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Spatial and Genetic Structure within Populations of Wild American Ginseng (*Panax quinquefolius* L., Araliaceae)

J. M. Cruse-Sanders and J. L. Hamrick

From the Department of Plant Biology, 2502 Plant Sciences Building, University of Georgia, Athens, GA 30602 (Cruse-Sanders and Hamrick); and Department of Genetics, 2502 Plant Sciences Building, University of Georgia, Athens, GA 30602 (Hamrick).

Address correspondence to Jennifer M. Cruse-Sanders, Rancho Santa Ana Botanical Garden, 1500 North College Ave., Claremont, CA 91711-3157, or e-mail: jennifer.cruse-sanders@cgu.edu.

Abstract

Spatial structure and fine-scale genetic structure were analyzed for the medicinal plant American ginseng (*Panax quinquefolius* L.) to more fully understand biological processes within wild populations. *P. quinquefolius* has been harvested for more than 250 years and is now considered threatened or rare throughout its range. Plants within four protected and four unprotected populations were significantly clumped based on Ripley's univariate analysis. Analysis with Ripley's bivariate test determined that juvenile plants were significantly clumped with adult plants at the shortest distance classes in all populations. Although plants were highly clumped, we found that significant fine-scale genetic structure was restricted to the shortest distance classes based on estimates of coancestry (f_{ij}). In most cases, estimates of f_{ij} were more significant among juveniles than among adults, especially at the shortest distance classes. The spatial structure of ginseng seems to result from the establishment and persistence of plants in favorable microhabitats coupled with limited seed dispersal around maternal individuals. There were no differences in patterns of fine-scale genetic structure between protected and unprotected populations.

Within a hierarchy of plant populations, subpopulations, and patches, genetically distinct groups of individuals can develop as a result of gene flow, drift, and/or selection. Among-population genetic structure is interpreted as evidence for low historical rates of gene flow with random changes in gene frequencies, and/or differential selection across the landscape (Heywood and Levin 1985; Rowland et al. 2001). Diminished gene flow throughout the species range is expected to result in isolation by distance (IBD), with nearby populations sharing more alleles at similar frequencies than geographically more distant populations (Heywood 1991). Levels of genetic structure also have been used to predict other relationships, such as the degree of structure in quantitative markers and divergence in genes coding quantitative traits (Merila and Crnokrak 2001).

Factors expected to lead to IBD among populations of plants are also predicted to produce genetic subdivision on a local, within-population scale. Local genetic subdivision, or "kinship structuring," results from the spatial clustering of individuals that are more closely related than would be expected if genotypes were distributed randomly (Heywood 1991). Kinship structuring can have evolutionary implications for the adaptive potential of the population (Wright 1970, 1977), as well as the development of breeding systems, ecological interactions among close relatives, and structured response to diseases, pollinators, and herbivores (Lande and Schemske 1985; Schall and Levin 1978).

Fine-scale genetic structure within populations not only impacts the evolutionary potential of a species, but it also indicates the role of evolutionary and ecological factors that shape plant populations. In some plant populations, significant genetic structure over small distances results from restricted seed dispersal, such that siblings or parents and offspring grow close to one another, sometimes despite long-distance pollen flow (Campbell and Dooley 1992; Loiselle et al. 1995). Plant species at low densities with discrete seed shadows are expected to develop a positive fine-scale genetic structure (Hamrick and Nason 1996). In contrast, plant species with overlapping seed shadows, high outcrossing rates, rare seedling establishment, and/or recruitment away from the maternal plant should have little genetic structure within populations (Hamrick and Loveless 1986; Ueno et al. 2000). Molecular markers have made it possible to discern genetic structure within and among

populations that shed light on patterns of gene flow, dispersal, and selection (Backmann 1994; Dow and Ashley 1996). Furthermore, studies of spatial genetic structure at different life-history stages make it possible to tease out the role of historical causes, seed dispersal, and local selection within populations (Epperson and Alvarez-Buylla 1997; Kalisz et al. 2001; Tonsor et al. 1993).

In natural environments, the spatial distribution of individual plants within populations often depends on environmental factors that influence seedling establishment, such as light, moisture, and soil nitrogen levels, as well as disturbance (Berlow et al. 2002; Parker et al. 1997). Spatial patterning of individuals not only results from the heterogeneous distribution of favorable microsites, but also seed dispersal patterns (Loiselle et al. 1995; Ueno et al. 2000). Among understory herbaceous species, sexual reproduction is often limited by low seed output, limited dispersal, and low germination rates, and can result in low population growth rates for many forest herbs (Meier et al. 1995; Verheyen and Hermy 2001). These factors can also produce high levels of local genetic structure within populations.

In this study we analyze fine-scale genetic structure within eight wild populations of the medicinal plant American ginseng (*Panax quinquefolius* L.), hereafter "ginseng," from the Appalachian Mountains in the southeastern United States. Ginseng roots have been harvested for export in the medicinal trade since the early 1700's (Carlson 1986). In states where ginseng currently is harvested, the U.S. Fish and Wildlife Service (USFWS) mandates that the oldest plants, those with three and four leaves, should be collected after mid-August. This sampling scheme is designed to give plants a chance to reproduce, set seed, and replace themselves in the population (Robbins 1998). Ginseng hunters are required to plant seeds from collected plants at the population site, but anecdotal information indicates that most harvesters remove seeds to plant in more favorable sites closer to their homes (Nazarea V, personal communication).

Ginseng plants are nonclonal, with a single stem, 20–60 cm high, arising in late April to early May from a short rhizome with a whorl of one to four (or more) palmately compound leaves. Ginseng reproduces exclusively by seed after a prereproductive period of approximately 3 years (Nantel et al. 1996; Schlessman 1985). Ginseng plants with three or more leaves, or rarely two leaves, produce an umbel of small white flowers between late May and July. Breeding occurs by a mixed mating system (Carpenter and Cottam 1982; Schlessman 1985). Pollination is by generalists, primarily by small bees of the Halictidae, and Syrphid flies (Carpenter and Cottam 1982; Lewis and Zenger 1982). Berries with one to three seeds are produced in July and August, and then redden and mature from August through October (Anderson et al. 1993; Lewis and Zenger 1982). There is evidence for IBD among populations within the southeastern Appalachian Mountains based on a survey of allozyme diversity (Cruse-Sanders and Hamrick 2004). Reproductive output in ginseng is positively related to population size, with low fruit set in small populations likely due to pollinator limitations (Hackney and McGraw 2001).

Ginseng populations should have a significant fine-scale genetic structure as a result of the microhabitat requirements of the plants as well as restricted seed movement. We also expected to see different patterns of coancestry between unprotected (populations where ginseng harvest is permitted) and protected populations. Unprotected populations should have less fine-scale genetic structure as a result of disturbance by harvesters collecting the oldest (largest) plants from populations. Epperson and Chung (2001) noted differences in populations of *Pinus strobus*, where adults in undisturbed populations had significant genetic structure attributed to pollen and seed dispersal, whereas logged populations were nearly randomly distributed. Our objectives were to map and analyze the spatial distribution of plants within southern Appalachian forests; compare spatial dispersion and patterns of coancestry within populations and among age classes within populations; and compare fine-scale genetic structure within protected and unprotected populations.

Materials and Methods

Study Species and Site Description

Panax quinquefolius L. (Araliaceae) is a perennial herb native to coves and hillsides in the understory of rich deciduous woods of the Appalachian Mountains and adjacent areas from Quebec, Canada, south to Georgia, and west to Nebraska (Anderson et al. 1993; Robbins 1998). Wild plants can be readily grouped into five size classes that reflect age class stages: seedling, one leaf (1–3 years), two leaves (3–5 years), three leaves (5–9 years), four (or more) leaves ($\geq$9 years) (Anderson et al. 1993). For purposes of this study, we categorized seedlings with one or two leaves as juveniles and those with three or four leaves as adults.

