

The formation and loss of genetic variance among newly-founded lupine populations on Mount St. Helens

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Abstract: Cohorts of newly-founded populations are expected to have higher among-population genetic variance than older populations, thereby increasing genetic variance of the entire metapopulation. However, such effects may be transient if colonization is episodic, and short-lived if gene flow is high. We investigate the genetic consequences of episodic metapopulation expansion in the plant Lupinus lepidus, a pioneer species on Mount St. Helens. We ask: 1) Do newly-founded populations exhibit high among-population genetic variance? and 2) What is the likely fate of such variance? Based on 532 genotypes at two locus-specific PCR-amplified markers, we estimated F_{st} for 15 newly-founded populations (1-4 years-old), 3 older colonizing (7-10 years), 14 pre-eruption (>10 years) populations, and for the oldest individuals in newly-founded populations (including founders of 8/15 populations). Colonization was associated with strong founder effects, with $F_{st}=0.35$ among the oldest individuals and $F_{st}=0.26$ for all individuals in newly-founded populations. Observed F_{st} 's at each locus agreed with those from computer-simulated founding, based on estimates of number of founders and of allele frequencies in source populations. Older colonizing and surviving populations had very low among-population variance ($F_{st} = 0.05$ and 0.02 , respectively). Thus, age structure among populations, developed by 11 years of colonization, is associated with differences between age classes in among-population genetic variance, such that colonization increases the overall genetic variance. However, rapid erosion of genetic structure due to gene flow suggests that the effects of colonization may be transient.

INTRODUCTION

Population bottlenecks figure prominently in evolutionary theories of speciation (Mayr 1954, Carson 1968, Carson and Templeton 1984), mating system evolution (Lande and Schemske 1985, Charlesworth and Charlesworth 1987), and in Wright's shifting balance theory of adaptive evolution (Wright 1940, 1983), and are also a major issue in the management of declining species (Frankel and Soulé 1981). Sewall Wright (1931, 1978) pointed out that by sampling only a small number of gametes to form the succeeding generation, bottlenecks cause the stochastic loss of genetic variation within populations (genetic drift) and divergence between them. Later population genetic models demonstrated that the consequences of a bottleneck depend not only the degree of restriction in population size, but also upon subsequent population demography and the type of genetic variation considered (Nei et al 1975, Lande 1980, Sirokkomaa 1983, Maruyama and Fuerst 1984, 1985, Goodnight 1987, 1988, Willis and Orr 1993). In particular, rapid population growth after founding may prevent loss of heterozygosity and quantitative genetic variation, but not the loss of alleles (Nei et al 1975, Lande 1980, Sirokkomaa 1983).

Population bottlenecks are expected to be common during founder events or colonization (Mayr 1963, Baker and Stebbins 1965, Brown and Marshall 1981, Barrett and Husband 1990). Based on this expectation, high levels of differentiation among populations in combination with low levels of variation within them are often attributed to bottleneck effects accompanying colonization (examples for plants include Schwaegerle and Schaal 1979, Ledig and Conkle 1983, Critchfield 1984, Barrett 1985, Systma and Schaal 1985, Les et al 1991, Husband and Barrett 1992, and Westerbergh and Saura 1994). This pattern also is found frequently in genetic studies of invasive species (Brown and Marshall 1981). These explanations are usually retrospective inferences based on contemporary patterns of genetic variation, with little direct knowledge of population history. As a result, we have little direct evidence for how demographic processes during colonization affect the distribution of genetic variation within and between populations.

Until recently, most theoretical consideration of colonization or founder events neglected their metapopulation context. Metapopulation genetic models are concerned with the consequences of repeated migration, extinction, and recolonization for the maintenance of genetic variation and its distribution among a set of populations (McCauley 1991). Model parameters are usually judged in terms of their effects on F_{st} , Wright's measure for the among-population component of genetic variation (i.e. a measure of population differentiation). One important result of these models is that extinction/recolonization (e/r) may increase or decrease F_{st} , and the direction and magnitude of this effect depends on the mode of colony formation, i.e. not only on the severity of a population bottleneck (number of colonists), but on the source of colonists and degree of relatedness (Slatkin 1977, Wade and McCauley 1988, Whitlock and McCauley 1990). However, under most modes of colony formation, e/r will increase F_{st} : given some level of extinction ($e > 0$), population turnover will increase overall F_{st} provided

$$k < \frac{2Nm}{(1 - \phi)} + \frac{1}{2},$$

where k is the number of colonists, N the equilibrium effective population

size, m the migration rate, and ϕ the relatedness of colonists, provided the metapopulation is at equilibrium, the founding population grows instantly to N , and $m \ll 1$ (Whitlock and McCauley

1992, Whitlock 1992b). Wade and McCauley (1988) point out that e/r creates an age structure at the level of populations, in which younger cohorts of populations are further from the equilibrium between migration and population size. An increase in the extinction rate leads to a higher proportion of young populations, shifts populations further from migration-drift equilibrium, and thereby increases the overall F_{st} (Wade and McCauley 1988, Whitlock and McCauley 1990, McCauley 1991). If colonists are few and more closely related within founding groups than between, F_{st} among founding groups ($F_{st,0}$) may be large, and the metapopulation is shifted further from migration-drift equilibrium.

