

GENETIC CONSEQUENCES OF SEED DISPERSAL IN THREE SYMPATRIC FOREST HERBS. I. HIERARCHICAL POPULATION-GENETIC STRUCTURE

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Abstract.—To examine the effects of seed dispersal on spatial genetic structure, we compare three sympatric species of forest herbs in the family Apiaceae whose fruits differ widely in morphological adaptations for animal-attached dispersal. *Cryptotaenia canadensis* has smooth fruits that are gravity dispersed, whereas *Osmorhiza claytonii* and *Sanicula odorata* fruits have appendages that facilitate their attachment to animals. The relative seed-dispersal ability among species, measured as their ability to remain attached to mammal fur, is ranked *Sanicula* > *Osmorhiza* > *Cryptotaenia*. We use a nested hierarchical sampling design to analyze genetic structure at spatial scales ranging from a few meters to hundreds of kilometers. Genetic differentiation among population subdivisions, estimated by average genetic distance and hierarchical *F*-statistics, has an inverse relationship with dispersal ability such that *Cryptotaenia* > *Osmorhiza* > *Sanicula*. In each species, genetic differentiation increases with distance among population subdivisions. Stochastic variation in gene flow, arising from seed dispersal by attachment to animals, may partly explain the weak relationship between pairwise spatial and genetic distance among populations and heterogeneity in estimates of single locus *F*-statistics. A hierarchical island model of gene flow is invoked to describe the effects of seed dispersal on population genetic structure. Seed dispersal is the predominant factor affecting variation in gene flow among these ecologically similar, taxonomically related species.

Key words.—Apiaceae, comparative approach, *Cryptotaenia canadensis*, gene flow, hierarchical *F*-statistics, isozymes, *Osmorhiza*, population genetic structure, *Sanicula odorata*, seed dispersal.

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Pollen and seed dispersal have important consequences for the spatial genetic structure and thus the evolutionary dynamics of plant populations. Plant populations may become genetically differentiated over short distances because of spatially heterogeneous selection, often in opposition to high rates of gene exchange (Jain and Bradshaw 1966; Antonovics 1968; McNeilly 1968; Antonovics and Bradshaw 1970; Snaydon 1970; Turkington and Harper 1979; Waser and Price 1985; Tonsor 1990). Perhaps more commonly, spatial genetic structure at selectively neutral loci may arise because of restricted dispersal and genetic drift (Wright 1931, 1943, 1946, 1951; Malecot 1948; Rohlf and Schnell 1971; Turner et al. 1982; Sokal et al. 1989). The “shifting-balance” model of Wright (1932, 1988) predicts that such spatial genetic structure facilitates adaptive evolution. How often appropriate conditions exist in nature for the shifting-balance process to occur remains under debate (Crow et al. 1990; Wade and Goodnight 1991).

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Both direct and indirect measures of gene flow indicate that dispersal is limited in many plant species and leads to spatial genetic differentiation (Ehrlich and Raven 1969; Levin and Kerster 1974; Brown 1979; Handel 1983; Crawford 1984; Loveless and Hamrick 1984; Slatkin 1985; Hamrick and Loveless 1986; Hamrick and Godt 1990; Heywood 1991). Comparisons of allozyme variation among many unrelated plant taxa have shown that the amount of gene flow, and hence the extent of genetic differentiation among population subdivisions, is broadly correlated with several ecological and life-history characteristics, particularly breeding system, life form, and mode of seed dispersal. However, seed dispersal appears to exert a much weaker influence than the breeding system on population genetic structure. Moreover, mode of seed dispersal has not proved a strong predictor of the relative degree of population differentiation compared among plant species. In particular, species with animal-mediated seed dispersal, via ingestion by or external attachment to birds and mammals, tend to have high levels of genetic differentiation similar to those with gravity-dispersed seeds. Wind-dispersed species tend to exhibit the least genetic differentiation among population subdivisions

