

Life on the edge for limber pine: Seed dispersal within a peripheral population¹

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Abstract: Interactions within populations at the periphery of a species' range may depart from those in populations more centrally located. Throughout its core range, limber pine (*Pinus flexilis*, Pinaceae) depends on Clark's nutcrackers (*Nucifraga columbiana*, Corvidae) for seed dispersal. Nutcrackers, however, rarely visit the Pawnee National Grassland peripheral population of limber pine on the eastern Colorado plains. Using live mammal trapping, fluorescent pigment tracking of disseminated seeds, and limber pine seed germination experiments in the field, we tested the hypothesis that limber pine seeds in this peripheral isolate are dispersed by nocturnal rodents. Live trapping and tracking indicated that deer mice (*Peromyscus maniculatus*, Muridae) and Ord's kangaroo rats (*Dipodomys ordii*, Heteromyidae) are the likely seed dispersers in this population. Rodents cached seeds in surface caches on tree leaf litter, rock, or soil substrate or buried them under soil, tree litter, or plants. Seeds cached under soil and plants, as opposed to surface caches, accounted for the greater number of stored seeds. Numbers of seeds per cache for buried caches was significantly higher than numbers of seeds for surface caches. The largest caches on average were those buried under plants. Also, we found that some rodent caches contained one or more seeds up to 27 d after the caches were made. In experiments simulating observed cache types, we determined that most cache types, but especially buried caches, had some germination potential. Rodents disseminated seeds over shorter distances than do nutcrackers, possibly explaining previously observed genetic substructuring in the Pawnee population. Seed dispersal by rodents also precludes the metapopulation dynamics typical of limber pine in its core range, and may lead to the loss of peripheral populations over time.

Keywords: Clark's nutcracker, *Dipodomys ordii*, limber pine, nocturnal rodents, *Nucifraga columbiana*, peripheral population, *Peromyscus maniculatus*, *Pinus flexilis*, seed dispersal.

Résumé : Les interactions qui se produisent à l'intérieur des populations situées à la périphérie de l'aire de répartition d'une espèce peuvent différer de celles qui se trouvent au cœur de l'aire. Dans la partie centrale de son aire de répartition, le pin flexible (*Pinus flexilis*, Pinaceae) est tributaire du casse-noix d'Amérique (*Nucifraga columbiana*, Corvidae) pour la dissémination de ses graines. Les casse-noix visitent toutefois rarement la population périphérique de pin flexible de Pawnee National Grassland, dans les plaines de l'est du Colorado. Nous avons testé l'hypothèse selon laquelle les graines de pin de cette population isolée sont disséminées non pas par des casse-noix, mais plutôt par des rongeurs nocturnes. Pour ce faire, nous avons utilisé trois méthodes, soit la capture de mammifères vivants, le pistage des graines disséminées qui ont été marquées par des pigments fluorescents et des expériences de germination de graines de pin sur le terrain. La capture de mammifères et le pistage des graines indiquent que la souris sylvestre (*Peromyscus maniculatus*, Muridae) et le rat kangourou d'Ord (*Dipodomys ordii*, Heteromyidae) étaient les agents probables de dissémination dans cette population. Les rongeurs ont dissimulé les graines dans des caches de surface sur la litière des feuilles d'arbres, sur les rochers ou sur le sol, ou bien les ont enfouies dans le sol sous la litière ou dans le sol à proximité de plantes. Les caches dans le sol et sous les plantes comportaient la majorité des graines emmagasinées. Le nombre de graines par cache étaient supérieurs dans les caches enfouies que dans celles de surface. En moyenne, les plus grandes caches étaient creusées sous les plantes. Nous avons également trouvé que certaines caches de rongeurs contenaient une ou plusieurs graines jusqu'à 27 jours après la constitution des caches. Lors des expériences où il y avait eu simulation des différents types de caches, nous avons observé que la plupart des types de caches, mais en particulier celles enfouies dans le sol, favorisaient d'une certaine façon la germination. Les rongeurs disséminent les graines sur de plus courtes distances que les casse-noix, ce qui pourrait expliquer la structure génétique qui est observée dans la population de Pawnee National Grassland. La dissémination des graines par les rongeurs empêche également l'établissement d'une dynamique de métapopulation identique à celle que l'on trouve dans le centre de l'aire de répartition du pin flexible, ce qui pourrait se traduire par la perte éventuelle de populations périphériques.

Mots-clés : casse-noix d'Amérique, *Dipodomys ordii*, dissémination des graines, *Nucifraga columbiana*, *Peromyscus maniculatus*, pin flexible, *Pinus flexilis*, population périphérique, rongeurs nocturnes.

Nomenclature: Flora of North America Editorial Committee, 1993; Fitzgerald, Meaney & Armstrong, 1994; American Ornithologists' Union, 1998.

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Introduction

Populations at the periphery of a species' range may differ from those more centrally located because of different community composition, demographics, selection pressures, and marginal environments. Marginal environments alone may change population characteristics. For example, Volis *et al.* (1998) found that peripheral populations of wild barley (*Hordeum spontaneum*) initiated reproduction earlier, grew faster, developed lighter seeds, and were phenotypically more variable than plants from core populations—a pattern apparently associated with unpredictable climate. In contrast, the annual plant *Corrigiola litoralis* showed reduced genetic variation and higher levels of inbreeding in peripheral populations (Durka, 1999). Nantel and Gagnon (1999) determined that a clonal herbaceous plant (*Helianthus divaricatus*) and clonal shrub (*Rhus aromatica*) showed greater demographic variability at the northern periphery of their ranges. Peripheral environments thus appear to engender differences in life history traits.

Differences in population characteristics and community composition between the core and periphery of a range may alter species interactions. Community composition, in particular, determines the numbers and kinds of species interactions that are possible, especially for potentially interdependent relationships, such as mutualisms and predator–prey interactions (Benkman, 1995; Armbruster, 1997; Thompson, 1997). For example, the relationship between the pollinating floral parasite *Greya politella* and its host plants in genus *Lithophragma* varies geographically from predator–prey to mutualism, depending in part on the abundance of other pollinators (Thompson, 1997). The dynamics of geographic variation in coevolved interactions have been addressed both theoretically and empirically by Thompson (1994). Here, we describe for limber pine (*Pinus flexilis*, Pinaceae) the replacement of its major seed disperser, Clark's nutcracker (*Nucifraga columbiana*, Corvidae), by nocturnal, seed-caching rodents at the eastern periphery of its range, which is 100 km from core limber pine populations.

