Molecular Ecology (2008) **17**, 731–740 doi: 10.1111/j.1365-294X.2007.03625.x

Colonization genetics of an animal-dispersed plant (*Vaccinium membranaceum*) at Mount St Helens, Washington

S. YANG,* J. G. BISHOP† and M. S. WEBSTER*
*School of Biological Sciences, Washington State University, PO Box 644236, Pullman, WA 99164-4236, USA,
†School of Biological Sciences, Washington State University, 14204 NE Salmon Creek Avenue, Vancouver, WA 98686-9600, USA

Abstract

Population founding and spatial spread may profoundly influence later population genetic structure, but their effects are difficult to quantify when population history is unknown. We examined the genetic effects of founder group formation in a recently founded population of the animal-dispersed *Vaccinium membranaceum* (black huckleberry) on new volcanic deposits at Mount St Helens (Washington, USA) 24 years post-eruption. Using amplified fragment length polymorphisms and assignment tests, we determined sources of the newly founded population and characterized genetic variation within new and source populations. Our analyses indicate that while founders were derived from many sources, about half originated from a small number of plants that survived the 1980 eruption in pockets of remnant soil embedded within primary successional areas. We found no evidence of a strong founder effect in the new population; indeed genetic diversity in the newly founded population tended to be higher than in some of the source regions. Similarly, formation of the new population did not increase among-population genetic variance, and there was no evidence of kin-structured dispersal in the new population. These results indicate that high gene flow among sources and long-distance dispersal were important processes shaping the genetic diversity in this young *V. membranaceum* population. Other species with similar dispersal abilities may also be able to colonize new habitats without significant reduction in genetic diversity or increase in differentiation among populations.

Keywords: AFLP, colonization, dispersal, founder effect, Mount St Helens, *Vaccinium membranaceum*

Received 29 April 2007; revision received 28 August 2007; accepted 26 October 2007

Introduction

Founding of new populations may occur in a variety of ecological circumstances, including invasion or range expansion, re-colonization of previously occupied habitat in a classic metapopulation system, or colonization of new habitat created by disturbance, such as during primary succession. A major genetic consequence of these episodes is the creation of age structure among populations in which young populations are further from the equilibrium between migration and drift, which can lead to either an increase or decrease in the average differentiation among populations (Wade & McCauley 1988). Whether colonization increases or decreases divergence depends primarily on the processes that create groups of founders.

Several population genetic models have examined whether or not founding leads to differentiation, depending on the mode of colony formation and the resulting strength of founder effects. These models indicate that dispersal to the new habitat from many sources is less likely to result in strong founder effects, as compared to when dispersal is from very few sources, because colonizing individuals are mixed from different populations (Slatkin 1977; Whitlock & McCauley 1990). This mixing may also prevent differentiation among populations, depending on its magnitude (Slatkin 1977). Similarly, movement of individuals between source populations (migration), as well as movement of pollen between these sources (mating), reduces among-population genetic variance (Slatkin 1977; Whitlock & McCauley 1990). In contrast, increased genetic variance among populations can result when founders share a

Correspondence: Suann Yang, Department of Biology, Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802 USA. Fax: 1 (814) 865 9131; E-mail: suy14@psu.edu

common origin, as when founder groups formed via kin-structured movement (Whitlock & McCauley 1990). This situation may occur when seeds are dispersed as a unit, such as within a fruit (as full- and half-sibs) or as a group of fruits from a single plant or population. Genetic differentiation of populations also may depend on the number of founders relative to the level of migration between sources (Wade & McCauley 1988). When colonization from multiple sources occurs significantly more often than does migration between sources, genetic differentiation should decrease. This situation is especially likely when establishment at a new site is much easier than at existing sites, such as when founders escape density-dependent mortality during establishment (Wade & McCauley 1988). These models indicate that understanding both dispersal biology and the ecological context of establishment is critical to predicting the effects of disturbance and colonization on the formation of population genetic structure.

Informed by these theoretical predictions, and by simulation studies showing that long-distance dispersal may maintain genetic diversity in new populations (Ibrahim *et al*. 1996; Le Corre *et al*. 1997; Davies *et al*. 2004; Bialozyt *et al*. 2006), contemporary patterns of population genetic structure are often attributed to colonization processes, especially dispersal. Generally, these conclusions are drawn despite lack of knowledge regarding population history and age, and often the role of founder group formation cannot be directly tested. Only a few studies have directly examined the connection between colonization events and plant population genetics (Erickson *et al*. 2004; Ingvarsson & Giles 1999; Litrico *et al*. 2005; Sezen *et al*. 2005; Jones *et al*. 2006; Raffl *et al*. 2006), and these have supported a causal relationship between the ecological circumstances of colonization and genetic diversity and population differentiation. However, the role of founder group formation remains unclear because most of these studies were not conducted immediately after the colonization events (but see Erickson *et al*. 2004; Sezen *et al*. 2005; Jones *et al*. 2006). Because other processes can lead to population structure, such as local selection following dispersal (Kalisz *et al*. 2001) and competition (Travis *et al*. 2004), we can most clearly understand the theoretical predictions regarding the role of colonization processes by examining patterns soon after the founding event.

