www.sciencemag.org/cgi/content/full/313/5789/966/DC1

Supporting Online Material for

Plant Genotypic Diversity Predicts Community Structure and Governs an Ecosystem Process

Gregory M. Crutsinger,* Michael D. Collins, James A. Fordyce, Zachariah Gompert, Chris C. Nice, Nathan J. Sanders

*To whom correspondence should be addressed. E-mail: gcrutsin@utk.edu

Published 18 August 2006, *Science* **313**, 966 (2006)
DOI: 10.1126/science.1128326

This PDF file includes:

Supporting Online Material

Materials and Methods

Study Site

We initiated this research in Spring of 2005 in an old-field site at Freel's Bend at the Oak Ridge National Laboratory (ORNL) National Environmental Research Park (NERP) near Oak Ridge, Tennessee (35º58' N, 84º17'W). The site was abandoned from agricultural use in 1943, and has been extensively managed for open-space and wildlife habitat by ORNL and the Tennessee Wildlife Resource Agency (TWRA). The soil, classified as a Typic Hapludult, has a silty clay loam texture and is moderately well drained and slightly acidic. Precipitation is generally evenly distributed throughout the year with an annual mean of 1322 mm; the mean annual temperature at the site is 13.9°C. The fields surrounding the experimental area are typical of other old fields in eastern Tennessee in terms of plant community composition. Besides *Solidago altissima,* dominant plant species include *Verbesina occidentalis* L. (yellow crownbeard), *V. virginica* L. (white crownbeard), and *Rubus* spp. (blackberry); sub-dominants include about 60 other herbaceous and woody species.

Plant Propagation

We collected rhizomes from 21 *S. altissima* ramets in natural patches growing 50-150 m apart in several old fields surrounding the study site. Rhizomes were excavated with a hand trowel and only rhizomes directly attached to one another and to the stem from the previous year's growth were considered to be part of the same genet. Experimental ramets were propagated directly after excavation by cutting rhizomes into 3-cm sections and planting sections from each genotype in separate flats of sterilized potting soil (Pro-Mix BX, Premier Brands, New Rochelle, NY). Ramets were established in a common greenhouse environment set at 25° C for 9 weeks, watered as needed, and fertilized monthly using water-soluble fertilizer (15:20:25, N:P:K, Scotts Sierra Horticultural Co. Marysville, OH). Ramets were initially given a root stimulator (Roots 2, Roots inc. OSIA Independence, MO, 1 tsp per gal). Using small rhizome fragments and an extended greenhouse time period minimized any maternal effects carried over from growing in previous local environments (*S1*). One week prior to planting in the field, all genotypes were transferred to benches outside the greenhouse to adapt to natural light conditions and to minimize transplant shock.

We created treatments of 1, 3, 6, or 12 genotypes in May 2005, which are directly comparable to natural levels of genotypic diversity (*S2*). All 21 genotypes were planted in two replicate monocultures. Mixtures were created by randomly sampling from the pool of 21 genotypes with the constraint that no two patches in a treatment could have identical composition (7 replicates each). Each plot contained 12 ramets arranged in a 75-cm diameter circle in 1-m^2 plots spaced 1 m apart and randomized in a grid. A circular planting pattern ensured equal chance of colonization of any given plant in a plot (*S3*). Patches were spaced 1 m apart and arranged in a 15 m X 20 m grid. Trenches were cut around each plot (6 cm wide x 30 cm) using an EZ9000 Groundsaw trencher (E-Z Trench, Loris, SC.). Each plot was staked at the corners with 30 cm wooden stakes and lined with 12 mil heavy plastic (K-501R greenhouse film, Klerk's Plastic Inc., Richburg, S.C.) 30 cm deep to prevent rhizomes from spreading into neighboring plots. Three weeks prior to planting, all plots were sprayed with a broad-spectrum, post-emergent,

systemic herbicide (Round-Up Pro, Monsanto Co., St. Louis, MO, 5 % solution) to eliminate any vegetation previously established in the plot. Plots were weeded by hand biweekly for the remainder of the growing season. Ramets were watered for the first 3 weeks as needed (2 gal per plot) from collected rainwater. Seven plants died during the first week and were replaced with the same genotypes. After this, mortality was noted (though minimal, 0.5% or 4 ramets). A 3-m tall fence made of 1-in poultry wire was built around the experiment to exclude deer (Plate S1).