In the Rocky Mountains, limber pine ranges from southern Canada south to New Mexico and Arizona (Critchfield & Little, 1966). The large, wingless seeds of this pine are primarily disseminated by a bird, Clark's nutcracker, which caches them in late summer and fall (Tomback, 1998 and references therein). As limber pine cones open in late August and early September, some of the seeds fall to the forest floor. At the same time, nutcrackers remove limber pine seeds from cones in tree canopies or from detached cones and place seeds in their sublingual pouch for transport. They bury seeds in substrate 1 to 3 cm deep, in groups of 1 to 15 seeds, with means ranging from 2.6 to 5.2 seeds per cache (Table 5 in Tomback & Linhart, 1990; Tomback, 2001). Nutcrackers carry seeds as far as 22 km to storage sites, but more typically over distances from a few metres to several kilometres (Tomback, 1998). Dispersal of limber pine seeds by Clark's nutcracker is well studied within the core range of *P. flexilis* (Lanner & Vander Wall, 1980; Tomback & Kramer, 1980; Vander Wall, 1988) and accounts for many aspects of limber pine population genetic structure and ecology (Linhart & Tomback, 1985;

Tomback & Linhart, 1990; Rebertus, Burns & Veblen, 1991; Schuster & Mitton, 1991; Carsey & Tomback, 1994; Latta & Mitton, 1997; Feldman, Tomback & Koehler, 1999; Mitton, Kreiser & Latta, 2000; Webster & Johnson, 2000). In general, populations of nutcracker-dispersed pines are not typically highly differentiated or substructured (Schuster, Alles & Mitton, 1989; Schuster & Mitton, 2000; Bruederle *et al.*, 2001) because of both long-distance pollen flow and seed transport.

In northern Colorado and southern Wyoming, limber pine demonstrates remarkable ecological amplitude, ranging in elevation from > 3,500 m at treeline to as low as 1,600 m on the eastern plains, where it forms small, isolated populations on rocky escarpments (Hess & Alexander, 1986; Schuster *et al.*, 1995; Schoettle & Rochelle, 2000). These limber pine isolates occur nearly 100 km east of the closest lower-elevation (~ 2,300 m) populations and are peripheral geographically (see Figure 1), climatically, and demographically (Schuster, Alles & Mitton, 1989; Schuster *et al.*, 1995; Schoettle & Rochelle, 2000). Patterns of precipitation and average temperature differ greatly between the plains and mountains (Schoettle & Rochelle, 2000). Whereas only 7% of the limber pine trees in the Pawnee National Grassland isolate are older than 100 y, limber pine on the eastern slope of the Colorado Front Range typically achieve ages of 335 to 1,500 y at high elevations (Schuster *et al.*, 1995). Possible remnants of a more continuous distribution of limber pine, or established recently by Clark's nutcrackers, these peripheral populations remain enigmatic in origin and ecology despite extensive analysis (Potter & Green, 1964; Wells & Stewart, 1987; Schuster *et al.*, 1995).

Nutcrackers are considered rare winter visitors to the eastern plains, including the Pawnee National Grassland (Pawnee) (Andrews & Righter, 1992; USDA Forest Service, 2000a). None have been observed, even when cones were available (Schuster & Mitton, 2000; A. W. Schoettle, unpubl. data from 1996–2000). Nutcrackers do not cache seeds in winter (Tomback, 1998), and very few seeds are likely to remain in cones by then. Furthermore, pinyon jays (*Gymnorhinus cyanocephalus*), which cache seeds within their breeding home ranges (Marzluff & Balda, 1992), are considered migrant and irruptive in the Pawnee National Grassland and rare visitors to the eastern plains in general; other pine seed-caching jays do not occur in these peripheral isolates (Andrews & Righter, 1992; USDA Forest Service, 2000a). These woodlands may be too limited in area and in resources to sustain a breeding population of jays or nutcrackers.

These isolated limber pine populations are genetically distinct from core populations with respect to numbers of polymorphic loci and observed heterozygosity (Schuster & Mitton, 2000). In addition, Schuster and Mitton (2000) discovered genetic substructure within the Pawnee population in the form of differentiated subpopulations. Gene flow values among the subpopulations were as low as those found among limber pine core populations separated by longer distances. Schuster and Mitton (2000) attributed the Pawnee population substructure to restricted seed-dispersal distances. The absence of nutcrackers in the Pawnee population during seed availability, in conjunction with genetic substructure,

