Ecological and genetic correlates of long-term population trends in the Park Grass Experiment

Ecological and Genetic Correlates of Long-Term Population Trends in the Park Grass Experiment

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abstract: The Park Grass Experiment (PGE) is the longest-observed set of experimental plant communities in existence. Although the gross composition of the vegetation was at equilibrium over the 60-yr period from 1920 to 1979, annual records show that individual species exhibited a range of dynamics. We tested two hypotheses to explain why some species initially increased and why subsequently some of these (the outbreak species) decreased again. The study was designed around eight phylogenetically independent contrasts (PICs), each containing related species with different dynamics. Our first hypothesis was that persistent increasers and outbreakers have higher intrinsic rates of natural increase than control species (species without trends), allowing them to spread when interspecific competition is reduced by drought. This was tested by measuring establishment and seed production of species in field experiments, with and without interspecific competition. Seed production in outbreak species responded more strongly to release from interspecific competition than it did in either of the other groups of species. Our second hypothesis was that outbreak species eventually declined because they lacked the genetic variation necessary to adapt to the novel habitats to which they had initially spread. We tested this by measuring mating systems and genetic diversity in persistent and outbreak species in the PGE. In seven out of seven PICs tested, the outbreak species was more selfing than its persistent relative. There was a significant positive correlation between outcrossing rate and gene diversity. These results support roles for both ecological and genetic traits in long-term dynamics.

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It is clear from recurring relationships between vegetation composition and environmental variables that many plant communities, particularly herbaceous ones, possess a recognizable equilibrium state on which they converge (e.g., Ellenberg 1988; Rodwell 1992; Barbour and Billings 2000). It is equally clear that this state is frequently perturbed by climate (Sala et al. 1992; Silvertown et al. 1994; Dunnett et al. 1998). The study of equilibrium plant community dynamics encounters at least two important practical problems. The first is the paucity of long-term data, and the second, exacerbated by the first, is the difficulty of generalizing beyond the specifics of individual cases. Our understanding of nonsuccessional vegetation dynamics will be advanced if the traits that determine how a species behaves can be identified. On the basis of an analysis of 60-yr population trends for plants in the Park Grass Experiment (PGE), Dodd et al. (1995) suggested that demographic and genetic traits both influenced long-term population behavior. In this article, we report tests of these hypotheses.

Population Trends in the Park Grass Experiment

The Park Grass Experiment at Rothamsted Experimental Station in England is perhaps the best-documented, and certainly the longest-observed, set of equilibrium plant communities. The PGE was set up between 1856 and 1872 when a hay meadow of uniform vegetation composition and soil type was divided into 20 plots of between 0.1 and 0.2 ha (Williams 1978). A variety of fertilizer treatments were established and continue to be applied on a regular schedule to the present day. There are also three control plots that are not fertilized. The number of plots has been increased and their area decreased as a result of subdivision for liming, so there are now over 80 plots. (The soil on some plots is now highly acidic, and these have not been included in this study.) By 1920, the major changes induced in the original meadow community by fertilizer

treatments had stabilized, and a distinct, recognizable equilibrium composition was present on most plots (Williams 1978; Silvertown 1980, 1987; Dodd et al. 1994*b*). Though now very different from each other, the plant communities in the PGE have a common origin in the flora of the original meadow and also share the same climate and original soil type (Dodd et al. 1994*a*). These communities therefore provide perhaps the best model system we have for understanding the long-term dynamics of plant populations and communities, particularly for the period between 1920 and the present.

In a unique 60-yr time series recording species' presences on each plot, Dodd et al. 1995 (fig. 1) discovered that nearly a quarter of the commonest species in the experiment showed outbreaks in their distribution by increasing and then decreasing again. Between 1920 and 1979, 10 of 43 species recorded on the nonacidified plots showed outbreaks, seven increased and persisted, five decreased, and 21 showed no trend. It should be emphasized that the classification of the species into these different categories was determined by statistical curvefitting and is not arbitrary.

The discovery of the outbreaks is of general interest because outbreaks occurred within communities with stable biomass and guild composition over the same time period (Silvertown 1980, 1987) and because the data show that even a 40-yr time series would be too short to detect the pattern. In other words, outbreaks might well be common phenomena in herbaceous plant communities, even those otherwise thought to be stable, but this will be very difficult to detect unless records have been kept continuously for 60 yr or more.

Causes of Population Trends: A Hypothesis

In seeking an explanation for these trends, Dodd et al. (1995) investigated whether population behavior was habitat or life-history specific. In theory, one would certainly expect life history, and particularly the intrinsic rate of increase *r*, to influence dynamics (e.g., Hassell et al. 1976), but habitat may have an influence too. For example, Tilman and Wedin (1991) found habitat-specific dynamics in populations of the grass *Agrostis scabra*, in which chaos only occurred on plots receiving high nitrogen. In the PGE, there is good evidence that edaphic conditions and fertilizer treatments do not explain why some species showed outbreaks. First, the outbreaks occurred between 1920 and 1979, well after the biggest changes in composition following fertilization at the start of the experiment had already stabilized (Silvertown 1980), so a response to the commencement of fertilization in the 1850s to 1870s can be ruled out as a cause. Second, species' dynamics did not differ between limed and unlimed plots or between plots

