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Contrasting Patterns of Pollen and Seed Flow Influence the Spatial Genetic Structure of Sweet Vernal Grass (*Anthoxanthum odoratum*) Populations

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Abstract

The spatial genetic structure of plant populations is determined by a combination of gene flow, genetic drift, and natural selection. Gene flow in most plants can result from either seed or pollen dispersal, but detailed investigations of pollen and seed flow among populations that have diverged following local adaptation are lacking. In this study, we compared pollen and seed flow among 10 populations of sweet vernal grass (*Anthoxanthum odoratum*) on the Park Grass Experiment. Overall, estimates of genetic differentiation that were based on chloroplast DNA (cpDNA) and, which therefore resulted primarily from seed flow, were lower (average $F_{ST} = 0.058$) than previously published estimates that were based on nuclear DNA (average $F_{ST} = 0.095$). Unlike nuclear DNA, cpDNA showed no pattern of isolation by adaptation; cpDNA differentiation was, however, inversely correlated with the number of additions (nutrients and lime) that each plot had received. We suggest that natural selection is restricting pollen flow among plots, whereas nutrient additions are increasing seed flow and genetic diversity by facilitating the successful germination and growth of immigrant seeds. This study highlights the importance of considering all potential gene flow mechanisms when investigating determinants of spatial genetic structure, and cautions against the widespread assumption that pollen flow is more important than seed flow for population connectivity in wind-pollinated species.

Key words: *chloroplast DNA, gene flow, genetic diversity, local adaptation, Park Grass Experiment*

The spatial genetic structure of plant populations is ultimately determined by the synergistic effects of gene flow, genetic drift, and natural selection. Gene flow is influenced by historical factors, such as postglacial range expansion and past colonization events (Taberlet et al. 1998; Templeton 1998; Hewitt 2000) and also by more recent processes, such as long-distance dispersal events or physical barriers to gene flow (Sax et al. 2005; Freeland et al. 2011). The rate at which genetic drift alters allele frequencies depends on effective population size, which in turn is partly influenced by gene flow (Slatkin 1987). Selection pressures continually change, and local adaptation will alter allele frequencies to an extent that depends on the strength of selection relative to the amount of gene flow and the effective population size (Griswold 2006; Andre et al. 2011). All of these processes are further influenced by ecological factors (Lexer et al. 2005; Pilot et al. 2006). Unraveling these synergistic influences can be daunting, and the task is tractable only if each investigation is limited to the examination of relatively few variables.

In most plants, these interactions are further complicated by the fact that gene flow can result from at least 2 processes: pollen dispersal and seed dispersal. However, the contributions of seed and pollen dispersal to ongoing gene flow are well documented for relatively few plant populations, and characterization of each is essential if we are to understand the respective roles that they play in shaping the spatial genetic structure of plant populations (Schnabel and Hamrick 1995; Bossart and Prowell 1998; Heuertz et al. 2003; Garcia et al. 2007). A number of studies have shown pronounced differences in patterns of seed versus pollen flow across a landscape (e.g., Garcia-Verdugo et al. 2010; Dixon et al. 2011), although other studies have found the 2 to be comparable (e.g., Barluenga et al. 2011). In general, pollen-mediated gene movement influences genetic diversity within and among plant populations, whereas seed

dispersal is important for the colonization of new sites, the reestablishment of extinct populations, and local migration (Barluenga et al. 2011 and references therein); however, for some species, this may be an overly simplistic view that underestimates the potentially important contributions that seed flow makes to ongoing population connectivity (Bacles et al. 2006). Contrasting patterns of seed and pollen flow critically influence virtually all aspects of population genetics. For example, although the effective numbers of contributing male parents consistently exceeded the numbers of contributing female parents in Californian valley oak (*Quercus lobata*), seed dispersal strongly influenced local seedling neighborhood size (Grivet et al. 2009); this in turn impacts effective population size and hence genetic drift, genetic diversity, and inbreeding.