Arthropod Surveys

We visually surveyed every ramet 5 times from May-October 2005. Although more time-consuming than destructive sampling methods, visual sampling allows for repeated measurements with minimal impact on the arthropod community (*S3-S5*). We identified and counted all herbivorous, omnivorous, and predatory arthropods down to morphospecies by looking over the entire genet, including all new ramets that were produced throughout the growing season. One individual of each morphospecies was taken back to the lab for further identification to the lowest taxonomic level possible. Arthropods were assigned to trophic levels and feeding guilds based on field guides and relevant literature. Because of logistical difficulties in field surveying, we lumped parasitoids and bees other than honeybees (*Apis mellifera)* or bumblebees (*Bombus sp.*) into size classes. Flowering obscured many arthropods during the last survey in October. To avoid under sampling, after visually surveying the entire stem, we shook each flower head three times onto white paper and counted all arthropods that fell off.

We used linear regression to determine overall effects of genotypic diversity on total arthropod, herbivore, and predator plot-level cumulative richness and abundance. We also used linear regression to determine the relationships of these variables with plot-level Aboveground Net Primary Productivity (ANPP). We used individual-based rarefaction to obtain rarefied total richness, herbivore richness, and predator richness (Ecosim 7.0) (*S6*). Rarefied richness was log-transformed to achieve normality. We used linear regression to determine overall effects of genotypic diversity on rarefied total, herbivore, and predator richness. To assess the relative effects of ANPP and genotypic diversity on rarefied herbivore richness, we used stepwise regression. We also used stepwise regression to test the relative effects of ANPP, genotypic diversity, and rarefied herbivore richness on rarefied predator richness.

Non-additive Effects

To test for non-additive effects of genotypic diversity on arthropod diversity, we used Monte Carlo simulations using data from genotype monoculture plots to construct null genotype mixtures and their associated arthropod communities. We compared the observed arthropod communities to these null communities. Each null mixture consisted of 3, 6, or 12 genotypes sampled to match the exact identities corresponding to a particular plot combination (e.g., for a 3-genotype plot containing G3, G13, and G19, we sampled only from monoculture plots containing these three genotypes) (*S7*). For each sampled genotype, the appropriate number of individual plants (4, 2, or 1) was randomly sampled without replacement from a randomly selected replicate monoculture plot. This process was repeated 5,000 times for every mixed genotype plot. To calculate statistical differences between arthropod diversity in observed versus null mixtures, we used a bootstrap approach. For each of 10,000 iterations, we sampled seven null mixtures and calculated mean number of arthropod species at the plot-level. We measured *P* values as the fraction of iterations in which the null mean arthropod richness was equal to or

exceeded the observed mean richness. We calculated 95% confidence intervals using the percentile method (2.5^{th} and 97.5^{th} percentiles). If the effects of genetic diversity on arthropod richness were additive, we would expect no difference between observed and predicted means ($P > 0.05$). All Monte Carlo simulations were coded in Microsoft Visual C++ 6.0 (Microsoft, Redmond, WA, USA).

For the expected values, we did not use the average of the monocultures, but instead constructed null mixtures based on individual plants drawn repeatedly. This test is more robust than simply taking the average as it takes into account the species turnover due to variation in susceptibility among genotypes. By doing so, this method is a more conservative test for non-additive effects of arthropods in response to genotypic diversity than simply taking plot averages. Our findings suggest that the bulk of our pattern in driven by differences in species composition among genotypes.

Plant Productivity

We estimated ANPP as plant biomass at the peak of the growing season (late July) using an allometric equation developed specifically for *Solidago altissima*, but averaged across haphazardly selected genotypes. Thirty individual ramets from patches growing near the study site were measured to the nearest mm, harvested, oven-dried at 60° C for 48 hours, and weighed to the nearest 0.1 g. This equation accurately predicts aboveground biomass ($r = 0.77$). Allometric methods allowed repeated arthropod sampling throughout the year. We used linear regression to determine overall effects of genotypic diversity on plot-level ANPP. Analyses were done in JMP statistical software (version 5.1, SAS Institute Inc., 2004)

Partitioning Selection and Complementarity

Using standard methods to partition effects in biodiversity experiments (*S8*), a positive complementarity effect occurs if genotype yields in a mixture are on average higher than the weighted average monoculture yield of component genotypes. Selection effect is measured by the covariance between the monoculture yield of genotypes and the deviation from expected relative yield in a mixture. We used ANOVA to determine if complementarity and selection effects differed from zero. We used linear regression to determine the relationship of these effects with genotypic diversity.