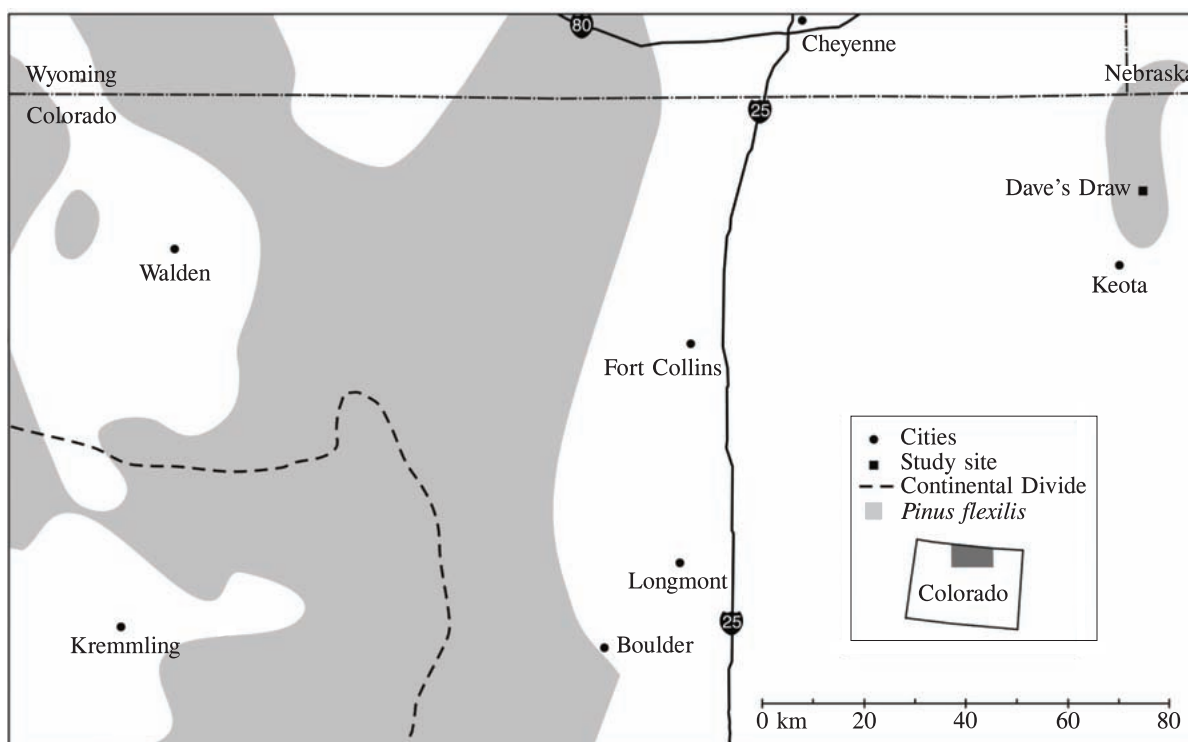


FIGURE 1. Map showing the location of the Pawnee study area in Dave's Draw, US Department of Agriculture Research Natural Area within the Pawnee National Grassland. The Pawnee limber pine population is located about 100 km east of the core range of limber pine in the Front Range of the Colorado Rocky Mountains (grey shading). Limber pine in its core range has a patchy distribution.

suggests that other agents, such as rodents or gravity, are dispersing limber pine seeds in the Pawnee population but over shorter distances than do nutcrackers.

Additional differences related to seed-dispersal ecology occur between the Pawnee and core populations. In the core range, mature limber pines frequently grow in "tree clusters", in which two or more different genets are fused or contiguous at the base, a consequence of seed dispersal by nutcrackers (Linhart & Tomback, 1985; Carsey & Tomback, 1994). Few tree clusters were found within the Pawnee population (Schuster and Mitton, 1991). This difference could result either from mode of seed dispersal in the Pawnee population or from harsher environmental conditions reducing survival of cluster members (Feldman, Tomback & Koehler, 1999), as we see for the piñon pines (Vander Wall & Balda, 1977; Hollander & Vander Wall, 2004). Furthermore, absence of genetic substructure within core populations is suggested by the pattern of relatedness within and among limber pine tree clusters: Adjacent tree clusters tend to show little to no relatedness (Carsey & Tomback, 1994). Similar random fine population structure among tree clusters has been found for other nutcracker-dispersed pines, which comes from many birds caching seeds from different parent trees in a haphazard spatial pattern within a population (Tomback & Linhart, 1990; Rogers, Millar & Westfall, 1999; see Bruederle *et al.*, 2001, for review). Thus, the substructure in the Pawnee population strongly suggests a different mode of seed dispersal than nutcrackers.

Studies have implicated seed-caching rodents in the secondary dispersal of pine seeds, both with and without seed wings (Vander Wall, 1992; 1994; 1997). Rodents for-

age on the forest floor for seeds that have fallen from or been blown from open cones and cache them within their home ranges, carrying them in cheek pouches or holding them between their incisors. Chipmunks (*Tamias* spp.) and other potential diurnal seed dispersers or predators, (e.g., *Tamiasciurus hudsonicus*) (Vander Wall, 1992; 1993; Benkman, 1995) are not present in the Pawnee National Grassland, but deer mice (*Peromyscus maniculatus*) and other nocturnal seed-cachers are (Fitzgerald, Meaney & Armstrong, 1994; USDA Forest Service, 2000b).

We focused on the hypothesis that nocturnal rodents disseminate limber pine seeds in the Pawnee isolate and may account for genetic substructure. To test this hypothesis, we determined (1) whether nocturnal rodents would cache limber pine seeds presented under tree canopies, (2) the identities of caching nocturnal rodents, (3) how far seeds are transported by nocturnal rodents, (4) the cache types made by nocturnal rodents, (5) whether caches are always completely recovered by rodents, and (6) whether any of the cache types might result in seed germination. This study did not attempt to determine the relative contribution of different cache types and particular rodent species to limber pine regeneration in the Pawnee population, although some inferences are possible.

Methods

POPULATION GENETICS OF LIMBER PINE POPULATIONS: BACKGROUND INFORMATION

Schuster and Mitton (2000) found the Pawnee limber pine population to have the lowest percentage of polymor-

phic loci, the lowest average number of alleles per polymorphic locus, and the lowest observed heterozygosity compared to three core mountain populations and one larger peripheral isolate (Wilson/Foster) to the north. The three core mountain populations shared high genetic similarity, but both peripheral populations “were more differentiated and lacked some rare alleles present in the mountain populations” (Schuster & Mitton, 2000). Although allozyme allele frequencies differ significantly among limber pine populations in general (Schuster, Alles & Mitton, 1989; Schuster & Mitton, 2000), Schuster and Mitton (2000) also found genetic substructure within the Pawnee population, manifested as significant differences in allele frequencies among five “topographic” subpopulations.

Seed dispersal by nutcrackers in the core ranges frequently results in mature limber pines growing in “tree clusters”, in which two or more different genets are fused or contiguous at the base (Linhart & Tomback, 1985; Carsey & Tomback, 1994). With each single or clustered limber pine tree growth form considered a “tree site”, tree clusters were estimated to occur at about 20% of tree sites in each of four populations at different elevations in the Front Range of Colorado (Carsey & Tomback, 1994) but at fewer than 10% of tree sites in the Pawnee population (Schuster & Mitton, 1991). Furthermore, absence of genetic substructure within core populations is suggested by the pattern of relatedness within and among limber pine tree clusters: in the Rainbow population, Colorado Front Range, tree cluster members, which usually come from the same parent trees, were related on average between half and full sibs ($r = 0.43$). When tree cluster members were compared to members of nearby clusters, the mean r was 0.01, indicating little or no relatedness (Carsey & Tomback, 1994).

STUDY AREA

From August through December 2000, and then in June 2001, we studied seed dispersal in Dave’s Draw, a USDA Forest Service Research Natural Area within the Pawnee National Grassland (Figure 1). Limber pine at Dave’s Draw occurs on the north-facing rim of an east–west trending escarpment with an elevation of 1,630 m (see Figure 1 in Schuster & Mitton, 2000; see Schoettle & Rochelle, 2000 for study site details). The escarpment woodland comprises both limber pine and Rocky Mountain juniper (*Juniperus scopulorum*), with understory including *Yucca glauca*, *Artemisia* spp., *Rhus triloba*, *Chrysothamnus nauseosus*, and *Ribes* spp. We initially studied seed dispersal in two areas below the escarpment but soon focused on the area with greatest dispersal activity.

