6. D. Sutton, *A Revision of the Tribe Antirrhineae* (Oxford Univ. Press, Oxford, 1988).
7. I. Mateu-Andres, J. G. Segarra-Moragues, *Ann. Bot. (Lond.)* **92**, 647 (2003).
8. P. Vargas, J. A. Rosselló, R. Oyama, J. Güemes, *Plant Syst. Evol.* **249**, 151 (2004).
9. Z. Schwarz-Sommer, B. Davies, A. Hudson, *Nat. Rev. Genet.* **4**, 657 (2003).
10. T. Gubitz, A. Caldwell, A. Hudson, *Mol. Biol. Evol.* **20**, 1537 (2003).
11. J. Hackbarth, P. Michaelis, G. Scheller, *Z. Indukt. Abstammungs- Vererbungslehre* **80**, 1 (1942).
12. H. Stubbe, *Genetik und Zytologie von Antirrhinum L. sect. Antirrhinum* (Veb Gustav Fischer Verlag, Jena, Germany, 1966).
13. K. Schwinn *et al.*, *Plant Cell* **18**, 831 (2006).
14. E. Baur, *Bibl. Genet.* **4**, 1 (1924).
15. G. W. Horgan, *Comput. Electron. Agric.* **31**, 169 (2001).
16. T. F. Cootes, G. J. Edwards, C. J. Taylor, *IEEE Trans. Pattern Anal. Mach. Intell.* **23**, 681 (2001).
17. N. B. Langlade *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 10221 (2005).
18. N. H. Barton, G. M. Hewitt, *Annu. Rev. Ecol. Syst.* **16**, 113 (1985).
19. N. H. Barton, B. O. Bengtsson, *Heredity* **57**, 357 (1986).
20. D. Luo *et al.*, *Cell* **99**, 367 (1999).
21. E. S. Coen, R. Carpenter, C. Martin, *Cell* **47**, 285 (1986).
22. W. S. Moore, J. T. Price, in *Hybrid Zones and the Evolutionary Process*, R. G. Harrison, Ed. (Oxford Univ. Press, Oxford, 1993), pp. 196–225.
23. D. W. Schemske, H. D. Bradshaw Jr., *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11910 (1999).
24. L. Chittka, J. D. Thomson, Eds., *Cognitive Ecology of Pollination* (Cambridge Univ. Press, Cambridge, 2001).
25. H. D. Bradshaw Jr., D. W. Schemske, *Nature* **426**, 176 (2003).

26. We thank C. Martin and J. Venail for providing the *ROS* sequence before publication; M. Burrus, L. Copsey, J. Bowers, C. Cazettes-Vicedo, and Z.-L. Liu for their help with carrying out genotyping and genetics; M. Bernardet, M. Cruzan, and J. Leneveu for their help in the field; and G. Hewitt for helping to initiate this project. This research was funded by grants from the Biotechnology and Biological Sciences Research Council, UK. Sequences are deposited in GenBank; accession numbers DQ866629 to DQ866657 for *ROSEA1*, DQ866658 to DQ866676 for *PALLIDA*, and DQ866677 to DQ866701 for *DICHOTOMA*.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5789/963/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
References

25 April 2006; accepted 21 July 2006
10.1126/science.1129161

Plant Genotypic Diversity Predicts Community Structure and Governs an Ecosystem Process

Gregory M. Crutsinger,[1]* Michael D. Collins,[1] James A. Fordyce,[1] Zachariah Gompert,[2] Chris C. Nice,[2] Nathan J. Sanders[1]

Theory predicts, and recent empirical studies have shown, that the diversity of plant species determines the diversity of associated herbivores and mediates ecosystem processes, such as aboveground net primary productivity (ANPP). However, an often-overlooked component of plant diversity, namely population genotypic diversity, may also have wide-ranging effects on community structure and ecosystem processes. We showed experimentally that increasing population genotypic diversity in a dominant old-field plant species, *Solidago altissima*, determined arthropod diversity and community structure and increased ANPP. The effects of genotypic diversity on arthropod diversity and ANPP were comparable to the effects of plant species diversity measured in other studies.