Between 1999 and 2000 we recorded the number of leaves and presence of fruits and flowers on plants in eight populations at sites in Maryland, North Carolina, and Georgia (Figure 1). More populations were studied in these areas to analyze the among-population genetic structure (Cruse-Sanders and Hamrick 2004). Sampling sites ranged from 600 m^2 to 21,000 m^2, and every individual within each patch was mapped. Four populations were protected from harvest and four were unprotected (Table 1). Populations for mapping studies were chosen based on our ability to identify the entire population and population size ($N > 15$ individuals).

Spatial Analysis

Individual ginseng plants were labeled and their position relative to other ginseng within the population was recorded. All plants within the population were located by searching the area and flagging all individuals (including stems affected by herbivory) before mapping them. We mapped populations with one of two methods: the first was to establish a grid and locate plants along x and y axes; the second was to record the distance and compass direction between plants. For one population, NC15, x and y coordinates were determined

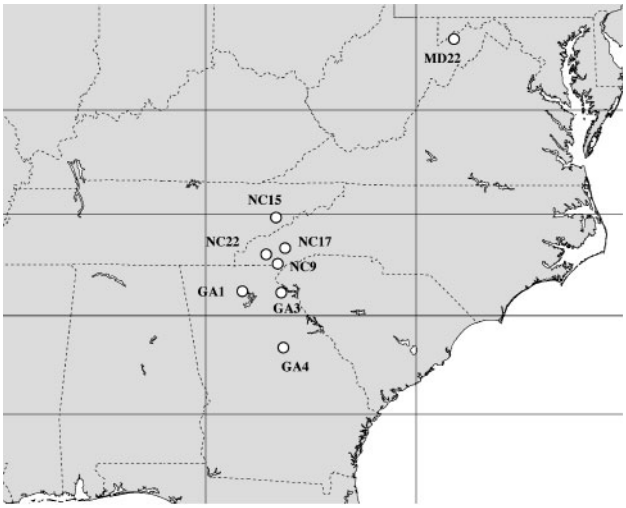

Figure 1. Map of the southeastern United States with the location of *P. quinquefolius* sampling sites. Refer to Table 1 for site descriptions.

from a scale drawing on graph paper. From these data we determined the location of each plant, the area of each focal population, and the density of individuals within populations.

Spatial analysis of *P. quinquefolius* within populations was performed with Ripley's K [Ripley 1977; analysis program written by Richard Duncan (1995)], with

$$K(t) = A\sum_{i=1}^{n}\sum_{j=1}^{n} w_{ij} I_{t(i,j)}/n^2,$$

where n is the sample size, A is the area of the plot, w_{ij} corrects for edge effect, I_t is a value of one if the distance between plants is less than or equal to t, otherwise it is zero (Diggle 1983; Haase 1995). For this analysis, a circle of radius t was centered on each individual and the number of neighbors within the circle was determined for each individual and for each value of t. Ripley's $K(t)$ analysis measured distances from each plant in our sample plots to every other plant, and these empirical functions were compared to hypothetical functions assuming spatial randomness of plants in the plot. To plot the results, generated values for $K(t)$ were square-root transformed into an $L(t)$ function, where $L(t) = t - [K(t)/\pi]^{1/2}$ and plotted against t (t = distance). The equation for $L(t)$ transforms the expected $K(t)$ function under a Poisson distribution to a value of zero, with regularly dispersed plants being positive and clumped individuals negative. A bivariate measure, Ripley's $K_{12}(t)$, was used to describe the distribution of adult and juvenile plants relative to one another (Diggle 1983; Ripley 1977). The bivariate spatial function was transformed as described for $K(t)$, resulting in $L_{12}(t)$, which was then plotted against t. Both the bivariate and univariate functions were calculated for 2 m distance classes up to half the distance of the shortest axis of the study sites. This corrected for bias associated with edge effect.

The degree to which the observed values differed from expected values determined the intensity of nonrandom spatial distribution. Significance was tested using Monte Carlo simulations that defined a 95% confidence interval (CI) based on 19 simulations of random point distributions (Stewart and Rose 1990). When the observed values, $L(t)$, lay outside the CI at any spatial scale, the distribution of ginseng was significantly different than random; values below the line indicated clumping, otherwise they were overdispersed relative to each other.

Table 1. Sampling locations and descriptions of *P. quinquefolius*

ID	Protection status	N	Area (m^2)	Density/m^2	Altitude (m)	Rainfall (mm/year)	Location
GA1	U	61	600	0.102	451	1583	Blue Ridge Mountains, GA, Chattahoochee National Forest
GA3	U	66	2800	0.024	223	1336	Blue Ridge Mountains, GA, Chattahoochee National Forest
GA4	U	104	4800	0.022	124	1194	Oconee National Forest, Piedmont province, GA
MD21	U	94	21000	0.004	558	931	Savage River State Forest, Appalachian plateau, MD
NC9	P	32	2400	0.013	1027	2122	Coweeta Long-Term Ecological Research Site, Blue Ridge Mountains, NC
NC15	P	106	4550	0.022	999	1489	Great Smoky Mountains National Park, Blue Ridge Mountains, NC
NC17	P	96	2499	0.027	1217	2122	Joyce Kilmer-Slickrock Wilderness Area, Blue Ridge Mountains, NC
NC22	P	48	3600	0.019	4250	2122	Natahala Wilderness Area, Blue Ridge Mountains, NC

Protection status designates whether sites were protected from harvest (P) or if harvest was permitted (U).

The population size is indicated by N, which also refers to the sample size for that site.

Electrophoretic Studies

To determine the multilocus genotypes of the plants, we sampled one leaflet to an entire leaf from every mapped individual for use in allozyme electrophoresis. Freshly clipped leaves were placed in individually labeled plastic bags and kept in a cooler or refrigerator until they were brought back to the University of Georgia. Plant material was crushed in a mortar within 48 h of sampling with a phosphate-polyvinylpyrrolidone extraction ("camellia") buffer and a pinch of sea sand (Wendel and Parks 1982). Enzyme extracts were then absorbed onto chromatography paper wicks and stored at −70°C until analysis.

Allozyme analysis followed procedures outlined in Cruse-Sanders and Hamrick (2004). Briefly, electrophoresis was conducted on 10% potato starch gels, and we consistently resolved seven enzyme systems with 16 putative loci. We used the following method for each gel and electrode buffer system (abbreviation, number of loci): With a modification of system 34 we ran florescent esterase (FE, 1), menedione reductase (MNR, 2); using system 4, we ran isocitrate dehydrogenase (IDH, 2), malate dehydrogenase (MDH, 2), phosphoglucoisomerase (PGI, 4), triose phosphate isomerase (TPI, 3), and uridine diphosphatase (UGPP, 2). The test for significant differences in expected heterozygosity (H_e) was performed on jackknifed values (Weir and Cockerham 1984). Inbreeding coefficients per population were estimated from polymorphic loci, $F_{IS} = 1 - H_o/H_e$, and significance was determined with bootstrap analysis over loci with 1000 replicates (Weir 1990, 1996). Similarly we tested for significant differences in coancestry coefficients among populations, θ (Cockerham 1969), analogous to Wright's F_{ST} (1951), with bootstrap analysis over all loci.