AFLP Genotyping and Data Analysis

The AFLP technique generates large numbers of genetic markers throughout the genome providing data on overall genetic similarity and diversity (*S11*). AFLP markers were generated by use of four selective primer pairs*:* *EcoRI*-ACA and *MseI*-CTC, *EcoRI*-AGT and *MseI*-CTT, *EcoRI*-AGT and *MseI*-CTC, and *EcoRI*-AGT and *MseI*-CTA. Amplicons were separated and visualized on 6% denaturing polyacrylamide gels, using an ABI PRISM 377 DNA sequencer (Applied Biosystems Inc). GeneScan was used to visualize AFLP bands, which were sized by comparison to a size standard ladder (ROX standard, Applied Biosystems Inc) added to each lane. Bands < 100 bp in length and bands with peak heights < 250 relative fluorescent units were not scored. We scored the presence and absence of 206 AFLP amplicons for all 21 ramets (Table S1). Mean dissimilarity between genotypes was 25.1% (range: 14.1-32.5%). AFLP data were analyzed using non-metric multidimensional scaling and Bayesian clustering. Genotypic similarity was measured as Cavalli-Sforza and Edwards distances (*S12*) using PHYLIP (*S13*) and

NMDS was performed to illustrate patterns of similarity among ramets using the NCSS 97 statistical software package. The results of this analysis reveal little or no genetic structure among the 21 ramets (Fig. S6). The program STRUCTURE (*S13*) was used to cluster individuals based on their AFLP banding profiles. STRUCTURE employs a model-based Bayesian clustering algorithm to assign individuals probabilistically to clusters to minimize deviations from linkage equilibrium. The admixture model was run for 500,000 generations with an initial burnin of 50,000 generations. Bayesian clustering using STRUCTURE with number of clusters (*k*) set to 2 found no evidence of genetic structure among the 21 ramets (Fig. S7), supporting the results of the non-metric multidimensional scaling ordination.

Supporting Text

Herbivore Assemblages Among Genotypes

To examine how variation among plant genotypes influenced the structure of herbivore assemblages, we examined seperately the distribution of herbivore feeding guilds across the 21 unique *Solidago* genotypes using ANOVA. We found significant variation in abundance of four of six herbivore feeding guilds (Fig S4). To determine whether overall herbivore assemblage composition varied among genotypes, we used nonmetric multidimensional scaling (NMDS), a nonparametric analytical technique that is applied to the dissimilarity matrix calculated among genotypes using the Bray-Curtis dissimilarity coefficient (*S9, S10*). Comparisons between genotypes were made using an analysis of similarity (ANOSIM) statistical test (Primer version 5, Primer-E Ltd., Plymouth Marine Laboratory, Plymouth, UK). This analysis indicated that herbivore community composition differed among host-plant genotypes (ANOSIM: $R = 0.348$, $P = 0.01$) (Fig. S3).

To examine herbivore performance on particular genotypes, we initiated a bioassay using *Spodoptera exigua* caterpillars (a generalist herbivore) of similar size and mass. In early August, we excised one leaf from 10 randomly chosen ramets from each genotype across the two replicate plots. We chose full-sized leaves undamaged by herbivores. We placed the leaf on moist filter paper in plastic containers in the lab and allowed a randomly selected neonate caterpillar to feed for 5 days. We then recorded the weight of surviving caterpillars. We analyzed these data using an ANOVA. We found significant differences in caterpillar performance among genotypes (Fig. S5).

Host Plant Quality

We examined variation among plant genotypes in the ratio of carbon:nitrogen of green leaf tissue. In July, we excised five full-sized leaves from 6 randomly chosen ramets of each genotype. Leaves were air-dried, run through a ball grinder, and then oven dried at 60°C for 72 hours. We calculated C:N ratios using a Carlo-Erba Model 2500 CHN analyzer (Milan, Italy). We analyzed these data using ANOVA. We found significant differences among genotypes in C:N ratios (Fig. S5).

Supporting Figures

Plate S1 Photograph shows experiment in late July at the peak of the growing season (Photo credit: G. M. Crutsinger).