We examined the role of founder group formation in determining population genetic patterns using a newly formed population of the animal-dispersed *Vaccinium membranaceum* (black huckleberry) in primary succession at the volcano Mount St Helens (Washington, USA). The 1980 eruption of Mount St Helens created a 60-km^2 area of primary succession north of the volcano (the Pumice Plain and debris avalanche), surrounded by 135 km^2 of secondary succession (Lawrence 2005). In this landscape, we can unambiguously identify founders and their potential sources based on substrate (primary succession) and reproductive status (no founders are yet reproductive). Possible sources for these founders are the surrounding secondary succession, as well as adult *V. membranaceum* in the area of primary succession that survived the eruption. These survivors are located in small inclusions of pre-eruption soils primarily on north-facing slopes that face away from the volcanic blast. Though initially buried by the eruption, they recovered when their below-ground structures (rootstocks) were exposed by erosion. These survivor plants are isolated from one another and from the large, dense patches of *V. membranaceum* in secondary successional areas. The dispersal biology of *V. membranaceum* is complex; hence, expectations for the effect of founding are unclear. On the one hand, this species is strongly outcrossing (vander Kloet 1988; S.Y. unpublished data), and dispersed by highly mobile frugivorous dispersal agents (S.Y. unpublished data), traits that may promote high levels of gene flow and long-distance dispersal (Hamrick & Nason 1996). Kin-structured dispersal is also likely, as a single fruit contains numerous seeds. In addition, frugivores may forage longer in dense patches of *V. membranaceum*, which may further increase the kin-structure of migrants, but may decrease probability of dispersal to unoccupied sites. On the other hand, lack of density-dependent mortality in unoccupied sites, and the vast area of primary succession available for colonization, may result in high numbers of founders.

We use amplified fragment length polymorphisms (AFLP; Vos *et al*. 1995), and population assignment tests (Duchesne & Bernatchez 2002) to address two specific questions. (i) From where do founders of the new *V. membranaceum* population originate? (ii) How well do the population genetic patterns of this system fit with theoretical predictions regarding the role of founder group formation? We discuss our results in terms of dispersal and establishment ecology of *V. membranaceum* at Mount St Helens.

Materials and methods

Description of study system and population sampling

Vaccinium membranaceum (Ericaceae) is found in open and forested habitats between altitudes of 1000 m and 1800 m above sea level throughout the Pacific Northwest (vander Kloet 1988; Pojar & MacKinnon 1994). *V. membranaceum* is one of the few animal-dispersed plants beginning to colonize the primary successional Pumice Plain (Titus *et al*. 1998). Observed frugivorous visitors in secondary succession include coyotes (*Canis latrans*), cedar waxwings (*Bombycilla cedrorum*), varied thrushes (*Ixoreus naevius*), Townsend's solitaires (*Myadestes townsendi*), white-crowned sparrows (*Zonotrichia leucophrys*), black bears (*Ursus americanus*) and

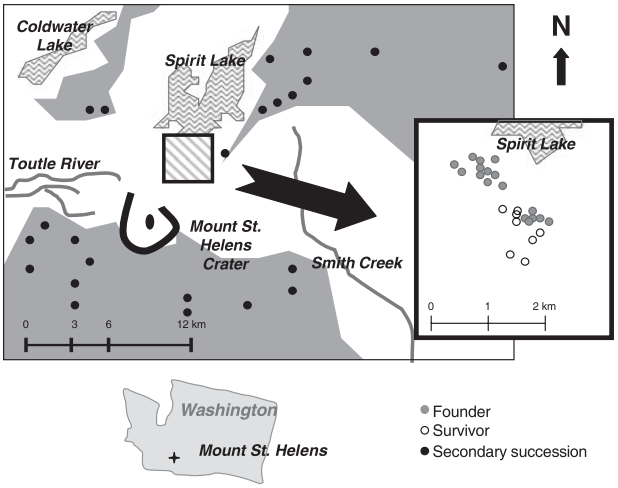

Fig. 1 Sample locations for AFLP genotyping. We sampled 15–20 individuals from each collection site in secondary succession. The grey shaded areas represent secondary succession, and the white areas primary succession. Survivors, indicated in the inset, are growing in small remnants of pre-eruption soils.

golden-mantled ground squirrels (*Spermophilous saturatus*). During the time when *V. membranaceum* fruit is available, numerous coyote scats containing partially and completely digested fruits can be found along coyote travel corridors. Because more than 3500 seeds can be found in a single coyote scat (S.Y., personal observation), we suspect that coyotes play a major role in seed dispersal of *V. membranaceum.* Bears can also remove high quantities of fruit, but they are not as common in our study area.

Our sampling scheme was designed to characterize both distant and local sources of propagules (Fig. 1). We considered potential sources to be adults located in the surrounding secondary succession, as well as the survivors within primary succession. We collected leaves from 15 to 20 individuals per sampling site from 23 sites in secondary succession with 11 of these sites located north of the Mount St Helens crater, and the remaining 12 located south of the crater. Through an intensive search of the eastern portion of the Pumice Plain (area of primary succession boxed in Fig. 1), we identified and sampled 21 survivors and 68 founders. Locations of sampling sites in secondary succession and of individuals in primary succession were obtained by GPS (Global Positioning System). Tissue was kept at 4 °C, and DNA extracted within three days of field collection following the procedure of Boches *et al*. (2005).

AFLP procedure

Initial screening with microsatellites (Boches *et al*. 2005) revealed a complex banding pattern of 1–4 bands per individual per locus (S.Y., unpublished data), confirming that *V. membranaceum* is a tetraploid (vander Kloet 1988; vander Kloet & Dickinson 1999). Because of this polyploidy, we chose to use AFLP instead of microsatellites, thus avoiding the difficult problem of estimating allele copy number (Nybom 2004). The AFLP procedure is a multilocus DNA fingerprinting technique utilizing a selective polymerase chain reaction (PCR) amplification of restriction fragments from a total digest of genomic DNA (Vos *et al*. 1995).