WOODLAND COMPOSITION AND REGENERATION SURVEYS

We selected for study an area near the southern boundary of Dave’s Draw that allowed reasonably safe access for night work. Along the escarpment, much of the bedrock was exposed, with shallow soil and scattered trees. Below the escarpment, soil development was better and supported denser woodland, with steep slopes leading down to the draw. Two 50- × 50-m plots, A and B, were established to assess variation in woodland composition and age structure within the study area. Plot A included part of the escarp-

ment rock; it was just west of Plot B and nearly contiguous. Initially, we set up seed-dispersal studies within both plots, but we had no seed-dispersal activity in Plot A; thus, seed dispersal was studied within Plot B.

We recorded all trees and all limber pine seedlings within Plots A and B. Limber pine trees were classified as seedlings if below 1.5 m in height, and any trees with multiple stems were considered a single “tree site”. Tree densities were adjusted to number of tree sites per hectare. We also surveyed the terrain above and along the escarpment rock (rimrock) for seedlings. Seedling ages were estimated by counting above-ground terminal bud scars on the main stem, which is a method that is well correlated with ring counts (Parent, Morin & Messier, 2000) and is non-destructive. Although the accuracy of this method declines with tree age, the smooth bark of the limber pine seedlings enabled the bud scars to be located and counted with confidence. This information revealed patterns of recruitment over time.

SEED-DISPERSAL TRACKING

We studied seed caching by nocturnal rodents using fluorescent pigment tracking (Longland & Clements, 1995) for six nights between 25 August and 21 October. Seed stations were placed under limber pine canopies at one to four locations each night by 1900. Each station provided 200 limber pine seeds (collected from Medicine Bow National Forest, Wyoming and microwaved to prevent germination) coated with fluorescent pigment (Radiant Color, Richmond, California). The seeds were placed in a petri dish in the centre of a sheet of sandpaper impregnated with the same pigment colour and stapled to an aluminum foil tray (~ 30 × 50 cm). This mode of seed presentation was intended to simulate natural accumulations of limber seeds under tree canopies.

Using a UV light (SuperBright 2010LW with 368 nm lamp and battery pack, UV Systems, Inc., Renton, Washington), we followed the tracks of animals from seed stations to cache sites or burrows. Tracking and data collection began by 0300 and continued until dawn. Sites with buried caches were usually marked by fluorescent blotches on the soil surface. All cache sites were examined in the dark, marked with surveyor’s flags, and revisited for additional description after sunrise. Caches were excavated, described (surface or buried cache, number of seeds per cache, depth of cache, substrate type, presence or absence of leaf litter, microsite), replaced, and usually revisited on succeeding field days; distinct pigmented rodent footprints were measured at night. Straight-line distances between caches and seed stations were determined with 50-m transect tapes. Pink, yellow, blue, and green pigments were used to determine the feeding station of origin of tracks and seeds. Beginning on 29 September, four seed stations (upper, lower, east, west) per study night were established within plot B, separated by 29.0 to 42.5 m distance. After 25 and 26 August, weekends with full or nearly full moons were avoided for fieldwork.

RODENT TRAPPING

On the nights of 29 September to 2 October, we trapped nocturnal rodents to identify potential seed-cachers, but

avoided trapping in our seed-dispersal study area. We established three traplines totalling 106 Sherman traps ($7.5 \times 7.5 \times 22.5$ cm), with each trapline consisting of paired traps spaced at 10-m intervals. One trapline consisted of 58 traps in grassy gullies below the escarpment east of our seed-dispersal study area; the second comprised 30 traps in vegetation primarily of *Rhus*, *Yucca*, and *Opuntia* along the rimrock above the escarpment; the third included 18 traps along a hillside west of our seed-dispersal study area. We set and baited traps with a mixture of rolled oats and peanut butter by 1800 and checked traps each morning at 0700. We measured the hindfoot of each individual captured. We released individuals unmarked at capture sites.

SIMULATED SEED-CACHING EXPERIMENT

To determine whether any of the nocturnal rodent cache types commonly observed in the fluorescent pigment tracking study had potential for limber pine recruitment, we set up an experiment using seeds collected in September 2000 at Dave's Draw. The experiment was not designed to be a rigorous examination of the relative contribution of different cache types, but rather to provide empirical evidence for germination potential. Observed surface caches tended to contain only one or two seeds, and when two seeds were present, they were usually side by side and touching. Observed buried caches contained an average of between four and five seeds (Table I).

On 6 December 2000, we established five replicates of the following cache types above the escarpment west of the seed-dispersal study area within Plot A and B: five caches buried 2 cm deep under soil (five seeds per cache), five caches on the soil surface (two seeds per cache), and five caches buried in soil at the base of herbaceous plants (in vegetation tussocks, five seeds per cache). For each cache type there were 25 caches total, with five groups of five caches each. Caches were spaced at least 10 cm apart. Each replicate set was covered by a hardware cloth cage to minimize seed theft by rodents and separated by a minimum of 3 m distance. In addition, we established three replicates of five caches (two seeds each) on surface tree litter, also

protected by hardware cloth and separated by at least 3 m distance, for a total of 15 caches in three groups of five caches. We examined each cache again on 11 June 2001 and recorded the number of seeds that had germinated or were missing, empty, or with dead embryos. Germination was defined as either radical emergence from the seed or cotyledon seedling emergence from the soil.

STATISTICAL ANALYSES

We compared seed-dispersal distances and numbers of seeds per cache for both buried and surface caches with Wilcoxon Rank Sum tests (Statistix 8 Analytical Software, Tallahassee, Florida, USA), a powerful non-parametric statistical procedure for comparing two samples. Non-parametric tests make no assumption about the specific distribution of data other than independence and are thus often useful for relatively small samples of unknown distribution (Siegal & Castellan, 1988).