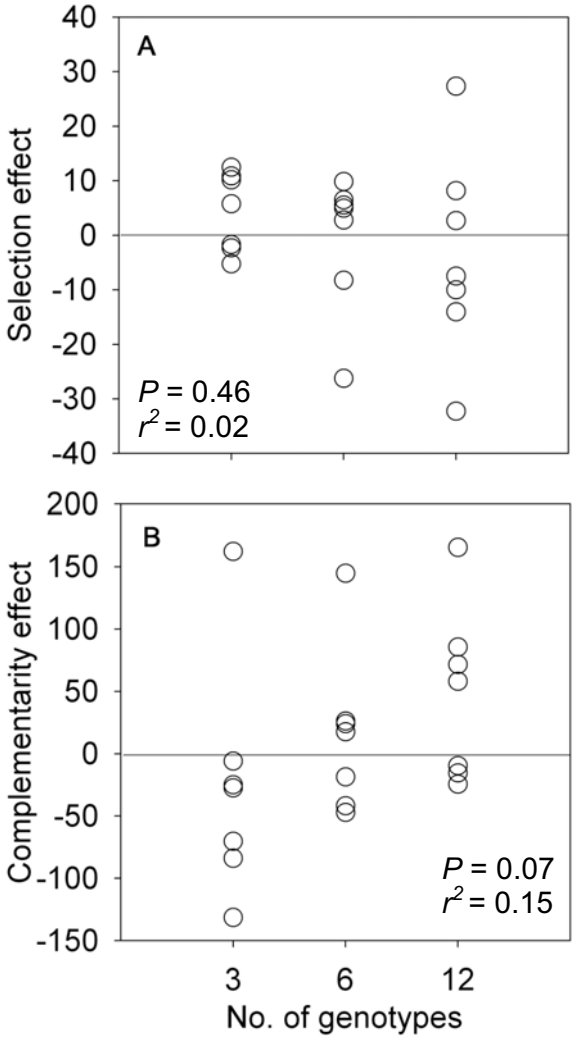

Fig. S1 Relationship between selection effects (**A**) and complementarity effects (**B**) and *Solidago altissima* genotypic diversity.

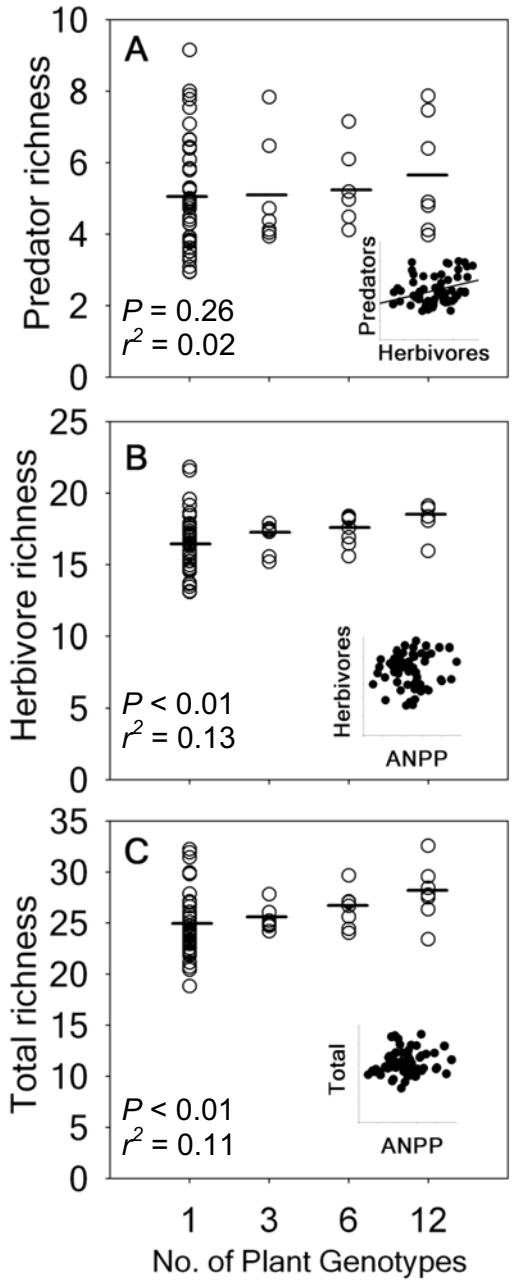

Fig. S2 Relationship between population-level genotypic diversity and rarefied predator species richness (**A**), rarefied herbivore species richness (**B**), and rarefied total species richness (**C**). Open circles indicate plot-level observations, and the horizontal lines indicate treatment means. The inset figure in (**A)** shows the relationship between rarefied herbivore species richness and rarefied predator species richness (r^2 = 0.10, P = 0.009). The inset in (**B**) shows the relationship between ANPP and rarefied herbivore richness (r^2 =0.0002, P = 0.95). The inset in (**C**) shows the relationship between ANPP and rarefied total richness (r^2 = 0.01, P = 0.28).