Ecological theory (*1*, *2*) and field experiments (*3*, *4*) have revealed a positive relationship between the diversity of plant species and the diversity of associated consumers. At least two mechanisms might explain this pattern. First, because approximately 90% of herbivorous insects exhibit some degree of host specialization (*5*), as plant species richness increases, so should the number of associated herbivore species. This resource specialization hypothesis has some theoretical support (*1*, *2*, *6*). Second, if aboveground net primary productivity (ANPP) increases as plant species richness increases (*7*), then more herbivore individuals, and therefore more species, will be supported by increases in available energy (this has been called the more individuals hypothesis) (*8*). An increase in the number of herbivore species by either of these mechanisms should support more predator species (*9*). Recent studies have shown that population genotypic diversity, like plant species diversity, can have extended consequences for communities and ecosystems (*10*–*14*). However, no studies to date have explicitly linked intraspecific genotypic diversity, the structure of associated communities, and the potential mechanisms driving these patterns, such as energy availability. This paucity of studies exists despite numerous calls for such research within the literature regarding biodiversity-ecosystem function (*7*, *15*). We tested whether host-plant genotypic diversity determines the structure of associated arthropod communities and governs an ecosystem process, ANPP, that influences arthropod species richness.

We manipulated the plot-level genotypic diversity (the number of genotypes per plot) of *Solidago altissima*, tall goldenrod, a common perennial plant throughout eastern North America. Twenty-one *S. altissima* ramets were collected from local *S. altissima* patches growing in fields near the study site, and each ramet was identified as a unique genotype by means of amplified fragment length polymorphism. From these 21 genotypes, we established 63 1-m^2 experimental plots, each containing 12 individuals and 1, 3, 6, or 12 randomly selected genotypes, mimicking the densities and levels of genotypic diversity found in natural patches of similar size. We censused arthropods on every ramet in each plot five times over the course of the growing season. In total, we counted 36,997 individuals of ~136 species. We estimated ANPP at the peak of the growing season using nondestructive allometric techniques (*16*).

Total cumulative arthropod species richness increased with plant genotypic diversity. The number of arthropod species was, on average, 27% greater in 12-genotype plots than in single-genotype plots (Fig. 1), indicating that plant genotypic diversity was an important determinant of arthropod diversity. When we examined the effects of genotypic diversity on community structure, we found that herbivore species richness (Fig. 2B) and predator richness (Fig. 2A) also increased with increasing genotypic diversity. The effects of genotypic diversity on arthropod communities were nonadditive (Fig. 1). That is, total arthropod richness and herbivore and predator richness were all greater in the 6- and 12-genotype plots than would be predicted by summing the number of arthropod species associated with the corresponding genotypes grown in monoculture ($P < 0.01$).

ANPP also increased with plant genotypic diversity and was 36% greater in 12-genotype plots than in single-genotype plots (Fig. 2C). The effect of genotypic diversity on ANPP could be due to increased niche complementarity (mixed genotypes used available resources more completely or mixed genotypes facilitated one another, thereby increasing ANPP in mixtures) (*7*, *15*) or to sampling or selection effects (increased ANPP caused by randomly assembled mixtures having a higher probability of containing highly productive genotypes) (*17*). Using standard techniques (*18*) we found that selection effects were highly variable and were not significantly different from zero ($P > 0.60$ for all treatments), indicating that highly productive genotypes do not dominate in mixtures and drive observed increases in ANPP. Selection

[1]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA. [2]Department of Biology, Texas State University, San Marcos, TX 78666, USA.

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

effects were not related to genotypic diversity (fig. S1A). We also found complementarity effects to be highly variable, generally positive, but not significantly different from zero ($P > 0.20$ for all treatments). We found a marginally significant increase in complementarity with increasing genotypic diversity (fig. S1B), indicating that positive interactions among genotypes in mixtures may lead to increases in ANPP with increasing genotypic diversity.