The proportion of seeds that germinated per cache was calculated based on the total number of recovered seeds per cache. For the simulated cache experiment, germination success was calculated in two ways: 1) missing seeds were excluded from the total seeds in each cache before calculations of proportion of seeds germinated, and 2) germination success was based on the proportion of caches of each type within a replicate with at least one germinated seed. These measures compensate for different experimental cache sizes. Chi-square Goodness of Fit tests for single samples (Siegal & Castellan, 1988) were used to examine the proportion of empty seeds, seeds with dead (withered) embryos, and missing seeds in separate tests across the different cache types. All seeds were originally randomly distributed from a bag into caches, and the seeds within the bag were constantly mixed. Consequently, we treated the distribution of seeds into cache types as independent events and assumed that any differences in seed condition arose from cache type.

We used two statistical approaches to compare numbers of germinated seeds among the different simulated cache types, as follows: for one statistical approach, we used the data for the two measures of germination success for each

TABLE I. Summary statistics from the fluorescent pigment tracking study conducted 25 August to 21 October 2000. Limber pine seeds were transported and cached by nocturnal rodents at the Pawnee study area, Pawnee National Grassland, Colorado. Means are presented with standard errors in parentheses.

Cache type	No. of located caches	Total no. of seeds cached	No. of seeds per cache Mean (SE)	No. of seeds per cache Range	Distance (m) from seed station Mean (SE)	Distance (m) from seed station Range
BURIED						
Under litter	2	3	1.5 (0.5)	1-2	7.05 (6.95)	0.10 - 14.00
Under soil	7	16	2.3 (0.4)	1-4	8.63 (1.59)	4.46 - 16.20
Under plant	6	50	8.3 (3.3)	1-22	4.20 (0.63)	2.34 - 6.06
In plant	5	18	3.6 (1.4)	1-9	12.82 (3.63)	1.50 - 20.57
SURFACE						
On litter	6	12	2.0 (0.8)	1-6	2.77 (1.38)	0.50 - 9.44
On rock	5	8	1.6 (0.2)	1-2	7.22 (1.32)	2.68 - 10.34
On soil	5	6	1.2 (0.2)	1-2	6.94 (2.68)	2.23 - 17.40
OVERALL						
Buried	20	87	4.4 (1.2)	1-22	8.2 (1.3)	0.1 - 20.6
Surface	16	26	1.6 (0.3)	1-6	5.5 (1.1)	0.5 - 17.4
Combined	36	113	3.1 (0.7)	1-22	7.0 (0.9)	0.1 - 20.6

cache type replicate, as described above. The sample size was $n = 5$ each for caches buried under soil, in soil under plants, and on soil surface and $n = 3$ for caches on litter. Germination success was compared between all buried cache replicates and all surface cache replicates with non-parametric Wilcoxon Rank Sum tests. The data based only on the five replicates of three cache types each were analyzed together with Friedman Two-way Analysis of Variance (Statistix 8 Analytical Software, Tallahassee, Florida, USA) (Siegel & Castellan, 1988). Whereas these applications of non-parametric statistics may not be perfect because of differences in the distribution of proportions between the five-seed and two-seed caches, the outcome was informative in combination with the second analysis.

For the second approach, we considered each seed to be independent and examined the exact probability of germination depending on cache type. Independence among seeds implies that one seed germinating has no effect on the others in the same cache. Dependence would imply that the germination of one seed in a cache increases or decreases the probability of other seeds germinating in the cache. Thus, independence, while not likely true in this case, simplifies calculations and is a moderate assumption that is not likely to have a great effect on the results.

Having assumed that the number of seeds that germinated in each cache followed a binomial distribution, we performed likelihood ratio tests to check for differences in the probability of germination between pairs of groups. In essence, the likelihood ratio test compared the two estimates of probability of germination for the two cache types under consideration, with the probability of germination estimated by combining information from both cache types to form a weighted average germination probability. If the individual cache-type germination probabilities were different from their average, the test was "significant". This test is akin to the typical z -test for a difference in two-proportions except that exact P -values were calculated based on the binomial distribution rather than a normal distribution approximation. The alpha value was shrunk to $0.05/3$ or 0.017 , because there were three pair-wise comparisons of cache type. These pairwise comparisons used two different data sets: (1) the number of seeds that germinated out of the total seeds cached in the five replicates for a given cache type (125 for seeds cached under soil and in soil under plants and 50 for seeds cached on the soil surface) and (2) the number of seeds that germinated out of the total seeds cached minus the number of missing seeds for each cache type.

Results

WOODLAND COMPOSITION AND REGENERATION SURVEY

Plot A supported an overall density of 184 tree sites per ha, with limber pine at 72% of the tree sites and seedlings at 43% of limber pine site. Plot B comprised 228 tree sites per ha, with limber pine at 61% of sites; only 7% of the limber pine were seedlings. Thus, regeneration was evident within both plots but not uniform in distribution.

During the study, we had seed-dispersal activity only within Plot B, despite the greater proportion of seedlings

in Plot A. Disperser distribution and conditions suitable for germination may vary over time. The presence of a higher proportion of mature trees in Plot B may have provided more suitable habitat for nocturnal rodents but perhaps less suitable habitat for seedling establishment.

We located a total of 36 single and clustered seedlings on the terrain above the rimrock. For the plot and rimrock survey combined ($n = 55$ seedlings), the distribution of seedling germination years was continuous, with the exception of two multi-year gaps, 1976 to 1980 and 1996 to 2000. Both gaps are evident from the rimrock sample (Figure 2).

Most limber pine seedlings did not occur directly under limber tree canopies, and many seedlings (especially on the rimrock) occurred upslope from mature limber pine trees, indicating that gravity (*i.e.*, seedfall) was not the typical form of seed dispersal in this isolate.

SEED-DISPERSAL TRACKING

The number of caches located per night grew from two to 20 as we focused our study within the area of Plot B. Nocturnal rodents removed 0 to 200 seeds per tray per night ($x = 149.9$ seeds ± 17.1 SE), leaving behind both eaten and uneaten seeds. Some tracks led into burrows, probably for larder hoarding, which does not result in seed germination (Vander Wall, 1997). Caching rodents either placed the seeds in surface caches ($n = 16$) on tree leaf litter, rock, or soil substrate or buried them ($n = 20$ caches) within plants or as deep as 3 cm under soil, tree litter, or plants (Table I). Buried caches accounted for the greater number of stored seeds (Table I). Cache sizes for buried caches were significantly higher than those for surface caches (Wilcoxon Rank Sum test, $z = 2.636$ with correction for continuity and ties, $P = 0.0084$, two-tailed) (Table I). The largest caches on average were those buried under plants. Travel distances from feeding stations to caches ranged from 0.10 to 20.6 m. Distances to buried caches *versus* surface caches, however, did not differ statistically ($z = 1.353$ with correction for continuity and ties, $P = 0.176$, two-tailed) (Table I).