Patterns of pollen and seed flow must be partly influenced by selection pressures, although few if any studies have investigated the interactions between local adaptation, seed flow, and pollen flow; such interactions are difficult to quantify because a thorough understanding of selection pressures in natural populations is often elusive. An alternative approach is to use long-term experiments, which can provide important insights into the processes that influence population genetic structure (Silvertown et al. 2006; Fischer et al. 2010; Silvertown et al. 2010). The Park Grass Experiment (PGE) in the United Kingdom, the world's longest-running ecological experiment, started in 1856 when 20 plots were demarcated from what was originally 2.8 ha of nearly level grassland (Tilman et al. 1994). Varying amounts and types of nutrients were added to these plots each year with the exception of 3 control plots, to which no nutrients were added. Between 1903 and 1965, each numbered plot was subdivided into 4 plots (a–d), 3 of which are limed to maintain pH values of approximately 7, 6, and 5 ("a"–"c"), with the fourth plot ("d") receiving no lime and hence maintaining a pH of between 3.5 and 5.7 depending on the fertilizer treatment (Rothamsted Research 2006). As a result, plots with the same number receive the same nutrient additions but, depending on their letter, have different pH values; for example, plot 1b receives annual nitrogen additions, plus a liming treatment that leaves it at pH 6.2, whereas plot 1d receives nitrogen but no liming treatment and hence has a pH of 4.1.

The PGE has provided insight into numerous ecological and evolutionary questions (e.g., Freville et al. 2007; Harpole and Tilman 2007; van den Berg et al. 2008; Kohler et al. 2010). Perhaps the most intensively studied species on the PGE is sweet vernal grass, *Anthoxanthum odoratum*, an allotetraploid, wind-pollinated, short-lived perennial. Adaptation of *A. odoratum* to changing edaphic conditions on the PGE was first demonstrated through a series of experiments in the 1970s (Snaydon 1970; Snaydon and Davies 1972; Davies and Snaydon 1973a, 1973b, 1974). A more recent study concluded, on the basis of population genetic data, that long-term resource additions have caused the local adaptation of *A. odoratum* to different plots on the PGE; furthermore, these adaptive changes have led to genetic differentiation across numerous loci and not just at candidate adaptive genes, which means that there must be a barrier to gene flow among proximate plots (Freeland et al. 2010). This barrier may be a result of flowering phenology: differences in *A. odoratum* flowering time have previously been documented at plot boundaries on the PGE (Snaydon and Davies 1976; Silvertown et al. 2005). Collectively, these studies show that local adaptation has occurred in *A. odoratum* populations in response to different combinations of resource additions.

Normally, wind-pollinated species show substantially greater pollen flow than seed flow (Petit et al. 2005; Garcia-Verdugo et al. 2010). If natural selection has indeed altered flowering times between plots, the genetic differentiation between *A. odoratum* plots that was previously identified from nuclear data (Freeland et al. 2010) may therefore be explained in 1 of 2 ways: restricted pollen flow despite ongoing seed flow or a combination of restricted pollen and restricted seed flow. If the former is true, population differentiation must be driven primarily by natural selection; if the latter, population differentiation can be explained by a combination of natural selection and limited seed flow. In this study, we therefore tested the hypothesis that genetic differentiation among *A. odoratum* populations on the PGE has resulted from natural selection that is limiting pollen flow despite ongoing seed flow.

Elucidating patterns of pollen and seed flow is not only relevant to our understanding of gene flow and adaptation among *A. odoratum* populations. Genetic diversity is highest in *A. odoratum* populations on enriched plots (Silvertown et al. 2009), and this finding is difficult to reconcile with the reductions in gene flow that are most pronounced for these same plots (Freeland et al. 2010). Seed flow may provide an explanation for this apparent paradox. A greater understanding of the different patterns of pollen flow and seed flow against a backdrop of known local adaptation is therefore critical to our understanding of how gene flow shapes the genetic diversity within, and genetic differentiation among, populations. Additional insight into these interlinked processes is particularly important in the rapidly changing environments of today, which may cause plant populations to increasingly suffer from reductions in genetic diversity because of disruption to either pollen or seed flow (Ellstrand and Elam 1993).