Arthropod richness might respond to genotypic diversity either because of increased productivity in plots with higher genotypic diversity, as the more individuals hypothesis predicts (*8*), or because genotypes vary in susceptibility to particular herbivores, as the resource specialization hypothesis predicts (*6*). Like species richness, arthropod abundances increased with plant genotypic diversity (*19*). In addition, there was a positive relationship between ANPP and both arthropod richness and abundance (*19*). Arthropod richness and abundance were positively correlated with one another (*19*). To test whether the effects of ANPP and genotypic diversity on arthropod species richness resulted from species-rich plots having more arthropod individuals, as the more individuals hypothesis predicts (*8*), we used rarefaction to examine the response of rarefied arthropod species richness to plant genotypic diversity. Rarefaction corrects for differences in the number of individuals among plots (*20*). There was no relationship between rarefied total arthropod richness and ANPP, or between rarefied herbivore and predator richness and ANPP ($P > 0.10$ in all cases), indicating that ANPP controls richness by affecting the number of individual arthropods. Rarefied total richness and rarefied herbivore richness instead increased as plot-level plant genotypic diversity increased, but rarefied predator richness did not (fig. S2).

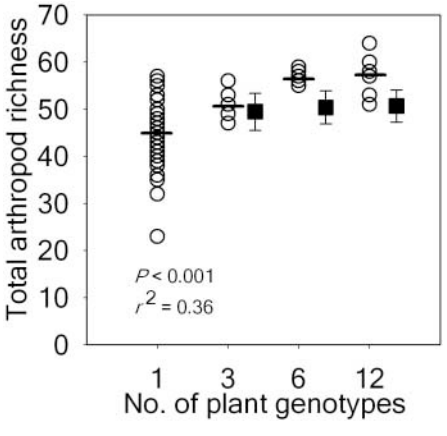

Fig. 1. Relationship between population-level genotypic diversity of *S. altissima* and total arthropod species richness. Circles indicate plot-level observations, and horizontal lines indicate treatment means. Squares indicate the number of arthropod species predicted by simple additive models. Error bars indicate 95% confidence interval.

However, rarefied predator richness did depend on rarefied herbivore richness, suggesting an indirect effect of host-plant genotypic diversity on predator diversity mediated by herbivore diversity (fig. S2). These results indicate that increasing genotypic diversity increases the amount of resources (ANPP) available to herbivores. As ANPP increased, so did arthropod abundance, resulting in increases in the number of species, as the more individuals hypothesis predicts (*8*). When we controlled for variation in arthropod abundance using rarefaction, genotypic diversity explained an additional 12% of the variation in rarefied total and rarefied herbivore richness, indicating a second mechanism by which genotypic diversity affects arthropod communities: by increasing the diversity of resources available, as predicted by the resource specialization hypothesis (*6*). Moreover, the abundance and composition of herbivore assemblages were more similar within *Solidago* genotypes than among genotypes, and particular genotypes were more susceptible to herbivory than were others (supporting online text and figs. S3 to S5). Taken together, these results suggest that particular herbivores are associated with particular host-plant genotypes.

To compare our results to studies that have examined how plant species diversity affects arthropod diversity and ANPP, we calculated the standardized effect sizes (SESs) (*21*) of genotypic diversity using our data and the SESs of plant species diversity using data from the Cedar Creek Long Term Ecological Research Biodiversity II experiment (*3*). A SES measures the number of standard deviations that the most diverse plots (12 genotypes in our case, 16 species from Cedar Creek) is above or below the single-genotype or single-species plots. The SES of plant genotypic diversity on arthropod diversity in our study (SES = 1.80) was nearly two times the SES of plant species diversity on arthropod diversity from Cedar Creek (SES = 0.93). The SES of plant genotypic diversity (SES = 1.33) on ANPP in our study was similar to the SES of plant species diversity on ANPP at Cedar Creek (SES = 1.35). Our results indicate that the effect of genotypic diversity within a host-plant population on associated arthropod communities and ANPP is directly comparable to the effect of species diversity within a plant community (*3*, *4*). A field experiment that orthogonally manipulates genotypic diversity and species diversity in concert could