One condition of these models can be tested easily when the relative age of populations is known. F_{st} in newly-founded populations ($F_{st,0}$) should differ from that of older populations ($F_{st,t+0}$). The condition $F_{st,0} > F_{st,t+0}$ has been shown to occur in several systems, including milkweed beetles (McCauley 1989), fungus beetles (Whitlock 1992), tidepool copepods (Dybdahl 1993), and two species of the plant Silene differing in their population dynamics (McCauley et al 1995, Giles 1996), although there was no difference between young and old populations of the plant Primula veris (Antrobus and Lack 1993), and Harding and Mankinen (1972) found lower levels of variation among colonizing populations of Lupinus succulentus than among stable populations. Some invasive species also exhibit lower F_{st} 's among invading populations compared to populations in their native range, but these systems have the unusual feature of drawing colonists from throughout the invader's native range to form colonizing populations (Brown and Burdon 1983, Novak and Mack 1993).

Even if $F_{st,t+0} > F_{st,0}$, the overall effect of e/r on F_{st} depends on other model parameters and assumptions. In particular, if migration rates are high founding effects are quickly swamped, i.e. the system returns rapidly to migration-drift equilibrium, and e/r has a trivial effect on overall F_{st} . In the case of milkweed beetles (McCauley 1989) and fungus beetles (Whitlock 1992b) measurement of model parameters k , N , m , and e , provided parameter combinations under which F_{st0} was likely to enhance overall F_{st} . However, it has been pointed out that species having high rates of population turnover are also likely have good dispersal ability (Barrett and Husband 1990), resulting in little effect of e/r on the distribution of genetic variation in most species in which turnover is observed (Harrison and Hastings 1996).

Most metapopulation genetic theory assumes a classical metapopulation at equilibrium, where extinction rates are constant and equal to recolonization rates, and where individual populations instantly reach demographic equilibrium. However, fluctuations in demographic parameters and in rates of extinction and recolonization rates may affect F_{st} in complex ways (Whitlock 1992a). For example, slow population growth after founding exacerbates the effect of population bottlenecks, but a given level of migration may lead to much higher levels of gene flow (Nm) in small populations, swamping the effect of $F_{st,0}$ (Whitlock 1992a). Episodic e/r , due perhaps to occasional large scale disturbances, results in a transient increase in F_{st} , whose persistence time depends on Nm . Currently there are few empirical data regarding longer term effects of extinction/recolonization processes, or how transient the effect of founding is likely to be, under either equilibrium or non-equilibrium metapopulation conditions.

In this study, we examine the population genetic consequences of metapopulation expansion in Lupinus lepidus var. lobbii on Mount St. Helens volcano. L. lepidus is a short-lived perennial legume characteristically found at high altitudes on volcanoes in the Pacific Northwest, and whose populations are strongly affected by extinction/recolonization dynamics. It grows

primarily on recently deposited volcanic material such as pumice and mudflows, on glacial moraines, and in smaller scale disturbances such as small landslides and goat wallows. Populations of *L. lepidus* are frequently eliminated by these disturbances, through successional displacement, and occasionally by lepidopteran herbivores (Bishop 1996; O. Edwards, personal communication). As it has only a short-lived seed bank (Edwards 1980, Bishop 1996), it often must recolonize through dispersal. The 1980 eruption of Mount St. Helens created a wide range of disturbed landscapes favorable for lupine population growth, including a 60 km² region of primary successional habitat on the volcano's north slope, known as the Pumice Plains. *L. lepidus* rapidly colonized many of these areas (del Moral and Wood 1993, Wood and del Moral 1987). In 1981 a single lupine became established on the Pumice Plains, over 4 km from the nearest surviving population, and produced a local population of over 16 000 individuals within 5 years (Crisafulli et al unpublished manuscript). Despite initially rapid population growth and the founding of many other patches, *L. lepidus* has been slow to colonize most of the Pumice Plains, apparently due to poor dispersal ability (Wood and del Moral 1987, del Moral and Wood 1993) and the effects of herbivory (Bishop 1996, Fagan and Bishop unpublished data). At the onset of this study in 1991 the region uphill of the original population contained one additional large population, founded circa 1984, and approximately 30 small, discreet populations founded between 1987 and 1990.

Here, we examine the among population genetic variance (measured by F_{st}) in a substantial subset of the colonizing populations on the Pumice Plains using novel, locus-specific, PCR-based markers. These populations have the subject of extensive demographic study (Bishop 1996, Bishop and Schemske unpublished manuscript, Crisafulli et al unpublished manuscript), and hence the age of the populations and the individuals within populations is known. We use this information to make comparisons among age classes of colonizing populations and investigate temporal changes in the youngest cohort of colonizing populations by calculating F_{st} among the oldest plants separately. Measurements of number of founders and allele frequencies in putative source populations, are used in simulations to evaluate the magnitude of founder effects and variation among loci. We also compare colonizing populations to a set of populations sampled at the metapopulation scale and known to have survived the 1980 eruption. These populations are sampled over a larger spatial scale than the colonizing populations and include the likely source of initial colonists. Lastly, we look for evidence of disequilibrium conditions and high gene flow by examining isolation by distance at the scale of the metapopulation and of colonization (Slatkin 1993). Our focus throughout is on the questions 1) do younger sets of populations exhibit higher among population genetic variance due to founding? and 2) How rapidly does the variance among populations change?