Analysis of Coancestry

Within populations, the relationship between genetic relatedness and spatial distance can be examined by analyzing the spatial autocorrelation for genotypes of mapped individuals as a means of evaluating consequences of pollen and seed dispersal within populations (Heywood 1991; Kalisz et al. 2001; Loiselle et al. 1995). To identify differences in fine-scale genetic structure among populations under different types of management, individuals within the mapped populations were analyzed using an index of spatial autocorrelation developed by J. D. Nason (Kalisz et al. 2001; Loiselle et al. 1995). For this analysis, multilocus allozyme genotypes from the 16 polymorphic loci were used to estimate coancestry between all possible pairs of individuals within mapped populations. The coancestry estimator, f_{ij}, measures the correlation in allele frequencies, p_i and p_j, at a locus in pairs of mapped plants i and j, and therefore is an estimate of relatedness between pairs of individuals (Kalisz et al. 2001). The estimate of coancestry was calculated as $f_{ij} = [(\sum(p_i - p)(p_j - p))/kp(1 - p)] + [1/2(N - 1)]$, $(i > j)$, where p is the population sample allele frequency, $k = n(n - 1)/2$ is the total number of pairwise connections between individuals located a discrete number of map units from each other, and $1/(2(N - 1))$ adjusts for the bias of sampling all possible pairs of individuals within a sample population (Kalisz et al. 2001; Loiselle et al. 1995).

To obtain the multilocus estimator, results for f_{ij} are combined over all loci by weighting by the polymorphic index, $\sum p_a(1 - p_a)$. If gene movement is restricted over distance within the population, then it is expected that estimates of coancestry should decline for pairs of plants located increasing map units from each other. We chose to analyze the data at the 2 m distance interval as a compromise between resolution at the expected level of seed dispersal within the population and increasing the number of pairs per distance class (Nason JD, personal communication). The power of the analysis depends on the number of polymorphic loci included and the number of pairs for comparison within each distance class. We only plotted those f_{ij} values that included a minimum of 10 pairs per distance class, because we wanted to maximize the number of independent samples while maintaining resolution at the distance class scale.

Significant values for estimated f_{ij} were determined with a randomization procedure (Kalisz et al. 2001; Loiselle et al. 1995). Map locations were randomly assigned multilocus genotypes drawn at random with replacement from the sample population. For each distance class, the results from 399 simulations were ordered and used to form a 95% CI around the null hypothesis of no spatial genetic structure. The null hypothesis of no genetic structure, $f_{ij} = 0$, was rejected when coancestry estimates were outside the 95% CI based on the 399 simulations.

Results

Spatial Patterning

Densities within the mapped populations ranged from 4 to 100 individuals per 100 m^2 (Table 1). The average proportion of juvenile plants (one to two leaves) in the unprotected populations was 0.65 (±0.167) and in protected populations it was 0.56 (±0.158) (Figure 2). The percentage of adult plants with fruits or flowers ranged between 10% and 56%, whereas among juvenile plants, less than 10% had reproductive structures (Figure 3). Based on visual inspection of the scatter plot of spatial distribution within the populations, the plants are highly clumped, with juvenile plants clustering significantly with adults at the shortest distance classes, as shown with the bivariate Ripley's analysis (Figure 4). Ginseng plants with reproductive structures were scattered throughout populations with the same patchy distribution as the entire population (data not shown).

Bivariate $L_{12}(t)$ analyses determined that, in all populations but GA4, at short distances, juvenile plants were closer to adults than expected by chance (Figure 4). The bivariate distribution indicates significant clumping of adults and juveniles, at the scale of 2–6 m in seven of the eight populations (Figure 4). Population GA4 does not have a significant relationship at the shortest distance class (95% CI), but does show that ginseng adults and juveniles are significantly more closely dispersed at 6 m.

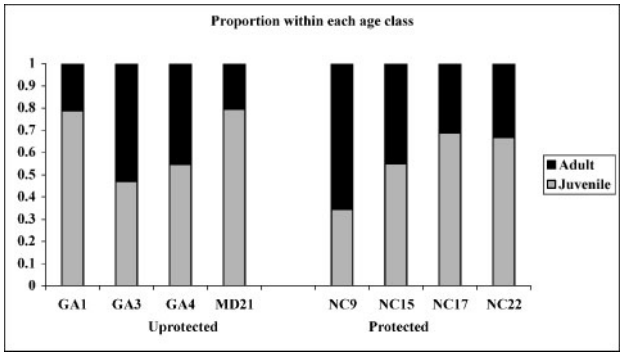

Figure 2. Proportion of juvenile (one- and two-leaf plants) and adult (three- and four-leaf plants) *P. quinquefolius* in each population.

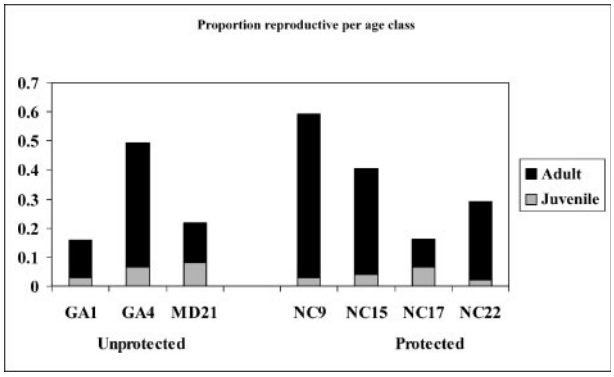

Figure 3. Proportion of *P. quinquefolius* plants with flowers or fruits in each age class within each population. The height of the bar indicates the total proportion of reproductive individuals in the population. The black portion of the bar is the proportion of reproductive plants that were adults. The gray portion of the bar refers to the proportion of total reproductive plants classified as juveniles (one- and two-leaf plants). Data for the presence of reproductive structures was not collected in population GA3.

General spatial patterning of all plants in the populations analyzed with univariate $L(t)$ indicated that ginseng plants were significantly clumped at most spatial scales. Plants within populations GA3, GA4, MD21, NC17, and NC22 were significantly clumped at all distance classes tested, ranging from 20 m to 120 m depending on the area sampled (Figure 5). Within population NC9, plants were randomly dispersed within the 16 m to 20 m distances classes. Whereas in population GA1, plants were significantly clumped in distance classes 0 m to 4 m, but had a random spatial pattern for the 6 m to 10 m distance class.

When adult and juvenile categories were separated for univariate $L(t)$ analyses, there was slightly different spatial patterning between the two age classes. Juvenile plants in most populations were significantly clumped at the majority of distance classes tested (Figure 5). Within populations GA1 and NC9, significant clumping of juvenile plants was restricted to the first few distance classes, 6 m and 9 m, respectively (data not shown). Adult plants were significantly clumped at all distance intervals in NC22 (Figure 5a). Populations GA3, MD21, and NC17 had a similar pattern. Adult plants in GA4 were significantly clumped at 2–6 m, 10–12 m, and 18–24 m (Figure 5b). In population GA1, adults were clumped at 2 m and 4 m, whereas in NC15 and NC9, adult plants were significantly clumped beyond 30 m and 12 m, respectively. We saw no clear pattern among protected and unprotected populations.

Fine-Scale Genetic Structure

Analysis of fine-scale genetic structure indicated that coancestry values (f_{ij}) did not differ significantly ($P < .05$) from zero in populations MD21 and NC22 (Figure 6). Within populations GA1, GA4, GA3, NC9, NC15, and NC17, plants were more closely related than expected with a random distribution at the shortest distance class, 2 m. We also found significant coancestry values for plants at further distance classes within populations GA1, GA3, NC9, and NC17. Populations GA1 and NC17 were the only populations with significantly negative values for coancestry at 10–12 m and 24–26 m, respectively.