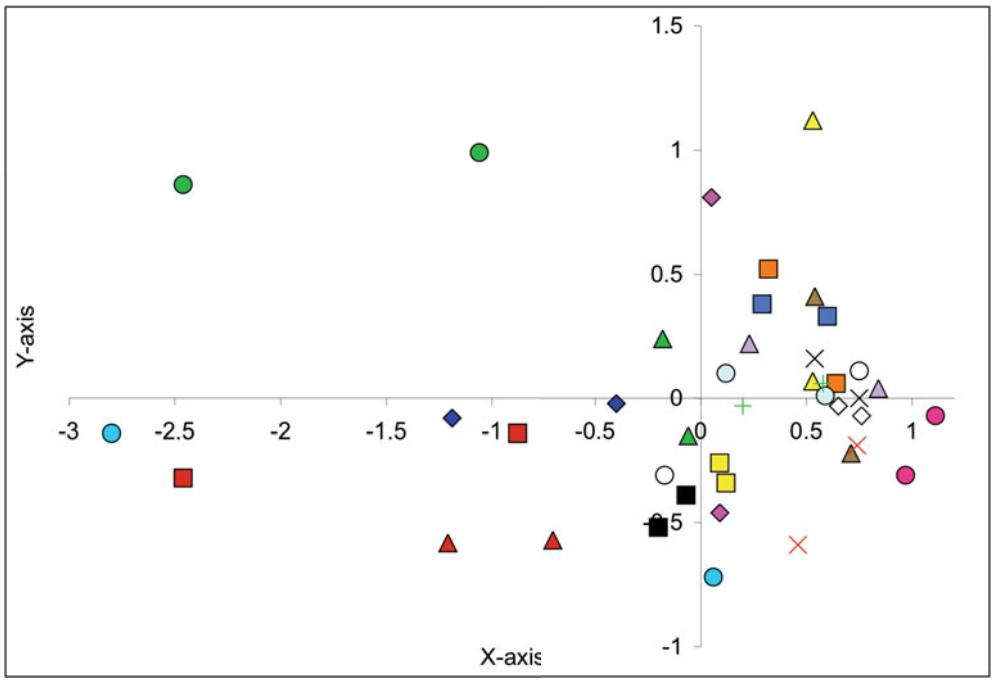

Fig. S3 NMDS (nonmetric multidimensional scaling) ordination demonstrates that the composition of herbivore assemblages on particular *Solidago altissima* genotypes differed significantly from one another (ANOSIM: $R = 0.348$, $P = 0.01$). Each point represents an herbivore assemblage for a given plot ($n = 2$ plots per genotype, matching in color and shape).