Materials and Methods

In angiosperms, nuclear DNA is biparentally inherited and therefore dispersed by both pollen and seeds, whereas chloroplast DNA (cpDNA) is normally maternally inherited and therefore largely dispersed by seeds but not by pollen. By comparing estimates of gene flow derived from the 2 different genomes, we can gain insight into relative rates of pollen flow and seed flow (Ennos 1994; Savolainen et al. 2007), which we can then compare to environmental factors. We based this study on an existing AFLP (nuclear) data set from Freeland et al. (2010), plus a novel cpDNA

data set. The 10 plots on the PGE that were sampled for this study included 4 pairs of plots to which various combinations of nutrients have been added (plots 1b and 1d, 4/2b and 4/2d, 8b and 8d, 9/1b and 9/2d) plus 2 control plots to which no nutrients have been added (plots 3b and 3d) (Table 1). Samples of leaf tissue from *A. odoratum* were taken along 4 transects placed the length of each sampled plot, evenly spaced across the plot, and at least 1 m from the plot boundaries. Individually, sampled plants were at least 0.5 m apart from one another. Two leaf samples approximately 1.5-cm long were taken from each plant, placed onto ice until the transect was complete, and then snap frozen into liquid nitrogen in the field. These were then stored at –80°C in the laboratory until DNA was extracted.

A Qiagen Tissue Lyser with 2-mm diameter tungsten beads was used at 25 Hz for 2 × 1 min to disrupt the leaf samples that were then extracted with Qiagen DNeasy96 plant kits, according to the manufacturer's instructions, and eluted with 100 + 50 µl of elution buffer. *S*amples were PCR amplified at 6 chloroplast microsatellite loci using primers WCt5, WCt10, and WCt13 (Ishii et al. 2001) and rpoC/rpsC2, atpB/rbcL, and atpI/atpH (Provan et al. 2004). Loci were amplified in one triplex (WCt5, WCt13, and atpB/rbcL), one duplex (atpI/atpH and rpoC2/rps2), and one single (WCt10) reaction. The reagents and cycling parameters followed the methods of Biss et al. (2003), with the exception of the primer concentrations in the multiplexes, which ranged from 5 to 20 pmol depending on the strength of the amplified product. Primers were labeled with IR800, and PCR products were visualized on an LI-COR Gene ReadIR 4200 sequencer using a Licor 300-bp size ladder for reference.

Each chloroplast haplotype was identified from its combination of 6 microsatellite alleles. Genetic diversity was calculated as the effective number of haplotypes ($N_e = 1/\sum p_i^2$) and unbiased haplotype diversity ($H_e = [N/(N-1)][1-\sum p_i^2]$), in which N is the sample size for each population and p_i is the frequency of haplotype i in each population (Nei 1978). The genetic similarity of populations was inferred from a bootstrapped neighbor-joining tree, which was reconstructed from haplotype frequencies with Phylip v. 3.5 (Felsenstein 2005), using 5000 randomizations. F-statistics were calculated in Arlequin (Excoffier et al. 2005), and the significance value tested using a nonparametric permutation test following the method of Excoffier et al. (1992). F_{ST} has known limitations as a measure of population divergence (Jost 2008; Meirmans and Hedrick 2010, but see also Ryman and Leimar 2009), and we therefore also calculated Jost's D as a measure of population differentiation using the software program SPADE (Chao and Shen 2009). Geographic distances between plots were calculated from the midpoint of each plot and compared with genetic distances using a Mantel test in IBDWS (Jensen et al. 2005) to determine if there was a pattern of isolation by distance.