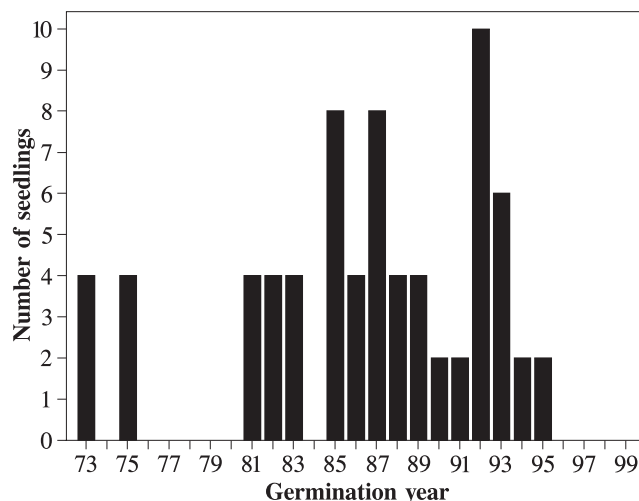


FIGURE 2. The distribution of seedling germination years among the 36 limber pine seedlings and saplings surveyed above the rimrock in the Pawnee limber pine population.

We revisited most caches ($n = 29$) from 1 to 27 d after they were made, with many revisited 7 d later ($n = 19$ caches). Of the caches revisited, four of 20 buried caches (20%) and three of nine surface caches (33%) still contained one or two seeds. Two buried caches examined 21 and 26 d after they were made still contained the original numbers of cached seeds.

RODENT TRAPPING

Nightly trapping success ranged from 16 to 29% per trapline; the highest trapping success for all three nights combined (37%) occurred along the hillside (compared with 13% for the rimrock and 24% below the escarpment). The 73 captures included 68 deer mice, three western harvest mice (Muridae: *Reithrodontomys megalotis*), and two Ord's kangaroo rats (Heteromyidae: *Dipodomys ordii*). Also, on October 27–28, we trapped a bushy-tailed woodrat (Muridae: *Neotoma cinerea*) next to one of the seed stations.

Hindfoot lengths for 66 deer mice ranged from 18 to 21 mm (mean = 19.4), and hindfoot measurements for the three western harvest mice were 16, 17, and 18 mm. Various fluorescent tracks, whether they led to caches or burrows, were compatible in size and character with the tracks of all four trapped species.

GERMINATION SUCCESS FOR THE SIMULATED SEED-CACHING EXPERIMENT

We were unable to relocate all of the sown seeds in 35% of the simulated caches, suggesting that the enclosures were not animal-proof. Seventy-five percent of the incomplete caches lacked only one (56%) or two (19%) seeds. However, there were a disproportionately high number of missing seeds from under plant caches (33% of seeds) compared to the other cache types ($\chi^2 = 16.8$, $df = 3$, $P < 0.01$), and the explanation is not clear.

Seeds cached under plants or in soil had higher germination success than those cached on the surface of soil or in litter (54% and 44% versus 10% and 0%, respectively) (Figure 3). Almost 90% of the buried caches, but fewer than 15% of the surface caches, resulted in at least one successful germinant. Results from the tracking study (Table I) suggest that greater proportions of cached seeds are in fact placed by rodents in the more favourable cache sites (under soil, under plant) for successful germination.

Germination success differed significantly between all surface cache replicates (on soil and on litter, $n = 8$) and all buried cache replicates (under soil and in soil under plant, $n = 10$), based on both the average proportion of germinants per cache, minus missing seeds, and proportion of caches with one or more germinants within a replicate (Wilcoxon Rank Sum tests: $z = 3.509$ with correction for continuity and ties, $P = 0.0004$, two-tailed, and $z = 3.394$, $P = 0.0003$, two-tailed, respectively). Using data from the five replicates of three cache types, we found significant differences among caches placed on soil surface, buried under plants, and buried under soil for both measures of germination success (Friedman Two-way Analysis of Variance: average proportion of germinants, total seeds minus missing seeds, $Fr = 7.600$, $P = 0.0224$, $df = 2$; proportion of caches with one or more germinants, $Fr = 7.895$, $P = 0.0193$, $df = 2$). Both

measures of germination success were significantly lower for surface caches ($n = 5$) versus either caches buried under soil ($n = 5$) or in soil under plants ($n = 5$) (Wilcoxon Rank Sum tests: z values between 2.432 and 2.562, all P -values between 0.010 and 0.015). In contrast, germination success for caches buried under soil compared to caches buried in soil under plants was not significantly different (Wilcoxon Rank Sum tests: average proportion of germinants, total seeds minus missing seeds, $Fr = 0.841$, $P = 0.400$; proportion of caches with one or more germinants, $Fr = 0$, $P = 1.00$).

Analyses based on probabilities of germination confirmed that seeds buried in soil under plants and under soil had significantly higher probabilities of germination than seeds placed on the soil surface (Table II). Mixed results were obtained when germination probabilities were compared between caches buried under soil and caches in soil under plants: when full cache sizes were used, the differences were statistically significant ($P = 0.0002$), suggesting that seeds in soil under plants had a lower probability of germination. However, when missing seeds were considered, the differences were not significant ($P = 0.0185$) (Table II).

