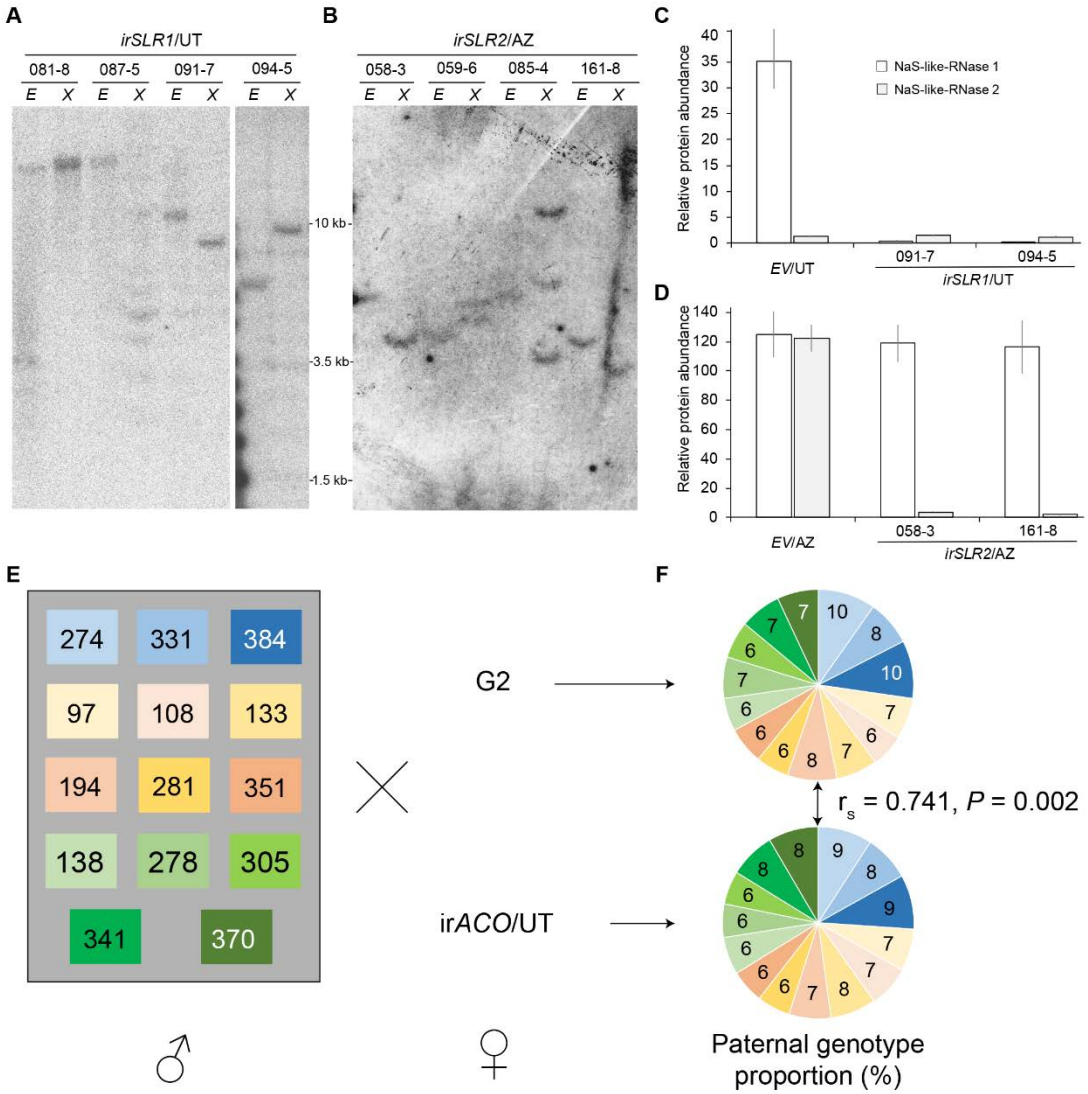


**Figure S4. Southern Blot and Silencing Efficiency Analysis of *NaS-like-RNase* RNAi Transgenic Lines; No Significant Difference of Progeny Paternity between G2 and ir*ACO*/UT Flowers in Mixture Pollinations. Related to Figure 4 and Table S1.** (A and B) Southern blot analysis was performed to detect the T-DNA copy number in transgenic lines silenced in (A) *NaS-like-RNase1* in the UT background (ir*SLR1*/UT) and (B) *NaS-like-RNase2* in the AZ background (ir*SLR2*/AZ). Genomic DNA was isolated from homozygous plants of the $T_2$ generation and digested overnight in separate reactions with *Eco*RV (*E*) or *Xba*I (*X*). A DIG-labeled fragment of the hygromycin resistance gene (*hptII*) served as probe. The blot indicates the presence of T-DNA insertion for transgenic lines: A-17-081-8, A-17-087-5, A-17-091-7, A-17-094-5, A-17-058-3, A-17-059-6, A-17-085-4 and A-17-161-8. The fragment size from the DNA markers is indicated. (C and D) Relative protein abundance (mean ± SE, n=3, relative to NaEF) of S-like-RNases in (C) *EV*/UT and two ir*SLR1*/UT or in (D) *EV*/AZ and two ir*SLR2*/AZ transgenic lines were quantified by mass spectrometry in styles. (E) Schematic representation of mixture pollinations with 14 non-self pollen genotypes. G2 or ir*ACO*/UT maternal genotypes (♀) were pollinated with equal-number mixed pollen loads containing equal contributions from 14 pollen donors (♂). (F) Pie charts (and numbers within slices) indicate percentage (mean of 3 replicates, each replicate represents a capsule resulting from

an independent pollination) of each paternal genotype of seeds fertilized in the equal-pollen grain mixture pollination. Slice colors correspond to the colors shown in (E). From each capsule, at least 50 seeds were germinated. Genomic DNA was extracted from two-week-old seedlings and analyzed for paternity by three microsatellite markers optimized to genotype these particular *N. attenuata* accessions. Spearman's correlations ($r_s$) quantify the consistency of mate selection between G2 and ir*ACO*/UT flowers.