We based our AFLP protocol on that of Vos *et al*. (1995). Genomic DNA was digested with *Eco*RI (Invitrogen) and *Mse*I (NEB) at 37 °C for 2 h, and ligated to *Eco*RI and *Mse*I adaptors at 16 °C for 3 h in two separate steps, with enzymes denatured after each step (65 °C for 20 min). To ensure correct ligation between fragments and adaptors, we followed with a third step where we combined restriction and ligation together (25 °C for 24 h). Fragments were then amplified using a series of selective PCR amplifications. The first selective PCR used unlabelled primers based on the adaptors plus a single nucleotide (sequences with + selective base outside the adaptor region — *Eco*RI: GACTGCGTACCAATTC + a and *Mse*I: GATGAGTCCTGAGTAA + c). PCR products were checked for complete digestion on 2% agarose gels. Reactions with fragments primarily larger than 600 bp in size (indicating incomplete restriction) were discarded and repeated until results were satisfactory. The second selective PCR used four primer pairs, fluorescently labelled (same sequences as first selective PCR primers, except for the following selective bases: *Mse*I + cat/*Eco*RI + aac, *Mse*I + cat/*Eco*RI + aca, *Mse*I + cat/*Eco*RI + agg, and *Mse*I + cat/*Eco*RI + atc). The first selective PCR was for 2 min at 72 °C, 20 cycles of 30 s at 94 °C, 30 s at 59 °C, 2 min at 72 °C, and 30 min 60 °C. The second selective PCR was for 2 min at 94 °C; one cycle of 20 s at 94 °C, 30 s at 66 °C, and 2 min at 72 °C; 7 cycles of 20 s at 94 °C, annealing starting at 65 °C and decreasing 1 °C per cycle, and 2 min 72 °C; 30 cycles of 20 s 94 °C, 30 at 59 °C, 2 min at 72 °C, with a final hold of 30 min at 60 °C. The fluorescently labelled PCR products were then analysed with LIZ size standard (Applied Biosystems) on an ABI 3730 automated sequencer. Fragments were scored using GENEMAPPER version 3.5 (Applied Biosystems). Only bands between 150 bp and 500 bp that appeared above 1000 relative fluorescent units were scored to minimize scoring of false (artefact) bands. To ensure repeatability, AFLPs were generated and scored from separate, duplicate DNA extractions for a subset of individuals in each set of fragment-generating reactions. We used AFLPOP (Duchesne & Bernatchez 2002) to identify redundant and correlated fragments; only independent fragments were included in subsequent analyses in order to avoid inflation of similarity indices (Bonin *et al*. 2007). Our scoring precautions and identification of correlated fragments caused us to discard almost 70% of fragments generated; however, the remaining fragments were consistently reproducible.

Analyses of genetic differentiation and dispersal

The boundaries between genetically distinct groups of individuals are not always obvious for most continuously distributed species (Manel *et al*. 2003). For *V. membranaceum* in our study area, primary succession separates northern from southern sampling sites, and Spirit Lake separates northwestern from northeastern sampling sites. We thus grouped sampling sites into four geographical regions (northwest, NW; northeast, NE; southwest, SW; and southeast, SE).

We chose statistical analyses that identify and assess the importance of several processes influencing founder group formation and its consequences. To assess the amount of gene flow between these geographical regions, we examined the fractional assignment of source individuals into genetic groups with the program STRUCTURE (Pritchard *et al*. 2000). STRUCTURE probabilistically assigns individuals into genetic clusters to minimize departures from Hardy–Weinberg equilibrium and linkage equilibrium. We explored the number of genetic clusters (K) of only the source populations by performing 10 replicates of each simulation from $K = 1$ to $K = 10$, with a burn-in 10^5, and MCMC of 10^6, assuming admixture and frequencies correlated as recommended by Pritchard *et al*. (2000). A simulation study by Evanno *et al*. (2005) suggested that the highest log likelihood does not always signify the most likely number of genetic groups. Instead, the second derivative of the likelihood function with respect to K (ΔK) more reliably predicts the actual K by identifying the break in the slope of the likelihood distribution (Evanno *et al*. 2005; but see Waples & Gaggiotti 2006). We used both of these selection criteria to estimate the number of genetic groupings in our data. In addition to estimating the number of genetic groups, STRUCTURE estimates Q, the proportion of membership of each individual into a genetic cluster. To examine the degree of admixture in our populations, we also calculated the number of individuals whose highest Q was greater than 0.9.

We used AFLPOP (Duchesne & Bernatchez 2002) to allocate founders to the geographical regions. AFLPOP uses likelihood to assign individuals to candidate source populations based on the frequency of fragments present (Duchesne & Bernatchez 2002). We first determined the most likely source for each individual, and then we repeated the procedure with increased stringency using a log-likelihood difference (LLD) of 0.2 (2× more likely — the log-likelihood analogue of a 95% CI). In addition, we estimated the dispersal distances of founders from source regions by comparing the geographical centre of sampling sites within each region to the geographical centre of the new population.

We also used multidimensional scaling analysis (MDS) to visualize genetic groupings of founders and sources. We first obtained a Nei-Li distance matrix of all individuals with PAUP version 4.0 (Swofford 2003), and performed an MDS on this matrix with PROC MDS in SAS version 8 (SAS Institute). Multidimensional scaling is similar to factor analysis or discriminant analysis, in that it does not assume linearity, unlike principal components analysis or principal coordinates analysis (Lessa 1990).

We then examined the spatial distribution of founders and survivors in the area of primary succession to explore spatial genetic patterns, and to identify possible kin-structured dispersal. We performed global autocorrelation using GENALEX (Peakall & Smouse 2006). Global autocorrelation calculates r, the autocorrelation coefficient as a function of distance classes. Global autocorrelation analysis identifies structuring across an entire study area. Significant r at short distances may occur when a founder dispersed from a nearby adult. Also, because *V. membranaceum* fruits are many-seeded, and dispersal agents typically consume multiple fruits per bush, neighbouring founders may be closely related. This kin-structured dispersal would also produce significant r at short distances. Unfortunately, relatedness estimators for polyploids using dominant markers do not exist (Kosman & Leonard 2005), meaning that we cannot definitively confirm these relationships.