All of the relocated seeds in the on-litter caches were empty or with dead embryos. Although we had distributed seeds at random across cache types, we found a disproportionately high number of empty seeds in caches on soil (40%) and on litter (67%) and disproportionately fewer under plants (18%) ($\chi^2 = 9.40$, $df = 3$, $P = 0.0244$). There were no differences across cache types for seeds with dead embryos ($\chi^2 = 1.11$, $df = 3$, $P = 0.775$), suggesting that

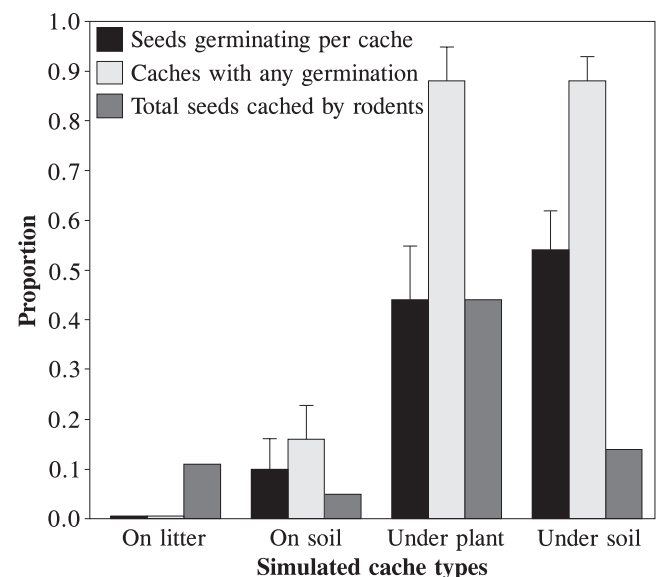


FIGURE 3. Germination success among four types of simulated rodent caches compared in two different ways. First, missing seeds are excluded from the total possible seeds in each cache, yielding an adjusted proportion per cache of germinated seeds, and second, the average proportion of caches with at least one germinated seed was calculated across replicates ($n = 3$ replicates for on-litter cache types and $n = 5$ replicates for other cache types). Finally, the proportion of total seeds placed by nocturnal rodents during the fluorescent pigment tracking study in each of the four cache types is also depicted in the histogram (other cache types occurred in the study, so the total does not equal 1.00).

TABLE II. Germination probabilities were compared between paired-simulated cache types. Probability data were based on seed germination within the five replicates of the following three simulated cache types: BP - buried under plants (five seeds per cache); BS - buried under soil (five seeds per cache); SS - soil surface (two seeds per cache). The probabilities were calculated in two ways: based on original cache sizes and based on original cache sizes minus missing seeds. Alpha value was established at $0.05/3 = 0.017$. See text (Statistical Analyses) for additional detail.

Cache types	Probability	
	All seeds	Minus missing seeds
Buried under soil	62/125 = 0.496	62/110 = 0.564
Buried under plant	37/125 = 0.296	37/84 = 0.440
On soil surface	5/50 = 0.100	5/44 = 0.114
SS versus BS	$P < 0.001$	$P < 0.001$
SS versus BP	$P = 0.0009$	$P = 0.0009$
BS versus BP	$P = 0.0002$	$P = 0.0185$ (ns)

these seeds had been randomly distributed when the experiment was constructed.

Discussion

Recent studies of plants have confirmed restricted gene flow, reduced genetic variation, and genetic differentiation among peripheral populations (Durka, 1999; Lammi, Siikamäki & Mustajärvi, 1999). Researchers have also found ecological, demographic, and physiological differences in plants between core and peripheral populations (Volis *et al.*, 1998; Nantel & Gagnon, 1999). The isolated limber pine population in the Pawnee National Grassland lends support to the contention that peripheral populations are ecologically and genetically distinct.

The dependence of limber pine on Clark's nutcracker for seed dispersal is well documented from several geographic regions within the core range of limber pine (Lanner & Vander Wall, 1980; Tomback & Kramer, 1980; Vander Wall, 1988). Nutcrackers, however, are rare visitors to the limber pine population in the Pawnee National Grassland. Here, we have demonstrated that the principal seed dispersers for limber pine in this peripheral population are most likely to be nocturnal seed-caching rodents rather than nutcrackers by (1) showing that nocturnal rodents cache limber pine seeds presented under tree canopies, (2) determining the presence and identity of seed-caching nocturnal rodents in the Dave's Draw study area, (3) showing that the dispersal distances for seeds transported by nocturnal rodents are short and thus could account for genetic substructure, (4) locating and describing the cache types made by nocturnal rodents, (5) showing that caches are not always recovered by rodents, and (6) demonstrating that some of the cache types might result in seed germination. In addition, the distribution of seedlings along the escarpment above cone-bearing trees indicates that gravity is not an important mechanism for seed movement.

Schuster and Mitton (2000) determined that the Pawnee limber pine population was more genetically differentiated and lacked several rare alleles relative to core populations to the west. Schuster and Mitton (2000) also found genetic substructure within the Pawnee population that was com-

parable in magnitude to differences among core populations and attributed it to restricted seed-dispersal distances. The short distances that rodents dispersed seeds in this study are consistent with the establishment of small genetic subpopulations within the Pawnee study area, as identified by Schuster and Mitton (2000). Therefore, consistent with Schuster and Mitton's (2000) suggestion, our study indicates that the Pawnee limber pine isolate has experienced a shift in seed-dispersal ecology compared to core populations.

The distribution of estimated seedling germination years was nearly continuous, with seedlings absent from two multi-year gaps, 1976 to 1980 and 1996 to 2000 (Figure 2). This provides circumstantial evidence for seed dispersal by agents other than or in addition to nutcrackers, because nutcrackers have rarely been recorded in the Pawnee National Grassland. If nutcrackers alone harvested and dispersed seeds in the Pawnee limber pine population every 5-10 y, their activity would still not account for the observed distribution of regeneration. The gaps in regeneration were probably caused either by low seed production or drought conditions. Schuster *et al.* (1995) reported poor recruitment at Pawnee in the 1930s and 1940s, corresponding to regional drought. Both higher summer temperatures and lack of rainfall in the Pawnee population compared to mountain populations (Schoettle & Rochelle, 2000) may lower survival of young seedlings, particularly in drought years.

Limber pine is considered a bird-dispersed pine (*sensu* Tomback & Linhart, 1990), and little is known about the contribution of rodents to seed dispersal for this species in general. However, the fine-scale genetic structure within and among tree clusters in core populations of limber pine (Carsey & Tomback, 1994) is very similar to that of whitebark pine (*P. albicaulis*) (Rogers *et al.*, 1999), which is essentially an obligate mutualist of Clark's nutcrackers (Tomback, 1982; Tomback & Linhart, 1990). Although rodents may contribute some limber pine recruitment within the core range, their more restricted seed-dispersal distances are not evident in the genetic structure of those populations.

