Evolutionary limits ameliorate the negative impact of an invasive plant

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Invasive species can quickly transform biological communities due to their high abundance and strong impacts on native species, in part because they can be released from the ecological forces that limit native populations. However, little is known about the long-term dynamics of invasions; do invaders maintain their dominant status over long time spans, or do new ecological and evolutionary forces eventually develop to limit their populations? *Alliaria petiolata* is a Eurasian species that aggressively invades North American forest understories, in part due to the production of toxic phytochemicals. Here we document a marked decline in its phytotoxin production and a consequent decline in their impact on three native species, across a 50+ year chronosequence of *Alliaria petiolata* invasion. Genetic evidence suggests that these patterns result from natural selection for decreased phytotoxin production rather than founder effects during introduction and spread. These patterns are consistent with the finding of slowing *A. petiolata* population growth and rebounding native species abundance across a separate chronosequence in Illinois, U.S. These results suggest that this invader is developing evolutionary limits in its introduced range and highlight the importance of understanding the long-term processes that shape species invasions and their impacts.

allelopathy | *Alliaria petiolata* | genetic diversity | glucosinolates | plant-soil feedbacks

Several dozen theories have been proposed to explain the success of introduced species in their new habitats (1); many of these theories posit that a mismatch of evolutionary histories between the invader and native community releases the invasive species from various ecological forces that limit native populations [e.g., herbivory, disease, competition (2)]. Subsequent evolutionary changes during or after introduction may allow invaders to further capitalize on this release, for instance, by shifting resources from defense to growth and reproduction in response to reduced herbivore loads (3, 4). Existing studies on this topic have primarily tested whether populations differ in the new vs. the old range and they do not consider how evolutionary forces may change during the invasion process in the introduced range. For instance, invasive cane toads in Australia from the leading edge of the invasion front show increased investment to dispersal traits (larger body sizes, relatively longer legs, and greater activity) compared to those in the interior of the new range (5), even though this comes at the cost of increased rates of arthritis (6). Some evidence suggests that releases from ecological limitations may be temporary; for instance, some plants can acquire diverse herbivore communities over time in their new range (7–10). Although rare, there are cases of highly abundant invaders experiencing gradual, or even rapid population declines over time (11). Some of these can be attributed to an ecological change, such as the introduction of a new competitor or pathogen, or community succession (12). However, for many others, the causes of decline are not clear and could involve evolved changes in either the introduced or native species (11). Invasive species can evolve rapidly in response to changing

ecological conditions; for instance, chemical defenses in *Pastinaca sativa* initially declined in its new range upon release from a specialist herbivore, but then rapidly increased when that herbivore was subsequently introduced (13). In addition to such external ecological stimuli, shifting selection pressures may also originate within the introduced population. For example, the traits favored by intense intra-specific competition in the dense monospecific stands of established populations of aggressive invaders may differ from those favored when invading new habitats, where competition is mostly inter-specific. Thus, it is not clear how, when, or if continued evolution in invasive species further solidifies their dominance by, for example, reinforcing their release from ecological limitations, or if evolution results in new limiting factors that ameliorate the invader's impact on native communities over time.

Allelopathy, in which plants produce toxic chemicals to reduce the growth of competitors, may play an important role in plant invasions, especially if the allelochemicals are novel to the new range where native species have no co-evolved defenses (14). Such traits may be under strong selection to increase during an invasion, if they are more effective in the introduced range because of the new competitors' naivety. However, allelopathic traits may incur costs (15) and may become less favorable over time as native species develop resistance mechanisms, or as native species are extirpated from invaded communities, such that intra-specific competition becomes the most important selective force (16).