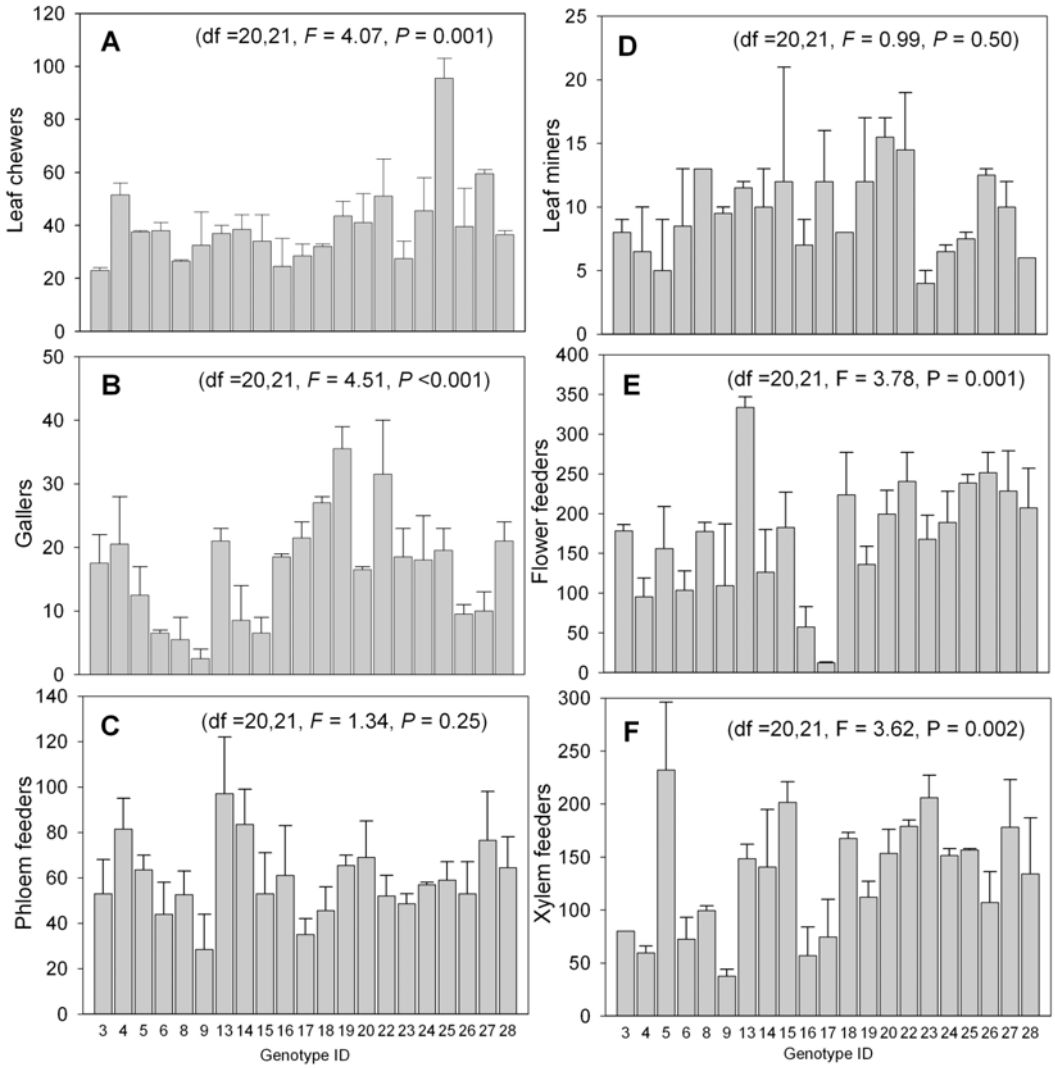

Fig. S4 Plot-level mean cumulative abundance (± SE) of six herbivore feeding guilds across 21 *Solidago altissima* genotypes, including leaf chewers (**A**), gallers (**B**), phloem feeders (**C**), leaf miners (**D**), flower feeders (**E**), and xylem feeders (**F**).

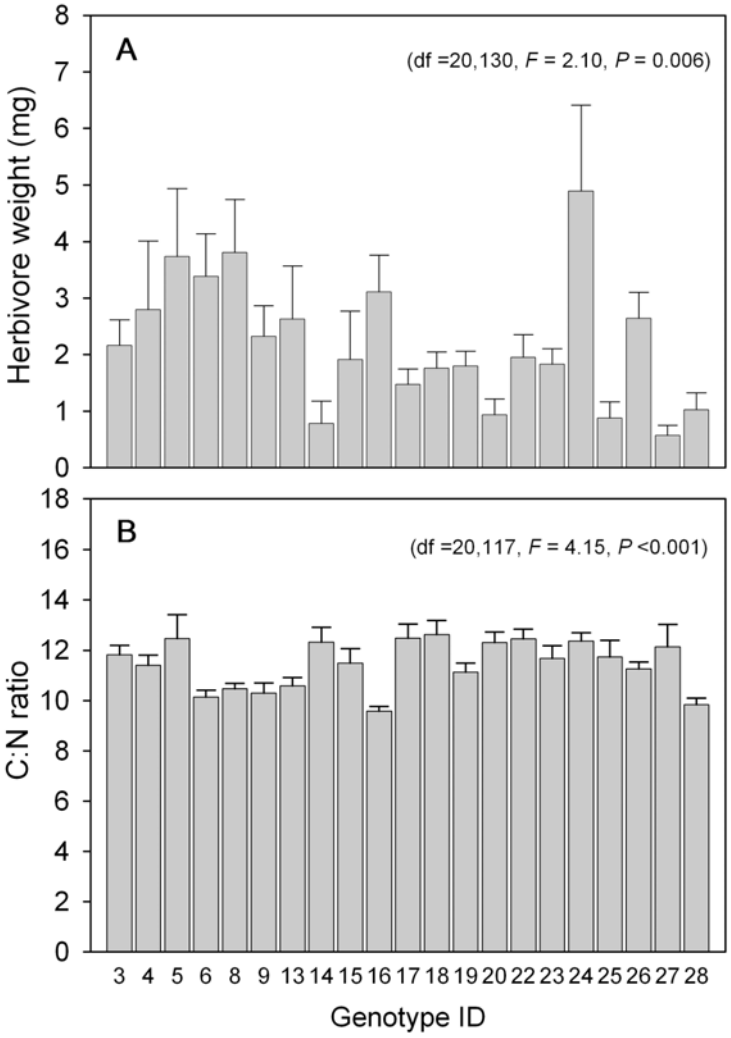

Fig. S5 Herbivore performance measured as mean final weight (± SE) of *Spodoptera* caterpillars during feeding trials (**A**) and mean C:N ratio (± SE) for green leaf tissue (**B**) for 21 *Solidago altissima* genotypes.