| Genetic background | Wild-type or transgenic line | Target gene | Related to figure | Relative protein abundance (to NaEF) | |
|---|---|---|---|---|---|
| | | | | S-like-RNase 1 | S-like-RNase 2 |
| UT | WT | - | 1 & 2 | 31.07 | 1.29 |
| | VIGS | Empty vector | 1 | 34.55 | 2.17 |
| | | SLR1 | | 0.74 | 1.99 |
| | RNAi | Empty vector | 4 | 35.11 | 1.37 |
| | | SLR1 (091-7) | | 0.43 | 1.49 |
| | | SLR1 (094-5) | | 0.32 | 1.26 |
| AZ | WT | - | 1 & 2 | 118.28 | 112.97 |
| | VIGS | Empty vector | 1 | 113.26 | 115.97 |
| | | SLR1 | | 6.03 | 112.80 |
| | | SLR2 | | 121.67 | 14.11 |
| | RNAi | Empty vector | 4 | 124.96 | 122.37 |
| | | SLR2 (58-3) | | 118.97 | 3.26 |
| | | SLR2 (161-8) | | 116.37 | 2.23 |
| G2 | WT | - | 1 & 2 | N.D. | 0.12 |
| G8 | | | | N.D. | 0.054 |
| 274 | WT | - | 2 & 4 | 0.013 | 0.017 |
| 331 | | | | N.D. | 0.57 |
| 384 | | | | 8.89 | 0.99 |
| 97 | | | | 173.07 | 0.23 |
| 108 | | | | 221.54 | 0.068 |
| 133 | | | | 35.15 | 0.65 |
| 194 | | | | 191.89 | 3.06 |
| 281 | | | | 135.59 | 0.52 |
| 351 | | | | 31.95 | 0.95 |
| 138 | WT | - | 2 & 4 | 209.99 | 234.08 |
| 278 | | | | 33.75 | 176.75 |
| 305 | | | | 24.22 | 189.44 |
| 341 | | | | 218.89 | 233.79 |
| 370 | | | | 60.17 | 74.07 |
| 83 | WT | - | 2 | 28.54 | 0.041 |
| 84 | | | | 25.36 | 0.33 |
| 138 | | | | 209.99 | 234.08 |
| 176 | | | | 154.31 | 0.91 |
| 179 | | | | 26.02 | 163.28 |
| 304 | | | | 161.71 | 1.59 |
| 351 | | | | 31.95 | 0.95 |

| | | |
|---|---|---|
| 382 | 193.22 | 1.61 |

**Table S1. Natural Accessions and Transgenic Lines with Corresponding Relative Protein Abundances of NaS-like-RNases. Related to Figures 1, 2, 4, S4 and STAR Methods.**