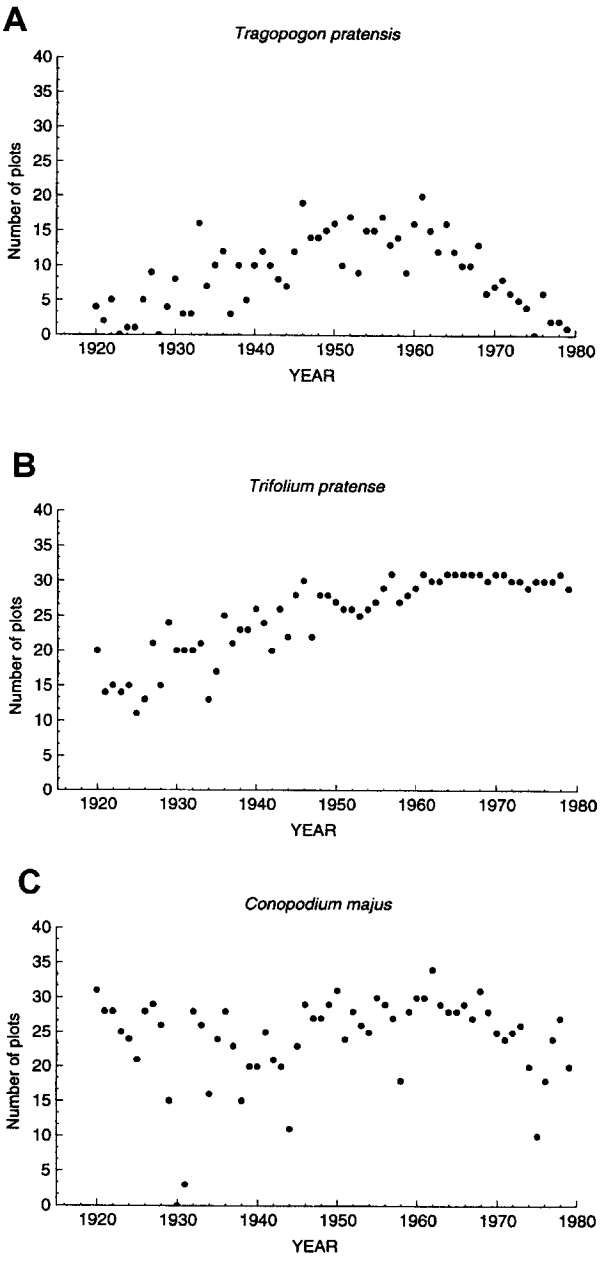

Figure 1: Examples of three population trends observed in the Park Grass Experiment over the period 1920–1979. *A*, Outbreak in *Tragopogon pratensis*. *B*, Persistent increase in *Trifolium pratense*. *C*, Control in *Conopodium majus*. From Dodd et al. (1995), with permission.

with and without nitrogen (Dodd et al. 1995). Third, plots acidified by the application of ammonium sulfate fertilizer are excluded from the plots referred to here, so extreme changes in soil reaction with time may also be ruled out.

As a hypothesis generator, Dodd et al. (1995) used discriminant analysis to determine which life-history and hab-

itat traits best distinguished between outbreak, persistent-increase, and control species. This showed that habitat characteristics were poor discriminators. In contrast, two life-history traits were identified as significant. Persistent-increase and outbreak species were more ruderal (sensu Grime 1979) than the rest, while outbreak species were more inbreeding than the persistent-increase species. One consequence of inbreeding is to reduce genetic heterozygosity and diversity. It should be stressed that the measures of ruderalness and mating system used in the correlations were obtained from literature sources and not from within the PGE, so these results were useful in hypothesis building but could not be regarded as definitive.

The correlations between population behavior and life-history traits suggest hypotheses to explain why certain species initially increased and why a subset of these (the outbreak species) decreased again (Dodd et al. 1995):

> H_1: Persistent-increase and outbreak species increased when there was a reduction in interspecific competition because these species have higher intrinsic rates of natural increase (r) than control species.
>
> H_2: Outbreak species eventually decreased because they lacked the genetic variation necessary to adapt and persist under conditions of interspecific competition. Persistent increasers possess higher genetic variation and can thus sustain themselves under conditions of interspecific competition in the wide variety of habitats present in the PGE.

If H_1 and H_2 are both correct, then the increases were primarily due to demographic traits, while decreases in outbreak species were due to low genetic diversity. H_1 is founded on the fact that ruderal species reproduce early and have high fecundity and short lives (Grime 1979), which are all traits that increase r. H_2 is based on the observation that mating system is the primary determinant of genetic diversity (e.g., Charlesworth and Pannell 2001) and that local adaptation has been demonstrated to have occurred in at least one persistent-increase species (*Anthoxanthum odoratum*) at Park Grass (Snaydon and Davies 1972).

To test H_1, we measured components of r (seedling emergence rate, survival, and fecundity) for species with control, persistent-increase, and outbreak dynamics in experimental field populations with and without interspecific competition. H_1 would be supported if r were higher in persistent-increase and outbreak species than in control species or if there were a dynamics type × competition interaction that showed r in persistent-increase and outbreak species to be more responsive to the removal of interspecific competition than control species. We tested H_2 by determining mating systems and genetic diversity for persistent-increase and outbreak species in the PGE. H_2 would be supported if populations of outbreak species were more selfing and less diverse genetically than persistent-increase species. Comparisons between species need to be designed with the problem in mind that, because of common descent, species are not independent data points and that unrecognized third variables may produce spurious correlations between the variables of interest if this nonindependence is ignored (Harvey and Pagel 1991). To overcome this problem, we used experimental designs that incorporated phylogenetically independent contrasts (Felsenstein 1985).