Fine-scale genetic structure differed among the age classes (Figure 7). Five of the six populations with significant estimates for coancestry at the shortest distance classes had significant fine-scale genetic structure among juvenile plants (GA1, GA3, GA4, NC9, NC15, and NC17) (Figure 7). Only two populations had significant fine-scale genetic structure among adult plants, but in one of these, NC9, it was exclusively adults with significant coancestry estimates.

The inbreeding coefficient among all plants, F_{IS}, was significantly greater than zero in both protected and unprotected populations (0.491 and 0.429, respectively; 95% CI, 1000 replicates). Genetic structure among populations, θ, was significantly different from zero in both protected and unprotected populations (95% CI, 1000 replicates). Furthermore, genetic structure among unprotected populations was significantly greater than among protected populations (0.638 and 0.526, respectively). A similar pattern was seen for θ among young plants in unprotected (0.807) versus protected (0.559) populations and among old plants in unprotected (0.752) and protected (0.462) populations (95% CI, 1000 replicates).

Discussion

Spatial Distribution

Overall *P. quinquefolius* exhibited a clumped spatial distribution in all populations. Juvenile plants were significantly clumped at all distance classes, except in populations GA1 and NC9. Adult plants in populations GA1, GA4, and NC9 had a slightly different spatial pattern than juveniles in the populations, with a random distribution at shorter distance classes (6 m, 14 m, and 12 m, respectively). Otherwise adult and juvenile plants had similar spatial clumping. There was

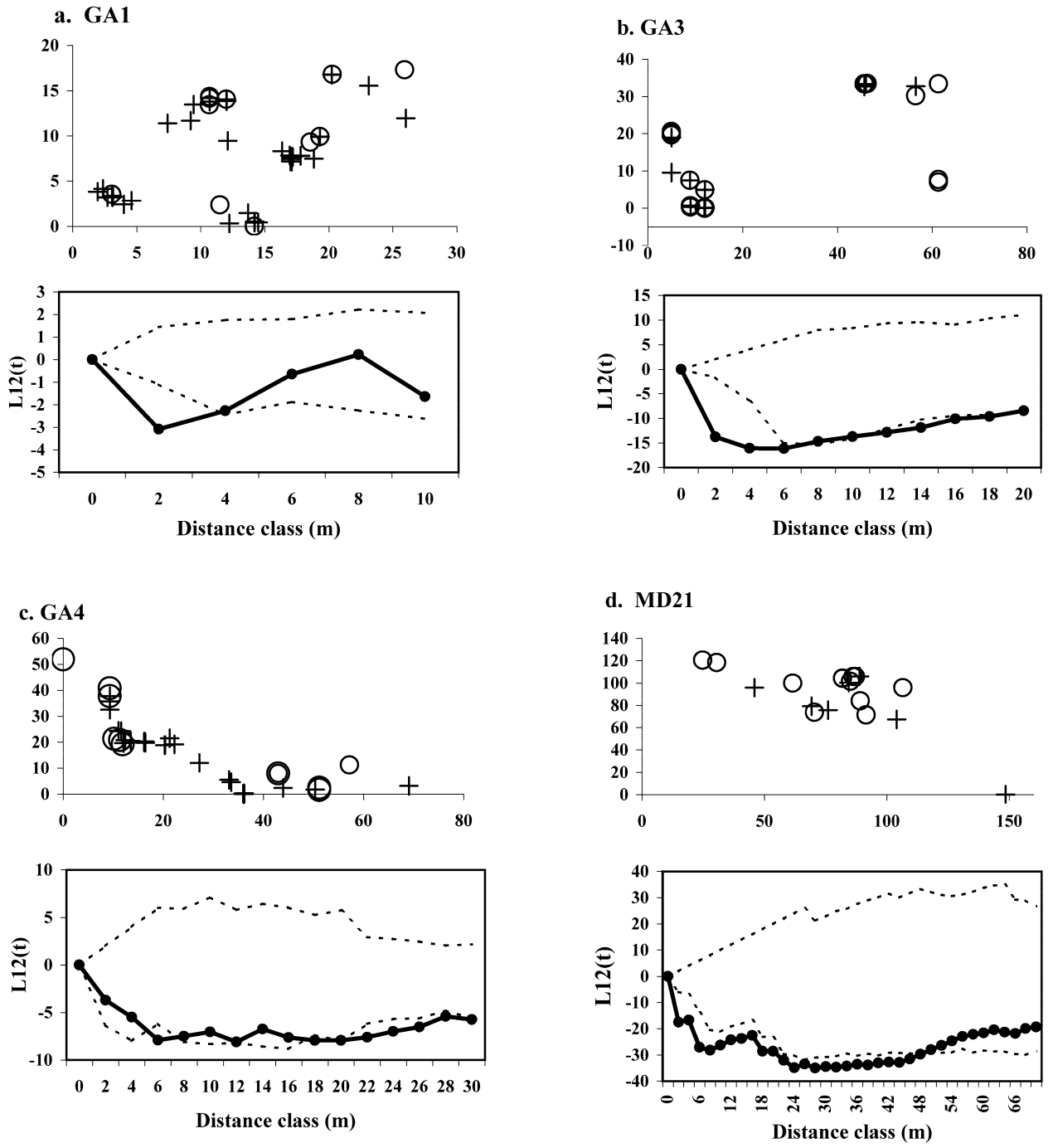

Figure 4. Maps of all *P. quinquefolius* in the study populations with bivariate $L_{12}(t)$ analysis of adult and juvenile plants. Both juvenile (+) and adult (o) plants are mapped for each population. Below the map for each population is a correlogram for bivariate $L_{12}(t)$ results. The dashed lines indicate the 95% CI, data points that lie below the CI show significant clumping of juvenile and adult plants; those points that lie above the CI are significantly hyperdispersed. Results are shown for population (**a**) GA1, (**b**) GA3, (**c**) GA4, (**d**) MD21, (**e**) NC9, (**f**) NC15, (**g**) NC17, and (**h**) NC22. See Table 1 for location descriptions.

significant bivariate clumping among adults and juveniles at the shortest distance classes in all populations but GA4. Significant spatial clumping within *P. quinquefolius* populations, especially at the shortest distance classes may result from a combination of limited seed dispersal and spatial heterogeneity in favorable germination niches. Seeds are dispersed primarily by gravity and pollen is moved by small insects, which should lead to a high degree of fine-scale genetic diversity resulting from clusters of related seedlings growing near the maternal plant and a high degree of shared