Because our system is not likely to be in Hardy–Weinberg equilibrium, we estimated θ^B (Bayesian estimator of F_{ST}), and hs (Bayesian estimator of H) using HICKORY (Holsinger *et al*. 2002), which does not assume Hardy–Weinberg equilibrium to estimate these parameters. We used the default parameters and the *f*-free model, which does not estimate f (within-population inbreeding) to obtain θ^B or hs. The use of this model prevents unreasonable estimates of f from influencing θ^B estimates, but increases the credible interval for θ^B. We also calculated average number of fragments for each population, and Shannon diversity ($I = -\Sigma\, p_i \ln p_i$, where p_i is the frequency of the presence or absence of each fragment based on the total fragments found over the entire data set). For AFLP, number of fragments reflects within-population genetic variation, and Shannon diversity encompasses both number of fragments and their frequency (Tremetsberger *et al*. 2003). In addition, both of these measures are sensitive to sample size. However, our apparently small 'sample' of survivors is a census of all individuals surviving the (1980) eruption; hence their diversity metrics are biologically meaningful.

Results

Our AFLP scoring method produced 95 repeatable markers from four primer pairs in 355 individuals. All markers were polymorphic, and all individuals had unique fragment combinations. The Bayesian estimate of $F_{ST}(\theta^B)$ among all populations was $\theta^B = 0.0860$ (95% credible interval = 0.0642–0.1044).

Our STRUCTURE analysis revealed our regional groupings did not completely explain population genetic structuring.

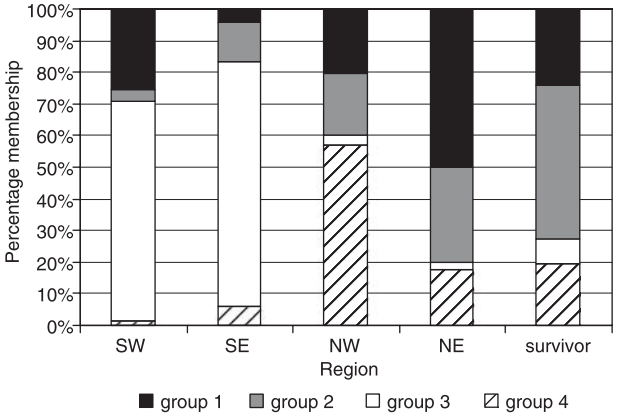

Fig. 2 Percentage membership of each region in the genetic groups determined by STRUCTURE.

The log-likelihood function appeared to plateau at $K = 5$, and we found greater variance between replicates as K increased (see Evanno *et al*. 2005), suggesting that five or more genetic clusters were needed to explain our data. The second derivative of the likelihood function with respect to K (ΔK) identified a peak at $K = 4$. Because the patterns of membership when $K = 4$ and $K = 5$ were similar, we show only the results for $K = 4$ (Fig. 2). Regional designations were not congruent with observed genetic clustering: individuals in a source region did not belong exclusively in a single genetic cluster. Indeed, only 52.1% of all individuals had a greater than 0.9 proportion of membership within a single genetic cluster. These results are indicative of high levels of gene flow across our study system. Nevertheless, some patterns did emerge. In particular, SW and SE were similar to each other in that they both had predominant membership in genetic group 3, in contrast to the remaining regions, which had very little membership in this group. In addition, NE and survivors belonged more to genetic groups 1 and 2 than to 3 and 4 (Fig. 2).

Where do founders originate?

Population assignment. Using regions as source populations, all 68 founders could be assigned using AFLPOP to a most likely source region (LLD = 0), but when the assignment was repeated at a 95% confidence level (LLD = 0.2), 11 individuals could not be assigned (Table 1). Of these 11 individuals, six cases could not be assigned because the LLD between NE and survivors was not large enough, reflecting the genetic similarity of these two sources (Fig. 2). Founders were allocated to all potential source regions, but approximately half were allocated to the population of remnant survivors on the Pumice Plain (Table 1). The average distance between the survivors and founders was approximately 1.5 km, suggesting that

Table 1 Proportion of the 68 founders assigned to each source by AFLPOP. Analysis was performed at two levels of stringency, most likely (log-likelihood difference = 0) and 95% confidence level (log-likelihood difference = 0.2)

Source	Founders assigned		Average dispersal (km)
	Most likely	95% CL	
NW	0.22 ($n = 15$)	0.21 ($n = 14$)	5.95
NE	0.16 ($n = 11$)	0.13 ($n = 9$)	6.67
SW	0.07 ($n = 5$)	0.03 ($n = 2$)	9.71
SE	0.06 ($n = 4$)	0.03 ($n = 3$)	10.1
Survivor	0.49 ($n = 33$)	0.43 ($n = 29$)	1.52
Not assigned		0.16 ($n = 11$)	

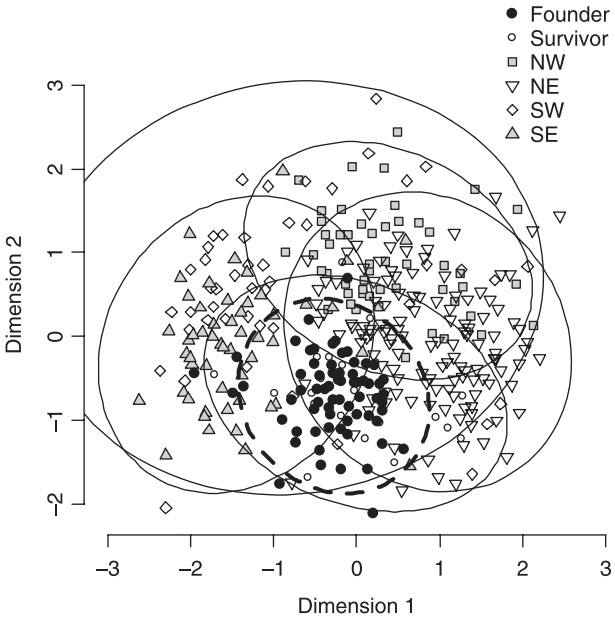

Fig. 3 Multidimensional scaling plot of founders, survivors, and secondary successional sources (NW, NE, SW, SE). Each symbol represents a single individual. Founders overlap with all sources, as shown by 95% confidence ellipses (solid lines for sources, dashed line for founders).

dispersal from nearby sources is more common than long-distance dispersal. Of the individuals assigned to secondary successional source populations, significantly more founders were allocated to northern regions than to southern regions ($x^2_{3,0.05} = 13.43$, $P = 0.004$). This pattern was confirmed by the multidimensional scaling analysis: the cluster of founders overlapped with all source clusters, but showed the most overlap with the survivors and the least overlap with southern region individuals (Fig. 3).