Chloroplast DNA differentiation was compared with nuclear (AFLP) differentiation from a previous study (Freeland et al. 2010) in 3 ways: 1) by simply comparing average F_{ST} or D values from each plot (i.e., determining which values were higher); 2) by using a Mantel test to compare all pairwise AFLP and cpDNA F_{ST} or D values; and 3) by calculating pollen- to seed-flow ratios for all pairs of plots based on F_{ST} or D values using the equation:

$$\text{Pollenflow/seedflow} = [(1/F_{ST(b)} - 1) - 2(1/F_{ST(m)} - 1)]/(1/F_{ST(m)} - 1),$$

in which $F_{ST(b)}$ is population differentiation calculated from biparentally inherited (AFLP) loci and $F_{ST(m)}$ is the population differentiation calculated for maternally inherited (cpDNA) loci (Ennos 1994) (note that D can be a substitute for F_{ST} in this equation).

We compared F_{ST} and D values to edaphic conditions in 3 ways, each of which was done separately for AFLP-based values and cpDNA-based values. In addition, each comparison was done with and without counting lime as

Table 1 Summary of added nutrients, sample size, chloroplast genetic diversity within, and genetic differentiation among, the 10 *Anthoxanthum odoratum* populations sampled for this study

Plot	Key nutrients added[a]	*n*	Total number of haplotypes	cpDNA unbiased haplotype diversity (H_E)	Effective number of cpDNA haplotypes (N_e)	Average AFLP F_{ST} [b]	Average AFLP D[b]	Average cpDNA F_{ST}	Average cpDNA D
1b	N	81	6	0.763	4.05	0.100	0.234	0.082	0.174
1d	N	94	6	0.760	4.04	0.083	0.302	0.048	0.143
3b	—	95	9	0.756	3.97	0.098	0.211	0.085	0.135
3d	—	95	10	0.534	2.12	0.079	0.227	0.157	0.316
4/2b	N, P	95	7	0.730	3.61	0.097	0.163	0.035	0.114
4/2d	N, P	94	9	0.741	3.75	0.092	0.189	0.042	0.164
8b	P, Mg	87	6	0.645	2.76	0.098	0.265	0.026	0.087
8d	P, Mg	92	7	0.742	3.75	0.098	0.172	0.04	0.117
9/1b	N, P, K, Mg	95	10	0.735	3.67	0.120	0.143	0.029	0.089
9/2d	N, P, K, Mg	86	7	0.688	3.13	0.085	0.166	0.032	0.109

[a] All "b" plots also received applications of lime.

[b] AFLP data were taken from Freeland et al. (2010).

an addition because lime is the only addition that alters soil pH. The 3 sets of comparisons were: 1) Mantel tests were used to compare pairwise F_{ST} or D values to the corresponding pairwise numbers of differences in additions (Table 1), for example, plot 8b had P and Mg added, whereas plot 3b received no nutrient additions, and therefore, the pairwise difference between plots 8b and 3b was the addition of P and Mg = 2. The comparison between plots 8b and 3b was therefore based on an F_{ST} value of 0.0314, and a nutrient difference of 2. 2) Average F_{ST} or D values for each plot (i.e., averaged across all pairwise F_{ST} or D values which involved that plot) were compared with the number of additions that plot had received. 3) The number of significant pairwise population differentiation values for each plot (i.e., based on all pairwise values which involved that plot) was compared with the number of additions that plot had received.

Results

A total of 19 cpDNA haplotypes was identified in the 10 plots, based on the 6 combined cpDNA microsatellite loci. The number of haplotypes per plot ranged from 6 to 11, with an average of 7.7 (Table 1). Six out of the 19 haplotypes were private (i.e., occurring on only 1 plot), although these were all at very low frequency (<5%) with the exception of one private haplotype that was found on plot 4/2b at a frequency of 10.5%. The majority of haplotypes, private or otherwise, occurred at low to moderate frequencies (0.01–0.16) with the exception of haplotypes 3 and 14, which were the only 2 haplotypes that were found in all 10 plots. In all plots, these were the 2 highest frequency haplotypes with the exception of plot 3d (a control plot), in which haplotype 3 was found at a frequency of 0.64 and haplotype 14 was found at a frequency of only 0.07. Genetic diversity was high: the effective number of haplotypes in each plot ranged from 2.12 to 4.05 and haplotype diversity ranged from 0.534 to 0.763 (Table 1).