| Gene | Sequence (5' to 3') | Purpose |
|---|---|---|
| SLR1-F | ATGTTTAAGTCACAGCTAATATC | Cloning, RT-PCR |
| SLR1-R | TTATCGGAACAAAATCTTCGTG | Cloning, RT-PCR |
| SLR2-F | ATGTTTAGATCGCAGCTCGTG | Cloning, RT-PCR |
| SLR2-R | TCATTGTGGAAAACTAATTTCATTCTTG | Cloning, RT-PCR |
| SLFL-F | ATGAAAGAGGTGGACGGTCAAAG | Cloning, RT-PCR |
| SLFL-R | TTAGGACCACTTCTCTCTCTCTTTC | Cloning, RT-PCR |
| SSK1-F | ATGGCAACCGAAGGCAAGAAA | Cloning, RT-PCR |
| SSK1-R | CTAATTAACAGTATCATCAATTTCAG | Cloning, RT-PCR |
| CUL1-F | ATGGAAGAGACTGAGGAGAAG | Cloning, RT-PCR |
| CUL1-R | TTAGGCAATGTACTTGTACGTG | Cloning, RT-PCR |
| SLR1-RT-F | CACATTTAACGAAACCAGAGATGCC | RT-qPCR |
| SLR1-RT-R | TTTATATGTTCGACGCACTTGAGGT | RT-qPCR |
| SLR2-RT-F | AGCGTATCCTAACCTCAATTGCATT | RT-qPCR |
| SLR2-RT-R | GTTGCCTCTCGGTTGAAACAGA | RT-qPCR |
| SLFL1-RT-F | AGCGGGTGATAATTGGATCGTTTC | RT-qPCR |
| SLFL1-RT-R | ACTCTTGCACGGTTCAATGTCTAAG | RT-qPCR |
| SLFL2-RT-F | GCTTCCTCCTATTGAATATCCGTTA | RT-qPCR |
| SLFL2-RT-R | ATTTGAGTTCCTTTACTTCATCGGA | RT-qPCR |
| SLFL3-RT-F | CGTGTGGGTCAACAGTTTCC | RT-qPCR |
| SLFL3-RT-R | AACACTTCCCATCGTAGCCA | RT-qPCR |
| SLFL4-RT-F | TGTCATAGCCTCGCAGTCTTAGATAA | RT-qPCR |
| SLFL4-RT-R | CGTTTCTTGTATTGGGCTACTCTCC | RT-qPCR |
| SLFL5-RT-F | TGGAGGGATCATCTGTTGTTTCTTC | RT-qPCR |
| SLFL5-RT-R | TGAACTCCTTGACTTCTTCCGAATG | RT-qPCR |
| SLFL6-RT-F | CCTCGTAGTCTTGGATGAGTCTCTA | RT-qPCR |
| SLFL6-RT-R | GCACACCTTCATTATCCAAATTTCCA | RT-qPCR |
| SSK1-RT-F | CGAGGACAAGGACAAGGACA | RT-qPCR |
| SSK1-RT-R | TCAGCTGCAGATTGACAACA | RT-qPCR |
| CUL1-RT-F | TGTCTTGTGCGTATAATGAAAGCCA | RT-qPCR |
| CUL1-RT-R | TTTGCTCAACTGCTCAACACACT | RT-qPCR |
| EF-RT-F | CCACACTTCCCACATTGCTGT | RT-PCR, RT-qPCR |
| EF-RT-R | CGCATGTCCCTCACAGCAAAAC | RT-PCR, RT-qPCR |
| TPI-F | CTTGTAAGCAATGTGGAACATGCAGATGCC | Molecular marker |
| TPI-R | TTAGGAAACAGCAACCCTAGACTTCTGGAG | Molecular marker |
| SLR2-dCAPS-F | AAAGAAAATTCTACTTTCCTTACACTTTGTGA | Molecular marker |
| SLR2-dCAPS-R | CCAAGATTCGATTACACGGG | Molecular marker |
| SLR1-VIGS-F | ATGTTTATGGGAACTTTGATTATC | VIGS, Southern blot |
| SLR1-VIGS-R | TTCATCGAACTTCAATTGAATCC | VIGS, Southern blot |
| SLR2-VIGS-F | GCATATTTTGATCTTGCCATG | VIGS, Southern blot |
| SLR2-VIGS-R | GTCTTACGTCGATGACAAGC | VIGS, Southern blot |
| SLFL1-VIGS-F | CGGGTTTGACCCCAACACTAATG | VIGS |
| SLFL1-VIGS-R | GGTTTGTTCCACGCCAATGAAATG | VIGS |
| SLFL2-VIGS-F | CCACTCATCGGTCCTTGTAA | VIGS |