Methods

Phylogenetically Independent Contrasts

Wherever possible, all experimental designs and sampling procedures were based on eight sets of phylogenetically independent contrasts (PICs). All available outbreak species were used. Relatives of the outbreak species were determined using the taxonomy of Stace (1997) to name species and assign genus membership, the phylogeny of Kellogg and Watson (1993) to pair grass genera, and the phylogeny of Chase et al. (1993) to identify related plant families. More recent phylogenies (Soltis et al. 1999) have not altered our understanding of the relationships among species in this study. Seven of the contrast sets each contained a triplet of related species chosen to include one persistent-increase species, one outbreak species, and one control species. Because there were only seven outbreak species present in the data set, an eighth comparison consisted of just one control species and one persistent-increase species (table 1); hence, 23 species were used in all. All species except *Bromus hordeaceus* were perennial. Tests of H_1 used all eight contrasts and 23 species. Tests of H_2 compared only persistent-increase and outbreak species and were therefore confined to seven contrasts and 14 species.

Estimation of r

Germinated seeds per plant approximates the finite rate of increase λ, so its natural logarithm provides a proxy for the intrinsic rate of increase ($r = \ln \lambda$). This was calculated as $\ln\{[(\text{mean percent seedling emergence}/100) \times \text{mean numbers of seeds/plant}] + 0.5\}$. The 0.5 was added to deal with the fact that the logarithm is otherwise not defined for plants that set no seed.

Seedling emergence rates with and without interspecific competition were measured in an experiment conducted at Long Hoos, an arable field on the Rothamsted Experimental Farm on a silty clay loam similar to the original soil of the PGE. Seeds of the 23 species used in this ex-

Table 1: Species used in the study, grouped into eight phylogenetically independent contrasts

Dynamics	Species	Family
1:		
C	*Achillea millefolium*	Asteraceae
I	*Leontodon hispidus*	Asteraceae
O	*Tragopogon pratensis*	Asteraceae
2:		
C	*Centaurea nigra*	Asteraceae
O	*Leucanthemum vulgare*	Asteraceae
3:		
C	*Rumex obtusifolius*	Polygonaceae
I	*Ranunculus acris*	Ranunculaceae
O	*Cerastium fontanum*	Carophyllaceae
4:		
C	*Lathyrus pratensis*	Fabaceae
I	*Trifolium pratense*	Fabaceae
O	*Lotus corniculatus*	Fabaceae
5:		
C	*Prunella vulgare*	Lamiaceae
I	*Plantago lanceolata*	Plantaginaceae
O	*Galium verum*	Rubiaceae
6:		
C	*Dactylis glomerata*	Poaceae
I	*Anthoxanthum odoratum*	Poaceae
O	*Bromus hordeaceus*	Poaceae
7:		
C	*Briza media*	Poaceae
I	*Alopecurus pratensis*	Poaceae
O	*Festuca rubra*	Poaceae
8:		
C	*Sanguisorba minor*	Rosaceae
I	*Heracleum sphondylium*	Apiaceae
O	*Agrimonia eupatoria*	Rosaceae

Note: Dynamics are control (C), persistent increase (I), and outbreak (O).

periment and the demographic experiment (see below) were collected from the PGE in 1997 or 1998 as they ripened, or, where this was not possible, freshly harvested material was obtained from a commercial supplier of British wildflower seeds. Note that H_1 (unlike H_2) does not suggest local adaptation, so this test does not require seeds sourced from the PGE itself. Between collection and sowing, all seed was stored under natural conditions protected from rodents and rain. The experiment was set up in four randomized complete blocks of two competition treatments × 23 species each, giving a total of 184 plots. A competition treatment was created by presowing plots with *Lolium multiflorum* at a rate of 32 g m^{-2} at the beginning of June 1998. *Lolium multiflorum* was used because it grew rapidly to produce a canopy similar in height and structure to that experienced by seedlings in the PGE. Plots that were allocated the no-competition treatment were kept bare by hand weeding. The experimental area was protected from rabbits by an electric fence.

In November 1998, just before sowing the first of the 23 species, *Lolium* in the competition treatments was cut to a level similar to the height of the vegetation prevailing at that time in the PGE. At the end of November, seeds of all the 23 species were sown onto their appropriate plots at a rate of 200 seeds per plot. Seeds were sown into the middle 100 × 100-mm area of each plot and, on the competition-free plots, were lightly covered with soil for protection from birds. The experiment was monitored for signs of seedling emergence at regular intervals from the date of sowing. Seedling emergence was first observed at the beginning of January 1999 and was monitored until May, by which time maximum seedling emergence appeared to have been reached in most species. In mid-May 1999, seedlings in the central sown area of each plot were counted, either in situ in the few cases in which numbers were low or by digging up the middle 100 × 100-mm area of each plot and counting seedlings in the lab.