METHODS

Populations were sampled from two distinct geographic groups: 1) The segment of the metapopulation that survived the 1980 eruption (hereafter termed "surviving populations"); and 2) The populations colonizing the Pumice Plains (hereafter termed the "colonizing populations"). In 1994 and 1995, 194 plants were collected from 14 surviving populations along a horseshoe-shaped section of the volcano's circumference at altitudes of 1100 to 1500 m (fig. 1). The circumference at this altitude is roughly 28km, and the diameter is about 11 km at its widest point. Rugged topographic features separate many of these populations and include the upper 1200 m of the truncated, cratered cone, and numerous canyons, lava flows, and ridges. The

history of these populations is not well-known, but most were growing in locations impacted by mudflows in the 1980 eruption, and appear to have expanded from *in situ* survivors (personal observation, del Moral personal communication). Therefore, although impacted by disturbance and heterogeneous in age, these populations represent the survivors of the 1980 eruption, and are the oldest set of populations sampled. In all cases these populations were discreet, with intervening spaces of 200 - 2000 m, and contained at least several hundred flowering individuals. The only populations that lie outside this circumference are those that have colonized the most recent mudflows and debris avalanches, which reach up to 10 km downhill. Elsewhere in southern Washington, *L. lepidus* is only found at altitudes exceeding 1850 m, and as a result the Mount St. Helens metapopulation is isolated by over 40 km from the next nearest metapopulation, on Mount Adams volcano. Within each population, plants were selected every 2 m on a 40 m transect, or at shorter intervals in smaller populations. Samples consisted of shoots, that is, meristems and young leaves, that were stored on dry ice or in silica grains for 1-4 days, then at -80° C until extraction.

The gap in the circumference sample (that is, the gap in the horseshoe) corresponds to the massive, 60 km² primary successional region created by the 1980 collapse of the north slope and the ensuing lateral, northward blast (fig. 1). All populations in this area were founded by long distance dispersal, or through secondary founding by propagules from colonizing populations. Most of these populations are immediate descendants of the oldest colonizing population, founded in 1981, or of several populations founded shortly thereafter, and are concentrated in an area of 6 km² in the vicinity of the 1981 population. As of 1994, these colonizing populations, including those not sampled, were still separated from surviving populations by large expanses of uncolonized terrain. In 1991, a sample of 338 plants was collected from 21 of the colonizing populations.

The age of colonizing populations are known to within one year. At the time of sampling (1991) they ranged in age from 0 to 10 years, i.e. these populations were founded between 1981 and 1991 (table 1). Our sample consisted of 18 populations founded between 1987 and 1990, and three populations founded in 1981 or 1984. These two groups will be referred to as “young” and “old” colonizing populations, respectively. The number of population sampled of each age is roughly in proportion to their occurrence through 1990 and the sample included a large proportion (30-50%) of all colonizing populations. The entire group was located in a 2 km by 4 km area; the young populations were more concentrated, with a maximum distance between pairs of 1.7 km. Population size corresponds closely to age: The oldest populations consisted in 1991 of up to several hundred thousand plants; some of the young populations consisted of only a single, isolated, reproductive individual. In 1-4 year old populations, which were all less than 105 plants, 32% of all plants were sampled, including 73% of all reproductives (table 1). In the smallest populations, all plants were sampled. In large populations samples were collected at regular intervals along one or more transects.

Demographic studies, begun in 1990 and 1991 in fifteen of the eighteen colonizing populations (Bishop and Schemske unpublished manuscript, Bishop 1996), allowed us to identify the oldest individuals based on plant size, retention of previous inflorescence stalks, persistence of dead individuals, and buildup of fine materials on the otherwise course substrates. This group was defined as reproductive plants greater than 1 year in age. In 8 of these populations, plus several others not studied genetically, this data allowed identification and

counting of population founders.

Marker development. Novel, PCR-based locus-specific markers were developed based on published gene sequences. Because the goal of this study was to detect population structure rather than to accurately characterize levels of nucleotide diversity, PCR primers were constructed so as to anneal in highly conserved regions but amplify across regions likely to be polymorphic (i.e. introns). Although some evidence suggests insertion-deletion polymorphisms within introns may be subject to selection, intron polymorphisms are more likely to be neutral with respect to selection than are allozymes (Karl and Avise 1992). PCR primers were chosen for loci meeting the following criteria: 1) Sequence information should be genomic (i.e. not just cDNA) and come from a species of *Lupinus* or from another legume; 2) Sequence must come from a locus thought to be single copy in *Lupinus*; 3) Two 15-20 bp sequences, 0.5-2.5kb apart and with an intervening intron, had to be highly conserved between *Lupinus* and another plant family, or across more distant taxonomic groups. To increase the probability of amplification, primer sequences also satisfied other PCR criteria, and were partially degenerate. Markers were developed for Aspartate aminotransferase-P2 (EC 2.6.1.1. Reynolds et al 1992, Mett et al 1993) and L-asparaginase (EC 3.5.1.1. Lough et al 1992, Dickson et al 1992). We refer to these as AAT and Asp, respectively.

Amplification products for AAT and Asp displayed size polymorphism, eliminating the need for restriction digests of PCR product. *L. lepidus* is tetraploid ($4n=48$, Braatne 1989) and bands at duplicate loci were often present, but for both AAT and Asp one locus was reliably amplified and scored. Three and four putative alleles were readily distinguished for the scoreable AAT and Asp loci, respectively. Restriction site analysis combined with the original sequence information suggested that product size polymorphisms in both loci result from insertions or deletions within introns. Inspection of F1 progeny of heterozygous parents confirmed that all alleles at both loci, except the largest Asp allele, segregated in a manner consistent with disomic inheritance, although the number of progeny available was insufficient for estimating segregation ratios. Because of the largest Asp allele, many individuals exhibited three Asp alleles, which we initially attributed to polyploidy. However, failure of this allele to segregate suggested a possible artifact of PCR amplification. The artifactual nature of this band was confirmed by the following experiment. PCR product from a "three-allele" heterozygote was separated by gel electrophoresis, and each of the three resulting bands was excised and its DNA eluted. The eluted bands were then used separately and in combination as templates for a new set of PCR reactions. In the resulting PCR products, heterozygotes (having three bands) were produced only when pairs of PCR products were used as templates, indicating that the longest band must be produced through misalignment of two other alleles, perhaps due to repeat sequences. These markers and several others, not discussed here, amplified readily not only in *L. lepidus*, but also in *L. latifolius*, *L. arboreus*, *L. nanus*, and *L. bicolor* (Bishop and Karoly unpublished data). Thus these primers may be generally useful for work in the genus *Lupinus* and are available upon request.