Spatial genetic patterns. Global autocorrelation analysis did not reveal any spatial pattern across the entire Pumice Plain study area (founders and survivors). The estimate

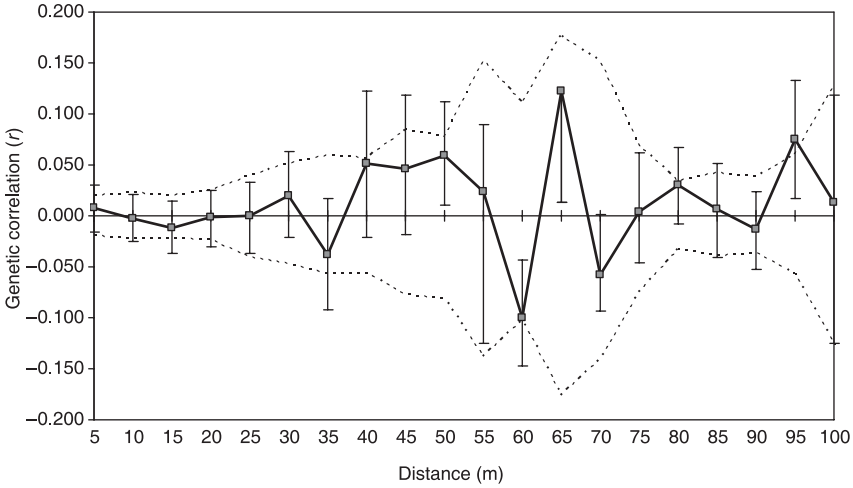

Fig. 4 Correlogram plot of the global genetic correlation coefficient *r* (bootstrapped 95% confidence error bars) for founders as a function of distance. The permuted 95% confidence interval (null hypothesis of no spatial structure) is between the dashed lines.

Table 2 Pairwise θ^B between secondary source regions (NW, NE, SW, SE), survivors, and founders. Credible intervals in parentheses following estimates

	NW	NE	SW	SE	Survivor
NE	0.046 (0.028–0.068)				
SW	0.090 (0.064–0.120)	0.118 (0.086–0.150)			
SE	0.124 (0.091–0.161)	0.158 (0.122–0.197)	0.289 (0.016–0.046)		
Survivor	0.034 (0.019–0.055)	0.033 (0.019–0.053)	0.076 (0.051–0.106)	0.108 (0.078–0.143)	
Founder	0.056 (0.036–0.080)	0.055 (0.037–0.080)	0.080 (0.056–0.108)	0.099 (0.070–0.131)	0.024 (0.012–0.042)

of *r*, the global autocorrelation coefficient, did not show a decrease with distance (Fig. 4). Thus, we found no evidence of kin-structured or restricted dispersal.

What impact does origin have on the genetics of early succession?

Genetic structure. When colonization is from multiple source populations, among-population genetic variance is expected to increase if the number of founders is small relative to the migration between sources (Wade & McCauley 1988). We found that θ^B of the five source populations (0.095, 95% credible interval = 0.072–0.116) overlapped with θ^B among all six populations (0.086, 95% credible interval = 0.064–0.104), indicating that the number of founding individuals was sufficient to prevent an increase in among-population genetic variance.

To see if there was an effect of source identity on genetic differentiation between populations, we examined pairwise θ^B between the founders and source populations. Because many founders come from the survivors, we expected that there would be more differentiation between the founders and the secondary successional sources than between the founders and survivors. In this comparison, the founders were more similar to the survivors than to the other sources (Table 2).

Founder effect. We did not observe lower genetic diversity in the newly founded population than in any of the putative source regions, suggesting the lack of a strong founder effect. First, the expected heterozygosity (*hs*) of founders and sources did not differ significantly (Table 3). Second, fragment number and Shannon diversity indices were comparable between the founders and source regions, with the exception of the lower Shannon diversity of the survivors (which likely experienced a genetic bottleneck during the 1980 eruption). Indeed, founders appeared to have somewhat higher genetic diversity than four of five source populations (Table 3).

Discussion

The new Pumice Plain population comprised founders originating from all five sampling regions, but the predominant source, contributing about 50% of founders, was the small population of survivors embedded within primary successional areas. More founders were assigned to the nearby survivors, fewer to the farther-away northern sources, and fewer still to the most distant southern sources on the far side of the volcano. In other words, source contributions were assortative by distance (Wade 1978) and by geographical barriers, factors that frequently promote genetic divergence of populations

Region	*N*	Avg frag (± SD)	H_{sh}	*hs* (95% credible interval)
NW	53	44.06 (± 7.41)	24.06	0.36 (0.34–0.38)
NE	130	45.03 (± 7.26)	32.65	0.36 (0.35–0.38)
SW	53	44.30 (± 9.68)	23.68	0.40 (0.37–0.42)
SE	47	45.77 (± 7.03)	24.55	0.37 (0.36–0.39)
Survivors	21	47.48 (± 6.70)	15.07	0.37 (0.36–0.40)
Founders	68	51.06 (± 6.18)	29.78	0.38 (0.37–0.39)

Table 3 Genetic diversity among secondary source regions (NW, NE, SW, SE), survivors, and founders. The number of individuals sampled (*N*), average number of AFLP fragments (avg frag), Shannon diversity (H_{sh}), and expected heterozygosity (*hs*) within each group are shown

(Davies *et al*. 2004). This pattern, combined with the opportunity for highly kin-structured dispersal of *Vaccinium membranaceum*'s many-seeded fruits, suggests a strong potential for colonization to increase the average differentiation of populations through founder effects. However, we detected no evidence of reduced genetic diversity in the new population. Indeed, genetic diversity in the new population was as high as or higher than source populations and tended to diminish population divergence.