Alliaria petiolata (garlic mustard, Brassicaceae) is a Eurasian biennial that has invaded forest understories throughout the northeastern and midwestern United States. Several traits of *A. petiolata* may contribute to its success in its introduced range, including its unusual phenology, high shade and cold tolerance, low herbivory rates, and allelopathic effects on native plant germination and mycorrhizal fungi growth (17). Like all members of the Brassicaceae, *A. petiolata* does not form connections with mycorrhizal fungi and it produces glucosinolates, a class of secondary compounds that break down into products toxic to herbivores, fungi, and other plants (18–20). *A. petiolata* also produces other unique secondary compounds, including iso-6-β-*o*-vitexin (in leaves), alliarinoside (in leaves and roots), and high levels of cyanide, which might also affect herbivores and soil microbes including mycorrhizal fungi (21–24). North American mycorrhizal fungi appear to be more sensitive to the allelochemicals of *A. petiolata* than fungi from its native range. If these allelochemicals weaken the mycorrhizal associates of native plants enough to reduce their ability to compete with the invader, this could explain *A. petiolata's* greater abundance in its introduced versus its native range (21).
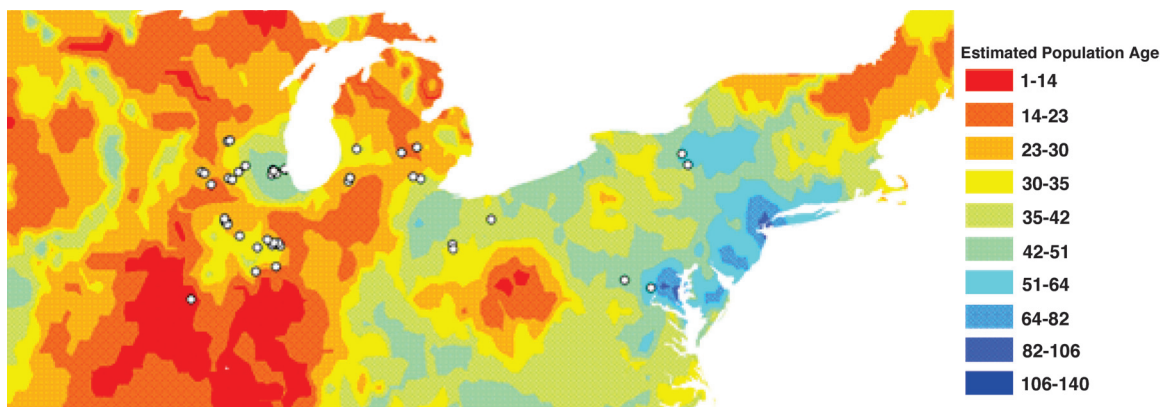
Fig. 1. Map of *A. petiolata* invasion history. Spatially kriged map of estimated *A. petiolata* population age (years since first report). White circles mark the populations used to measure allelochemicals concentrations and effects on native tree seedlings.

We evaluated a half century chronosequence of North American forest understory invasion by *Alliaria petiolata* to test whether investment in toxic allelochemicals and their impacts on three native species declined over time. We then compared the quantitative data to neutral genetic markers to determine whether any observed temporal clines were best explained by multiple introductions, stochastic processes, or by natural selection. Finally, we used field data from a separate chronosequence of forests in Illinois to test whether *A. petiolata* populations declined, while native woody species abundance increased, with invasion age as would be predicted if allelochemical concentrations tend to decline over time.

Results

We used data from >650 herbarium and literature records to create a spatially interpolated map of invasion ages across the eastern U.S. (Fig. 1). Using seeds collected in fall 2007 from 44 populations distributed across this range and grown in a common greenhouse environment, we found that investment in root tissue glucosinolate compounds declined significantly with increasing age of the source population (Fig. 2). A second putative allelochemical, alliarinoside, also showed a weaker decline with population age ($R^2 = 0.10$, $P < 0.05$). Concentrations of neither class of chemical in leaf tissue showed significant patterns across the chronosequence ($R^2 < 0.016$, $P > 0.41$). Since these plants were grown from seed in a common environment, these results most likely represent genetic differences between populations.
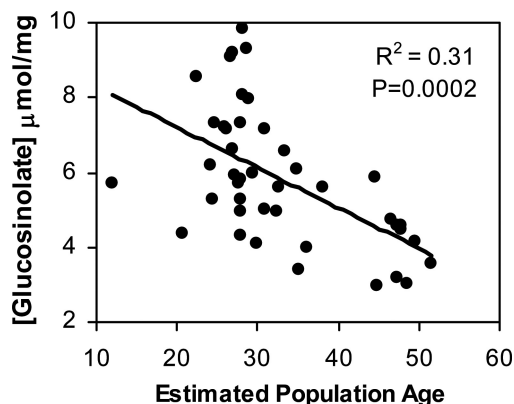

Fig. 2. Glucosinolate concentrations versus population age. Ordinary least squares regression of allyl + benzylglucosinolate concentrations in root tissue against the estimated age of the *A. petiolata* population. Each point is the mean of five replicates, and ages are estimated for seed sources.

These and the following results did not change when controlling for spatial autocorrelation. Both the age of *A. petiolata* populations and root glucosinolate concentrations showed significant trends across a North American longitudinal gradient ($R^2 = 0.26$, $P = 0.0005$ and $R^2 = 0.17$, $P = 0.006$, respectively). However, longitude could not explain the correlation between age and allelochemical concentration; when population age, latitude, and longitude were included in multiple regression models to explain glucosinolate concentrations the effect of age remained significant ($F_{1,43} = 6.38$, $P = 0.016$), while that of longitude did not ($F_{1,43} = 1.42$, $P = 0.24$; latitude had no significant effect in any model), indicating that age was the best predictor of glucosinolate concentrations. Additionally, glucosinolate concentrations showed a significant negative relationship with population age when the effect of longitude was statistically removed ($R^2 = 0.16$, $P = 0.008$).

