

LETTER

Community genetics: resource addition has opposing effects on genetic and species diversity in a 150-year experiment

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Abstract

We used the Park Grass Experiment, begun in 1856, to test alternative hypotheses about the relationship between genetic diversity and plant species diversity. The niche variation hypothesis predicts that populations with few interspecific competitors and hence broader niches are expected to contain greater genetic diversity. The coexistence hypothesis predicts that genetic diversity within species favours coexistence among species and therefore species and genetic diversity should be positively correlated. Amplified Fragment Length Polymorphism (AFLP) markers were used to measure the genetic diversity of populations of *Anthoxanthum odoratum* growing in 10 plots of differing species richness that lie along resource and soil pH gradients. Genetic diversity in *A. odoratum* was positively correlated with the number of resources added to a plot, but not correlated with species richness. However, separate analyses have shown a negative correlation between resource addition and species richness at Park Grass and elsewhere, so genetic and species diversity appear to respond in opposite directions.

Keywords

AFLP, *Anthoxanthum odoratum*, coexistence, community genetics, genetic diversity, niche variation hypothesis, Park Grass Experiment, resource limitation, species diversity.

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INTRODUCTION

The emerging field of community genetics seeks to elucidate the extent to which patterns of genetic variation within species affect the relationships between species and hence community composition at various trophic levels (Chase & Knight 2003; Vellend & Geber 2005; Johnson & Stinchcombe 2007). Operating in the reverse direction, interspecific interactions within a community may exert selection upon constituent populations, producing diffuse coevolution (Wade 2007). One of the earliest predictions made in this field was the niche variation hypothesis (Van Valen 1965), which proposed that the niche breadth of a species would be proportional to its genetic diversity. As, at equilibrium, species diversity is inversely proportional to average niche breadth, it follows from the niche variation hypothesis that genetic diversity and species diversity ought to be inversely related to each other and that high species diversity ought to constrain genetic diversity within species.

The opposite prediction follows from the competitive combining ability hypothesis (Aarssen 1983), which predicts that coexistence among competing plant species is facilitated by intransitive competitive relationships among genotypes belonging to different species. It is proposed that no one species is competitively superior to all others because each species' population contains some genotypes that are strong in interspecific competition and some that are weak. This scenario leads to intransitivity and introduces frequency-dependence that stabilizes competitive mixtures. Studies of experimental mixtures of genotypes and species have shown that such intransitivity can occur (Taylor & Aarssen 1990; Fridley *et al.* 2007; Whitlock *et al.* 2007). The prediction of the competitive combining ability hypothesis is that genetic diversity and species diversity ought to be positively correlated because the maintenance of the latter depends upon the existence of the former (Vellend 2006).

Species and genetic diversity may also be correlated with one another if both are dependent upon a third variable

(Vellend & Geber 2005). This is perhaps the most common cause of positive correlations between the variables because so many processes that reduce species diversity also cause attrition among genotypes. Thus, Vellend & Geber (2005) found that a positive correlation between species and genetic diversity was more common on islands, where small population sizes and bottlenecks caused by the colonization process are common, than in mainland communities. Cleary *et al.* (2006) found that disturbance of tropical rainforest forest habitat in Kalimantan produced a positive correlation between species and genetic diversity in butterfly communities there.

However, there are also reasons to doubt whether any correlation at all ought to exist between plant genetic diversity and species diversity because studies that have quantified these independently of each other have shown that quite different factors strongly influence each of them. Mating system is a very strong determinant of genetic diversity (Hamrick & Godt 1997; Charlesworth 2003), while resource supply has a dramatic effect upon species diversity in grasslands (Silvertown 1980; Tilman 1982). The conflicting predictions about the relationship between genetic and species diversity need to be resolved with experimental data.