In the Pawnee population, deer mice and Ord's kangaroo rats appear to be the primary seed cachers (scatter-hoarders), because harvest mice do not store seeds (Jones *et al.*, 1983; Vander Wall, 1990; Webster, 1999). Previous work has shown that singleleaf pinyon (*P. monophylla*) seeds, which are also large and wingless, may be dispersed by several diurnal and nocturnal rodent species, the latter including deer mice, piñon mice (*Peromyscus truei*), Great Basin pocket mice (*Perognathus parvus*), and Panamint kangaroo rats (*Dipodomys panamintinus*) (Vander Wall, 1997; Hollander & Vander Wall, 2004). Similarly, the large, wingless seeds of Colorado pinyon (*Pinus edulis*) are dispersed by the piñon mouse and brush mouse (*Peromyscus boylii*) (Pearson & Theimer, 2004). The seeds of both pinyons are also dispersed by pinyon jays and Clark's nutcrackers (Vander Wall & Balda, 1977; Ligon, 1978; Tomback, 1978).

Deer mice make mostly single-seed caches, whereas kangaroo rats carry 16 or more seeds in their cheek pouches and make larger caches (mean = 3.5 seeds) (Vander Wall, 1997). In our study, most surface caches were probably made by deer mice, and buried caches by both deer mice and kangaroo rats (Table I).

Our simulated caches indicated that the environmental characteristics of cache sites strongly affect the germination potential of seeds. We found that surface caches are unfavourable for sustaining seed viability, and they may be more vulnerable to predation in general. Intense solar radiation and high temperatures may have destroyed the contents of exposed seeds (Levitt, 1972). In general, caches that were buried in soil under plants or under soil had the greatest chance of germinating in the Dave's Draw study areas, probably because of greater moisture and protection from solar radiation. Thus, rodents that bury seeds are the most effective dispersers.

Hollander and Vander Wall (2004) found that seed germination was highest among caches buried at an intermediate depth, from 1 to 4 cm, rather than at more shallow or deeper depths, and Pearson and Theimer (2004) found in greenhouse experiments that caches placed in small particle soils had higher seed germination potential than caches placed in large-particle soils. Furthermore, both studies indicated that cache microsite affects seedling survival: seedlings from caches buried under shrubs (Hollander & Vander Wall, 2004) or near rocks survived longer than those buried in open microsites, because of higher soil moisture (Pearson & Theimer, 2004).

Additional impacts on seed germination in our study include the return of rodents either to eat or recache seeds. When we revisited caches, 20% of the buried caches and 33% of the surface caches still contained one or two seeds, and two buried caches were intact. Thus, in our study, caches made by nocturnal rodents were not always emptied and thus were conducive to seed germination. It is also possible that some of the retrieved seeds were recached rather than eaten and that the recached seeds may still germinate (Hollander & Vander Wall, 2004). The greatest proportion of seeds cached by rodents in our study were placed under small tussocks of vegetation, and these caches were also among the most successful for seed germination. However, rodent dispersers that make large caches, such as those of 9 to 22 seeds per cache (Table I), may reduce the probability of seedling survival, because of competition for moisture.

It could be argued for the simulated cache experiment that surface caches contained fewer seeds per cache and, consequently, had lower recruitment potential from a statistical perspective, although our measures of germination success and statistical analyses compensated for this. The condition of the surface seeds, however, suggests that seeds that are not buried become inviable faster than seeds that are buried. In contrast, the tussock microsite (seeds buried in soil under plants and in plants) appears to be favourable for seed germination and seedling survival, probably because of more favourable moisture conditions and protection from intense solar radiation. Observed cache types in soil under plants and in plants contained the highest proportion of seeds cached by rodents (Table I; Figure 3). Previous work in other semiarid steppe ecosystems has also found that tussock vegetation supports seed germination (Maestre *et al.*, 2001). In general, caches that were buried in soil, in soil under plants, or in plants had the greatest chance of germinating in the Dave's Draw study area. The intent of this

study, however, was simply to determine if any of the cache types made by rodents could lead to recruitment, which we confirmed. A rigorous study of the quality of limber pine seed dispersal, including determining whether nocturnal rodent species differ in their contribution as dispersers, awaits future investigation.

Nutcracker seed-caching behaviour and microsite selection in general may be more favourable to seed germination and survival than the caching behaviour and microsite use of nocturnal rodents, but this also requires further study. Nutcrackers bury most of their caches from 1 to 3 cm under substrate, never making surface caches, and also place many of their caches next to tree bases, snags, rocks, logs, and other shade-casting objects, which may increase seedling survival under conditions of water stress; only a small proportion of caches are placed under plants (Tomback, 1978; 1982). Disperser quality in the Pawnee limber pine population has apparently been sufficient to maintain recruitment over time, but climate change could bring additional challenges with higher temperatures and more variable precipitation patterns.

Although both limber and whitebark pine show large-scale population structure in mitochondrial DNA (mt-DNA) haplotypes, which may be explained by patterns of glacial refugia (Mitton, Kreiser & Latta, 2000; Richardson, Brunsfeld & Klopfenstein, 2002), local populations of nutcracker-dispersed pines are typically not very differentiated, nor do they tend to show family or subpopulation structure (*e.g.*, Schuster, Alles & Mitton, 1989; Bruederle *et al.*, 2001 and references therein). Schuster and Mitton (2000), however, determined that the Pawnee limber pine population has genetic substructure that is comparable in magnitude to differences among core populations. Furthermore, they could not account for this substructure by restricted pollen flow and suggested that restricted seed dispersal may be responsible for the genetic differences. Our study indicates that the Pawnee limber pine isolate has experienced a shift in seed-dispersal ecology compared to core populations. We also show that nocturnal rodents disperse seeds over shorter distances than do nutcrackers. Given the unique genetic substructure of the Pawnee population, our observations support the hypothesis that nocturnal rodents rather than nutcrackers are the main seed dispersers within this peripheral population.

Limber pine in its core range forms a metapopulation structure, with local extinction from successional replacement by shade-tolerant conifers on less extreme sites. New limber pine populations are initiated after fire by long-distance seed dispersal by Clark's nutcrackers (Rebertus, Burns & Veblen, 1991; Webster & Johnson, 2000). If long-distance seed dissemination is absent in eastern limber pine isolates, no metapopulation dynamics are possible to replace extinct populations. Each escarpment isolate may well expand and contract over time, with the possibility of extinction.

This study demonstrates that peripheral populations can experience shifts in mutualistic interactions, and specifically seed dispersers, relative to core populations. In summary, for a peripheral population of limber pine where the primary seed disperser is typically absent, we have shown that the

pattern of seed dispersal by nocturnal rodents is consistent with both continuous population recruitment and the unique genetic substructure found by Schuster and Mitton (2000).

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