Since the allelochemicals of *A. petiolata* are thought to indirectly affect competitive interactions via altered soil microbial communities, we tested the impact of population differences on native plants with a soil feedback experiment. We grew *A. petiolata* plants from seeds from the same 44 populations with a common soil community for 3 months and then used the resulting soil to grow three native tree species: *Platanus occidentalis* (sycamore), *Juglans nigra* (black walnut), and *Quercus rubra* (red oak). All species showed significantly greater growth in soils cultured by *A. petiolata* plants derived from older populations (Fig. 3*A*). For *P. occidentalis* and *J. nigra*, but not *Q. rubra*, this result could be partly attributed to differences in root glucosinolate concentrations (Fig. 3*B*). Leaf glucosinolate concentrations did not correlate with native species growth for any species, nor did alliarinoside concentration (in leaves or roots).

We genotyped 5–10 individuals from each of the 44 populations at 8 polymorphic microsatellite loci (25) and found that populations similar in age were not more likely to be similar genetically (correlation of genetic distance with temporal distance, $r = 0.074$, $P = 0.15$). Genetic diversity (expected heterozygosity across all loci) increased significantly with estimated population age (Fig. 4*A*). Population mean glucosinolate concentrations declined significantly with increasing genetic variation in neutral markers (Fig. 4*B*). The genetic composition of a population at the neutral loci was not related to glucosinolate concentrations ($r = -0.095$, $P = 0.92$). Indeed, when we restricted our analysis to a single widespread multilocus genotype (38 out of 270 genotyped individuals, present in 14 populations), we still found a significant decline in glucosinolate concentrations with population age ($R^2 = 0.62$, $P = 0.0008$, $n = 14$), indicating that glucosinolate concentrations may evolve rapidly without widespread changes in other areas of the genome.
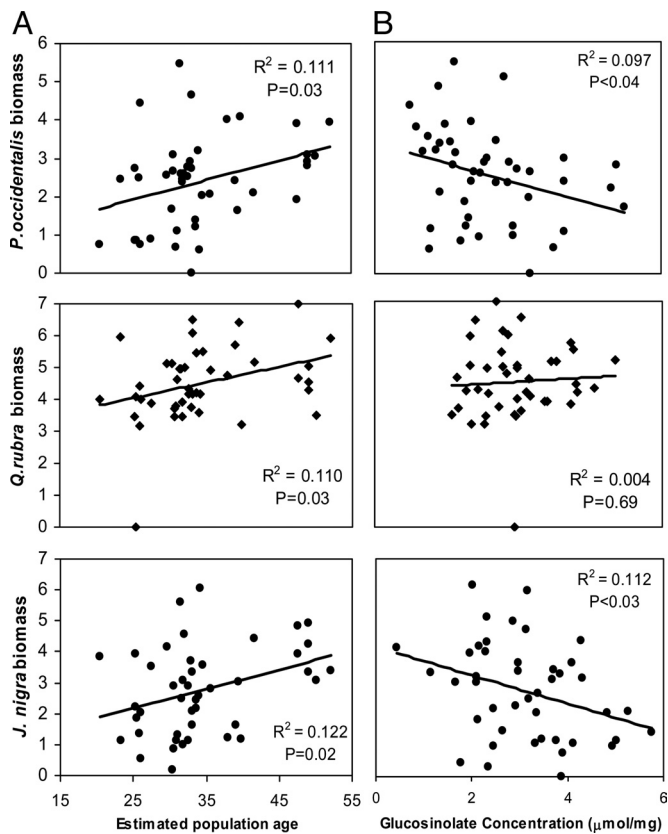
Fig. 3. Native tree seedling biomass in *A. petiolata* conditioned soils. Ordinary least squares regression of native seedling biomass (in grams) of three native tree species in soils cultivated by *A. petiolata* individuals from different source populations versus (*A*) the estimated age and (*B*) the mean root glucosinolate concentration of the *A. petiolata* source population.

We used field data from the Illinois Critical Trends Assessment Program, which performs repeated forest vegetation surveys at 5-year intervals in permanent plots at randomly selected sites throughout the state (26), to test whether *A. petiolata* abundance tends to grow more slowly, while the abundance of native species grows more rapidly, as *A. petiolata* populations age. *A. petiolata* was recorded at 30 forest sites in at least one of two sampling periods (none of these sites were included in the 44 populations used to measure allelochemistry). We calculated the difference in understory abundance of *A. petiolata*, native woody, and native herbaceous species at these sites over 5 years, and regressed this differ-

ence against the estimated age of the *A. petiolata* population. As predicted, *A. petiolata* populations showed larger increases in more recently invaded areas (Fig. 5*A*), while cover of native woody species decreased in recently invaded areas and increased in areas with a longer history of *A. petiolata* invasion (herbaceous species showed no effect) (Fig. 5*B*).