Number of seeds produced per plant was measured in a separate field experiment in Sawyer's Field, an arable field reserved for fertilizer-free experiments that lies adjacent to Long Hoos Field on Rothamsted Experimental Farm. The soil is a flinty clay loam over clay with sandy inclusions (Batcombe series with sandy variants). We used an experimental design of five randomized blocks, each containing 46 1 × 1-m plots. Half-meter-wide paths separating plots and 1-m-wide paths separating blocks were maintained free of vegetation. Half the plots in each block, chosen at random, were presown in September 1997 with a cultivar of *Festuca rubra* with a prostrate growth form to provide a competition treatment. *Festuca rubra* was selected for this purpose because it is the most widespread species on the nonacidified plots of the PGE and therefore the one most often encountered in interspecific competition. One competition-free and one *F. rubra* plot in each block was allocated at random to each of the 23 species. Twenty-five seedlings of the appropriate species, raised from seed in individual plugs in a glasshouse and then hardened off, were planted in each plot on a regular 5 × 5 grid. Data were collected from only the nine plants in the center of each plot, ignoring a guard row of 16 plants around the four sides. Dates of planting and recording were kept constant within PICs but varied between PICs, depending on their phenology. The group of three Asteraceae species and the two Poaceae groups were sown in the winter of 1997–1998 and other species in the spring of 1998. A rabbit-proof fence was erected around the experiment, and irrigation was provided during summer when required. Plots were regularly hand weeded. Seeds from each plant were collected before shattering and counted in 1998 and

1999 except in the case of the annual *B. hordeaceus*, which was recorded in 1998 only.

Mating Systems Estimation

Mating systems of 12 species were estimated with genetic markers (Ritland 1990). Maternal leaf tissue and seedlings were analyzed for up to 20 plants per species, with 20 progeny analyzed per maternal family wherever possible. Eight of the 14 species were sampled on the control plots of the PGE. Four species, not present in sufficient abundance on controls or not setting seed before the hay harvest, were sampled from unfertilized nearby vegetation similar to that on control plots (*F. rubra*, *Galium verum*) or other plots where they were abundant (*B. hordeaceus*, *Heracleum sphodylium*). Genetic self-incompatibility has been demonstrated in *Plantago lanceolata* (Ross 1973) and *Ranunculus acris* (Østerbye 1977), and accordingly, outcrossing rates (t) of one were used for these two species in comparative analyses.

Seeds were germinated in incubators or in pots placed outdoors over winter, and seedlings were raised in a greenhouse. Leaf tissue of 10 species (table 2) was prepared for allozyme analysis by grinding leaf samples in liquid nitrogen to produce a fine powder. This powder was then mixed with an homogenizing buffer: 15% (w/v) sucrose, 5% (w/v) PVP-40, 50 mM Tris HCl (pH 7.5) in 0.5% (w/v) Triton X-100 to which was added 0.1%–0.5% Mercaptoethanol (concentration dependent on species). Homogenates were then centrifuged for 3–5 min at 14,000 rev/min, and samples of the supernatants were then either stored in a −80°C freezer until needed or loaded onto allozyme gels and run. Schedules for enzyme staining and gel running were based on those described by Richardson et al. (1986).

Four species (*Tragopogon*, *Cerastium*, *Galium*, and *Bromus*) displaying no allozyme polymorphism and one (*Leontodon hispidus*) in which the allozyme protocol did not work were analyzed with inter-simple sequence repeat (ISSR) markers, using the same protocols used to measure genetic diversity (see "Genetic Diversity").

Genetic Diversity

Stratified random sampling was used to select plants for measurement of genetic diversity. Field sampling was stratified, as described below, to produce a collection of 80–120 samples per species from which a smaller number of plants was drawn at random for laboratory analysis. Stratified sampling of leaf material from the control plots and their four subplots, which received different liming treatments, was carried out as follows. Each of the four subplots was divided into 10 miniplots after we first discarded at least 0.5 m of edge. Each miniplot had an area of about 3.8 m^2. We searched miniplots for sample species, with the aim of getting one sample of the species from each miniplot. The location of each sample taken was recorded. For some species, noncontrol plots had to be sampled to get enough individuals, but the same sampling strategy using miniplots was also employed on these. Occasionally, where a species was sparse on the PGE plots, plants had to be sampled from the marginal vegetation. Each sample was placed in a cryotube and frozen in liquid nitrogen in the field before being brought back to the lab for longer-term storage in liquid nitrogen dewars until needed for ISSR analysis.

Inter-simple sequence repeat markers (Zietkiewicz et al. 1994) were scored using the following protocol: 50–100-ng template DNA was amplified by PCR using the appropriate ISSR primer (see app. A in the online edition of the *American Naturalist*) by one cycle of 94°C for 3 min; 40 cycles of 94°C for 30 s, of 46°–52°C for 1 min (depending on the primer used), and of 72°C for 1 min; and one cycle of 72°C for 5 min. PCR products were separated by electrophoresis on a 6% polyacrylamide minigel (Biorad Mini-Protean II) for 1 h at 200 V and visualized by silver staining.

We were unable to extract PCR-able DNA from *Trifolium pratense* and *Lotus corniculatus*, and a suitable ISSR primer could not be found for *H. sphondylium* among the 109 we tested. Genetic diversity was scored with ISSR markers for the remaining 11 species, including five pairs with contrasting long-term dynamics.