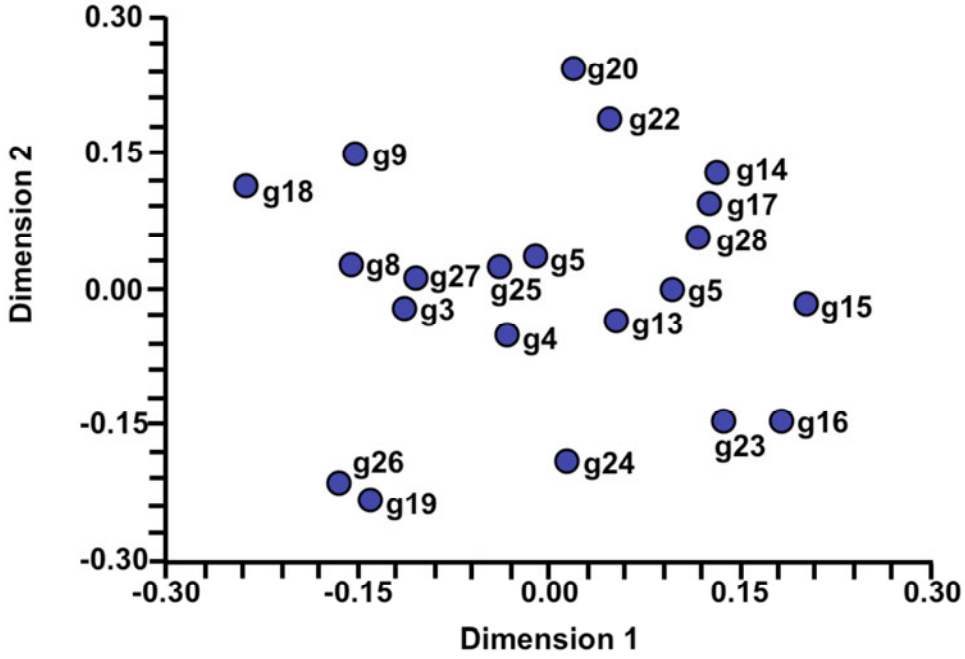

Fig. S6 Results of AFLP genotyping analysis using Non-Metric Multidimensional Scaling. Individual *Solidago altissima* ramet genotypes are illustrated in this ordination based on overall genetic similarity measured as Cavalli-Sforza and Edwards distances.

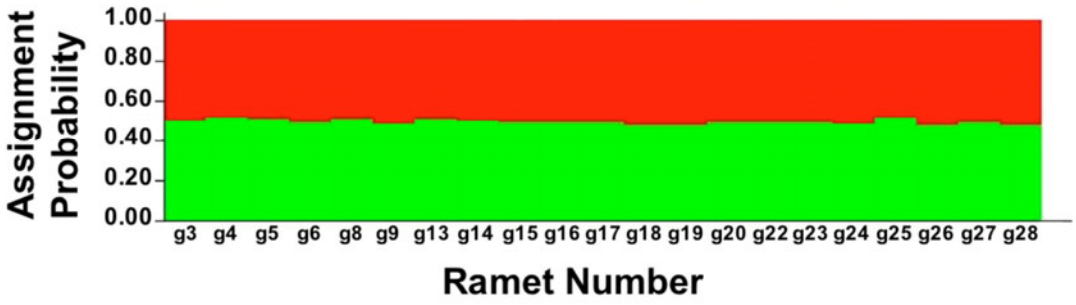

Fig. S7 Bayesian assignment probabilities for number of clusters, *k*=2. Each vertical bar corresponds to one individual *Solidago altissima* ramet. The proportion of each bar that is green represents an individual ramet's assignment probability to cluster 1, the proportion of each bar that is red represents an individual ramet's assignment probability to cluster 2. These results indicate that all ramets have approximately the same assignment probabilities and there is no significant structure among the 21 ramet genotypes.

Table S1 Presence (1) and absence (0) data from 206 AFLP amplicons for 21 *S. altissima* ramets labeled g3-g28.

g3
0111011111101010001010100001000011110111111011110001100001000001010111010100000
1011010010000001100000001001100000001000110010100011111100001111001100010110000
01000000001101001010100110011010000000000100000001

g4
0101011111101010001010100001000011110111011011110101100111000000011111010100011
1010010001100001010011011001100001010000110010100011011000001110000100010110000
01000000001110001100100101011010001000000100100001

g5
0111011111101010001010101011000011010111011001110001000101000000011001000101001
1010001010100001000000011001100001011000010000000001101000000010001110001011001
01000000001011001010100101111010000000000110100001

g6
0101111111101011001001100001001011110111011001110001100110010000011111010100101
1000010011100011000000001001110001011000000010100000001000010011001010000001001
01010000001110001011101100111010000000000000101001

g8
0101011111101010001011000001000111010111111011110111000101000000101100101000100
1010010010000000100000001100110000101110001000010001100100010110000110001010011
11000000001111101011000111011010001000001011010000 1

g9
0111011111111010101010000000100000001011101100111000100011101000101010100010100
1001010010110001011000001101101001011000010000100101001000100110111110010110011
01100000000111110101010110001110000000000101000000001

g13
0101011111101010001010000000100100111011101100111010100011000000101011100110001
1010000010100001000010011001100001010000110010100001010000000100000101110110000
11000010001110001000100100011000010000000100101001

g14
0101001111101010001001100001000011110111011001110101001101000100010110001100000
1000000010100001000110001101100001011100010000100001010110001111111110010110011
11100000000101010101110110111100001000000001010010001

g15
1111011111111010001110000101100011111111011011110001000101000000101111000110000
1000010010000000101010001100111000101000000000000000011100001101100101001100110 1
11000000001110011010100101111000010010010100001001

g16
0101111111101010001110100001100001111111111011110001000110000000101010111110001