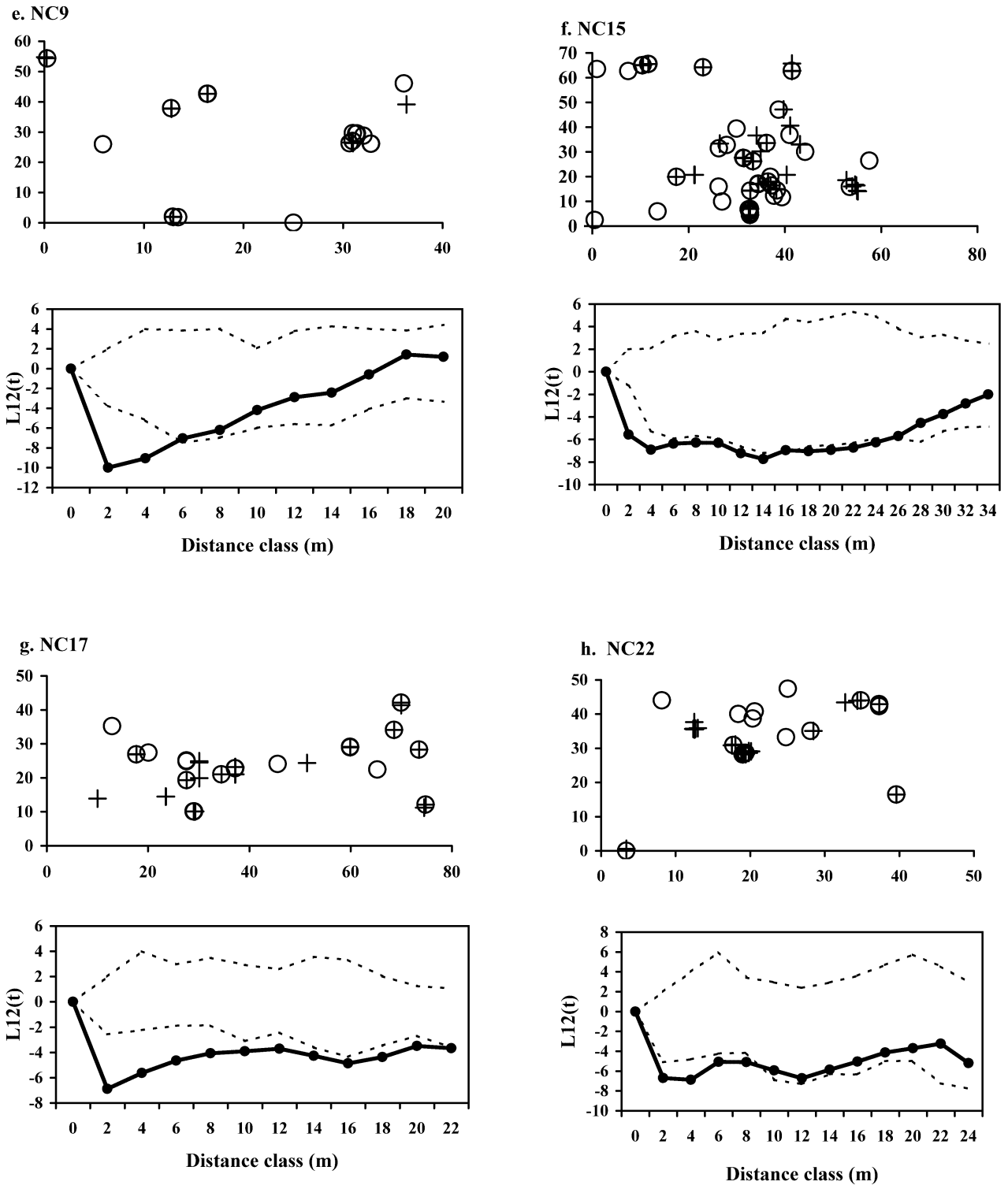

Figure 4. Continued.

alleles among plants at short distances (Dutech et al. 2002; Konuma and Terauchi 2001). Dispersal distances may be greater than expected, however, if bird dispersal is more prevalent than gravity dispersal and if humans play a large role in seed movement. In fact, many ginseng plants are found near turkey nests (Wilson M, personal communication) and people collect seeds and plant them in convenient locations (Bonds H, personal communication). Both types of

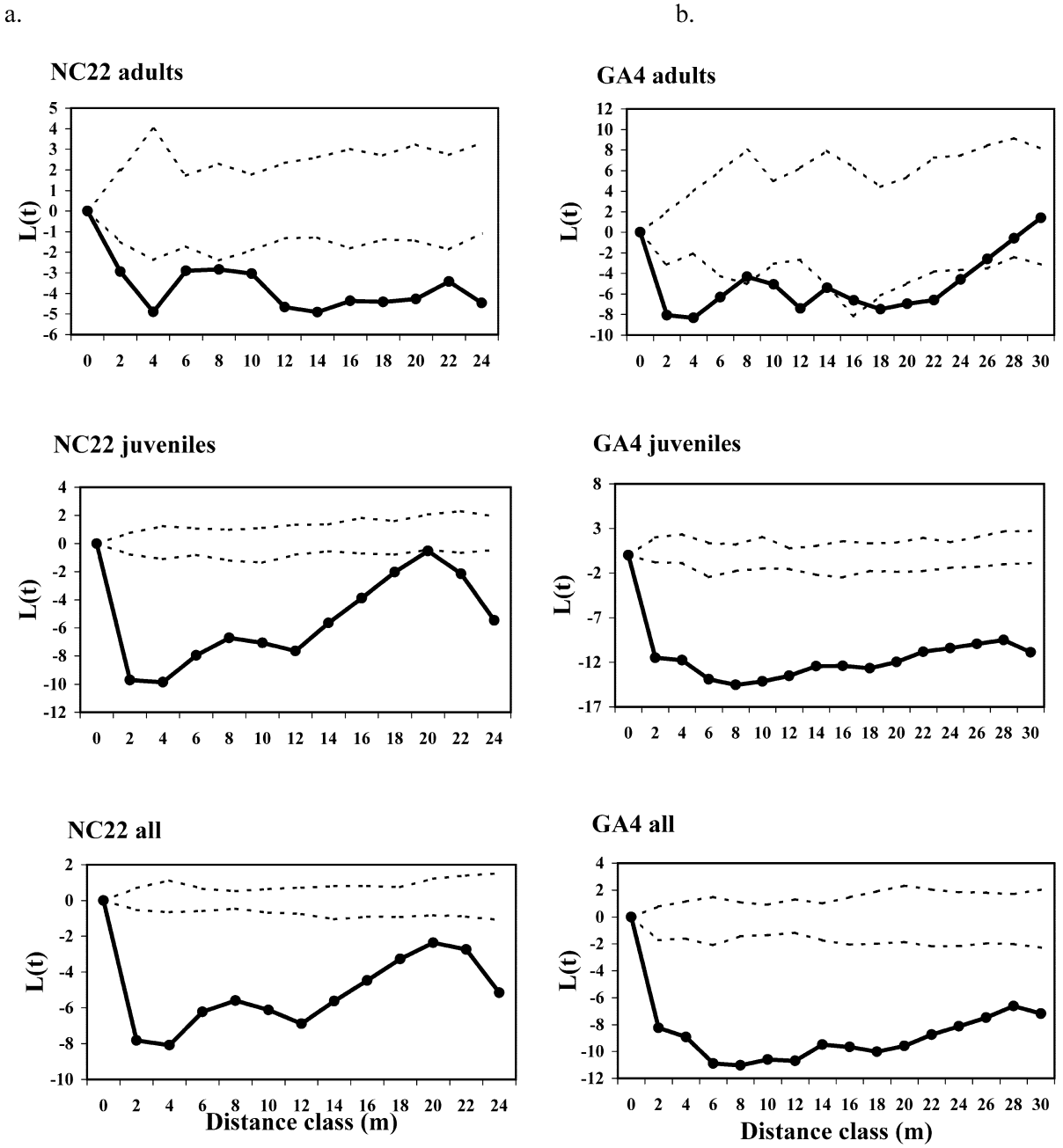

Figure 5. Correlogram for univariate $L(t)$ spatial analysis in two of the studied *P. quinquefolius* populations: (**a**) protected population NC22 adults, juveniles, and all plants; (**b**) unprotected population GA4 adults, juveniles, and all plants. Dashed lines are the 95% CI. Estimates of $L(t)$ that lie below the CI are significantly clumped; those that lie above the CI are significantly uniformly dispersed.

dispersal could lead to a clumped distribution of mixed genotypes.