| | | |
|---|---|---|
| SLFL2-VIGS-R | TAAGAGGATCCCAAAACAC | VIGS |
| SLFL4-VIGS-F | CAACTCACTGGTCCTTGCAATG | VIGS |
| SLFL4-VIGS-R | AGGGGGGTTCCCCGTAAAC | VIGS |
| SLR1-Pro-F | CTCTCTCTCTCTCTCTCTCTC | Promoter cloning |
| SLF2-Pro-F | ACTGGGCGAAAATTGGGATTGAC | Promoter cloning |
| SLR1-CDR1-F | TTTGTTTAGTTATGTAATGGTTATAAAGAG | Bisulfite sequencing |
| SLR1-CDR1-R | CCCAAACTAATTATTAAAATAATTTATATT | Bisulfite sequencing |
| SLR1-CDR2-F | AAGGTTTATGTGATAATGATAGGAGG | Bisulfite sequencing |
| SLR1-CDR2-R | AATACAATTCTAATTACAAAAATAAATCTC | Bisulfite sequencing |
| SLR1-CDR3-F | GTTTATAATTGTTTTGGTATTAATTATTT | Bisulfite sequencing |
| SLR1-CDR3-R | TATTAACCTAAATTCTTTCCCTTCC | Bisulfite sequencing |
| SLR1-CDR4-F | TGGGAATTTTGATTATTTGTAATTTG | Bisulfite sequencing |
| SLR1-CDR4-R | ACTTAAAAAAACACTCTATACTTTTTCATC | Bisulfite sequencing |
| SLR2-CDR1-F | AGAATGTGGTATATTTAAAGTGGTATAATT | Bisulfite sequencing |
| SLR2-CDR1-R | ACCTTTAACACATAAAATTTTCTTTACC | Bisulfite sequencing |
| SLR2-CDR2-F | TTATTTTTTTTATATTTTATGATTGTGTTT | Bisulfite sequencing |
| SLR2-CDR2-R | AAATATAACTCAATTTTAATAACAACTCC | Bisulfite sequencing |
| SLR2-CDR3-F | TTAGATTATATTTGATTTTATGTTAAGGTA | Bisulfite sequencing |
| SLR2-CDR3-R | ATTAAATATCTATCAATTTCATCTTTTAAT | Bisulfite sequencing |
| SLR2-CDR4-F | ATTATTTATATTTTATTGTGAATTTAGTTA | Bisulfite sequencing |
| SLR2-CDR4-R | ATTTTAATATTAAAACCACTCATCTCTTAT | Bisulfite sequencing |
| SLR2-CDR5-F | ATTTTAATGAGAAAAGAGTGTTATATATAG | Bisulfite sequencing |
| SLR2-CDR5-R | TAAAATAATATTATAATTATACCAAAATTC | Bisulfite sequencing |
| SLR1-AD-F | AACTTTGATTATCTGCAACTTGTTTTAAC | Y2H |
| SLR1-AD-R | TCGGAACAAAATCTTCGTGCTG | Y2H |
| SLR2-AD-F | GATTTCGACCAAATGCAACTCG | Y2H |
| SLR2-AD-R | TTGTGGAAAACTAATTTCATTCTTG | Y2H |
| SLFL1-C-BD-F | TTCACTCTGTTTAAGCGCTTCATC | Y2H |
| SLFL1-C-BD-R | GGACCACTTCTCTCTCTCTTTC | Y2H |
| SLFL1-N-AD-F | ATGAAAGAGGTGGACGGTCAAAG | Y2H |
| SLFL1-N-AD-R | ATTTGAAAGACAATCATGATTG | Y2H |
| SSK1-AD-F | ATGGCAACCGAAGGCAAGAAAATG | Y2H |
| SSK1-AD-R | ATTAACAGTATCATCAATTTCAG | Y2H |
| PhSSK-AD-F | ATGGCATCAGAAAAGAAAATG | Y2H |
| PhSSK-AD-R | ATTGACAGTATCATCAATTTC | Y2H |
| CUL1-BD-F | ATGGAAGAGACTGAGGAGAAG | Y2H |
| CUL1-BD-R | GGCAATGTACTTGTACGTGTTCG | Y2H |