Marker data. Total DNA was extracted using a Dellaporta preparation modified to allow rapid processing of samples (Dellaporta 1985, Bishop 1996). DNA extractions were stored in TE (10mM Tris, 1mM EDTA) at 4° C. DNA samples were then used as templates for PCR amplification of AAT and Asp markers. The PCR cycle was: 1 minute at 94°C, 1 minute at 51°C (AAT) or 46°C (Asp), and 1 minute 10 seconds + 1second/cycle at 72 °C, repeated for 34 cycles.

Precautions were taken to prevent spurious amplification due to contamination with PCR product. First, PCR reagents and PCR products always were handled using separate sets of pipettors. Second, the last tube of every set of reactions was a control in which template was replaced with an equivalent amount of TE (DNA samples were stored in TE), added after the rest of the reactions had been prepared. This control never produced product in several hundred sets of reactions. A number of DNA samples never produced product even upon multiple amplification attempts, due presumably to either a null allele or DNA degradation. Several lines of evidence point to the latter cause: 1) For a small number of these cases, extra tissue was on hand, allowing a second DNA extraction. In all these cases the second extraction produced PCR product; 2) DNA from greenhouse-grown plants, which was presumably in the best condition since extractions were performed immediately after collection, always produced product; and 3) DNA extractions stored for >1 year were much more likely to not amplify.

PCR products were separated on 1-1.5% NuSieve (FMC) agarose gels, stained with ethidium bromide, and examined under UV light. Products were run alongside size standards and/or against a mixture of PCR products for the appropriate locus. Products were often electrophoresed in several combinations in order to verify the identity of alleles.

Analyses. Genetic structure was quantified by calculating F_{st} , the among-population component of genetic variance, using the ANOVA method (Weir and Cockerham 1984, Weir 1990). This method corrects for unequal sample sizes and for sampling a small number of populations and individuals within populations. Our samples of newly colonizing populations included more than 30% of those founded before 1991 in the study region on the Pumice Plains. Since the largest of the new populations consisted of only 113 plants, with only 22 reproductive individuals, we were able to sample between 12% and 100% of the plants in any given population (32% of all plants and 73% of all reproductives in young populations). Therefore, in calculating F_{st} for young colonizing populations, we dropped the correction for small sample sizes (but retained corrections for unequal sample size). Since there were only two loci, F_{st} 's were calculated for each locus, and 95% confidence intervals were calculated for each locus by jackknifing across populations to estimate the standard error (Weir 1990). Confidence intervals were also calculated by drawing 1000 bootstrap data sets for each population, calculating F_{st} for each set of bootstraps for the set of populations, and using the percentile method to estimate confidence intervals. Programs for estimating F statistics and their variances under several sample size assumptions were written in Splus (Mathsoft Inc.) and are available upon request. When feasible, results of these programs were checked against Weir's genetic data analysis programs (Weir 1990).

To examine whether newly founded populations exhibit greater genetic structure than older ones, F_{st} was calculated separately at several levels. These levels were: The group of surviving (or circumference) populations, the colonizing populations on the Pumice Plains, the old colonizing populations (7-10 years old), the youngest set of colonizing populations (1-4 years old), and the oldest plants in 15 of the 18 youngest colonizing populations.

To gain a better idea of the expected F_{st} for each locus at the time of colonization, founding was simulated by randomly drawing individuals to found 40 new populations, repeating the process 1000 times. Since young colonizing populations are descended from the old colonizing populations, colonists were drawn at random from the pool of genotypes for those populations. The number of colonists for each population was modeled in two ways. First, it

was assumed that each population was founded by a single colonist ($k=1$). Second, data on the number of founders indicates that, in 1990-91, only about two-thirds of populations were founded by single founders, and the other one-third were founded by anywhere from two to five founders, so that the harmonic mean k is 1.33 (fig. 2). Thus, populations were founded by drawing between 1 and 5 colonists for each population, maintaining a harmonic mean $k=1.33$.

RESULTS

There was little evidence of genetic differentiation at the scale of the metapopulation (i.e. the circumference populations). F_{st} , which takes values between 0 (no differentiation) and 1 (complete differentiation, where all genetic variance is due to differences between populations), was indistinguishable from 0 based on 95% confidence intervals for both the AAT and Asp loci (table 2, fig. 3). Confidence intervals calculated by the bootstrap and jackknife procedures were similar in all sets of populations compared. At both loci a single allele predominated (>60%) in all the sampled populations, as well as in the old colonizing populations on the Pumice Plains. In addition, all surviving populations contain all three alleles at each locus, except for two cases at each locus where one or both of the minority alleles is missing.