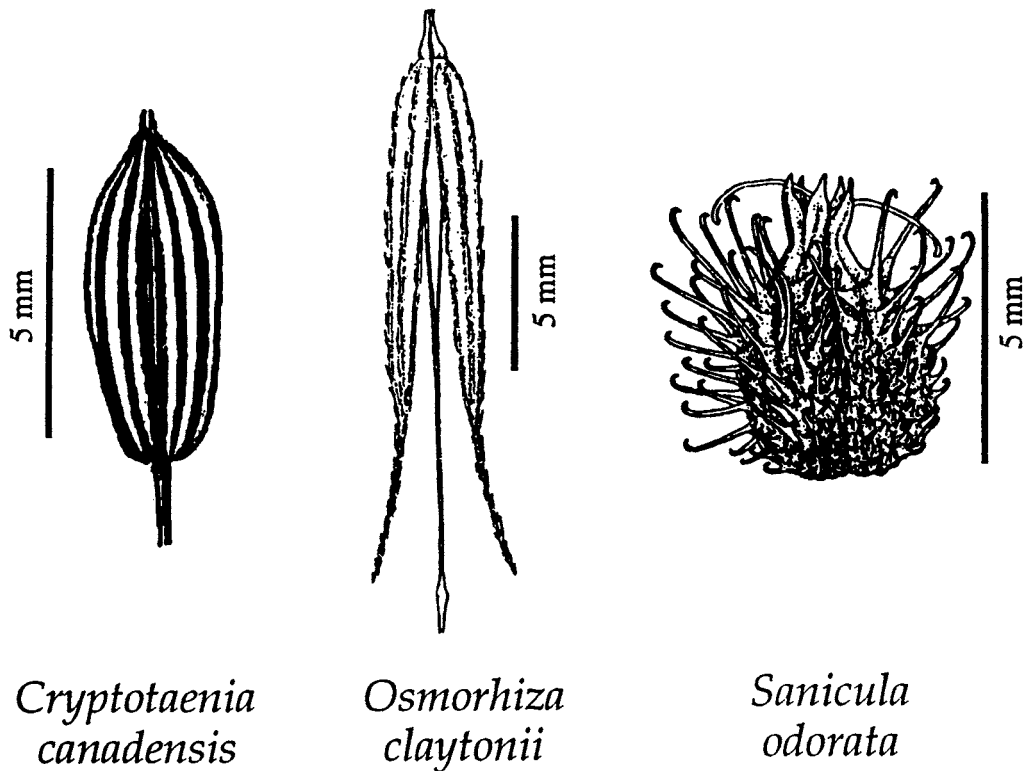


FIG. 1. Fruit morphologies of *Cryptotaenia canadensis*, *Osmorhiza claytonii*, and *Sanicula odorata* (adapted from Hiroe and Constance 1958; Constance and Shan 1948; Shan and Constance 1951).

(Loveless and Hamrick 1984; Hamrick and Godt 1990).

Broad taxonomic comparisons such as those cited above may present a biased picture of the effects of seed dispersal on population genetic structure for at least two reasons. First, the effects of correlated life-history traits may obscure the influence of seed dispersal on genetic structure. For instance, many species with wind-dispersed seeds also tend to be wind-pollinated, outcrossing trees, whereas many species with seed dispersal by gravity or attachment to animals are self-fertilizing herbs (Loveless and Hamrick 1984; Uma Shaanker et al. 1990). Second, comparisons across taxa often do not differentiate among the scales at which genetic structure is measured in different studies, such that their results may not be directly comparable. To examine the effects of seed dispersal on genetic structure, independent of variation in other ecological and life-history characters, it is necessary to study patterns of variation within and among populations of similar species (Loveless and Hamrick 1984). Only a few such comparative studies have been

made (Levin 1978; Schoen 1982; Sun and Ganders 1988; Van Dijk et al. 1988; Holtsford and Ellstrand 1989; Loveless and Hamrick 1988; Karron et al. 1988; Karron 1989; Pleasants and Wendel 1989), and none explicitly have examined seed dispersal.