**Table S2. DNA Primers Used in This Study. Primers for microsatellite markers see Key Resources Table and STAR Methods. Related to Figures 1, 2, 3, 4, S1, S2, S3, S4 and STAR Methods.**

| Gene | Species | GenBank accession number |
|---|---|---|
| AhS1-RNase | *Antirrhinum hispanicum* | CAD29435.1 |
| AhS2-RNase | *Antirrhinum hispanicum* | Q38716.1 |
| AhS3-RNase | *Antirrhinum hispanicum* | CAC41959.1 |
| AhS4-RNase | *Antirrhinum hispanicum* | Q38717.1 |
| AhS5-RNase | *Antirrhinum hispanicum* | CAA65318.1 |
| AoT2-RNase | *Aspergillus oryzae* | EIT82935.1 |
| AtRNS1 | *Arabidopsis thaliana* | NP_178399.1 |
| AtRNS2 | *Arabidopsis thaliana* | NP_030524.1 |
| AtRNS3 | *Arabidopsis thaliana* | NP_564264.1 |
| N. alata_S2-RNase | *Nicotiana alata* | P04007.1 |
| N. alata_S3-RNase | *Nicotiana alata* | AAB07492.1 |
| N. alata_S6-RNase | *Nicotiana alata* | AAB40028.1 |
| PhS1-RNase | *Petunia x hybrida* | AAA60465.1 |
| PhS2-RNase | *Petunia x hybrida* | Q40875.1 |
| PhS3L-RNase | *Petunia x hybrida* | AJ271065.1 |
| PhSv-RNase | *Petunia x hybrida* | BAA76513.1 |
| PiS1-RNase | *Petunia inflata* | AAA33726.1 |
| PiS2-RNase | *Petunia inflata* | AAG21384.1 |
| PiS21-RNase | *Petunia inflata* | AAG40753.1 |
| ShS1-RNase | *Solanum habrochaites* | AIG62994.1 |
| ShS2-RNase | *Solanum habrochaites* | AIG62995.1 |
| ShS4-RNase | *Solanum habrochaites* | AIG62997.1 |
| SlRNase LE | *Solanum lycopersicum* | NP_001234195.1 |
| SpS3-RNase | *Solanum peruvianum* | CAA53666.1 |
| SpS6-RNase | *Solanum peruvianum* | CAA81334.1 |
| SpS7-RNase | *Solanum peruvianum* | CAA81333.1 |
| SpS13-RNase | *Solanum peruvianum* | BAA04147.1 |
| SpS22-RNase | *Solanum peruvianum* | BAC00930.1 |
| SpS24-RNase | *Solanum peruvianum* | BAC00932.1 |
| SpS25-RNase | *Solanum peruvianum* | BAC00933.1 |
| AhSLF-S2 | *Antirrhinum hispanicum* | CAC33010.1 |
| AhSLF-S2L | *Antirrhinum hispanicum* | CAC33011.1 |
| AhSLF-S4 | *Antirrhinum hispanicum* | CAD56661.1 |
| AhSLF-S1 | *Antirrhinum hispanicum* | CAD56663.1 |
| AhSLF-S5 | *Antirrhinum hispanicum* | CAD56664.1 |
| N. alata-DD1 | *Nicotiana alata* | ABR18781.1 |
| N. alata-DD2 | *Nicotiana alata* | ABR18782.1 |
| N. alata-DD3 | *Nicotiana alata* | ABR18783.1 |
| N. alata-DD4 | *Nicotiana alata* | ABR18784.1 |
| N. alata-DD5 | *Nicotiana alata* | ABR18785.1 |
| N. alata-DD6 | *Nicotiana alata* | ABR18786.1 |
| N. alata-DD7 | *Nicotiana alata* | ABR18787.1 |
| N. alata-DD8 | *Nicotiana alata* | ABR18788.1 |