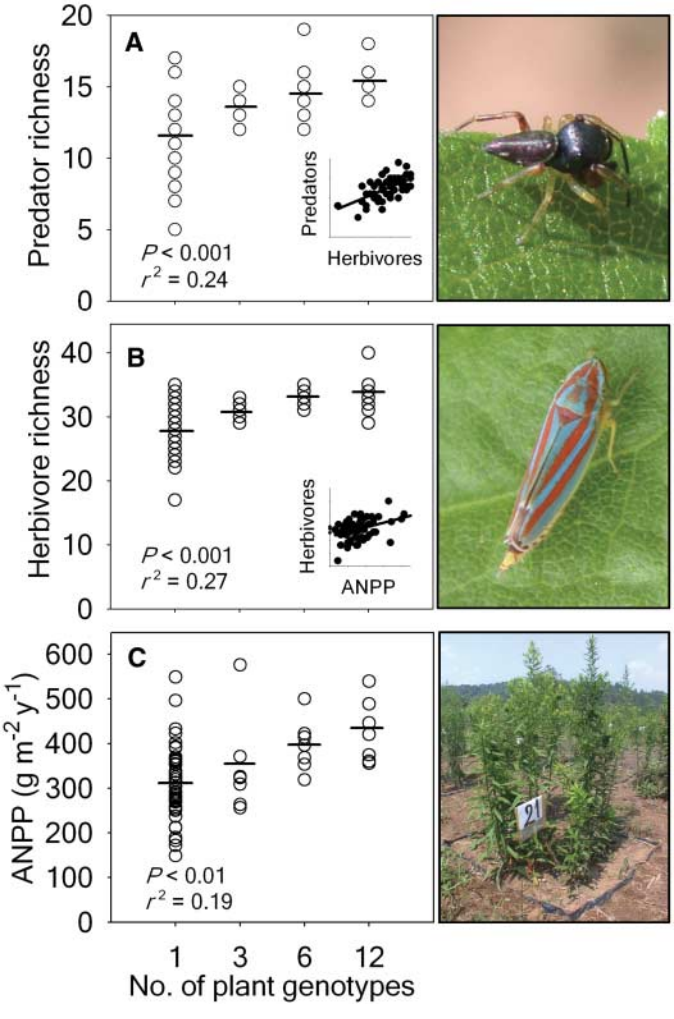

Fig. 2. Relationship between population-level plant genotypic diversity and predator species richness (**A**), herbivore species richness (**B**), and ANPP of *S. altissima* (**C**). Open circles indicate plot-level observations, and horizontal lines indicate treatment means. The inset in (A) shows the relationship between herbivore species richness and predator species richness ($r^2 = 0.36$, $P < 0.001$), and the inset in (B) shows the relationship between ANPP and herbivore richness ($r^2 = 0.17$, $P < 0.001$).

further elucidate the relative contributions of intra- and interspecific diversity on community- and ecosystem-level processes.

Our work indicates two mechanisms underlying the relationships among intraspecific genotypic diversity, the diversity of associated consumers, and ecosystem processes. We explicitly showed that the effect of genotypic diversity on arthropods does not occur simply because of increased ANPP in diverse plots. It also arises because of an increase in the diversity of resources available to herbivores. These effects are nonadditive and cascade across trophic levels to structure associated communities. Our results demonstrate the need to incorporate intraspecific variation into current ecological theory that has emphasized the importance of interspecific variation (*3*, *4*, *7*, *15*, *17*, *18*) or theory that ignores differences among species (*22*). Given the focus of conservation efforts on how the loss of species from communities affects ecosystem processes, our work suggests that the loss of genotypes from populations can no longer be overlooked (*14*, *23*–*25*).