Population genetic structure and genetic diversity during early succession

Our results demonstrate that the genetic consequences of colonization can be complex, and in some cases contrary to expectations. First, although the dispersal characteristics of *V. membranaceum* strongly suggested that seed dispersal should lead to local kin-structure in the newly founded population, we did not identify significant fine-scale spatial structure within the founders. Very low establishment rates on primary successional substrates may interfere with the development of local kin structure. Few if any seedlings have been observed on the Pumice Plain before 1990 (J.G.B. personal observation), suggesting that the 68 founders accumulated over at least 15 years. In addition, in four experiments over 12 years, coyote scats containing *V. membranaceum* seeds were placed in nearby primary successional plots, but few seedlings survived the first season and most of these eventually died (S. Yang, J.G. Bishop, J. H. Titus, unpublished data). Thus, although closely related seeds have arrived together, apparently they typically did not establish as a group in this system.

Second, we found no evidence of a strong founder effect in the newly founded population. Although founder effects are often predicted to occur during colonization, theory suggests that such effects will be minimal when founders derive from multiple sources, when the number of founders is large (Whitlock & McCauley 1990), and when some founders disperse from long distances (Davies *et al*. 2004; Bialozyt *et al*. 2006). Low levels of differentiation among our source populations (see MDS and STRUCTURE results) may also have contributed to the lack of founder effect. *V. membranaceum* is insect-pollinated and predominantly outcrossing (vander Kloet 1988; S.Y. unpublished data), so pollen flow between source populations has the potential of adding to genetic diversity of founders. This is consistent with the theoretical insight that gene flow between source populations can have disproportionate effects on the diversity of founders (Whitlock & McCauley 1990).

Third, this colonization event did not result in an increase in among-population genetic variance. Maintenance of genetic diversity during founding may be associated with increased among-population genetic variance despite weak founder effects (Whitlock & McCauley 1990; Ibrahim *et al*. 1996; Le Corre *et al*. 1997; Davies *et al*. 2004; Bialozyt *et al*. 2006), for example when establishment is density-dependent (Wade & McCauley 1988). In contrast, one of the few conditions that leads to reduced genetic variance among populations occurs when colonization from multiple sources involves a large number of founders relative to the number of migrants between existing populations, as when founders are freed from density-dependent mortality (Wade & McCauley 1988). *V. membranaceum* seeds able to establish in primary succession likely experience reduced competition relative to seeds moved between source populations where space is already occupied, and this may have contributed to a lack of an effect on among-population genetic variance.

In contrast to our results, a number of studies of colonizing plants have found evidence of strong founder effects. For example, abiotic seed dispersal of 2 m or less in the pioneer shrub *Helicteres brevispira*, combined with kin-structured seed banks, resulted in significant substructuring in secondary tropical forest succession despite an avian pollen vector (Franceschinelli & Kesseli 1999). Island colonization by *Silene dioica* occurred by similar matrilocal dispersal, producing a persistent family structure whose signal was still detectable after patches fused (Ingvarsson & Giles 1999). *Quercus rubra* seedlings in a secondary successional temperate forest showed fine-scale spatial genetic structure, which was attributed to seed-caching behaviour of seed dispersal agents (Jones *et al*. 2006). Strong founder effects also were found in the animal dispersed *Iriartea deltoidea*, apparently because most colonizing individuals were produced by a very small number of adults in nearby old growth (Sezen *et al*. 2005). Our results also contrast with those for *Lupinus lepidus* colonizing the Pumice Plain, in which young populations were highly

divergent from each other, whereas older and surviving populations exhibited little variation among populations (Bishop 1996). In all of these studies, founder effects and high genetic differentiation appear to have resulted from limited dispersal.

Our findings are more similar to results obtained from genetic studies of other plants with long-distance dispersal abilities. For example, *Spartina alterniflora* dispersed by water into restored wetlands maintained genetic diversity comparable to established populations and exhibited little genetic differentiation, probably owing to wind dispersal of pollen and seed dispersal along tidal margins (Travis *et al*. 2002). Likewise, the wind-dispersed *Hypochaeris tenuifolia* on Volcán Lonquimay, Chile, displayed no significant reduction in total number of fragments or Shannon diversity of younger populations relative to older populations (Tremetsberger *et al*. 2003). Our results also mirror the pattern found in *Antirhea borbonica*, where founder events from multiple sources do not enhance among-population genetic variance (Litrico *et al*. 2005). Similarly, most of the genetic diversity in younger populations of *Saxifraga aizoides* (Raffl *et al*. 2006) and *Myrica cerifera* (Erickson *et al*. 2004) was already present in early stages of colonization, apparently because of long-distance gene flow. Results from these studies, combined with our own results from *V. membranaceum*, strongly support the hypothesis that long-distance dispersal from multiple sources, combined with high gene flow among sources, is an important element needed to maintain genetic diversity during colonization events.

Dispersal

Vaccinium membranaceum founders derived from multiple sources because both long distance and local dispersal occur. Colonizing propagules travelled an average of 4.2 km (from 1.5 to over 10 km) from source populations, suggesting that the distribution of *V. membranaceum* dispersal distances is characterized by an extremely long tail. Rare, long-distance migration events, typically difficult to detect and quantify, are a problem for which population assignment tests are well-suited. This method has been employed in a few other cases. For example, He *et al*. (2004) found a maximum dispersal distance of 2 km for wind- and gravity-dispersed *Banksia hookeriana* (He *et al*. 2004), whereas Tero *et al*. (2003) documented maximum dispersal distances of nearly 30 km for *Silene tatarica*, which can be dispersed by wind, water, and animals (including humans).