Data Analysis

Ecological Data

Appropriately transformed data from the two field experiments were analyzed using ANOVA and general linear model–fitting commands in the statistical software R (Ihaka and Gentleman 1996). Some analyses were also performed in GenStat (GenStat Committee 1993). Models contained terms for the blocks and factors for the eight PICs (to allow for phylogeny), three dynamics types (control, persistent increase, or outbreak), and two competition treatments (competition present or absent) as well as for interactions between dynamics type and competition treatment. Separate models were used for each of the response variables: mean percent seedling emergence, percent of plants setting seed, mean numbers of seeds per plant, and germinated seeds per plant. Percent of plants setting seed, mean numbers of seeds per plant, and germinated seeds per plant were calculated separately for the 1998 and 1999 seasons. In comparisons between species, *Bromus* seed counts from 1998 were used in 1999 since we had no 1999 data for this annual species.

Table 2: Estimates of outcrossing rate (t) and genetic diversity (gene diversity [H], Shannon function [I], and percent loci polymorphic) for seven phylogenetically independent pairs of species with contrasting long-term dynamics

		Outcrossing rate					Genetic diversity					
Dynamic	Species	t	Marker	Estimation method	n loci	n fam.	n ind.	Primer no.	n loci	Percent poly.	Gene diversity (H)	Shannon function (I)
Outbreak	*Tragopogon*	0	ISSR	Monomorphic	11	10	22	888	11	18.2	.0757 (.1718)	.1100 (.2473)
Increase	*Leontodon*	.813 (.121)	ISSR	MLDT	13	9	17	888	12	83.3	.3735 (.1808)	.5328 (.2539)
Outbreak	*Cerastium*	.002 (.014)	ISSR	MLDT	6	10	38	828, 850	12	83.3	.3764 (.1825)	.5358 (.2555)
Increase	*Ranunculus*	1		SI: Østerbye 1977			27	828, 850	11	100	.4266 (.0786)	.6153 (.0862)
Outbreak	*Lotus*	.976 (.107)	PGI	MLTET	2	20	…	…	…	…	…	…
Increase	*Trifolium*	.997 (.171)	PGI	MLTR	1	18	…	…	…	…	…	…
Outbreak	*Galium*	.476 (.114)	ISSR	MLDT	7	10	21	825, 827	12	75	.3254 (.2013)	.4683 (.2864)
Increase	*Plantago*	.95		SI: Tonsor et al. 1993			33	825, 827	12	75	.2905 (.2067)	.4259 (.2866)
Outbreak	*Bromus*	.001 (.000)	ISSR	MLDT	13	10	27	807	9	78	.2570 (.1743)	.3940 (.2490)
Increase	*Anthoxanthum*	.970 (.057)	PGI, PGM	MLTET	2	18	27	807	13	100	.4236 (.1085)	.6094 (.1231)
Outbreak	*Festuca*	.668 (.064)	PGI	MLTET	1	19	32	842	15	80.0	.2763 (.1953)	.4139 (.2652)
Increase	*Alopecurus*	.912 (.045)	PGI, PGM	MLTET	2	20	33	842	15	73.3	.2297 (.1695)	.3567 (.2464)
Outbreak	*Agrimonia*	.00 (.004)	PGI	MLTET	1	20	21	1,423	11	54.6	.1899 (.2178)	.2821 (.3070)
Increase	*Heracleum*	.946 (.077)	PGM	MLTR	2	16	…	…	…	…	…	…

Note: Outbreak species are compared with persistent increasers to test the hypothesis that outbreak species have the lower outcrossing rate and the lower genetic diversity of the two groups. Sample sizes (n) for the number of loci, families (fam.), and individuals (ind.) scored are given. Standard errors of estimates shown in parentheses. ISSR = inter-simple sequence repeat; SI = self-incompatible; PGI = phosphoglucoisomerase; PGM = phosphoglucomutase.

Residuals and other standard diagnostics were calculated to check the appropriateness of the modeling assumptions. An alternative approach to the analysis of the seedling emergence data, treating the seedling emergence counts as binomially distributed and fitting generalized linear models, was also explored. However, the responses turned out to be extremely overdispersed (i.e., to have far greater variances than the binomial model predicts), and allowing for this overdispersion by the standard method of introducing a multiplicative dispersion parameter (McCullagh and Nelder 1989) still did not lead to a particularly well fitting model.

Some of the responses analyzed had distributions that were considerably different from the normal form assumed by these analyses; for instance, many plants set no seed at all, particularly on the demographic plots sown with the competition treatment, so the distributions of seed counts had an excess of zeros. Thus, P values for the statistical tests were calculated by a randomization method as well as by the conventional method of comparing them with an F distribution. In practice, the two sets of P values did not differ in any respect important to the interpretation, and it is the conventional P values that are reported here.

Because a large number of plants grown with competition in the demographic experiment did not set any seed at all, the percent of plants setting seed was analyzed as a binary variate using logistic regression. These F-tests take account of the overdispersion of these data mentioned above.

Genetic Data

Mating systems were estimated using the maximum likelihood programs of Ritland (http://genetics.forestry.ubc.ca/ritland/programs.html) appropriate for diploids (program MLTR) or tetraploids (program MLTET) and dominant (ISSR; program MLDT) or codominant (allozyme) markers (MLTR).

Gene diversity, percent polymorphic loci, and Shannon index of diversity were calculated with POPGENE 1.31 (http://www.ualberta.ca/~fyeh/fyeh) for diploid, dominant markers with 1,000 bootstraps.