In contrast to the surviving metapopulation segment, colonizing populations showed clear evidence of interpopulation differences, or structure, with the greatest amount of structure in the youngest cohort of colonizing populations. For all colonizing populations taken together, F_{st} was 0.28 for AAT and 0.07 for Asp, both significantly different from 0.0 (fig. 3). However, F_{st} was quite different between old colonizing and young colonizing populations. The three old populations (7-9 year old) had F_{st} 's of 0.06 and 0.03 for AAT and Asp, respectively ($N=92, 118$) (table 2, fig. 3). The 18 young (1-4 year old) populations had F_{st} 's of 0.37 and 0.10 for AAT and Asp, respectively ($N=112, 161$). At each locus, 95% confidence intervals indicate that the old and young groups are significantly different from each other. The large discrepancy between the two loci is probably not just a result of a larger sample size for Asp (which is expected to capture more within population variation): Removing three populations for which no AAT information was available made the sample sizes more comparable, but did not change F_{st} 's.

The pie diagrams in fig. 4 illustrate the variation in allele frequencies at each locus among the 21 populations. The three old populations form the top row. At the AAT locus, with $F_{st}=0.28$, three of the colonizing populations have frequencies of >75% for one of the minority alleles, i.e. alleles that are less than 50% in all other populations. At asparaginase, with $F_{st}=0.07$, the identity of the majority allele does not switch between populations, although the allele frequencies do vary widely between 50 and 100%.

To further investigate the effect of founding on genetic structure, F_{st} was calculated for the oldest individuals in the 15 young colonizing populations for which demographic information was available. 73% (61/83; table 1) of the reproductive individuals were sampled, with 63% for AAT and 71% for Asp. F_{st} calculated for these "founder" individuals rose to 0.49 and 0.19 at the two loci, a rise of about 0.10 at each locus (fig. 3, table 2). Given that the generation time in these populations is approximately 2 years (Bishop 1996), and that the oldest individuals were not > 2 years older than the rest of the population, the among population genetic variance declined between 20 and 50% in less than one generation. In summary, older populations displayed lower among-population variances, with potentially very high F_{st} 's among founders, but rapid decline in F_{st} and only weak evidence of $F_{st}>0$ among the oldest colonizing populations or the surviving metapopulation segment.

Simulations of the founding process showed that a process of colonization by single founders would produce much higher F_{st} 's than those observed. However, a more realistic founding process, based on the observed number of founders, produced levels of differentiation in accordance with those observed:

	k = 1	k = 1.33
AAT	0.80	0.35 (0.24 - 0.49)
Asp	0.54	0.25 (0.16 - 0.38)

Here, K is the harmonic mean number of colonists, and 95% confidence intervals are in parentheses. Interestingly, differences between loci in the magnitude of F_{st} are seen in both the simulated and observed F_{st} s, indicating that these differences probably arise due to different allele frequencies in the source populations.

Inbreeding. Circumference populations showed evidence of high inbreeding (table 2). The average inbreeding coefficient across populations, F_{is} , is a measure of deviation from Hardy-Weinberg equilibrium due to inbreeding within populations. F_{is} was 0.39 for AAT and 0.58 for Asp in the surviving populations. Inbreeding levels were also high among the “founder” set of plants in the young colonizing populations (ca. 0.33), but much lower when calculated for all plants in these populations (ca. 0.11). However, 95% confidence intervals on F_{is} were very large in young populations, indicating that the level of inbreeding varies greatly among populations. Among the old colonizing populations F_{is} also varied tremendously between loci, although the sample size for AAT was rather small.

Isolation by distance. Neither regression nor graphical inspection provided any evidence of a relationship between M and geographic distance. This was true for both the largest spatial scale, i.e. the circumference populations, and for the young colonizing populations, which had high F_{st} 's. For both analyses, M assumed a wide range of values (< 0.01 and > 10) indicating that pairwise relatedness does vary greatly between populations, but that its variation is unrelated to distance.

DISCUSSION

Founder effects. What magnitude of differentiation among newly-founded populations constitutes a founder effect? F_{st} averaged 0.35 (0.49 for AAT, 0.19 for Asp) among the oldest individuals in young colonizing populations, a group that was known to include all the founders in 8 of 15 populations, and which consisted of the immediate offspring of founders (and possibly some of the founders themselves) in the other 7 populations. This is a very high level of differentiation given that the greatest distance between any pair of these populations is 1.7 km. By way of comparison, Hamrick and Godt (1989) found the mean level of differentiation in 119 studies of herbaceous perennials to be $F_{st} = 0.23$. However, these studies sampled across a much greater proportion of species' range; therefore, in a comparative sense, F_{st} among old plants is high and constitutes a founder effect.

Nevertheless, the expected F_{st} among founders could be lower or much higher depending on the number (k) and relatedness (ϕ) of original colonists, and additional differentiation is perhaps likely to occur soon after founding due to self-fertilization and small population size. Our simulations of founding by single colonists resulted in mean F_{st} s of 0.80 and 0.54 for AAT and Asp, respectively, far higher than the observed F_{st} s. When simulations were based on the

measured number of founders (harmonic mean $k=1.33$, range=1-5), simulated F_{st} s were 0.35 for AAT and 0.25 for Asp with observed values falling within the simulation confidence intervals. Thus, given allele frequencies in the two most likely source populations, and that about 50% of populations are founded by single founders (fig. 2), the observed F_{st} s are approximately predicted. It is also noteworthy that the simulation predicts a lower F_{st} for Asp than AAT, indicating that the rather large interlocus variance is expected given differences in allele frequencies among loci in the source populations.