Dispersal vectors play a central role in creating the pattern of founder origin and number. Hence, by considering the behaviour of *V. membranaceum* seed dispersal agents, we can more fully explain the genetic variation in our system. Although distance alone can produce dispersal patterns that are assortative-by-distance, landscape features between the seed source and deposition site and variation in frugivore movement behaviour may also enhance distance-dependent dispersal. For example, the volcano itself is a barrier to movement for some dispersal agents, and thereby may reduce south- to-north movement of seeds. Larger frugivores, such as the coyotes in our system, may move seeds longer distances than smaller frugivores, such as birds, as found by Jordano *et al*. (2007). In addition, dispersal agents may not often move between successional stages, making it more likely that source plant and seed deposition site are within the same successional stage. Specifically, preference of frugivorous dispersal agents for vegetated habitat may deter travel across primary successional areas, decreasing the probability that founders derive from secondary successional sources. Although some of the vertebrate dispersal agents of *V. membranaceum* may cross barren primary successional areas, this movement is probably rare. For example, when given a choice between crossing open territory or remaining in forested cover, many forest birds are more likely to remain within or at the edge of forest (Bélisle & Desrochers 2002). This behaviour has been shown to restrict seed dispersal to patches connected by corridors in an experimental landscape (Haddad *et al*. 2003), suggesting that birds may contribute a smaller portion of the seed rain into primary succession. Thus, despite the large size of secondary successional source populations, relatively few seeds may actually have moved from secondary to primary succession.

Our finding that the survivors were an important source of the founders revealed a new role for pre-eruption vegetation at Mount St Helens, and other successional landscapes resulting from cataclysmic disturbance. In general, surviving vegetation should be an important source of founders to the surrounding disturbed landscape (Cousins & Eriksson 2001); however, thorough vegetation surveys at Mount St Helens demonstrated that sites with surviving vegetation (including *V. membranaceum*) embedded in primary succession rarely contributed to the developing vegetation in the immediately adjacent (within 128 m) area of primary succession (Fuller & del Moral 2003; del Moral & Eckert 2005). Although we confirmed this lack of patch-scale dispersal and colonization for *V. membranaceum* in this study, we did detect a role of *V. membranaceum* survivors as a predominant source of founders on a larger landscape scale. Patches of surviving vegetation and pre-eruption soils within the Pumice Plain were important as safe sites for colonizing early successional species, but their role as contributors of late-successional species was thought to be minor because of the inability of these species to establish on primary surfaces (del Moral *et al*. 2005; S. Yang, J.G. Bishop, J. H. Titus, unpublished data). Using molecular markers to identify dispersal of other plant species from these surviving patches may also reveal similar larger-scale patterns.

Other considerations

As a rule of thumb, restoration projects are urged to utilize local materials for reintroduction, a demand that often must be carefully balanced against the need to ensure adequate variation for adaptation to changing environments, and against the practical issue of procuring large numbers of plants (Lesica & Allendorf 1999; Hufford & Mazer 2003). Restoration ecologists have begun delineating seed transfer zones, within which material can be transferred without detrimental effects, based on geographical and life-history considerations (Hufford & Mazer 2003). Our results suggest that natural populations of *V. membranaceum* are commonly composed of diverse founders derived from long-distance dispersal. For these and other species with similar dispersal biology, we suggest that restoration projects can justify relatively large seed transfer zones encompassing diverse locations. Reproducing the genetic diversity present during natural colonization will require managers to obtain a significant fraction of their source material from both distant and nearby sources.

In summary, by capturing genetic patterns soon after the founding event, we were able to find unambiguous support for the importance of population founding processes. In particular, long-distance dispersal, combined with harsh establishment conditions, leads to colonization from multiple source populations, lack of a founder effect in the new population, and no increase in fine-scale and landscape-scale population genetic structure for *V. membranaceum* at Mount St Helens. By focusing on the processes of founder group formation, we can gain critical insights into the population genetic patterns produced by post-disturbance colonization events.

Acknowledgements

We are indebted to S. Yang for invaluable field assistance, to N. Bassil and P. Boches for allowing us to use their facilities and expertise for DNA extraction, to G. Colbeck for considerable help with the AFLP protocol, and E. Jongejans for statistical assistance. We also thank D. McCauley and two anonymous reviewers, whose comments improved a previous version of this manuscript. This work was funded by grants to S. Yang from the WSU Higinbotham Awards for Research and Travel, and an NSF Graduate Research Fellowship.

References

Bélisle M, Desrochers A (2002) Gap-crossing decisions by forest birds: an empirical basis for parameterizing spatially-explicit, individual-based models. *Landscape Ecology*, **17**, 219–231.

Bialozyt R, Ziegenhagen B, Petit RJ (2006) Contrasting effects of long distance seed dispersal on genetic diversity during range expansion. *Journal of Evolutionary Biology*, **19**, 12–20.

Bishop JG (1996) *Demographic and genetic variation during colonization by the herb Lupinus lepidus on Mount St Helens*. PhD Dissertation, University of Washington.

Boches PS, Bassil NV, Rowland LJ (2005) Microsatellite markers for *Vaccinium* from EST and genomic libraries. *Molecular Ecology Notes*, **5**, 657–660.

Bonin A, Ehrich D, Manel S (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology*, **16**, 3737–3758.

Cousins SAO, Eriksson O (2001) Plant species occurrences in a rural hemiboreal landscape: effects of remnant habitats, site history, topography and soil. *Ecography*, **24**, 461–469.

Davies S, White A, Lowe A (2004) An investigation into effects of long-distance seed dispersal on organelle population genetic structure and colonization rate: a model analysis. *Heredity*, **93**, 566–576.

Duchesne P, Bernatchez L (2002) AFLPOP: a computer program for simulated and real population allocation, based on AFLP data. *Molecular Ecology Notes*, **2**, 380–383.

Erickson DL, Hamrick JL, Kochert GD (2004) Ecological determinants of genetic diversity in an expanding population of the shrub *Myrica cerifera*. *Molecular Ecology*, **13**, 1655–1664.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Franceschinelli EV, Kesseli R (1999) Population structure and gene flow of the Brazilian shrub *Helicteres brevispira*. *Heredity*, **82**, 355–363.