Discussion

Across a 50+ year chronosequence of *A. petiolata* population ages, we found that populations tended to evolve lower concentrations of several allelochemicals over time. Soil mediated impacts on three native species also declined along this sequence, and for two of the three natives could be attributed to declining *A. petiolata* allelochemical concentrations. While many studies have documented evolved differences in invasive species between their introduced and native range (3, 4), this study indicates that reduced invasiveness may evolve during the invasion process, suggesting that evolutionary forces may provide limits to invasive species when ecological limits have been removed during introduction.

Clines in quantitative traits of introduced species may form due to differential selection or to multiple introductions from genetically distinct demes in the native range (27). In our case, the temporal cline in allelopathic traits could have arisen if multiple introductions of *A. petiolata* have occurred over time, such that younger invasive populations tend to be derived from different source populations than older ones. However, we found that populations similar in age were not more likely to be similar genetically. Therefore multiple, temporally distinct introductions are not sufficient to explain the temporal cline, implicating a role for natural selection.

There may be more than one mechanism favoring lower allelochemical concentrations in older populations. Since *A. petiolata* was originally recorded (and assumedly introduced) in just a few locations on the east coast and subsequently spread westward over the last century, population age is partially confounded with longitude and all of the environmental variables that vary with longitude (e.g., precipitation, temperature, soil type). However, long-distance dispersal events have also created substantial variation in population age independent of longitude, for example, creating islands of relatively old populations around large cities like Chicago, IL and Toledo, OH (see Fig. 1). This pattern allowed us to statistically separate the effect of population age and latitudinal and longitudinal clines on allelochemical concentrations, which confirmed that allelochemicals are more directly attributable to population age. Thus, geographic variation in an unmeasured environmental variable that selects for higher or lower allelochemical concentrations would be insufficient to explain all observed patterns. Never-
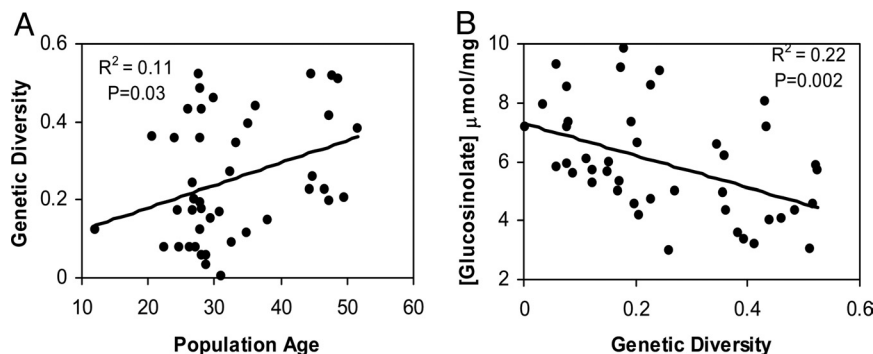

Fig. 4. Relationships between neutral genetic diversity, population age, and glucosinolate concentrations. Ordinary least squares regression of (*A*) genetic diversity of an *A. petiolata* population (expected heterozygosity corrected for sample size) versus the estimated age of the population and (*B*) the mean root glucosinolate concentration of an *A. petiolata* population versus its genetic diversity.
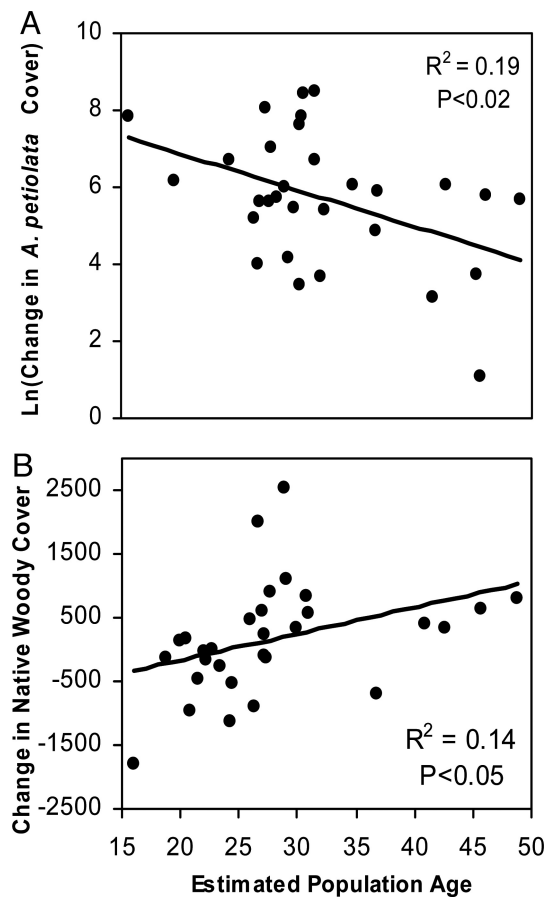
Fig. 5. Changes in *A. petiolata* invaded plant communities over time. Ordinary least squares regression of (*A*) the change in *A. petiolata* cover over 5 years (ln transformed) and (*B*) change in cover of native woody species in the understory over 5 years versus the estimated age of the *A. petiolata* population at that site.