Fine-Scale Genetic Structure

We found significant fine-scale genetic structure at the shortest distance class (2 m) in six of the eight populations examined. This finding is consistent with expectations if kin structure was present in the population, particularly where offspring were clustered around their parents. This spatial pattern has been reported for self-compatible species and plants with mixed mating systems, including *Polygonum thunbergii* (Konuma and Terauchi 2001), *Trillium grandiflora* (Irwin 2001; Kalisz et al. 2001), and the tropical tree

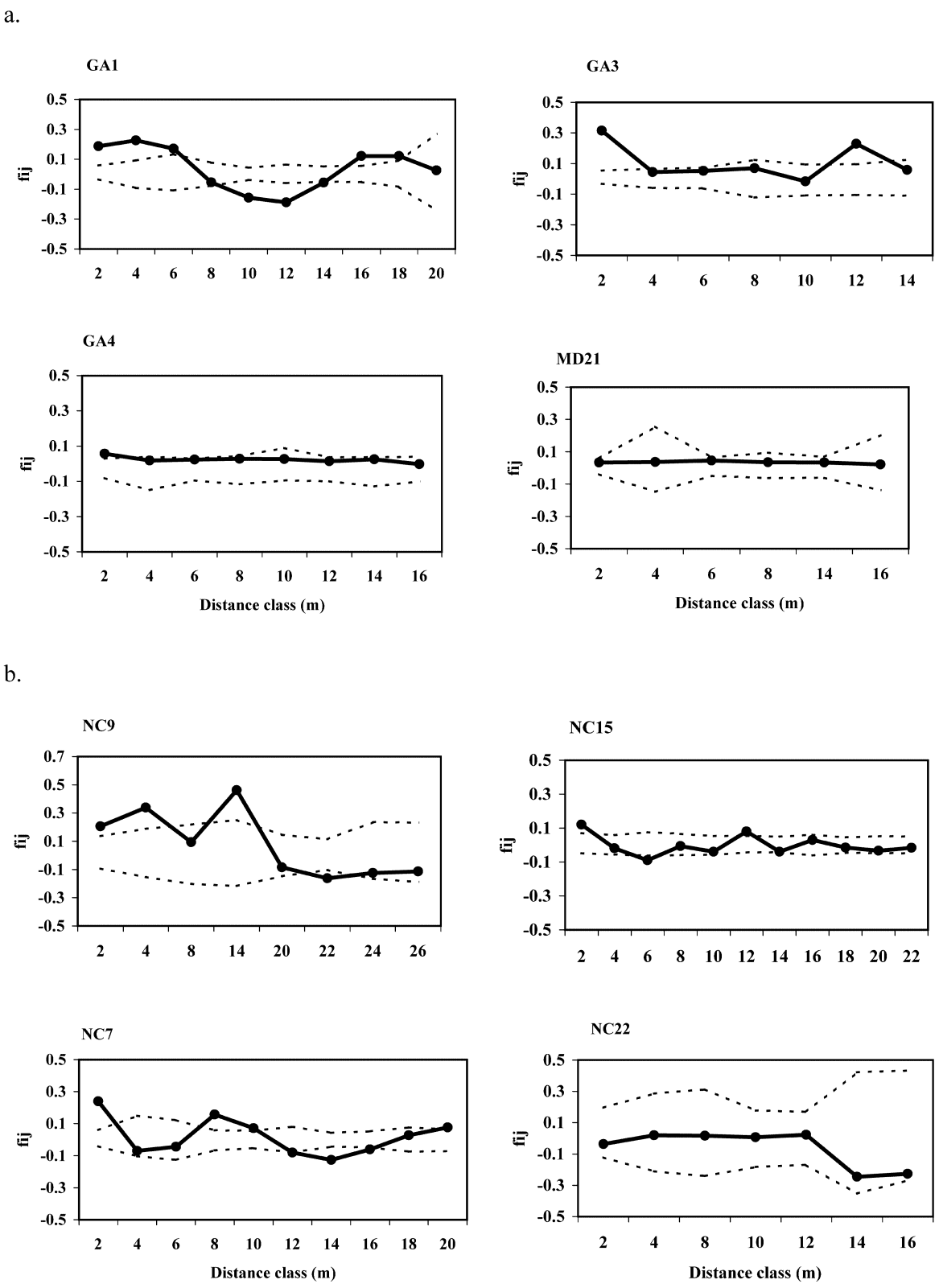

Figure 6. Spatial autocorrelation analysis of kinship, f_{ij}, for pairs of *P. quinquefolius*. (**a**) All plants within each unprotected population. (**b**) All plants within each protected population. Distance intervals are 2 m in all populations. Certain distance intervals within three populations did not contain enough pairs for valid coancestry estimation, therefore they were left out of the final plot. These include in MD21, the 10 m distance class; in NC9, the 12 m, 16 m, and 18 m distance classes; and in NC22, the 6 m distance class. The dashed lines represent upper and lower 95% CIs around zero relationship.

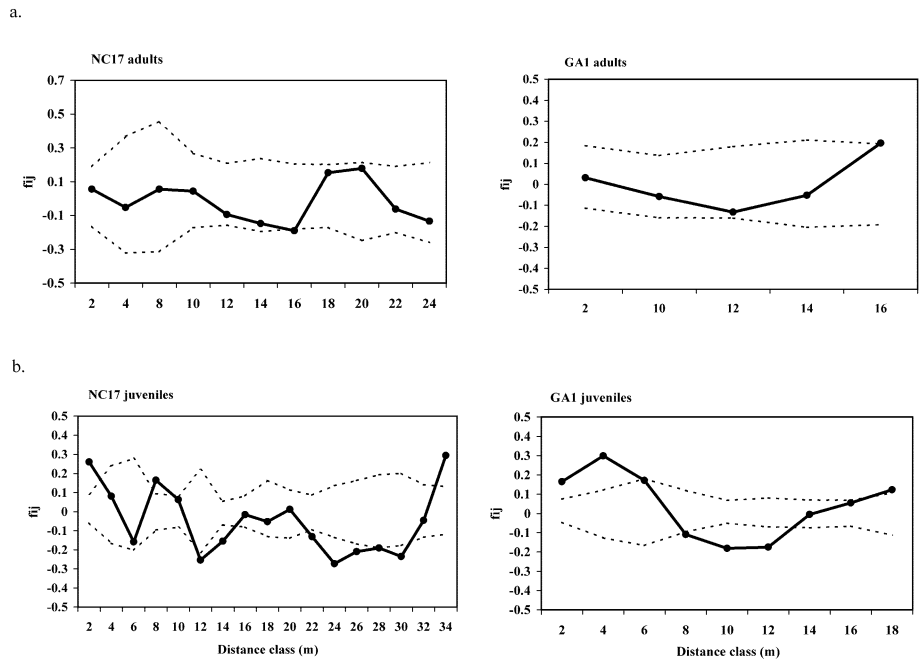

Figure 7. Correlogram of estimated coancestry, f_{ij}, for age classes in one protected and one unprotected population. (**a**) Protected population (NC17) adults and juveniles. (**b**) Unprotected population (GA1) adults and juveniles. Dashed lines represent upper and lower 95% CIs around the null hypothesis of no genetic structure, $f_{ij} = 0$.

Vouacapoua americana, which is pollinated by small insects and dispersed by rodents in French Guiana (Dutech et al. 2002). Significant fine-scale genetic structure has also been seen in species with high outcrossing rates, such as *Psychotria officinalis* in Costa Rica (Loiselle et al. 1995), *Quercus rubra* in temperate forests (Sork et al. 1993), and *Ipomopsis aggregata* from alpine herbaceous communities (Campbell and Dooley 1992).