Metapopulation consequences. Metapopulation genetic theory predicts that under many modes of colony formation, F_{st} among newly founded populations will be greater than among older populations, and that this will increase the overall F_{st} (Wade and McCauley 1988, Whitlock and McCauley 1990). The results of this study document such a pattern among colonizing populations of *Lupinus lepidus* at Mount St. Helens (fig. 3). The oldest plants in young colonizing populations had an average (across loci) F_{st} of 0.35. Compare this to 0.26 for all plants in the young populations, 0.05 for old colonizing populations, and 0.02 for the surviving metapopulation segment (“surviving populations”) (table 2). Although estimates of F_{st} differ significantly between the Asp and AAT loci, confidence intervals for each locus indicate that most of the differences between population age classes are significant. Assuming that the various cohorts of populations were sampled approximately in proportion to their occurrence, $F_{st}=0.09$ for the entire metapopulation. Taking the baseline F_{st} as 0.01-0.04 based on the surviving populations, the immediate effect of colonization is to increase F_{st} by a factor of between 2 and 9. In fact, colonizing populations may be underrepresented relative to surviving populations, so that the actual increase is greater; moreover, a major segment of the entire metapopulation, i.e. the colonizing populations, shows a high level of genetic structure (fig. 3).

Given the magnitude of among-population genetic variance in newly-founded populations, it is surprising that the surviving metapopulation segment, which is on a much larger spatial scale (fig. 1), exhibits virtually no among-population variance. This is not due to the absence of variation, since at least three alleles occur at each locus. Instead, several lines of evidence suggest that gene flow in *L. lepidus* may erode high levels of structure in the course of only a few years. First, at both loci, F_{st} in newly-founded populations fell by approximately 0.1 when young individuals were added to the oldest individuals (fig. 3). This represents a single generation or less (Bishop 1996). Second, F_{st} among the three older colonizing populations, which are only 3-9 years older than the young colonizing populations, was only 0.03-0.06, indistinguishable from F_{st} in the surviving populations. While the small number of loci and the large inter-locus variance in F_{st} may preclude confidence in the average magnitude of F_{st} , the finding that the loci change in parallel between age classes of populations lends confidence to these results. Third, the lack of any relationship between M and geographic distance in the surviving metapopulation segment probably represents high-levels of gene flow (Slatkin 1993). Lupine populations, especially newly-founded ones, present a small target on the landscape at Mount St. Helens. Given the relatively poor dispersal ability of lupine seeds (Wood and del Moral 1987), the high levels of gene flow necessary for a rapid decrease in F_{st} are probably due to pollen movement. Detailed observation of bumblebee activity in these populations indicates that, while most movements are within populations, bees are not nesting in newly-founded populations and they move frequently between them (Bishop et al, unpublished data). Bees are also seen occasionally at small lupine populations hundreds of meters from any other vegetation,

indicating the potential for long-distance pollen flow in this system. If this hypothesis is correct then maternally-inherited markers, such as chloroplast DNA, should show much higher among-population variance, as is the case in Silene alba (McCauley et al 1995).

Inbreeding. The levels of inbreeding observed in surviving populations were surprisingly high (table 2), especially in view of outcrossing rates in 1990 of approximately $t=1$ (Bishop 1996). However, the variance in F_{is} , estimated by jackknifing across populations, was also generally high. Since F_{is} is the average of inbreeding across populations, this indicates that there is a great deal of variability among populations in the level of inbreeding. We have continually noted great geographic and temporal variation, both within seasons and between years, in pollinator abundance (Bishop et al unpublished data) and have measured large differences among surviving and colonizing populations in outcrossing rates (Bishop 1996). Therefore, high variation in the levels of inbreeding are probably to be expected. Inbreeding was also particularly high in the oldest plants of young colonizing populations. Since bumblebees have had to recover from local extinction caused by the 1980 eruption, many founding propagules may have been produced by autogamy, which this species is capable of (Bishop et al unpublished data).

The effects of extinction/recolonization measured here are obviously episodic. Eruptions of Mount St. Helens occur about every 150 years and rarely cause devastation on the scale of the 1980 eruption (Harris 1988). Therefore the current number of colonizing populations, and their degree of isolation from surviving populations, is probably somewhat unusual even on a geological time scale. On the other hand, high-levels of gene flow also could be episodic. Populations of L. lepidus are eventually displaced by succession, and prior to the 1980 eruption, L. lepidus in the blast zone was generally found on patches of pumice isolated by strips of forest or other vegetation (Kruckeberg 1986). This could have the effect of reducing migration among patches. Furthermore, post-eruption populations of bumblebees may be unusually large due to a massive increase in floral resources in surrounding tracts of secondary successional landscape and escape from avian and mammalian predators in primary successional areas, and L. lepidus has little in the way of floral competitors in primary successional areas. Although we cannot know the genetic structure of the pre-eruption metapopulation, metapopulations with similar geographic distribution exist on surrounding Cascade volcanoes. The picture that emerges on Mount St. Helens is that eruptions may cause episodes of large-scale extinction/recolonization leading to the formation of high among-population genetic variance; variance which is transient due to its elimination by gene flow.

The evolutionary importance of extinction/recolonization has recently been questioned on the grounds that species having metapopulations with high population turnover are also likely to be excellent dispersers, since persistence of the metapopulation depends on colonizing disturbed sites, and as a result any increase in genetic structure due to extinction/recolonization dynamics is likely to be short-lived (Harrison and Hastings 1996). The foregoing results appear to support this argument. On the other hand seeds of L. lepidus are not especially well dispersed (Wood and del Moral 1987, del Moral and Wood 1993), and its success in colonization at Mount St. Helens is due partly to the vastness of suddenly-available primary successional habitat which other species find nearly uninhabitable. High levels of gene flow may be due more to pollinator movement, which may itself be unusual. Furthermore, several generations of increased structure among a large number of populations may provide novel opportunities to respond to selection.