It is in principle straightforward to design interspecific competition experiments in which genetic diversity is a treatment (Booth & Grime 2003), although the consequences of such manipulations may be slow to manifest themselves in perennial plant communities, even under controlled conditions (Whitlock *et al.* 2007). Another approach is to survey genetic diversity in established plant communities that have different species diversities (Odat *et al.* 2004). The drawback of this approach is that the communities compared are likely to have very different histories from each other and that these unknown differences could be confounded with the variables of interest. Here, we have used a study system that combines most of the advantages of a designed experiment with the long-term perspective associated with studying natural plant communities.

The Park Grass Experiment at Rothamsted in England is the longest-running ecological experiment known (Silvertown *et al.* 2006), with some 90 plots containing meadow plant communities that all originate from the same recognized plant community that existed when the experiment began in 1856 (Dodd *et al.* 1994). A variety of fertilizer (chiefly N, P, K, Mg) and liming treatments applied to different Park Grass plots during its 150-year history have created plant communities that differ in the identity and relative abundance of the species belonging to the original community that are now present. There have been no significant additions to the flora. Several plots that have received little or no fertilizer and have been moderately

limed approximate to controls and have species-rich communities of *c.* 40 species growing in them. Acidification and eutrophication caused by addition of fertilizer treatments have reduced the species richness of communities on other plots in a very regular and well-documented manner (Silvertown 1980; Crawley *et al.* 2005).

The meadow grass *Anthoxanthum odoratum* is found on a wider range of Park Grass plots than any other species and occurs in communities where it is almost the only species present as well as on species-rich control plots. *Anthoxanthum odoratum* is an outcrossing, non-clonal perennial, maintains a high level of genetic diversity (Silvertown *et al.* 2002) and has evolved local adaptation to different treatments (Davies & Snaydon 1976). It is therefore the ideal experimental subject for community genetics. To test the predictions of the alternative hypotheses, we used AFLP markers to measure the gene diversity of *A. odoratum* on 10 plots that differed in the species diversity of each plant community.

METHODS

Plant material

Forty-eight *A. odoratum* plants were sampled from regularly spaced locations along four transects evenly spaced across each plot and at least 1 m from the plot boundaries. The plants sampled were at least 500 mm apart from one another to avoid possible duplicate sampling of individuals. A 1.5-cm long piece of leaf was taken from each plant sampled and placed onto ice and then snap-frozen into liquid nitrogen in the field. These were stored at -80°C until DNA extraction. Plots sampled are shown in Table 1. Plots were chosen to give a range of pH and resources additions (nil, N, P, K, Mg).

Laboratory procedures

A QiagenTM (U.K.) Tissue Lyser with 2-mm diameter tungsten beads was used at 25 Hz for 2×1 min to disrupt the samples. DNA from leaf samples was extracted with the QiagenTM (U.K.) DNeasy96 plant kit, according to the manufacturer's instructions, and eluted with $100 + 50$ μL of elution buffer.

DNA samples were digested and ligated according to the method of Vos *et al.* (1995) except that Hind III was used as the rare-cutter enzyme and reactions were carried out in 96-well plates as 20 μL reactions containing 250 ng DNA, 2.5 U of each enzyme and correspondingly reduced amounts of other reagents. Digest was for 3 h at 37°C and ligation occurred overnight at room temperature. Samples were diluted to 200 μL with water, and 5 μL of the diluted digest was used for pre-amplification.

Table 1 Plot treatments, soil pH and diversities for 10 Park Grass plots

Plot	Nei's diversity h^*	Soil pH	No. resources added	Resources added	<i>Anthoxanthum odoratum</i> mean abundance (g m^{-2})*	Species richness*
1b	0.1166	6.2	1	N	1.023	28
1d	0.1659	4.1	1	N	6.462	4
3b	0.1322	6.3	0	–	0.672	34
3d	0.1396	5.3	0	–	0.783	31
4/2b	0.1706	6.3	2	N,P	1.724	11
4/2d	0.1658	3.7	2	N,P	20.08	7
8b	0.1785	6.1	2	P,Mg	1.009	32
8d	0.1685	5.1	2	P,Mg	1.492	26
9/1b	0.2057	6.4	4	N,P,K,Mg	4.357	20
9/2d	0.1952	3.6	4	N,P,K,Mg	26.762	4