Population NC9 had the highest proportion of adult plants within the population (Figure 2) and the highest proportion of reproductive adults (Figure 3). We found that adult plants in this population demonstrated significant fine-scale genetic structure, although juvenile plants did not. This pattern has also been noted for *T. grandiflora*, where history and selection were invoked to explain present fine-scale genetic structure (Kalisz et al. 2001), and for *Camelia japonica* (Ueno et al. 2002), in which fine-scale structure among adults was attributed to microhabitat differences. Loiselle et al. (1995) also determined that microgeographic selection was responsible for the pattern of genetic structure seen among subpopulations of *Psychotria officinalis* in Costa Rica. A similar pattern was noted for *Lupinus arboreus*, whose seedlings did not exhibit genetic structure as a result of high gene flow, but juveniles did have significant fine-scale genetic diversity attributed to microhabitat selection (Kittelson and Maron 2001).

In contrast, populations GA1, MD21, NC15, and NC17 had significant fine-scale genetic structure among juveniles, but not among adults. This result is consistent with expected patterns if there is some inbreeding and limited seed dispersal in the population with subsequent random mortality (Epperson and Alvarez-Buylla 1997; Hamrick et al. 1993). Estimates of coancestry among these four populations ranged from 0.17 to 0.24, and were equivalent to expectations for half and full sibs. Estimates of coancestry are equal to one half relatedness, therefore a coancestry value of 0.25 indicates a relatedness value of 0.5 (i.e., full sibs). In population GA1, the average pairwise coancestry value among juvenile plants at 2 m was 0.29, higher than expected for siblings and similar to what we might expect with clonal plants, although vegetative reproduction in ginseng is reportedly very rare (Lewis and Zenger 1982) and we did not see any evidence for clonality. Coancestry values greater than 0.25 among juveniles could be due to selfing within the population, as is suggested by significant F_{IS} values in the populations, or could result from low numbers of individuals or loci within a distance class.

Although our analysis of spatial distribution indicated that plants in all populations were significantly clumped at almost all distance classes, patterns of fine-scale genetic diversity varied among populations. As a result, it is difficult to make broad predictions for fine-scale genetic diversity within populations based on the spatial clumping of plants or on coancestry patterns observed within a small subset of populations. Unpredictable fine-scale structure among populations has been found in other species. Combined estimates of seed dispersal patterns and variations in fertility were sufficient to describe the within-population genetic structure of the tree *Gleditsia triacanthos*, however, Schnabel et al. (1998) attributed differences in fine-scale genetic structure among populations to unpredictable secondary seed dispersal. Similarly, in a study of the predominantly selfing plant *Medicago truncatula*, Bonnin et al. (2001) found varying

patterns of fine-scale genetic structure among subpopulations, suggesting that different mechanisms may be acting at different sites.

Year-to-year variability in seed production and dispersal could reduce fine-scale genetic structure and lead to different patterns among ginseng populations. Viability in the seed bank is expected to be short and seedling mortality is always high, thus the stability of populations is more sensitive to the survival of adult individuals (Charron and Gagnon 1991). In a 10-year study of *P. quinquefolius*, Dunwiddie and Anderson (personal communication) found that yearly fruit production varied considerably among adult plants, ranging from 0.5% to 33%. Such variation means that among ginseng populations there can be a different proportion of individuals contributing to the observed genetic patterns. If fine-scale genetic structure in this species primarily results from local seed dispersal and is often lost among adult plants, patterns of spatial genetic structure might be obscured after a period of low seedling recruitment. This suggests that conclusions about fine-scale genetic structure should be based on data from several populations.

Fine-Scale Genetic Structure Within Protected and Unprotected Populations

Among populations of *P. quinquefolius* subject to harvest pressure, Cruse-Sanders and Hamrick (2004) found significant IBD and a high level of genetic structure among populations. Approximately 50% of the total genetic diversity was explained by differentiation among populations. Based on these estimates, at least five populations throughout the southeastern range of *P. quinquefolius* would need to be preserved to protect 95% of the genetic diversity within the species. Human impact could reduce fine-scale genetic structure within populations. Indeed, the degree of fine-scale genetic structure observed was less than predictions based on the significant clumping of individuals and expectations for limited seed dispersal. In addition, there was no clear pattern of spatial genetic structure between protected and unprotected populations of *P. quinquefolius*. Despite significant differences in levels of genetic diversity and genetic structure among protected and unprotected populations, we were unable to attribute differences in fine-scale genetic structure to how these populations were managed. This is in contrast with what was found for populations of *Pinus strobus* in logged and old-growth forests, where significant genetic structure was found in the latter and randomly distributed genotypes in logged populations (Epperson and Chung 2001).

Most state ginseng regulations mandate that seeds from harvested ginseng plants be planted back into the population (Robbins 2000). This could result in the mixing of genotypes and blurring of genetic patterns, if harvesters bulk a collection of seeds before planting. In Europe, Konig et al. (2002) found a decrease in population genetic differentiation for *Quercus robur* and *Quercus petrea* with increasing human impact as a result of reforestation efforts and seed transfers. Furthermore, ginseng harvesters may create holes in natural population genetic structure by collecting the oldest, presumably maternal plants from patches of individuals. Our results were consistent with this expectation since adults generally demonstrated less fine-scale genetic structure than juveniles.

Conservation Implications

We found evidence for local seed dispersal in the majority of ginseng populations studied. The pattern of fine-scale genetic structure was seen in populations with significant clustering of juvenile plants with adults at the same distance intervals. This suggests that there are family-structured groups of individuals within certain populations. Our results indicate that spatial clumping of plants within populations is probably also due to microhabitat heterogeneity, because adult plants were spatially clumped but lacked significant coancestry values. Therefore the spatial structure of ginseng seems to result from the establishment and persistence of plants in favorable microhabitats coupled with limited seed dispersal around maternal individuals. In populations with significant fine-scale genetic heterogeneity, harvesting patterns that coarsely remove entire patches of plants may eliminate significant portions of the genetic diversity within ginseng populations.

Results from this study, along with high among-population genetic structure and significant IBD found within this species (Cruse-Sanders and Hamrick 2004), suggest that protection of genetic diversity within *P. quinquefolius* must preserve not only populations throughout its range, but also variation across an entire population. Similar measures have been recommended for other species of rare plants exhibiting fine-scale genetic structure (Chung et al. 2001). Finally, variation in fine-scale genetic structure among populations in this study, despite significant clumping of individuals in all cases, indicates that spatial patterning may not be closely associated with the distribution of genetic variation.

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References

Anderson RC, Fralish JS, Armstrong JE, and Benjamin PK, 1993. The ecology and biology of *Panax quinquefolium* L. (Araliaceae) in Illinois. Am Midl Nat 129:357–372.

Backmann K, 1994. Molecular markers in plant ecology. New Phytol 126:403–418.

Berlow EL, D'Antonio CM, and Reynolds SA, 2002. Shrub expansion in montane meadows: the interaction of local-scale disturbance and site aridity. Ecol Appl 12:1103–1118.

Bonnin I, Ronfort J, Wozniak F, and Olivieri I, 2001. Spatial effects and rare outcrossing events in *Medicago truncatula* (Fabaceae). Mol Ecol 10:1371–1383.

Carlson AW, 1986. Ginseng: America's botanical drug connection to the Orient. Econ Bot 40:233–249.

Campbell DR and Dooley JL, 1992. The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. Am Nat 139:706–734.