The neighbor-joining tree did not provide any evidence of clustering according to either edaphic conditions or geographical proximity of plots (Figure 1). F_{ST} values ranged from 0 to 0.289 (average = 0.0527) and were not correlated with the AFLP F_{ST} values (Mantel test: P = 0.656). There was no pattern of isolation by distance (R^2 = 0.0242, P = 0.8642). D values were generally higher than F_{ST} values, ranging from 0.011 to 0.516 (average pairwise value = 0.144) and were not correlated with the AFLP values (Mantel test: P = 0.724). There was no pattern of isolation by distance (R^2 = 0.0149, P = 0.188). Although estimates of genetic differentiation tended to be higher when based on D than when based on F_{ST} (see Table 1 for average values), the trends that we found were overall comparable when calculated from either F_{ST} or D values (i.e., comparisons that were significant when based on F_{ST} values were also significant when based on D values); therefore, we report only the results based on F_{ST} values unless there is a difference in the 2. Although genetic differentiation was illustrated by 23/45 significant pairwise F_{ST} values, only 2 of the 10 populations had their lowest pairwise F_{ST} values with their plot "pair" (a plot pair is the b and d plots with the same number, i.e., plots that had received the same nutrient additions; see Table 1). This was slightly higher (4/10 comparisons) when based on D. Furthermore, only 2 of the 10 plots had their lowest pairwise F_{ST} values with the plot to which they had the closest pH value (3/10 based on D), and only 2/10 plots had their lowest pairwise F_{ST} values with their nearest geographical neighbor, which is not necessarily their plot pair (this value was 3/10 when based on D).

Estimates based on comparisons of pollen and seed flow for 40/45 plot pairs, according to pairwise F_{ST} values, showed greater seed than pollen flow (0 and −2.09, with an average value of −1.03). When based on D, 29/45 comparisons showed higher seed flow than pollen flow, with an average value of 0.933. The highest ratio was between the 2 control plots (3b and 3d), which had a pollen-seed flow ratio of 10.15 when based on F_{ST} and 22.05 when based on D. Mantel tests showed a positive relationship between AFLP pairwise F_{ST} values and the corresponding pairwise number of differences in nutrient additions (P = 0.015 without lime included as an addition, P = 0.033 with lime included), but no such relationship when this comparison was based on cpDNA F_{ST} values (P = 0.483 without lime included in the analysis, P = 0.536 with lime) (Figure 2). The total number of added nutrients for each plot was not correlated with its average AFLP-based pairwise F_{ST} when lime was excluded (r = 0.435, n = 10, P = 0.104) but was positively correlated when lime was counted as a resource addition (r = 0.653, n = 10, P = 0.041). The total number of added nutrients for each plot was negatively correlated with the average cpDNA differentiation values (r = −0.737, n = 10, P = 0.015 without lime, r = −0.638, n = 10, P = 0.047 with lime) and also with the number of significant pairwise cpDNA F_{ST} comparisons involving each plot (r = −0.845, n = 10, P = 0.001 without lime, r = −0.881, n = 10, P = 0.0004 with lime) (Figure 3). We could not compare the total number of added nutrients to the number of significant pairwise AFLP F_{ST} values because all AFLP F_{ST} values were significant.