All main plots were established between 1856 and 1863, and fertilizer treatments have been applied since that time. The subdivision into b and d plots was made by liming the b plots from 1903 (Williams 1978). Sodium was also added to plots 8b, 8d, 9/1b, 9/2d but it is a substitutable rather than an essential resource (Subbarao *et al.* 2003) so it was not included in the analysis, although we did establish that including it did not qualitatively change the results reported.

*Averaged between 1991 and 1999.

Pre-amplification was carried out using +A for the Hind III pre-primer and +CA for the Mse I pre-primer as in Vos *et al.* (1995), except that reactions were carried out in 96-well plates as 25 μL reactions and formamide (as HiDi, supplied by Applied Biosystems, U.K.) was added to the PCR mix to a final concentration of 2%. PCR product was diluted to 200 μL , and 5 μL of this used for selective amplification. This was carried out with three +3 primer combinations according to the method of Vos *et al.* (1995) except in 96-well plates as 25 μL reactions and with the addition of HiDi as above. The three primer combinations were chosen from a screening of 9 that had previously been used successfully with other grass species. Forward primers were labelled with 6-FAM, NED or PET and PCR products visualized on an ABITM 3730 DNA Analyser. PCR products were visualized and assessed using the GeneMarkerTM programme from SoftGenetics, PA, U.S.A. A total of 225 polymorphic bands were scored from the three primers.

Statistical analysis

Nei's index of genetic diversity was computed using Popgene (Yeh *et al.* 1999). Nei's index values were used as the dependent variable in generalized linear models with a normal log-link function run in STATISTICA 8 (StatSoft). Independent variables were: mean species richness of the 10 plant communities calculated over the period 1991–1999 (Crawley *et al.* 2005), soil pH, the number of resources added in fertilizer treatments (values 0, 1, 2, 4) and the mean abundance of *A. odoratum* on each plot, measured by \log_e mean biomass of dry tissue collected in samples over the period 1991–1999 (Crawley *et al.* 2005). Mean biomass of

A. odoratum was used as a surrogate for population size. To test whether the same independent variables affected both species diversity and genetic diversity, a model was also run using species richness as a dependent variable, with soil pH, number of resources added and mean biomass of *A. odoratum* as independent variables.

Harpole & Tilman (2007) analysed the relationship between species richness and number of resources added in a different set of 10 Park Grass plots than ours that excluded acidified plots. They also used species richness data for a period nearly 50 years earlier than our data, and so to facilitate comparison between our results and theirs, we re-analysed data from the plots chosen by Harpole and Tilman using updated species richness values that were contemporaneous with those for our sample of plots. We have no genetic diversity data for most of the plots analysed by Harpole & Tilman (2007).

RESULTS

Nei's index of genetic diversity depended only upon the number of resources added to a plot (d.f. = 1; Wald statistic, $W = 27.29$; $P < 10^{-6}$). The effect was positive (Fig. 1). All other independent variables were not significant (mean species richness: $W = 0.87$, $P = 0.351$; soil pH: $W = 0.44$, $P = 0.508$; mean abundance of *A. odoratum*: $W = 0.81$, $P = 0.369$; all d.f. = 1).

Species richness was significantly negatively correlated with the biomass of *A. odoratum* (d.f. = 1, $W = 15.98$, $P = 0.000064$). Number of resources added ($W = 2.58$, $P = 0.108$), soil pH ($W = 0.79$, $P = 0.373$) and genetic diversity ($W = 0.253$, $P = 0.615$) were all non-significant.