10000001101000110001000111011000010000000000000000001111000101111000100011010011
1101000000111000101110010101000101001000010010000 1

g17
01110111111110100010101000010000010101110110101100010011010000010111010011000010
00001011000000100010000100110001001100001000010001110100011110000111010001101
0100010000111000101010010111101001100000001011111 1

g18
0101011111101010001011000000100001101011101100111100000100100010101111101010000
1011100010000001101000000100111010101010001001100100100000000111000100010011000
1110000000111100110111111000111000000000000100100001

g19
0110111111111010101010010001000111010111111001110001101110000010011101000101101
0101100100010010001001110111000010100000000111001010110001011100000000101100
10110100001111000100110011101100001001000110000100 1

g20
0011101111101100001011100001000111110111011000110001001101010001001110000101001
1110000100000001100100011001100001010000000000010001101100010111000100001111001
1110000001101000110010110011100001000000001001000 01

g22
0111111111101010001010000101000101111111011001110000000101000000010101000000000
1000010101000000101000000110011010010110000000100101001011000000110000000010110 01
11000000100110010110000001001110000100000101101010 01

g23
0111011111111010001010100001010011110111011011110100000111000100011111000100000
1000010110100001010101011011100001010000000000000000111001100010000001000101100 0
1100000000111100010111011011111100101111110000101000

g24
0110101111101010011010110001101011010111011111111000100010100000000101010101010 0
1010010010100001000000000100110000101110001100101000111000000001111000000010010 00
0110000001011100010011001010110000100100001101010 01

g25
0111011111101010001001010001010011011111101100110110100011100010001010100010000
1010000001000010100000000110011000010110000000000100011001000000110000001000101100 1
010000000010110010101001011100001000100001001000 00

g26
00110111111010100010101010010000010111111010111101010111010011110101111101010 1
1010010010000101010110001011000000101010001000000000101101000011001010001010010
010000000111101010111011110100000000000000000100001

g27
0110111111101010001010000000100001100011101100111001010010101000101010101010010
1010010010100001000100001001111001010111010000100001011000000111001010001011001
010000001001010001111101101010110000000000000000001

g28
0100101111101010001010100101100011000111011001110001101101100001110111010110 00
1000010111100101001100001001110001110001100000100001011000000110001000010010 01
11000000000111101101110010011101000101001000110000 0

Supporting References and Notes

S1. A. E. Weis, H. G. Hollenbach, W. G. Abrahamson, *Am. J. Bot.***74**, 1476 (1987).

S2. G. D. Maddox, R. E. Cook, P. H. Wimberger, S. Gardescu, *Am. J. Bot.* **76**, 318 (1989).

S3. M. T. J. Johnson, A. A. Agrawal, *Ecology* **86**, 874 (2005).

S4. R. B. Root, *Ecology* **77**, 1074 (1996).

S5. G. D. Martinsen, K. D. Floate, A. M. Waltz, G. M. Wimp, T. G. Whitham, *Oecologia* **123**, 82 (2000).

S6. N.J. Gotelli, G.L. Entsminger. *EcoSim: Null Models Software For Ecology. Version 7* (Acquired Intelligence Inc. & Kesey-Bear. Jericho, VT, 2006) **http://garyentsminger.com/ecosim.htm**

S7. M. T. J. Johnson, M. J. Lajeunesse, A. A. Agrawal, *Ecol. Lett.* **9,** 24 (2006).

S8. M. Loreau, A. Hector, *Nature* **413**, 548 (2001).

S9. D.P. Faith, P. R. Minchin, L. Belbin. *Vegetatio* **69**: 57–68 (1987).

S10. K.E. Haskins, C.A. Gehring, *Ecology* **85**, 2687 (2004).

S11. U. G. Mueller, L. L. Wolfenbarger, *Trends Ecol. Evol.* **14**: 389 (1999).

S12. L.L. Cavalli-Sforza, A.W. F. Edwards, *Evolution* **3**, 550 (1967).

S13. Felsenstein, J. (Dept. of Genetics, Washington University, Seattle. 1993).

S14. J. K. Pritchard, M. Stephens, P. Donnelly, *Genetics* **155**, 945 (Jun, 2000).