Discussion

Anthoxanthum odoratum is wind pollinated and outcrossing (Silvertown et al. 2002) and these facts, combined with the small size of the PGE, mean that there is a high potential for pollen-mediated gene flow; as a result, nuclear genetic differentiation (via seeds and pollen) should be lower than chloroplast genetic differentiation (largely via seeds). Nevertheless, our data show that in the majority of cases pollen flow is similar to, or lower than, seed flow. These results are comparable to those of an earlier study that compared pollen and seed flow on a different set of

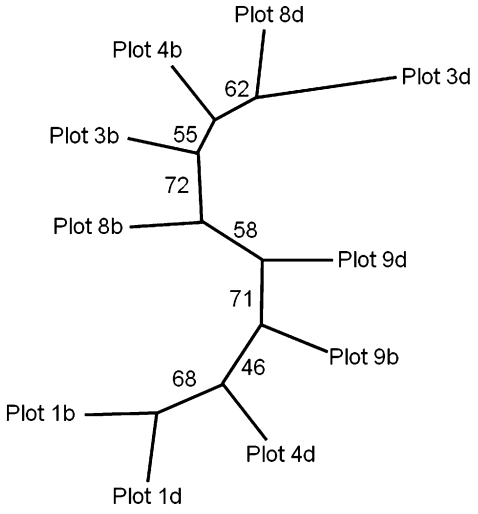

Figure 1. Neighbor joining tree reflecting the genetic distances between plots based on the distribution of cpDNA haplotypes. Numbers adjacent to branches represent bootstrap values.

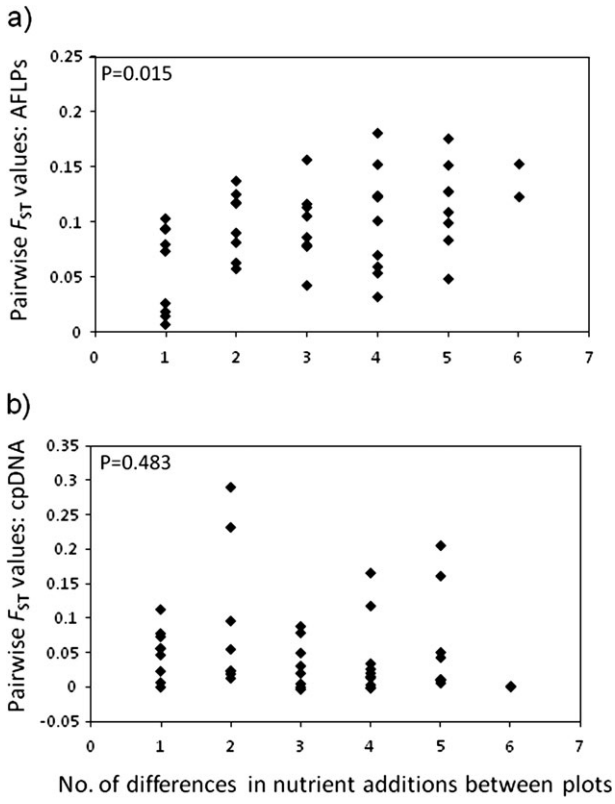

Figure 2. Results of Mantel test comparisons showing (**a**) a positive relationship between AFLP pairwise F_{ST} values and the corresponding pairwise number of additions (nutrients plus lime) and (**b**) cpDNA pairwise F_{ST} values and the corresponding pairwise number of additions (nutrients plus lime). In both figures, plot pairs that differed by only one addition included those that had the same nutrients added and differed only in whether or not they had received lime.

A. odoratum populations (Silvertown et al. 2005). Higher seed flow than pollen flow is very unusual, particularly in a wind-pollinated species: The median pollen to seed flow ratio found in a survey of 93 studies by Petit et al. (2005) was 17. Even more striking is the average pollen to seed flow ratio of nearly 150 in wind-pollinated, animal dispersed tree species that was identified by Garcia-Verdugo et al. (2010).