| | | |
|---|---|---|
| N. alata-DD9 | *Nicotiana alata* | ABR18789.1 |
| PpSFBB4-u2 | *Pyrus pyrifolia* | BAJ52223.1 |
| PpSFBB4-u1 | *Pyrus pyrifolia* | BAJ52224.1 |
| PpSFBB4-d1 | *Pyrus pyrifolia* | BAG07418.1 |
| PpSFBB4-d2 | *Pyrus pyrifolia* | BAJ52227.1 |
| PiS12-SLF-Like2 | *Petunia inflata* | AIK66494.1 |
| PiS12-SLF11 | *Petunia inflata* | AIK66455.1 |
| PiS12-SLF-Like1 | *Petunia inflata* | AIK66490.1 |
| PiS12-SLF14 | *Petunia inflata* | AIK66470.1 |
| PiS12-SLF12 | *Petunia inflata* | AIK66460.1 |
| PiS12-SLF13 | *Petunia inflata* | AIK66465.1 |
| PiS12-SLF16 | *Petunia inflata* | AIK66480.1 |
| PiS12-SLF17 | *Petunia inflata* | AIK66485.1 |
| PhSSK1 | *Petunia x hybrida* | ACT35733.1 |
| ShSSK1 | *Solanum habrochaites* | AIG62999.1 |
| SlSSK1 | *Solanum lycopersicum* | AIG62966.1 |
| PaSSK1 | *Prunus avium* | XP_021834044.1 |
| AhSSK1 | *Antirrhinum hispanicum* | ABC84197.1 |
| PbSSK1 | *Pyrus x bretschneideri* | CCH26217.1 |
| PbSSK2 | *Pyrus x bretschneideri* | CCH26218.1 |
| MdSSK1 | *Malus domestica* | NP_001281293.1 |
| MdSSK2 | *Malus domestica* | CCH26220.1 |
| ASK1 | *Arabidopsis thaliana* | NP_565123.1 |
| ASK2 | *Arabidopsis thaliana* | NP_568603.1 |
| ASK3 | *Arabidopsis thaliana* | NP_565604.1 |
| ASK4 | *Arabidopsis thaliana* | NP_564105.1 |
| ASK5 | *Arabidopsis thaliana* | NP_567091.1 |
| ASK6 | *Arabidopsis thaliana* | NP_566978.1 |
| ASK7 | *Arabidopsis thaliana* | NP_566693.1 |
| ASK8 | *Arabidopsis thaliana* | NP_566692.1 |
| ASK9 | *Arabidopsis thaliana* | NP_566694.1 |
| ASK10 | *Arabidopsis thaliana* | NP_566695.1 |
| ASK11 | *Arabidopsis thaliana* | NP_567959.1 |
| ASK12 | *Arabidopsis thaliana* | NP_567967.1 |
| ASK13 | *Arabidopsis thaliana* | NP_567090.1 |
| ASK14 | *Arabidopsis thaliana* | NP_565296.1 |
| ASK15 | *Arabidopsis thaliana* | NP_566773.1 |
| ASK16 | *Arabidopsis thaliana* | NP_565297.1 |
| ASK17 | *Arabidopsis thaliana* | NP_565467.1 |
| ASK18 | *Arabidopsis thaliana* | AAD32873.1 |
| ASK19 | *Arabidopsis thaliana* | NP_565295.1 |
| ASK20 | *Arabidopsis thaliana* | NP_001078065.1 |
| ASK21 | *Arabidopsis thaliana* | NP_567113.1 |
| PhCUL1-B | *Petunia x hybrida* | BAW00384.1 |

| | | |
|---|---|---|
| PhCUL1-C | *Petunia x hybrida* | BAW00386.1 |
| PhCUL1-G | *Petunia x hybrida* | BAW00387.1 |
| PhCUL3A | *Petunia x hybrida* | BAW00389.1 |
| PhCUL3B | *Petunia x hybrida* | BAW00390.1 |
| PhCUL4 | *Petunia x hybrida* | BAW00391.1 |
| PhCUL1-P | *Petunia x hybrida* | BAO58961.1 |
| PiCUL1-C | *Petunia inflata* | ABB77428.1 |
| PiCUL1-G | *Petunia inflata* | ABB77429.1 |
| PiCUL1-P | *Petunia inflata* | AHF49537.1 |
| SpCUL1 | *Solanum pennellii* | NP_001310370.1 |
| PbCUL1 | *Pyrus x bretschneideri* | NP_001289253.1 |
| AtCUL1 | *Arabidopsis thaliana* | NP_567243.1 |
| AtCUL2 | *Arabidopsis thaliana* | NP_171797.2 |
| AtCUL3A | *Arabidopsis thaliana* | NP_174005.1 |
| AtCUL3B | *Arabidopsis thaliana* | NP_177125.3 |
| AtCUL4 | *Arabidopsis thaliana* | NP_568658.1 |
| MdCUL1 | *Malus domestica* | XP_008390685.1 |
| ShCUL1 | *Solanum habrochaites* | AIG63002.1 |