References and Notes

1. G. E. Hutchinson, *Am. Nat.* **93**, 145 (1959).
2. R. H. MacArthur, *Geographical Ecology* (Harper & Row, New York, 1972), pp. 169–194.
3. E. Siemann, D. Tilman, J. Haarstad, M. Ritchie, *Am. Nat.* **152**, 738 (1998).
4. N. M. Haddad, D. Tilman, J. Haarstad, M. Ritchie, J. M. H. Knops, *Am. Nat.* **158**, 17 (2001).
5. E. Bernays, M. Graham, *Ecology* **69**, 886 (1988).
6. P. W. Price, in *Variable Plants and Herbivores in Natural and Managed Systems*, R. F. Denno, M. S. McClure, Eds. (Academic Press, New York, 1983), pp. 559–596.
7. D. U. Hooper *et al.*, *Ecol. Monogr.* **75**, 3 (2005).
8. D. S. Srivastava, J. H. Lawton, *Am. Nat.* **152**, 510 (1998).
9. M. D. Hunter, P. W. Price, *Ecology* **73**, 724 (1992).
10. Y. Y. Zhu *et al.*, *Nature* **406**, 718 (2000).
11. A. R. Hughes, J. J. Stachowicz, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 8998 (2004).
12. J. A. Schweitzer, J. K. Bailey, S. C. Hart, T. G. Whitham, *Ecology* **86**, 2834 (2005).
13. T. B .H. Reusch, A. Ehlers, A. Hämmerli, B. Worm, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2831 (2005).
14. M. T. J. Johnson, M. J. Lajeunesse, A. A. Agrawal, *Ecol. Lett.* **9**, 24 (2006).
15. M. Loreau, S. Naeem, P. Inchausti, Eds., *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives* (Oxford Univ. Press, Oxford, 2002), pp. 237–242.
16. See supporting material on *Science* Online.
17. M. A. Huston, *Oecologia* **110**, 449 (1997).
18. M. Loreau, A. Hector, *Nature* **413**, 548 (2001).
19. Abundances were positively related to genotypic diversity (total: $r^2 = 0.27$, $P < 0.001$; herbivores: $r^2 = 0.29$, $P < 0.001$; predators: $r^2 = 0.07$, $P = 0.03$). There was a positive relationship between ANPP and arthropod richness (total: $r^2 = 0.24$, $P < 0.001$; herbivores: $r^2 = 0.17$, $P < 0.001$; predators: $r^2 = 0.15$, $P = 0.001$) and total abundance ($r^2 = 0.19$, $P < 0.001$) and herbivore abundance ($r^2 = 0.23$, $P < 0.001$). Arthropod richness and abundance were correlated ($r = 0.74$, $P < 0.001$; herbivores: $r = 0.70$, $P < 0.001$; predators: $r = 0.29$, $P = 0.02$).
20. N. J. Gotelli, G. R. Graves, *Null Models in Ecology* (Smithsonian Institution, Washington, DC, 1996), pp. 21–46.
21. S. M. Scheiner, J. Gurevitch, Eds., *The Design and Analysis of Ecological Experiments* (Chapman & Hall, New York, 1993), pp. 347–369.
22. S. P. Hubbell, *A Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, NJ, 2001).
23. T. G. Whitham *et al.*, *Ecology* **84**, 559 (2003).
24. G. W. Luck, G. C. Daily, P. R. Ehrlich, *Trends Ecol. Evol.* **18**, 331 (2003).
25. G. M. Wimp *et al.*, *Ecol. Lett.* **7**, 776 (2004).