Our pollen to seed flow ratio is among the lowest reported in the literature but must be interpreted with several cautionary notes. First, estimates of pollen to seed flow ratios are based on equations that are derived from the island model of migration, which may often be unrealistic (Ennos 1994; Whitlock and McCauley 1999). Equilibrium between gene flow and genetic drift in *A. odoratum* populations on the PGE is unlikely because of the variable patterns of seed and pollen flow and the local adaptation of populations to different edaphic conditions (Freeland et al. 2010). In addition, we cannot rule out the possibility that the putatively high levels of seed flow are inflated by the retention of multiple cpDNA haplotypes that have remained widely distributed across the PGE for the past 150 years. Our second note of caution comes from our assumption of maternal inheritance of cpDNA which, although widespread, is not universal: Paternal inheritance of cpDNA has been detected in several species, albeit at very low frequencies, and it has been suggested that rare paternal inheritance is universal in plants (Azhagiri and Maliga 2007; Ellis et al. 2008).

Our third note of caution comes from the fact that our comparison of nuclear and cpDNA differentiation may also be influenced by the types of markers that we used: The nuclear data are dominant, bi-allelic markers, whereas the cpDNA are mononucleotide microsatellite markers. We chose to characterize cpDNA microsatellites partly because of their variability and partly because we wished to characterize a larger sample size than could be easily accommodated by sequencing each individual at multiple loci. Our cpDNA characterization of *A. odoratum* on the PGE was based on 6 loci genotyped from between 81 and 95 plants from each population, an approach that provides robust estimates of intrapopulation variability but which would not have been feasible based on multiple cpDNA sequences per individual. However, we must acknowledge the possibility that homoplasy has influenced our results because repetitive sequences are more likely to experience homoplasy than nonrepetitive sequences (reviewed in Vachon and Freeland 2011). This issue is not necessarily limited to our cpDNA sequences because AFLP markers are anonymous, and some may contain microsatellite or minisatellite sequences, although is much more likely to impact the cpDNA markers that we used because of their known repetitive nature. Collectively, these cautionary notes mean that we should interpret the pollen-seed flow ratios as guidelines and not as accurate measures of contemporary pollen and seed flow; however, these cautions are less applicable to the interpopulation patterns that are inferred

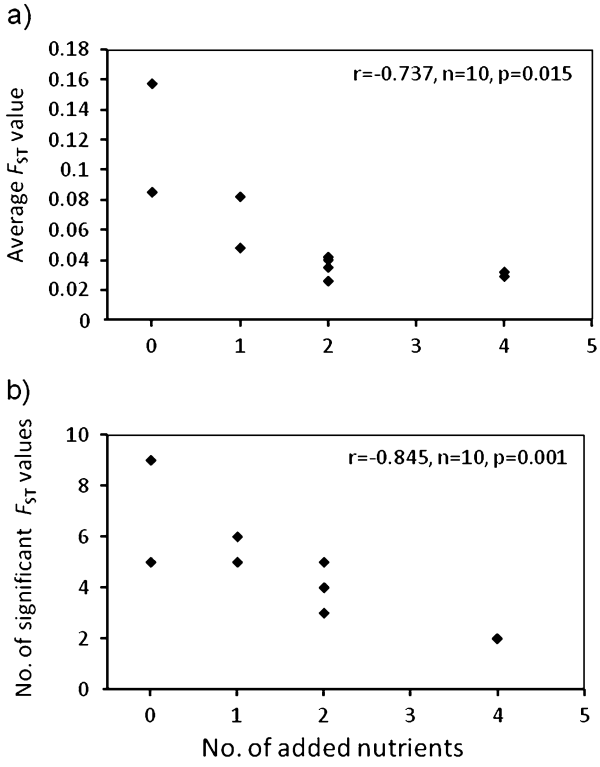

Figure 3. Correlations between the total number of additions (nutrients plus lime) for each plot and (**a**) the average cpDNA-based pairwise F_{ST} value for each plot and (**b**) the number of significant pairwise cpDNA F_{ST} comparisons involving each plot.

from either nuclear or microsatellite markers, which we will now discuss.