Results

Estimation of r

Practically no mortality was observed in either competition treatment, and the majority of plants in the competition-free treatment flowered in the second year (1999). Interspecific competition significantly reduced seedling emergence ($F = 78.00$, df $= 1, 155$, $P < .0001$), seed set (1998: $F = 660.8$, df $= 1, 200$, $P < .0001$; 1999: $F = 852.7$, df $= 1, 200$, $P < .0001$), seeds/plant (1998: $F = 38.3$, df $= 1, 200$, $P < .0001$; 1999: $F = 99$, df $= 1, 200$, $P < .0001$), and emerged seeds/plant (1998: $F = 32.7$, df $= 1, 20$, $P < .0001$; 1999: $F = 98.2$, df $= 1, 20$, $P < .0001$) in all dynamics types. A significant interaction between competition and dynamics type affected seeds/plant in 1999 and in the combined data for 1998 + 1999 (1999: $F = 6.47$, df $= 2, 200$, $P < .002$; 1998 + 1999: $F = 4.40$, df $= 2, 200$, $P < .013$). The interaction resulted from a significantly greater effect of release from competition on the seed production of outbreak species compared with control or persistent-increase species (fig. 2). There was no significant main effect of dynamics or phylogeny. All the experimental results were combined into the surrogate measure of $r = \ln$(germinated seeds/plant). This showed no significant effects of phylogeny and no difference between dynamics types in either 1998 or 1999 (fig. 3). A full ANOVA table and variance accounted for by phylogeny are given in the online version of the *American Naturalist*.

Mating Systems

Mating system estimates for the 12 species analyzed are given in table 2. Quantitative estimates were possible in 11 species except *Tragopogon pratensis*. This species showed no polymorphism at ISSR or allozyme loci, so no quantitative estimate of its mating system could be made. A value of $t = 0$ was assumed, since this is consistent with the complete lack of polymorphism. In all seven comparisons, the outbreak species was less outcrossing than its related persistent-increase species, a highly significant result ($P = .5^7 = .0078$, one-sided sign test). In six of the PICs, the difference between the species was large and

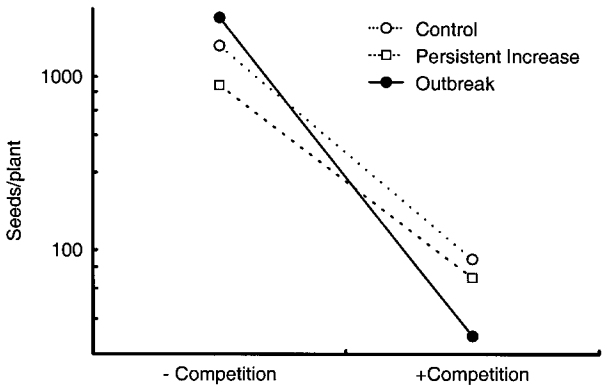

Figure 2: Interaction diagram for the mean number of seeds produced per plant in 1999 by species, with control, persistent-increase, and outbreak dynamics in the presence and absence of competition from *Festuca rubra*. The interaction term for ANOVA was significant ($F = 6.47$, df $= 2, 200$, $P < .002$).