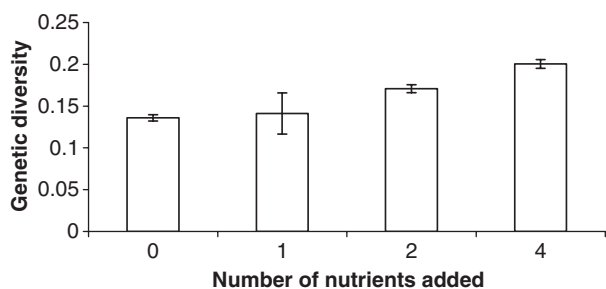


Figure 1 Mean (\pm standard error) of Nei's index of genetic diversity for plots receiving a fertilizer treatment containing nil, 1, 2 or 4 mineral nutrients.

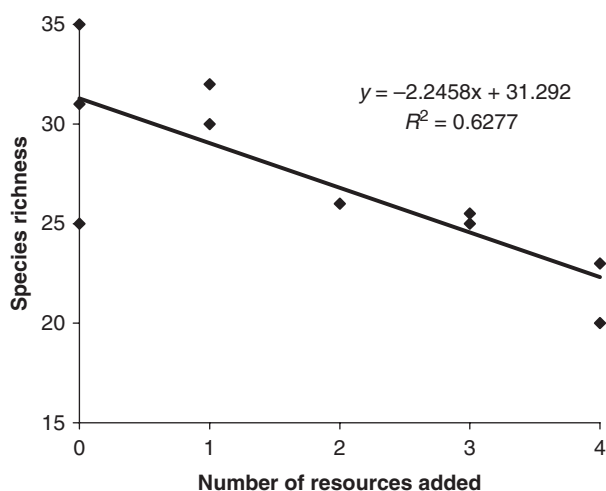


Figure 2 Relationship between species richness per plot between 1991 and 1999 and number of resources added for 10 non-acidified, unlimed Park Grass plots (nos. 2d, 3d, 4.1d, 7d, 8d, 12d, 14d, 15d, 16d, 17d). Plots 7d and 15d have identical values (three resources, 25 species), but one marker has been shifted to make both points visible in the graph.

The re-analysis of species-richness data from Harpole & Tilman's (2007) sample of Park Grass plots using values for the period 1991–1999 showed a negative correlation with the number of resources ($R^2 = 0.64$, Fig. 2), which was even stronger than the one they found ($R^2 = 0.55$) for species-richness data collected in the late 1940s.

DISCUSSION

We found that genetic diversity in *A. odoratum* was highly significantly correlated with the number of resources added to experimental plots (Fig. 1). Species richness was also correlated with the number of resources, though only in a sample of non-acidified plots sampled by Harpole & Tilman (2007; Fig. 2). The signs of the two correlations with resource number were opposite to one another. As expected

from resource competition theory (Tilman 1982), resource addition progressively reduced species diversity, but it increased genetic diversity in *A. odoratum*. Genetic diversity in plots receiving four nutrients (N, P, K, Mg) was 48% higher than in control plots receiving none (Fig. 1). This was not an effect of the increased population of *A. odoratum* on fertilized plots, as *A. odoratum* biomass (used as a surrogate for population size) was included as a covariate in the analysis and in any case was non-significant.

The niche variation hypothesis (Van Valen 1965) predicts that species and genetic diversities should be negatively correlated. Although no such negative correlation was found within the 10 plots where we measured genetic diversity, the opposing signs of the correlations of genetic diversity and species diversity with resource number, albeit in different Park Grass plots, does offer some support to the niche variation hypothesis. These correlations of opposite sign do not support the coexistence hypothesis (Aarssen 1983), which predicts that genetic diversity and mean species richness should be positively correlated.

Harpole & Tilman (2007) obtained the same results in experimental grassland plots in California as they found in the Park Grass Experiment. We have confirmed that their result, obtained with species-richness data from sampling Park Grass plots in the 1940s, still held good 50 years later (Fig. 2). They interpreted the negative relationship between species richness and number of resources added as indicating the progressive competitive exclusion of species, as the number of niche dimensions was reduced with each additional plant resource that was added, and hence became non-limiting to plant growth.