Regardless of the extent of seed flow on the PGE, the genetic differentiation of cpDNA across the experiment does not suggest isolation by adaptation, a pattern that had previously been identified from AFLP data (Freeland et al. 2010). In other words, cpDNA differentiation is not higher among increasingly manipulated (more enriched) plots (Figure 2). This is true regardless of whether genetic differentiation is calculated as F_{ST} or D: Overall, the 2 sets of values (F_{ST} and D) identified comparable trends, even though the former tended to be lower than the latter. Nevertheless, the pattern of cpDNA differentiation across the PGE is not random: Genetic differentiation of cpDNA is influenced by the overall nutrient richness of plots, resulting in an inverse correlation between the number of additives a plot receives and its average cpDNA F_{ST} value (Figure 3) (the relationship is upheld when comparisons are based on D). *Anthoxanthum odoratum* seeds are dispersed by wind, water, and mammals (awns on fur) and may also be dispersed during the annual mowing of the PGE, although care is always taken during mowing to minimize the spread of plant material between plots (Yeoman D, personal communication). However, we sampled only mature plants, and our data therefore reflect not seed flow but a combination of seed flow and subsequent germination and growth. The different cpDNA F_{ST} values among plots may therefore be explained by more frequent germination and growth of immigrant seeds in enriched plots, which could be influenced by 2 factors. The first is simply the possibility that higher nutrient levels have enabled a greater proportion of immigrant seeds to successfully germinate and grow. The second explanation is based on earlier findings that these high resource plots have lower species diversity and hence relatively low levels of interspecific competition (Harpole and Tilman 2007; Silvertown et al. 2009). More successful establishment of immigrant seeds on enriched plots could explain the correspondingly high levels of genetic diversity that were identified by Silvertown et al. (2009), despite the restricted pollen flow that was highlighted in Freeland et al. (2010).

Collectively, this and earlier studies describe a system in which adaptation to resource additions has restricted pollen flow to an extent that is correlated with nutrient additions (Freeland et al. 2010; Figure 2a). Despite being increasingly divergent, populations on enriched plots have the highest level of genetic diversity (Silvertown et al. 2009), and the data presented in this current study show that patterns of seed flow (and subsequent establishment of immigrant plants) can provide a plausible explanation for this apparent discrepancy. Less clear is how natural selection is acting on immigrant plants. Although flowering time can be influenced by nutrient additions (Wielgolaski 2001; Dahlgren et al. 2007), it is a quantitative trait with a heritable component (Devaux and Lande 2010). Indeed, Snaydon and Davies (1976) previously found heritable differences in flowering time between *A. odoratum* plants on adjacent plots, and the flowering time of immigrant plants is therefore likely to be somewhat asynchronous with that of their plot mates. This suggests that immigrant plants should be selected against during the flowering season, although further work, for example, common garden experiments will be needed to test this hypothesis. Selection against immigrant plants during the flowering season could explain why nuclear differentiation shows a pattern of isolation by adaptation despite the potentially homogenizing effect of high seed-mediated gene flow.

Overall, our results demonstrate some of the challenges that are associated with understanding population connectivity and highlight the importance of considering different dispersal mechanisms that can influence the distribution and abundance of alleles across a landscape. In addition, our study adds to the growing body of literature that quantifies the distinct roles of seed and pollen flow in a number of contexts including species' range expansions (Hu and Li 2003), fine-scale spatial genetic structure (Barluenga et al. 2011), and responses to habitat fragmentation (Sebbenn et al. 2011). More specifically, our findings may be considered consistent with an earlier study which concluded that in some wind-pollinated species seed flow may actually be more important than pollen flow for population connectivity (Bacles et al. 2006). Finally, our results further highlight some of the insights into evolutionary and ecological interactions that can be obtained from long-term experiments.

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