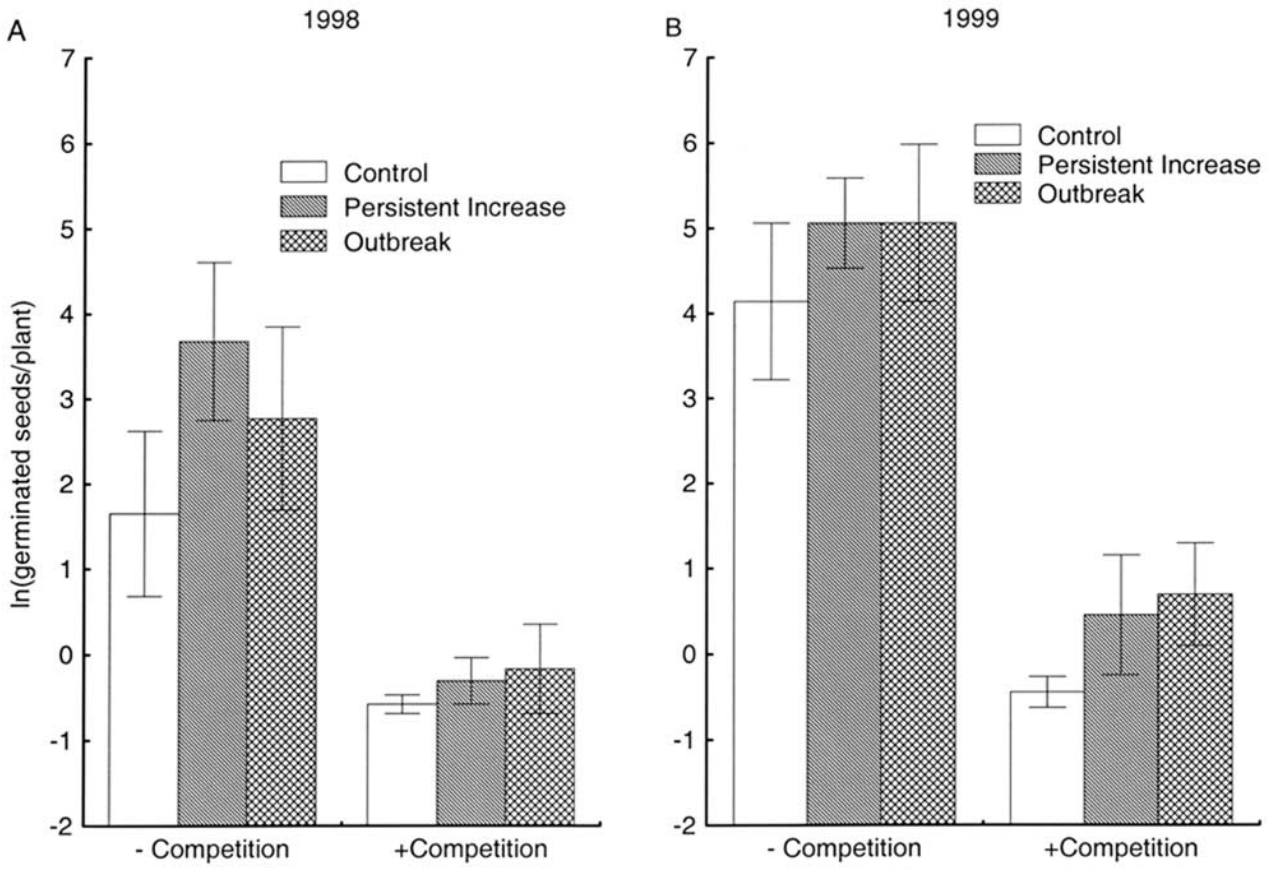

Figure 3: The ln(germinated seeds/plant) in control, outbreak, and persistent-increase species in experimental treatments with and without competition from *Festuca rubra*. Bars indicate SEM. Differences among dynamics groups are not significant ($P > .05$).

significant, the only exception being between the two Fabaceae, *Lotus corniculatus* and *Trifolium pratense*, where it was slight though still in the same direction as in the other pairs (table 2).

In ANOVA for outcrossing rate (t), dynamics type was significant ($F = 18.86$, df $= 1, 6$, $P = .005$), but phylogeny (PIC) was not ($F = 1.19$, df $= 6, 6$, $P = .42$; the full ANOVA table is given in the online version of the *American Naturalist*). This confirms that there is a difference between outbreak and persistent-increase species in terms of outcrossing rate. It should be borne in mind that the assumption of normal distributions, necessary for ANOVA, may not hold for these data; however, the main result has been confirmed by nonparametric analysis ("Data Analysis"). The variance in outcrossing rate accounted for by phylogeny was only 9%.

Genetic Diversity

Genetic diversity at ISSR loci was determined for 11 species (table 2). In three out of five pairs for which we have data for both species, the outbreak species had lower genetic diversity (by all three measures) than the persistent-increase species. The two exceptions were both pairs in which the outbreak species, *Galium* in one case and *Festuca* in the other, had a mixed mating system (table 2). Taking phylogeny into account, we found that genetic diversity did not vary significantly among dynamics types (ANOVA in app. D). Variance accounted for by phylogeny was 13% for percent loci polymorphic and 0 for gene diversity (H) and Shannon function (I). It is therefore valid to treat the species as independent data points for comparisons involving genetic diversity. Treating the 11 species as independent, we found there was a significant correlation between outcrossing rate (t) and gene diversity (H; Spearman rank correlation: $n = 11$, $R_S = 0.66$, $P = .027$).

Discussion

Ecological Causes of Initial Increase

We proposed that the initial increase phase observed in the long-term dynamics of 15 species (seven of which later

decreased and eight of which persisted) in the PGE was due to these species having higher values of r than control species, which allowed the 15 species to spread when drought caused a reduction in interspecific competition. Our surrogate measure of r was ln(germinated seeds/plant), and this failed to show the predicted difference between controls and the other species (fig. 3). However, one of the components of r indicates a more positive result that suggests it would be premature to dismiss the hypothesis. We found a significant ($P < .002$) interaction between competition treatment and dynamics that indicates that fecundity responds more strongly to release from interspecific competition in outbreak species than in others (fig. 2). This is consistent with our hypothesis that these species increased by colonizing following a disturbance, though it does not explain how persistent-increase species were apparently also able to take advantage of this. Interspecific competition had a strong and highly significant main effect on all variables measured for all three groups, and so the different responses of the species to competition may merely be a matter of degree. The seedling emergence rates of persistent-increase and outbreak species were, as expected, both higher than rates for control species with or without competition, though this difference was not significant. Field seedling emergence rates vary between years, and so this trend cannot be dismissed (or confirmed) on the basis of a 1-yr experiment.

A likely scenario that is consistent with our present and earlier findings is that the long-term outbreaks and persistent increases observed at Park Grass were triggered when drought checked competition from grasses. Silvertown et al. (1994) found a positive relationship between rainfall and the dominance of grasses in the historical record of hay biomass at Park Grass. Brenchley and Heintze (1933) noted that a severe frost in the winter of 1928–1929 followed by "exceptional drought" in the spring and summer of 1929 created bare patches on the acidified plots of the PGE that were invaded by *Chamerion angustifolium* (synonym: *Epilobium angustifolium*), which had been virtually absent before. This species subsequently increased on the acidified plots in typical outbreak fashion and did not finally disappear until 40 yr later in 1969 (Dodd et al. 1995). The dynamics of the species in this study were recorded on the nonacidified plots of the PGE that were not invaded by *C. angustifolium*. However, the timing of the outbreaks among species on the nonacidified plots was remarkably similar to that observed for *C. angustifolium*, suggesting that the trigger may have been the same on both plot types but that the initial effects on the nonacidified plots were less obvious to contemporary observers, or were thought to be less noteworthy, because the species that spread on them were natives of the PGE.