**Table S3. GenBank ID of Genes for Phylogenetic Analysis. Related to STAR Methods, Figure S1 and Figure S3.**

| Position of SNPs | Accession | | | |
|---|---|---|---|---|
| | UT | AZ | G2 | G8 |
| -726* | C | T | T | T |
| -606 | T | G | G | G |
| 62 | T | A | A | A |
| 162 | G | A | A | A |
| 204 | G | T | T | T |

**Table S4. SNPs of the Predicted Promoter (1280 bp) and ORF of *NaS-like-RNase2* in UT, AZ, G2 and G8. Related to Figures 2, S1, S2 and STAR Methods.** Start codon was taken as position 0. The SNP with asterisk was used in dCAPS markers in Figure S1F.

| | Cytosine-dense region | Methylation type | Methylation rate (%) (mean ± SE, n=10) | | *t*-test (*P* value) |
|---|---|---|---|---|---|
| | | | UT | AZ | |
| *NaS-like-RNase1* | 1 | CG | 98.75 ± 1.25 | 100 ± 0 | 0.33 |
| | | CHG | 96 ± 2.21 | 95 ± 2.24 | 0.75 |
| | | CHH | 22.04 ± 2.85 | 27.4 ± 3.42 | 0.24 |
| | 2 | CG | 99.5 ± 0.5 | 100 ± 0 | 0.33 |
| | | CHG | 100 ± 0 | 98 ± 2 | 0.42 |
| | | CHH | 34.97 ± 6.06 | 32.5 ± 5.62 | 0.77 |
| | 3 | CG | 92 ± 3.27 | 62 ± 8.14 | 0.003 |
| | | CHG | 85 ± 7.64 | 40 ± 10 | 0.002 |
| | | CHH | 59.38 ± 7.05 | 14.4 ± 1.875 | 7.99E-06 |
| | 4 | CG | 17.5 ± 3.82 | 0 ± 0 | 0.00023 |
| | | CHG | 7.15 ± 2.38 | 0 ± 0 | 0.0077 |
| | | CHH | 2.85 ± 0.78 | 0 ± 0 | 0.0017 |
| *NaS-like-RNase2* | 1 | CG | 94 ± 3.4 | 96 ± 1.63 | 0.60 |
| | | CHG | 85.56 ± 4.4 | 91.11 ± 3.23 | 0.32 |
| | | CHH | 18.21 ± 3.14 | 14.81 ± 1.83 | 0.36 |
| | 2 | CG | 98.57 ± 1.429 | 100 ± 0 | 0.33 |
| | | CHG | 84.45 ± 4.74 | 68.69 ± 5.44 | **0.045** |
| | | CHH | 16.59 ± 1.69 | 7.575 ± 1.98 | **0.0044** |
| | 3 | CG | 100 ± 0 | 24 ± 4 | **2.33E-13** |
| | | CHG | 93.33 ± 4.44 | 9.99 ± 5.09 | **3.25E-10** |
| | | CHH | 27.69 ± 3.84 | 2.31 ± 1.17 | **5.83E-06** |
| | 4 | CG | 99.9 ± 0.1 | 0 ± 0 | **3.75E-44** |
| | | CHG | 82.5 ± 7.5 | 0 ± 0 | **2.02E-09** |
| | | CHH | 10.14 ± 1.24 | 0 ± 0 | **1.77E-07** |
| | 5 | CG | 97.27 ± 1.39 | 0 ± 0 | **2.16E-23** |
| | | CHG | 90 ± 3.08 | 0 ± 0 | **1.25E-16** |
| | | CHH | 19.49 ± 1.34 | 0 ± 0 | **2.15E-11** |