theless, it is not possible to completely exclude these possibilities in a correlational study. For instance, *A. petiolata* may invade some habitat types more readily than others, which could cause population age to correlate with environmental variables in ways independent of geography.

On the other hand, there are plausible mechanisms by which temporal changes within populations and communities could lead to selection for reduced toxicity. In a related species, high glucosinolate concentrations were selectively favored in interspecific competition, but disfavored when competing with conspecifics (15, 16). Under intra-specific competition, producing the chemicals entailed a cost but accrued no benefit (since the growth of conspecifics was not impacted by the chemical) (15). As *A. petiolata* can form dense patches in just a few generations (28), selection pressures may quickly switch to favor lower glucosinolate levels. The selective benefit of allelopathic traits may also decrease over time if native plants or soil microbes evolve resistance, and this resistance is great enough to render the allelochemical ineffective (29). Ultimately, confidently determining the primary mechanisms creating the patterns that we have observed will require experimental approaches.

We found that glucosinolate concentrations declined with increasing levels of neutral genetic diversity, even though concentrations were unrelated to genetic composition. We also found that genetic diversity increased significantly with population age. Since *A. petiolata* is self-compatible, a single individual can find a new population (17), resulting in initially genetically

depauperate populations that gain diversity over time with mutation and immigration. A plausible scenario that could explain both the high allelochemical concentrations and low genetic diversity observed in young populations is that highly toxic founders may be more likely to establish self-sustaining populations, due to their strong interspecific competitive abilities at new sites. In contrast, populations founded with less toxic genotypes may grow more slowly and be more susceptible to stochastic extinction in the early phases of establishment. If selection pressures switch to favoring lower allelochemical levels as populations grow, then increased genetic variation over time due to mutation or immigration would allow for a more rapid response to selection. Of course, other explanations are also possible; for instance, genes encoding high allelochemical concentrations may be initially favored directly, but may be negatively genetically linked with other traits favored in older, denser populations. Further research is needed to elucidate the direct and indirect selection pressures acting on allelopathic traits at different stages of a local invasion.

The decline in allelochemistry and soil-mediated impacts on native species with population age leads to certain predictions about how plant communities with different histories of invasion by *A. petiolata* would change over time. Specifically, one would predict that abundances of *A. petiolata* should show the strongest increases, and those of native species the strongest decreases, in recently invaded areas compared to those with older populations. Among 30 forest sites in Illinois, we found this predicted pattern for *A. petiolata* populations and abundance of native woody species (but not native herbaceous species). This is consistent with studies showing woody species to be more sensitive to the allelopathic/anti-mycorrhizal effects of *A. petiolata* (30, 31).

Predictable declines in an invader's impact on native species could have important implications for our basic understanding of how new species are integrated into ecological communities and how we can best mitigate the environmental damage that may result from that process. In this case, eradication may be most important in newly established populations, which are poised to quickly spread and outcompete native species, while restoration efforts that reintroduce native plants and soil microbes may be more critical, and more effective, where populations are older and have reduced investment to allelopathic traits. In a broader sense, these results indicate that the extreme dominance exhibited by some invasive species may not be sustainable in evolutionary time, as shifting selection pressures may ultimately result in feedbacks that limit population growth. While it is not yet clear whether similar processes commonly occur with other invasive species, these results highlight the importance of considering the long term evolutionary dynamics of invaders and potentially adapting management efforts accordingly.

Materials and Methods

Estimation of Population Ages. We used 650 dated herbarium specimens of *A. petiolata* along with literature records and personal surveys to construct a map of the report of the invasive across the eastern half of the United States [see (32) for details of data collection]. We used spatial kriging (ArcMap 9.3, ESRI) to interpolate population ages between data points. Cross validation showed the kriged map to be a reasonable predictor of the known data (*r* = 0.51), with most of the error involving under prediction of the oldest sites and over prediction of the youngest sites. We used this map to predict the ages of the 44 populations from which seeds were obtained (Fig. 1). Since none of the 44 populations used in this study fell at either age extreme, the map did a considerably better job of predicting their ages (a subsequent spatial krig of cross-validation errors predicted error rates of ± 4 years for the 44 sampled populations). Herbarium records may not provide an exact measure of the initial data of invasion at a site, since it may take some time for a species to become abundant enough to be collected. However, over large spatial and temporal scales, date of first report can provide a useful predictor of relative population ages for comparative studies.