In particular, fixation of advantageous recessive mutations, which is difficult to achieve in large random mating populations (“Haldane’s sieve”), may be facilitated by this kind of population structure, as well as by self-fertilization (Charlesworth 1992). Differentiation caused by short-lived founder effects might result in high frequencies of advantageous recessive alleles, thereby exposing them to selection and avoiding “Haldane’s sieve”.

In summary, this study demonstrates that colonization by lupines at Mount St. Helens is associated with strong founder effects. The population level-age structure created by extinction and by colonization over a period of 10 years is associated with differences between population age-classes in the among-population genetic variance, such that colonization increases the overall amount of genetic structure. However, the increased structure caused by colonization may be transient due to high levels of gene flow. Given the unusual magnitude, even on a geological scale, of this episode of colonization, *L. lepidus* metapopulations might be undergoing a rare episode of increased genetic structure.

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Table 1. Characteristics of colonizing populations. Year founded, total population size, number of old plants, and sample sizes for each, are shown for young (Pop. 1-18) and old (19-21) colonizing populations sampled in 1991.

Pop.	Year Founded	Population		Old plants	
		Size	Sample	Size	Sample
1	91	4	3	0	0
2	90	1	1	1	1
3	90	1	1	1	1
4	90	4	4	2	2
5	90	5	5	1	1
6	90	6	4	4	4
7	90	8	8	NA	NA
8	90	20	13	NA	NA
9	89	4	4	2	1
10	89	7	5	1	1
11	88	16	7	NA	NA
12	88	24	8	1	1
13	88	25	13	2	2
14	88	31	31	7	7
15	88	90	22	20	13
16	88	91	17	10	10
17	87	64	13	24	10
18	87	105	11	7	7
Total		506	201	83	61
19	84	>20 000	7	NA	NA
20	84	>20 000	55	NA	NA
21	81	>80 000	65	NA	NA
Total		>1.2 E5	137		

Table 2. Genetic structure. Values of F_{st} and F_{is} are accompanied by 95% confidence intervals; Avg. denotes the two-locus estimate.

Group	F_{st}			F_{is}			Sample Size		
	AAT	Asp	Avg. al	AAT	Asp	Avg.	AAT	Asp	Pop
Surviving	0.04 ± 0.03	0.01 ± 0.02	0.02	0.39 ± 0.08	0.58 ± 0.08	0.49	194	194	14
Colonizing	0.27 ± 0.06	0.07 ± 0.03	0.17	0.28 ± 0.15	0.11 ± 0.11	0.19	154	279	21
Old	0.06 ± 0.03	0.03 ± 0.02	0.05	0.51 ± 0.22	0.11 ± 0.15	0.31	42	118	3
Young	0.37 ± 0.08	0.10 ± 0.02	0.26	0.10 ± 0.16	0.12 ± 0.15	0.16	112	161	18
Old Plants	0.49 ± 0.06	0.19 ± 0.02	0.35	0.32 ± 0.10	0.36 ± 0.11	0.34	52	59	15

FIGURE LEGENDS

Fig. 1. Location of sampled populations on Mount St. Helens volcano. Populations 1 and 2 correspond to two old colonizing populations, and 3-16 are surviving populations. All colonizing populations are within the yellow rectangle.

Fig. 2. Histogram of number of founders in 11 populations.

Fig. 3. F_{st} for different population age classes at each locus, with 95% confidence intervals.

Fig. 4. Allele frequencies at the a) AAT and b) Asp loci in colonizing populations. Old populations form top row (corresponding to numbers 19-21 in Table 1), the rest are young populations in the order given in table 1 (corresponding to 1-18).

Fig. 1.

Fig. 2.

Fig. 3.