**Table S5. Comparison of Cytosine Methylation in Four Cytosine-Dense Regions of *NaS-like-RNase1* between UT and AZ or Five Cytosine-Dense Regions of *NaS-like-RNase2* between G2 and AZ, Respectively. Related to Figures 2 and S2.**

| Accession | Allele size (bp) of PCR products amplified by different primer pairs | | | Latitude | Longitude |
|---|---|---|---|---|---|
| | Primer pair with 6FAM | Primer pair with HEX | Primer pair with AT550 | | |
| UT | 181 | 163 and 513 | 225 | N37°19'36.26" | W113°57'53.05" |
| AZ | 169 | 147 and 495 | 223 | N35°12'56.07" | W111°27'41.29" |
| G2 | 178 | 124 and 473 | 197 | N37°04'33.53" | W113°49'58.74" |
| 97 | 140 | 124 and 473 | 209 | N37°21'35.24" | W113°56'38.68" |
| 108 | 175 | 150 and 498 | 203 | N37°13'52.699" | W113°50'37.113" |
| 133 | 160 | 150 and 498 | 209 | N37°06'12.5" | W113°49'36.6" |
| 138 | 181 | 150 and 498 | 225 | N37°8'19.58" | W114°1'35.10" |
| 194 | 137 | 124 and 473 | 205 | N37°20'22.52" | W114°2'40.86" |
| 274 | 178 | 150 and 498 | 209 | N37°21'07.103" | W114°05'50.298" |
| 278 | 181 | 124 and 473 | 237 | N37°21'02.580" | W114°05'53.661" |
| 281 | 175 | 163 and 513 | 219 | N37°19'35.48" | W113°57'38.28" |
| 305 | 134 | 147 and 495 | ND | N37°45'19.61" | W118°35'41.82" |
| 331 | 178 | 124 and 473 | 197 | N37°13'15.83" | W113°48'20.86" |
| 341 | 181 | 153 and 502 | 209 | N37°9'45.30" | W114°0'58.52" |
| 351 | 117 | 124 and 473 | 183 | N37°17'09.1" | W114°07'31.5" |
| 370 | 137 | 150 and 498 | 189 | N37°9'1.30" | W113°47'43.36" |
| 384 | 175 | 163 and 513 | 215 | N37°14'27.05" | W113°49'36.71" |
| G8 | N/A | N/A | N/A | N37°04'33.53" | W113°49'58.74" |
| 83 | N/A | N/A | N/A | N37°19'35.48" | W113°57'38.28" |
| 84 | N/A | N/A | N/A | N37°19'35.48" | W113°57'38.28" |
| 149 | N/A | N/A | N/A | N35°12'56.07" | W111°27'41.29" |
| 176 | N/A | N/A | N/A | N37°16'38.65" | W113°53'35.18" |
| 179 | N/A | N/A | N/A | N37°21'1.04" | W113°57'5.17" |
| 304 | N/A | N/A | N/A | N37°20'22.52" | W114°2'40.86" |
| 308 | N/A | N/A | N/A | N37°13'5.50" | W113°48'24.25" |
| 382 | N/A | N/A | N/A | N37°14'27.05" | W113°49'36.71" |

**Table S6. Characterization of Three Microsatellite Markers and full GPS Coordinates for the collection sites of *N. attenuata* Natural Accessions. Related to Figures 2, 4, S2, S4 and STAR Methods.** Not detected, ND; not available, N/A.

| | Pollen tube growth rate | | | |
|---|---|---|---|---|
| | H1: detrimental effect | | H2: promoting effect | |
| pollen tube | SLR (+) style | SLR (-) style | SLR (+) style | SLR (-) style |
| SLFL (+) | a | a | a | b (b<a) |
| SLFL (-) | b (b<a) | a | b | a |

**Table S7. Predicted Results of Pollen Tube Growth Rates for Two Alternative Mechanistic Hypotheses. Related to Figure 5.** Predictions for different combinations of pollen and styles with high (+) or experimentally reduced (-) SLF-like (SLFL) and S-like-RNase (SLR) protein abundance, respectively. SLFL and SLR proteins are known to interact directly and are functional in the mate selection process. Predicted results of pollen tube growth rates are shown for two alternative mechanistic hypotheses: H1) S-like-RNases might have a detrimental effect on unfavored pollen tubes; H2) the interactions between S-like-RNase and SLF-like promotes or protects the growth of favored pollen tubes.

**Supplemental References**

S1. Goldberg, E.E., Kohn, J.R., Lande, R., Robertson, K.A., Smith, S.A., and Igic, B. (2010). Species selection maintains self-incompatibility. Science *330*, 493-495.

S2. Robertson, K., Goldberg, E.E., and Igic, B. (2011). Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. Evolution *65*, 139-155.