26. We thank K. Crawford, C. Engel, J. Hite, J. Ledford, and K. McFarland for help in the field and lab and W. Abrahamson, J. Bailey, M. Cadotte, A. Classen, R. Dunn, V. Eviner, N. Gotelli, M. Johnson, J. Schweitzer, D. Simberloff, J. Weltzin, J. Williams, and three anonymous reviewers for helpful comments. This research was funded by an Environmental Protection Agency Science to Achieve Results graduate fellowship, a Hilton Smith Graduate Fellowship, and the University of Tennessee.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5789/966/DC1
Materials and Methods
SOM Text
Figs. S1 to S8
Table S1
References

4 April 2006; accepted 12 June 2006
10.1126/science.1128326

p53-Mediated Inhibition of Angiogenesis Through Up-Regulation of a Collagen Prolyl Hydroxylase

Jose G. Teodoro, Albert E. Parker, Xiaochun Zhu, Michael R. Green*

Recent evidence suggests that antiangiogenic therapy is sensitive to p53 status in tumors, implicating a role for p53 in the regulation of angiogenesis. Here we show that p53 transcriptionally activates the α(II) collagen prolyl-4-hydroxylase [α(II)PH] gene, resulting in the extracellular release of antiangiogenic fragments of collagen type 4 and 18. Conditioned media from cells ectopically expressing either p53 or α(II)PH selectively inhibited growth of primary human endothelial cells. When expressed intracellularly or exogenously delivered, α(II)PH significantly inhibited tumor growth in mice. Our results reveal a genetic and biochemical linkage between the p53 tumor suppressor pathway and the synthesis of antiangiogenic collagen fragments.

The tumor suppressor activity of p53 results from its ability to transcriptionally activate a wide variety of target genes that in turn regulate cell cycle arrest, apoptosis, and suppression of angiogenesis (*1*). Although a number of p53 target genes involved in growth arrest and apoptosis have been identified, the role of p53 in the regulation of angiogenesis is less well understood. Using a polymerase chain reaction (PCR)–based subtractive hybridization strategy (*2*), we identified α(II) collagen prolyl-4-hydroxylase [α(II)PH] as a p53-stimulated gene. In this screen, ecdysone-inducible p53 expression was established in the $p53^{-/-}$ human cell line Saos-2 (Saos-2/Ec-p53 cells). Figure 1A (top) demonstrates that induction of p53 expression in these cells (bottom) stimulated transcription of α(II)PH as well as p21, a known p53 target gene (*3*). Up-regulation of α(II)PH was also observed when endogenous p53 expression was induced in wild-type HCT116 cells by the DNA damage-inducing agent camptothecin, but not in a matched $p53^{-/-}$ cell line (fig. S1). α(II)PH expression was also up-regulated upon expression of p53 from an adenovirus vector in $p53^{-/-}$ H1299 human cancer cells (Fig. 1B). By contrast, expression of p53 had no effect on transcription of another collagen prolyl-4-hydroxylase isoform, α(I)PH, or three other human prolyl hydroxylases, PHD1, 2, and 3.

The α(II)PH promoter region contains three partially overlapping putative p53-binding half sites (see below). We derived reporter constructs by cloning sequences upstream of the α(II)PH transcription start-site or, as a control, the p21 promoter, upstream of the chloramphenicol acetyltransferase (CAT) gene. Ectopic p53 expression increased activity of both p21-CAT and α(II)PH-CAT (Fig. 1C). The chromatin immunoprecipitation (ChIP) experiment of Fig. 1D shows that p53 was recruited to the α(II)PH promoter in vivo. Collectively, the results of Fig. 1 show that α(II)PH is a direct p53 target gene.

Prolyl hydroxylation is a required, rate-limiting step in collagen biosynthesis (*4*), suggesting that p53-mediated stimulation of α(II)PH expression might increase collagen levels. We therefore investigated the effect of p53 expression on endogenous collagen 18 levels. Unexpectedly, the level of full-length collagen 18 was diminished in H1299 cells following expression of wild-type p53 but not a transcriptionally defective p53 mutant (Fig. 2A, left, and fig. S2) (*5*). One explanation for this result is that under these conditions, collagen breakdown was also stimulated. To test this hypothesis,

Howard Hughes Medical Institute, Programs in Gene Function and Expression and Molecular Medicine, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA.

*To whom correspondence should be addressed. E-mail: michael.green@umassmed.edu