Resource competition may also explain why the genetic diversity of *A. odoratum* increased with each addition of a resource. In a community of plant species that coexist through resource partitioning of essential elements such as N, P and K, all species consume some quantity of all the resources, though in different amounts. Thus, if one species is eliminated by the removal of, for example, K as a limiting resource, two things happen: (1) niche dimensionality reduces by one, and (2) the elimination of the species that was a superior competitor on the now absent resource dimension releases resources that it formerly consumed on the two remaining dimensions. Thus adding K increases the availability of N and P for the species that are left. In other words, niche breadth on N and P axes increases for the surviving species. According to the niche variation hypothesis, a greater niche breadth will enable more genotypes to persist. This scenario is potentially consistent with our finding that genetic variation in *A. odoratum* at Park Grass increases as interspecific competitors are eliminated by the addition of resources. However, there is an important *caveat*.

The hypothesis that genetic variation is positively correlated with a broader niche implies that the genetic loci

in question are functional, or at least closely linked to functional genes. It is usual to assume that molecular markers are neutral and if this is true of all of our AFLP loci, then our results would offer no support to the niche variation hypothesis. How many, if any, of the 225 AFLP bands scored in our analysis might belong to, or be correlated with, functional genes is an open question, although some putatively functional fragments have been sequenced and are being investigated (Biss submitted).

Operation of the niche variation hypothesis at Park Grass is supported by other studies of *Anthoxanthum odoratum* conducted in the experiment. The hypothesis predicts that generalist species such as *A. odoratum* with wide ecological amplitude will contain specialist genotypes. This has been confirmed by Davies & Snaydon (1976; Snaydon & Davies 1982) who found that selection was strong and produced rapid local adaptation by *A. odoratum* to conditions on different plots, including the ability to respond to added resources such as P (Davies & Snaydon 1974). While these differences have arisen between populations on different plots, such divergence could only have occurred if genetic variation for resource use was present within the larger population of *A. odoratum* in the original Park Grass meadow before different fertilizers were applied.

We need to consider a limitation of this study. There was some confounding between the number of resources applied (0–4) and the identity of those resources (N, P, K, Mg). Of the three resource elements added, only N was applied to some plots at all three non-zero levels of resource addition (Table 1). Ideally, the experimental design would have balanced the number and identity of resources, and so we cannot rule out the possibility that the element K, which was added only to the plots receiving four resources, was responsible for the correlation between genetic diversity and resource number. However, there is no obvious mechanism by which this would occur for K alone.

There are about 80 perennial species in the Park Grass Experiment as a whole and so it is worth speculating about whether the one species that we studied might be typical or exceptional. Earlier work has shown that a number of other species in the experiment are outcrossing and have high genetic diversity (Silvertown *et al.* 2002), like *A. odoratum*, although none has the very wide ecological amplitude of this species. There is no reason to think that genetic diversity in these other species will not respond to resource addition in the same way as it apparently has in *A. odoratum*, although the response would necessarily be manifested across a narrower range of resource addition treatments.

Taking into account all the evidence about the evolution of *A. odoratum* at Park Grass (Silvertown *et al.* 2006; Harpole & Tilman 2007), there is some tentative support for the niche variation hypothesis, even though we cannot be sure that it explains the correlation we found between genetic

variation measured using AFLPs and resource addition. Support for the hypothesis has also recently been confirmed in various animal species (Bolnick *et al.* 2007). A corollary of the niche variation hypothesis is that species living in species-rich communities are less likely to be able to evolve in response to environmental change than species living in species-poor communities because of the constraint of interspecific competition on genetic diversity (de Mazancourt *et al.* 2008). Our finding that genetic variation, the raw material of evolution, is lower in resource-poor, species-rich plant communities also supports this conclusion.

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