Measurement of Allelochemicals Concentrations. We measured allelochemical concentrations from leaf and root tissue from one individual from each of five maternal families for each of 44 populations grown in a common greenhouse environment. Plants grew in 500 mL pots that were filled with 400 mL of a sterilized potting soil/sand mix and 100 mL of field soil collected from a remnant oak-hickory forest in eastern Illinois with no history of *A. petiolata* invasion (Vermillion River Observatory, University of Illinois). Approximately 5 mg of leaf tissue was collected from each plant after 6 weeks and placed immediately into 95% methanol. After 3 months, plants were removed and a sample of fine root tissue was placed into 95% methanol. The tissue was then weighed for fresh mass and ground by bead beating. The resulting solution was passed through a sephadex anion exchange column; the flow through was collected for later analysis of glycosides and flavonoids, while desulphoglucosinolates were extracted from the retained fraction [see (33) for methods]. Both fractions were analyzed by HPLC and peaks identified by LC-MS (alliarinoside and iso-6-O-β-vitexin) or comparison to standards (allyl- and benzylglucosinolate). Glucosinolate and alliarinoside concentrations per mg of plant tissue were then regressed against the estimated population age. Results did not change when using various spatial regression techniques (spatial autoregressive models or generalized least squares models with spatially autocorrelated errors), for this or any subsequent analysis. To further explore spatial patterns, we correlated both allelochemical concentrations and the estimated ages of our 44 populations with latitude and longitude. We then used multiple regression to test the direct effects of age, latitude, and longitude on allelochemical concentrations by controlling for covariance among the predictors. Finally, for a highly conservative test of whether spatial trends could explain temporal trends in allelochemistry, we took the residuals of a simple regression of population age on longitude and then correlated allelochemical concentrations with those residuals. All statistical analyses were performed in R with the base (34), spdep (35), and nlme packages (36).

Test of Soil Feedbacks on Native Species. We tested the indirect allelopathic effects of the 44 *A. petiolata* populations (as mediated by effects on soil microbes), by using the resulting soil from the above experiment to grow three native tree species; *Platanus occidentalis* (sycamore), *Juglans nigra* (black walnut), and *Quercus rubra* (red oak). The soil from each *A. petiolata* individual was divided into three new pots (167 mL each), which were then filled with an additional 333 mL of a sterilized sand/soil mix. Each pot then received a germinated seed of one of the three native species. The seedlings were grown for 4 months and then harvested for above- and belowground biomass. Total biomass (using the mean value for the five replicates per population) was then regressed against the estimated population age from which the initial *A. petiolata* individual was derived or against the mean glucosinolate or alliarinoside concentrations of the initial *A. petiolata* population.

Analysis of Neutral Markers. To account for deterministic versus stochastic forces in creating among population patterns in quantitative traits, we genotyped 270 individuals from the 44 populations at eight microsatellite loci. Microsatellites are short, non-coding regions of repeated nucleotide motifs-genotypes vary in the number of repeats at a given locus–that make useful genetic markers since they are co-dominant and highly polymorphic (37). Methods were followed from (25). In brief, we extracted DNA using the DNeasy 96 Plant Kit (Qiagen) and performed two multiplexed PCR (five and three loci each) for each individual using identical conditions to (25). PCR products were separated on an ABI Prism 3730xl Analyzer with a ROX 500 internal size standard. Individuals were genotyped with GeneMapper ™ software (Applied Biosystems).

To determine whether populations similar in age were also similar genetically (indicating that multiple introductions at different times could explain temporal clines in allelochemical concentrations), we used a Mantel test to test for a significant correlation between Nei's genetic distance and the difference in predicted age between two populations. This approach is analogous to testing for isolation by distance, where we have substituted temporal for spatial distance. Analyses were performed with the adegenet (38) and vegan (39) packages in R.

We calculated genetic diversity as the expected heterozygosity averaged across all loci, after statistically removing any variation due to different sample sizes between populations. We then used ordinary least squares regression to compare genetic diversity versus population age and population mean allelochemical concentrations versus genetic diversity. Results did not change when using alternative measures of genetic diversity (allelic richness or number of multilocus genotypes).