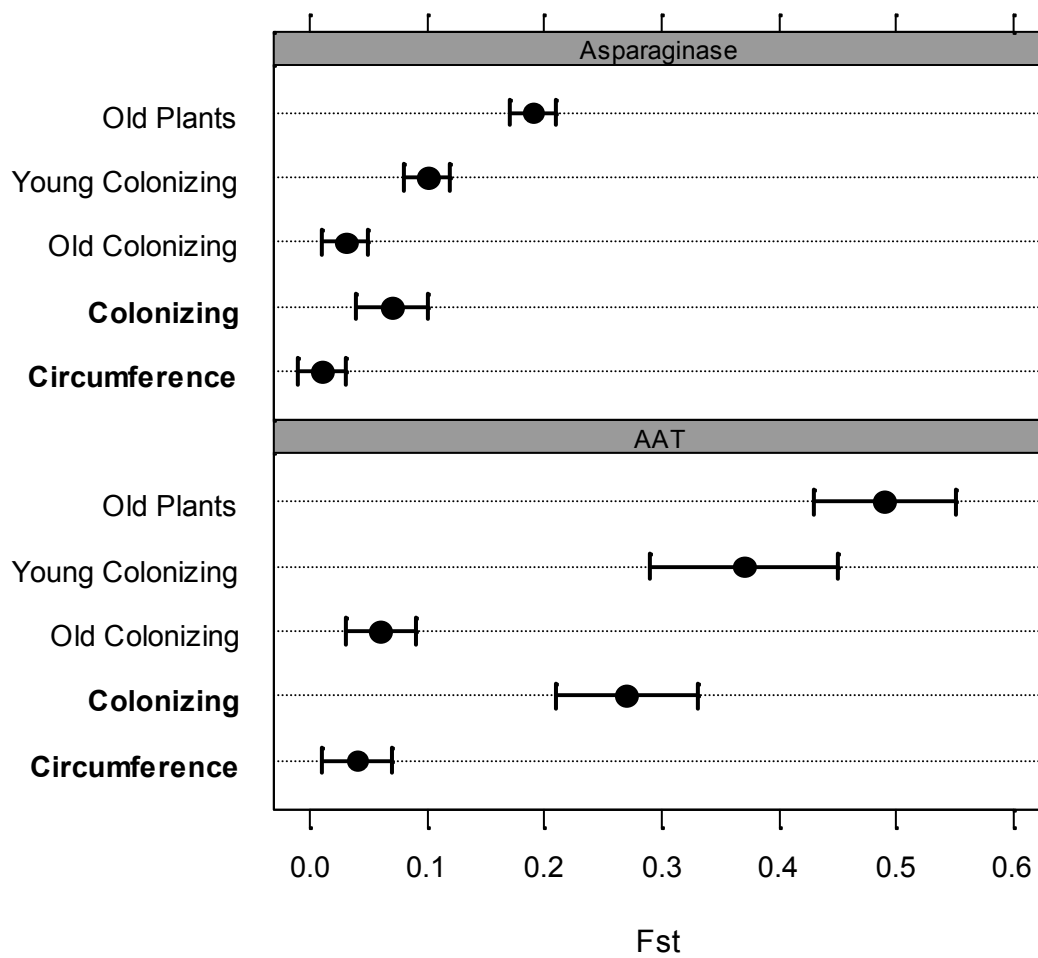


Fig. 4a.

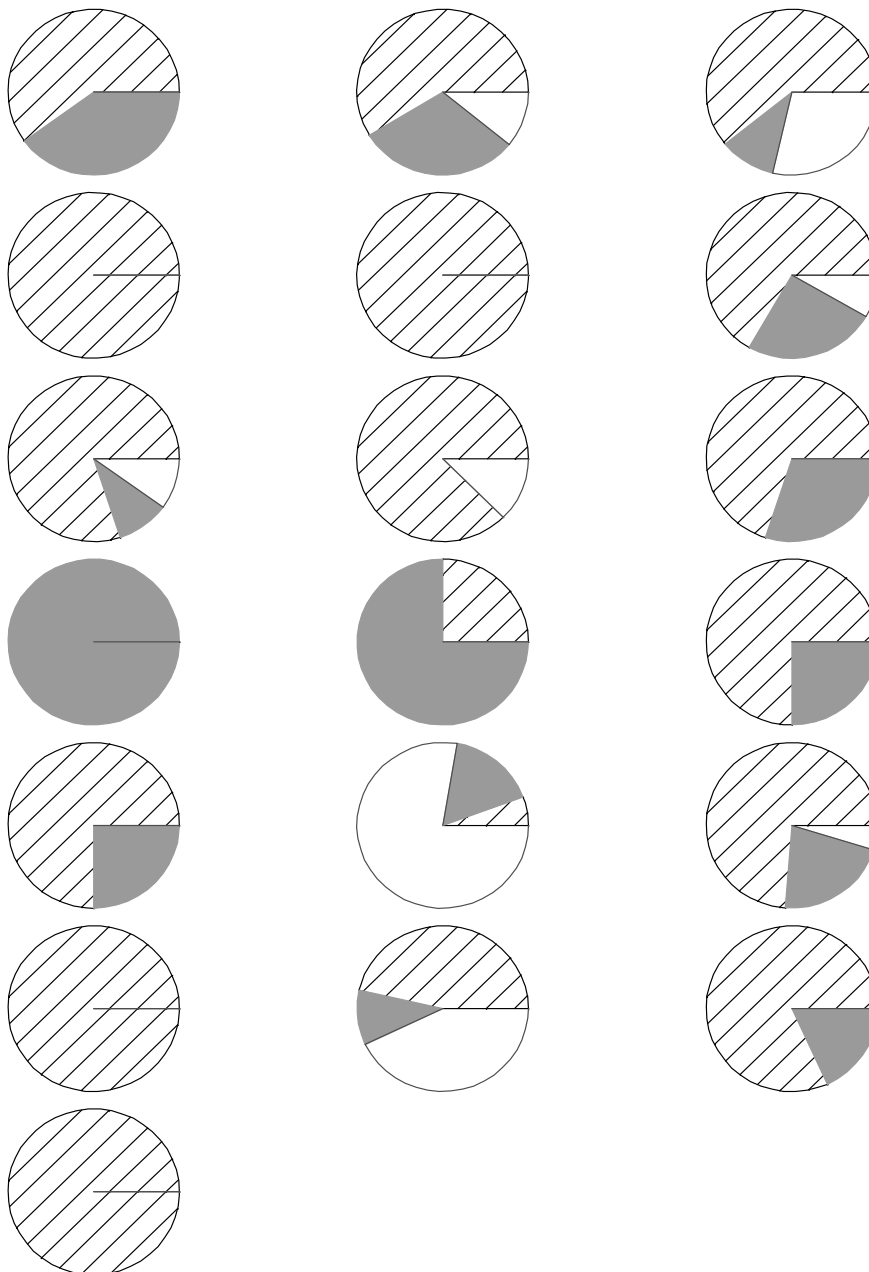


Fig. 4b.