If the long-term trends were triggered by extreme events such as drought, this raises the question of how the increases were maintained for so long. This is a question about transient dynamics. The simplest explanation for the long duration of the aftereffects of disturbance would be that the plants in question are long-lived, but this explanation will not suffice because one of the outbreak species (*Bromus hordeaceus*) is an annual that peaked in abundance in 1948 after nearly 20 generations of spread (Dodd et al. 1995), while other outbreak species, such as *Tragopogon pratensis*, *Cerastium fontanum*, and *Linum catharticum*, and persistent-increase species, such as *Anthoxanthum odoratum*, *Plantago lanceolata*, and *Ranunculus acris*, are all relatively short-lived perennials. Once the initial cohort of individuals has died, the transient dynamics of a population is governed by its age-dependent vital rates (Law and Edley 1990). This question will be investigated further using age-structured population models.

Genetic Causes of Population Maintenance or Decline

We proposed that species with selfing mating systems might be unable to sustain populations in the long term in (for them) marginal environments because they would lack the genetic diversity required to evolve locally adapted populations. This study has confirmed that there is a strong relationship between outbreak dynamics and selfing in the PGE. All of the outbreak species were less outcrossing than their relatives with persistent-increase dynamics. This supports the prediction of population genetics theory that selection in a spatially heterogeneous environment favors recombination and, hence, outcrossing (Lenormand and Otto 2000).

Our data showed the expected positive relationship between outcrossing rate and genetic diversity at ISSR loci, although only when species were treated as independent data points. This procedure was validated by the finding that phylogeny did not account for a significant amount of variation in outcrossing rate. Charlesworth and Pannell (2001) made comparisons of allozyme diversity between congeneric species pairs in nine genera and found that selfing species had lower diversity than outcrossing congeners in all cases. In general, mating system is the single most important determinant of genetic diversity in plants (Hamrick and Godt 1997; Silvertown and Charlesworth 2001). It is possible that the mating system of some species may have been subject to recent evolutionary change at Park Grass and that genetic diversity has not yet reached equilibrium in these populations. This seems quite likely in the case of *Festuca rubra*, which we found had a mixed mating system and anomalously high diversity but which is self-incompatible in other British populations (Harberd 1961).

In addition to the statistically significant correlation between mating system and genetic diversity in species at Park Grass, there is circumstantial evidence that genetic diversity may play a mechanistic role in the dynamics of particular species. We found very low genetic diversity in *T. pratensis* (table 2), an outbreak species that is especially susceptible to pathogen attack. A dramatic attack by a rust was observed in the PGE in 1993 when *T. pratensis* reached high density on several plots. More recent work in the PGE has confirmed that this rust infection is spatially density dependent (G. R. Edwards and M. J. Crawley, personal communication). We also know that, at least in the case of mildew, disease incidence does vary with plot treatment (N fertilizer) and that one outbreeding, increasing species (*A. odoratum*) has evolved increased resistance to this disease on some high nitrogen plots (Snaydon and Davies 1972). Pathogens provide a plausible explanation as to how mating system and genetic diversity may have ecological consequences (Burdon and Thrall 2001). The relationship between genetic diversity and resistance to disease is the foundation of the Red Queen hypothesis for the evolution of sex (e.g., Levin 1975; Hamilton et al. 1990), and the considerable volume of evidence supporting that idea also supports our hypothesis.

Conclusions

Park Grass has a uniquely long history for an ecological experiment. The 60-yr period investigated here covers much less than half the total duration of the PGE and yet is five times longer than a typical long-term ecological study of 12 yr. Perhaps the single most important lesson contained in this living archive is that a large part of the change that can be observed during an individual investigator's lifetime will be transient dynamics. Though on first realization this fact may seem discouraging, rather, it should be seen as a challenge to explain larger, grander scales of ecological change. This study has demonstrated that the task is difficult, but progress may be made. Experimental measurements of demographic variables made over a short period, equivalent to less than a generation for any of the perennials in the study, indicate that release from competition from dominant grasses probably triggered long-term persistent increases. Our results support the suggestion made by Davis et al. (2000) that plant invasions may in general be triggered by temporary increases in resource availability. In the PGE, this would have occurred when interspecific competition was temporarily reduced by drought.

We have also confirmed that species able to sustain populations across a wide range of environments are invariably more outcrossing than related species unable to achieve this. Further experiments are needed to test whether the explanation for this is that selfing constrains the ability of a population to adapt and survive in the mosaic of environments found in the PGE. The practical implication of this would be that local extinction rates (which are by definition higher in the outbreak species) would be expected to be higher in selfing species than outcrossers. This is also relevant to the conservation of outcrossing species. There is growing evidence that population fragmentation increases the short-term extinction risk for outcrossing species because it increases inbreeding depression and pollinator limitation of seed set (Newman and Pilson 1997; Fischer and Matthies 1998; Kery et al. 2000). To this can now be added the possible threat that reduced genetic diversity as a result of inbreeding may increase the longer-term extinction risk because it limits local adaptation. Local adaptation has so far only been tested for at Park Grass in *Anthoxanthum odoratum*, in which it proved to be very strong (Davies and Snaydon 1976). Our results now call for further investigation of the potential link between local adaptation and local extinction, a question for which the Park Grass Experiment is an ideal study system.

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