Change in *A. Petiolata* and Native Species Abundance in Illinois. We used data from Illinois' Critical Trends Assessment Program (CTAP) to determine how changes in *A. petiolata* and native species abundance related to the history of invasion at a site. CTAP botanists conduct repeated vegetation surveys at permanent plots in randomly selected sites throughout Illinois [see (26) for detailed protocols]. In forest understories, all vascular plants are recorded in thirty 0.25-m^2 quadrats spread across three randomly selected 50-m transects at each site. We used a subset of 30 sites that had been surveyed twice (5 years between surveys) and had recorded *A. petiolata* in at least one of the surveys. We calculated the difference in total abundance (estimated as m^2 cover ha^{-1}) between surveys for *A. petiolata*, native woody species, and native herbaceous species for each site and regressed this difference against the estimated age of the *A. petiolata* population at that site. The change in *A. petiolata* abundance was natural log transformed to meet assumptions of normality and homoschedasticity.

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Corrections

ECOLOGY

Correction for "Evolutionary limits ameliorate the negative impact of an invasive plant," by Richard A. Lankau, Victoria Nuzzo, Greg Spyreas, and Adam S. Davis, which appeared in issue 36, September 8, 2009, of *Proc Natl Acad Sci USA* (106:15362–15367; first published August 21, 2009; 10.1073/pnas.0905446106).
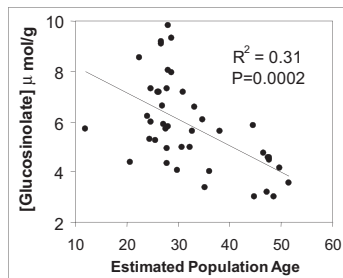
The authors note that Figs. 2, 3, and 4 incorrectly reported the units for glucosinolate concentrations as μmol/mg, but instead the units should appear as μmol/g. This error does not affect the conclusions of the article. The corrected figures and their legends appear below.

Fig. 2. Glucosinolate concentrations versus population age. Shown is the ordinary least squares regression of allyl + benzylglucosinolate concentrations in root tissue against the estimated age of the *A. petiolata* population. Each point is the mean of five replicates, and ages are estimated for seed sources.
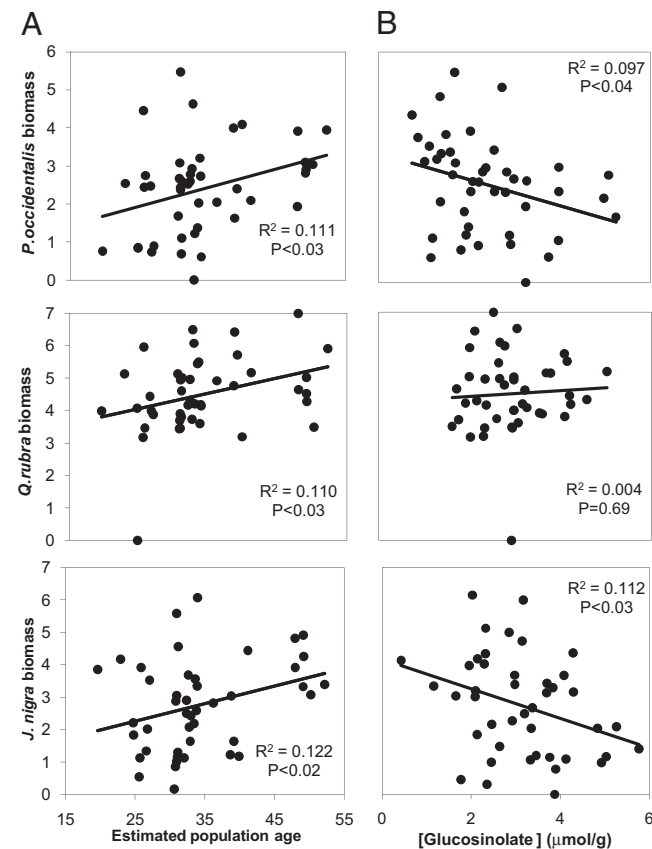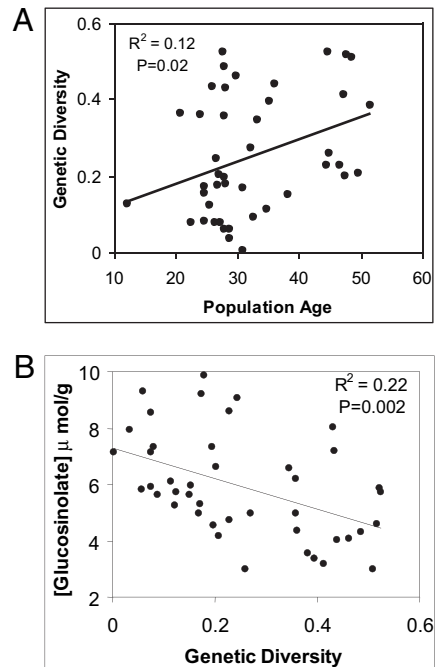
Fig. 4. Relationships among neutral genetic diversity, population age, and glucosinolate concentrations. Shown is the ordinary least squares regression of genetic diversity of an *A. petiolata* population (expected heterozygosity corrected for sample size) versus the estimated age of the population (*A*), and the mean root glucosinolate concentration of an *A. petiolata* population versus its genetic diversity (*B*).