Carpenter SG and Cottam G, 1982. Growth and reproduction of American ginseng (*Panax quinquefolius*) in Wisconsin, U.S.A. Can J Bot 60:2692–2696.

Charron D and Gagnon D, 1991. The demography of northern populations of *Panax quinquefolium* (American ginseng). J Ecol 79:431–445.

Chung MG, Chung MY, and Epperson BK, 2001. Conservation genetics of an endangered herb, *Hanabusaya asiatica* (Campanulaceae). Plant Biol 3: 42–49.

Cockerham CC, 1969. Variance of gene frequencies. Evolution 23:72–84.

Cruse-Sanders JM and Hamrick JL, 2004. Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius* L. (Araliaceae). Am J Bot 91:540–548.

Diggle PJ, 1983. The statistical analysis of spatial point patterns. London: Academic Press.

Dow BD and Ashley MV, 1996. Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. Mol Ecol 5:615–627.

Duncan R, 1995. Spatial analysis programs. Canterbury, New Zealand: Lincoln University.

Dutech C, Seiter J, Petronelli P, Joly HI, and Jarne P, 2002. Evidence of low gene flow in a neotropical clustered tree species in two rainforest stands of French Guiana. Mol Ecol 11:725–738.

Epperson BK and Alvarez-Buylla ER, 1997. Limited seed dispersal and genetic structure in life stages of *Cecropia obtusifolia*. Evolution 51:275–282.

Epperson BK and Chung MG, 2001. Spatial genetic structure of allozyme polymorphisms within populations of *Pinus strobus* (Pinaceae). Am J Bot 88:1006–1010.

Haase P, 1995. Spatial pattern analysis in ecology based on Ripley's K-function: introduction and methods of edge correction. J Veg Sci 6: 575–582.

Hackney EE and McGraw JB, 2001. Experimental demonstration of an allee effect in American ginseng. Conserv Biol 15:129–136.

Hamrick JL and Loveless MD, 1986. The influence of seed dispersal mechanisms on the genetic structure of plant populations. In: Frugivores and seed dispersal (Estrada A and Fleming TA, eds). Dordrecht, The Netherlands: Dr. Junk Publications; 211–223.

Hamrick JL, Murawski D, and Nason JD, 1993. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. Vegetatio 107–108:281–297.

Hamrick JL and Nason JD, 1996. Consequences of dispersal in plants. In: Population dynamics in ecological space and time (Rhodes OE, Chesser RK, and Smith MH, eds). Chicago: University of Chicago Press; 203–246.

Heywood JS, 1991. Spatial analysis of genetic variation in plant populations. Annu Rev Ecol Syst 22:335–355.

Heywood JS and Levin DA, 1985. Associations between allozyme frequencies and soil characteristics in *Gaillardia pulchella* (Compositae). Evolution 39:1076–1086.

Irwin RE, 2001. Field and allozyme studies investigating optimal mating success in two sympatric spring-ephemeral plants, *Trillium erectum* and *T. grandiflorum*. Heredity 87:178–189.

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

Kittleson PM and Maron JL, 2001. Fine-scale genetically based differentiation of life-history traits in perennial shrub *Lupinus arboreus*. Evolution 55:2429–2438.

Konig AO, Ziegenhagen B, vanDam BC, Csaikl UM, Coart E, Degen B, Burg K, de Vries SMG, and Petit RJ, 2002. Chloroplast DNA variation of oaks in western central Europe and genetic consequences of human influences. For Ecol Manage 156:147–166.

Konuma A and Terauchi R, 2001. Population genetic structure of the self-compatible annual herb, *Polygonum thunbergii* (Polygonaceae), detected by multilocus DNA fingerprinting. Am Midl Nat 146:122–127.

Lande R and Schemske DW, 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24–40.

Lewis WH and Zenger VE, 1982. Population dynamics of the American ginseng *Panax quinquefolium* (Araliaceae). Am J Bot 69:1483–1490.

Loiselle BA, Sork VL, Nason J, and Grahm C, 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officianalis* (Rubiaceae). Am J Bot 82:1420–1425.

Meier AJ, Bratton SP, and Duffy DC, 1995. Possible ecological mechanisms for loss of vernal-herb diversity in logged eastern deciduous forests. Ecol Appl 5:935–946.

Merila J and Crnokrak P, 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J Evol Biol 14:892–903.

Nantel P, Gagnon D, and Nault A, 1996. Population viability analysis of American ginseng and wild leek harvested in stochastic environments. Conserv Biol 10:608–621.

Parker KC, Parker AJ, Beaty M, Fuller MM, and Faust TD, 1997. Population structure and spatial pattern of two coastal populations of Ocala sand pine (*Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg. var. clausa D. B. Ward). J Torr Bot Soc 124:22–33.

Ripley BD, 1977. Modeling spatial patterns. J R Stat Soc B 39:172–212.

Robbins CS, 1998. American ginseng: the root of North America's medicinal herb trade. TRAFFIC North America Report B347. Washington, DC: TRAFFIC North America.

Robbins CS, 2000. Comparative analysis of management regimes and medicinal plant trade monitoring mechanisms for American ginseng and goldenseal. Conserv Biol 14:1422–1434.

Rowland DL, Beals L, Chaudhry AA, Evans AS, and Grodeska LS, 2001. Physiological, morphological, and environmental variation among geographically isolated cottonwood (*Populus deltoids*) populations in New Mexico. West N Am Nat 61:452–462.

Schall BA and Levin DA, 1978. Morphological differentiation and neighborhood size in *Liatris cylindracea*. Am J Bot 65:923–928.

Schlessman MA, 1985. Floral biology of American ginseng (*Panax quinquefolius*). Bull Torr Bot Club 112:129–133.

Schnabel A, Nason JD, and Hamrick JL, 1998. Understanding the population genetic structure of *Gleditsia triacanthos* L.: seed dispersal and variation in female reproductive success. Mol Ecol 7:819–832.

Sork VL, Stowe KA, and Hochwender C, 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L) expressed as resistance to leaf herbivores. Am Nat 142:928–936.

Stewart GH and Rose AB, 1990. The significance of life-history strategies in the developmental history of mixed beech (Nothofagus) forests, New Zealand. Vegetatio 82:101–114.

Tonsor SJ, Kalisz S, Fisher J, and Holtsford TP, 1993. A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*. Evolution 47:833–843.

Ueno S, Tomaru N, Yoshimau H, Manabe T, and Yamamoto S, 2000. Size-class differences in genetic structure and individual distribution of *Camellia japonica* L. in a Japanese old-growth evergreen forest, Tsushima, Japan. Mol Ecol 9:647–659.

Verheyen K and Hermy M, 2001. The relative importance of dispersal limitation of vascular plants in secondary forest succession in Muizen Forest, Belgium. J Ecol 89:829–840.

Weir BS, 1990. Genetic data analysis: methods for discrete population analysis. Sunderland, MA: Sinauer Associates.

Weir BS, 1996. Genetic data analysis II. Sunderland, MA: Sinauer Associates.

Weir BS and Cockerham CC, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wendel JF and Parks CR, 1982. Genetic control of isozyme variation in *Camellia japonica* L. J Hered 73:197–204.

Wright S, 1951. The genetical structure of populations. Ann Eugen 15:323–354.

Wright S, 1970. Random drift and the shifting balance theory of evolution. In: Mathematical topics in population genetics (Kojima K, ed). New York: Springer-Verlag; 1–31.

Wright S, 1977. Evolution and the genetics of populations. Vol. 4: Variability within and among natural populations Chicago: University of Chicago Press.

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