Fuller RN, del Moral R (2003) The role of refugia and dispersal in primary succession on Mount St Helens, Washington. *Journal of Vegetation Science*, **14**, 637–644.

Haddad NM, Bowne DR, Cunningham A *et al*. (2003) Corridor use by diverse taxa. *Ecology*, **84**, 609–615.

Hamrick JL, Nason J (1996) Consequences of dispersal in plants. In: *Population Dynamics in Ecological Space and Time* (eds Rhodes OE, Chesser RK, Smith MH), pp. 203–236. University of Chicago Press, Chicago, Illinois.

He T, Krauss SL, Lamont BB, Miller BP, Enright NJ (2004) Long-distance seed dispersal in a metapopulation of *Banksia hookeriana* inferred from a population allocation analysis of amplified fragment length polymorphism data. *Molecular Ecology*, **13**, 1099–1109.

Holsinger KE, Lewis PO, Dey DK (2002) A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology*, **11**, 1157–1164.

Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution*, **18**, 147–155.

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.

Ingvarsson PK, Giles BE (1999) Kin-structured colonization and small-scale genetic differentiation in *Silene alba*. *Evolution*, **53**, 605–611.

Jones FA, Hamrick JL, Peterson CJ, Squiers ER (2006) Inferring colonization history from analyses of spatial genetic structure within populations of *Pinus strobus* and *Quercus rubra*. *Molecular Ecology*, **15**, 851–861.

Jordano P, Garcia C, Godoy JA, Garcia-Castano JL (2007) Differential contribution of frugivores to complex seed dispersal patterns. *Proceedings of the National Academy of Sciences, USA*, **104**, 3278–3282.

Kalisz S, Nason JD, Hanzawa FM, Tonsor SJ (2001) Spatial population genetic structure in *Trillium grandiflorum:* the roles of dispersal, mating, history, and selection. *Evolution*, **55**, 1560–1568.

vander Kloet SP (1988) *The Genus Vaccinium in North America*. Agriculture Canada, Ottawa.

vander Kloet SP, Dickinson TA (1999) The taxonomy of *Vaccinium* section *Myrtillus* (Ericaceae). *Brittonia*, **51**, 231–254.

Kosman E, Leonard KJ (2005) Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. *Molecular Ecology*, **14**, 415–424.

Lawrence R (2005) Remote sensing of vegetation responses during the first 20 years following the 1980 eruption of Mount St Helens: A spatially and temporallly stratified analysis. In: *Ecological Responses to the 1980 Eruption of Mount St Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 111–126. Springer, New York.

Le Corre V, Machon N, Petit RJ, Kremer A (1997) Colonization with long-distance seed dispersal and genetic structure of maternally inherited genes in forest trees: a simulation study. *Genetical Research*, **69**, 117–125.

Lesica P, Allendorf FW (1999) Ecological genetics and the restoration of plant communities: mix or match? *Restoration Ecology*, **7**, 42–50.

Lessa EP (1990) Multidimensional analysis of geographic genetic structure. *Systematic Zoology*, **39**, 242–252.

Litrico I, Ronfort J, Verlaque R, Thompson JD (2005) Spatial structure of genetic variation and primary succession in the pioneer tree species *Antirhea borbonica* on La Reunion. *Molecular Ecology*, **14**, 1575–1584.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, **18**, 189–197.

del Moral R, Eckert AJ (2005) Colonization of volcanic deserts from productive patches. *American Journal of Botany*, **92**, 27–36.

del Moral R, Wood DM, Titus JH (2005) Proximity, microsites, and biotic interactions during early succession. In: *Ecological Responses to the 1980 Eruption of Mount St Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 93–110. Springer, New York.

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, **13**, 1143–1155.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pojar J, MacKinnon A (1994) *Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia, and Alaska*. Lone Pine Publishing, Redmond, WA, USA.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raffl C, Schonswetter P, Erschbamer B (2006) 'Sax-sess' — genetics of primary succession in a pioneer species on two parallel glacier forelands. *Molecular Ecology*, **15**, 2433–2440.

Sezen UU, Chazdon RL, Holsinger KE (2005) Genetic consequences of tropical second-growth forest regeneration. *Science*, **307**, 891–891.

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.

Swofford DL (2003) PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

Tero N, Aspi J, Siikamäki P, Jäkäläniemi A, Tuomi J (2003) Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Molecular Ecology*, **12**, 2073–2085.

Titus JH, Moore S, Arnot M, Titus PJ (1998) Inventory of the vascular flora of the blast zone, Mount St Helens, Washington. *Madroño*, **45**, 146–161.

Travis SE, Proffitt CE, Lowenfeld RC, Mitchell TW (2002) A comparative assessment of genetic diversity among differently-aged populations of *Spartina alterniflora* on restored versus natural wetlands. *Restoration Ecology*, **10**, 37–42.

Travis SE, Proffitt CE, Ritland K (2004) Population structure and inbreeding vary with successional stage in created *Spartina alterniflora* marshes. *Ecological Applications*, **2004**, 1189–1202.

Tremetsberger K, Stuessy TF, Samuel RM, Baeza CM, Fay MF (2003) Genetics of colonization in *Hypochaeris tenuifolia* (Asteraceae, Lactuceae) on Volcán Lonquimay, Chile. *Molecular Ecology*, **12**, 2649–2659.

Vos P, Hogers R, Bleeker M *et al*. (1995) AFLP — a new technique for DNA-fingerprinting. *Nucleic Acids Research*, 4407–4414.

Wade MJ (1978) A critical review of the models of group selection. *Quarterly Review of Biology*, **53**, 101–114.

Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.

Suann Yang is interested in the evolutionary ecology of colonization, and is currently using this perspective to study plant invasions. She conducted this study as part of her PhD, which combined foraging behaviour, community ecology and molecular genetics to understand geographical variation in successional plant populations. Mike Webster studies kinship and dispersal patterns in a variety of organisms using molecular genetic tools. John Bishop studies plant–insect interactions, successional mechanisms, and molecular evolution of plant defences.