Fig. 3. Native tree seedling biomass in *A. petiolata* conditioned soils. Shown is the ordinary least squares regression of native seedling biomass (in grams) of three native tree species in soils cultivated by *A. petiolata* individuals from different source populations versus the estimated age (*A*) and the mean root benzyl-glucosinolate concentration (*B*) of the *A. petiolata* source population.

PERSPECTIVE

Correction for "Tipping elements in the Earth System," by Hans Joachim Schellnhuber, which appeared in issue 49, December 8, 2009, of *Proc Natl Acad Sci USA* (106:20561–20563; first published December 7, 2009; 10.1073/pnas.0911106106).

Because of a printer error, an incorrect version of this article was published in the print issue; the online version appears correctly.

LETTER (ONLINE ONLY)

Correction for "Parameters that influence the prediction of epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnosis," by Janakiraman Vani, Mohan S. Maddur, Sébastien Lacroix-Desmazes, Srini V. Kaveri, and Jagadeesh Bayry, which appeared in issue 46, November 17, 2009, of *Proc Natl Acad Sci USA* (106:E129; first published October 26, 2009; 10.1073/pnas.0911020106).

The authors note that on page E129, right column, first full paragraph, second line, "One of the reports suggests that >80% of current smokers or ex-smokers are positive for tuberculin skin test as compared to <3% in nonsmokers (5)" should instead appear as "One of the reports suggests that >80% of current smokers or ex-smokers are positive for tuberculin skin test and this was significantly higher than for nonsmokers (5)."

MICROBIOLOGY

Correction for "Highly specialized microbial diversity in hyper-arid polar desert," by Stephen B. Pointing, Yuki Chan, Donnabella C. Lacap, Maggie C. Y. Lau, Joel A. Jurgens, and Roberta L. Farrell, which appeared in issue 47, November 24, 2009, of *Proc Natl Acad Sci USA* (106:19964–19969; first published October 22, 2009; 10.1073/pnas.0908274106).

The authors note that, on page 19964, left column, first paragraph, last sentence, "This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 20)" should instead read as "This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 64)." This error does not affect the conclusions of the article.

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

Correction for "Single molecule measurement of the 'speed limit' of DNA polymerase," by Jerrod J. Schwartz and Stephen R. Quake, which appeared in issue 48, December 1, 2009, of *Proc Natl Acad Sci USA* (106:20294–20299; first published November 11, 2009; 10.1073/pnas.0907404106).

The authors note that, due to a printer error, the legend for Fig. 4 appeared incorrectly. The second sentence, "The red curves are normalized single exponential fits given by $f = \tau^{-1} \exp(-\tau / \tau)$, where τ is the mean pause lifetime," should instead appear as "The red curves are normalized single exponential fits given by $f = \tau^{-1} \exp(-t / \tau)$, where τ is the mean pause lifetime." The figure and its corrected legend appear below.
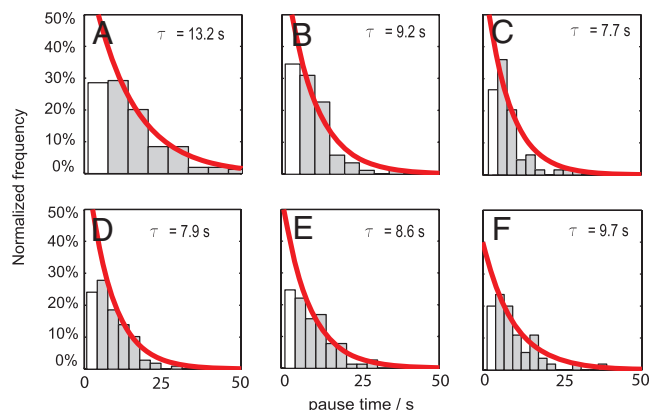

Fig. 4. Pause lifetimes for Pol I(KF) and φ29 were measured under different conditions for the sample and control sequences. The red curves are normalized single exponential fits given by $f = \tau^{-1} \exp(-t / \tau)$, where τ is the mean pause lifetime. Bins excluded from the fit due to undersampling are shown in white. (*A*) Pol I(KF) at 23°C with the sample template; (*B*) Pol I(KF) at 23°C with 1 M betaine with the sample template; (*C*) Pol I(KF) at 23°C with the control template, (*D*) φ29 at 23°C with the sample template, (*E*) φ29 at 23°C with 1 M betaine with the sample template, and (*F*) φ29 at 23°C with the control template.