To investigate the effects of seed dispersal on population genetic structure, we compare three ecologically similar species of forest herbs in the family Apiaceae whose fruits vary widely in morphological adaptations for dispersal (fig. 1). The fruits of *Sanicula odorata* and *Osmorhiza claytonii* have appendages that should facilitate their attachment to animals (ectozoochory), whereas *Cryptotaenia canadensis* fruits are smooth and do not appear to be dispersed by animals. Based on differences in fruit morphology, we predict that seed dispersal should be the most restricted in *Cryptotaenia* and least restricted in *Sanicula*. These differences in seed-dispersal ability are expected to result in the most genetic differentiation among population subdivisions in *Cryptotaenia* and the least in *Sanicula*. To test this prediction, we use a hierarchical sampling design

to examine patterns of genetic differentiation at several spatial scales. We address the following questions. (1) Do differences in fruit morphology among species correlate with their adherence to mammal fur, an analogue of dispersal ability? (2) Do species with more restricted seed dispersal have greater genetic differentiation among population subdivisions? (3) How does the apportionment of genetic variation within each species differ across spatial scales?

MATERIALS AND METHODS

Study Organisms

Cryptotaenia canadensis (L.) DC., *Osmorhiza claytonii* (Michx.) C. B. Clarke, and *Sanicula odorata* (Raf.) Pryer and Phillippe (formerly *Sanicula gregaria* Bickn.; Pryer and Phillippe 1989) (Apiaceae) are three common forest understory herbs that grow sympatrically in southern Wisconsin, where they have probably co-occurred since the last glacial retreat ca. 10,000 yr ago. They are found in a wide variety of forest habitats, but tend to occur most frequently in disturbances such as treefall gaps, forest edges, and streambanks. Their distribution is thus often patchy (Curtis 1959; Fassett 1976).

All three species are polycarpic perennials with little or no vegetative spread. *Cryptotaenia* reproduces from seed and by (usually single) vegetative offshoots from monocarpic ramets (Baskin and Baskin 1988 pers. obs.). *Osmorhiza* reproduces only by seed (Baskin and Baskin 1984, 1991), whereas *Sanicula* appears capable of limited vegetative spread as evidenced by occasional multiple flowering ramets from the same short rootstock (Shan and Constance 1951 pers. obs.).

Several lines of evidence suggest that pollen dispersal distances are similarly restricted in each species. They each have protogynous flowers and inflorescences that are andromonoecious (Constance and Shan 1948; Shan and Constance 1951; Hiroe and Constance 1958; Gleason and Cronquist 1963; Baskin and Baskin 1988). All are self-fertile and capable of mechanical self-pollination in the absence of insect visitation, as determined using pollinator exclosures (Williams 1991). Like other members of the Apiaceae, these species have relatively unspecialized flowers visited by a suite of small, generalist pollinators (Knuth 1908; Bell 1971; Lindsey 1984). Common flower visitors to all species in this study were small solitary bees (Halictidae, Andrenidae), bee flies (Syrphidae), and beetles (Mordellidae). These

visitors have been characterized as ineffective long-distance pollinators in an earlier study of related species of Apiaceae (Lindsey 1984). Limited observations suggest that patterns of pollinator visitation do not differ greatly among these plant species. Most pollinator movements were among flowers on a single plant or two adjacent plants. Estimates of mating system parameters are available only for *Cryptotaenia*, where outcrossing rates vary from 5%–40% among populations (C. F. Williams unpubl. data).

The fruits of these species vary widely in morphology and apparent adaptations for dispersal. The fruits of *Sanicula* and *Osmorhiza* have appendages (numerous recurved hooks and short spines, respectively) that should facilitate attachment to animals, whereas *Cryptotaenia* fruits are smooth and apparently gravity dispersed (fig. 1). Each hermaphroditic flower can produce two single-seeded, dry fruitlets comprising the schizocarp. On average, *Cryptotaenia* produces more seeds per plant ($\bar{x} = 42.7$, $SD = 27.3$, $N = 21$) than *Osmorhiza* ($\bar{x} = 28.0$, $SD = 22.5$, $N = 96$) or *Sanicula* ($\bar{x} = 25.1$, $SD = 18.0$, $N = 37$) (C. F. Williams unpubl. data). None of these species' seeds appear to be eaten by potential vertebrate dispersers, although herbivory and invertebrate seed predation are common (Williams 1991).

Study Sites and Sampling Design

We sampled 18 different sites (= populations) in Wisconsin and northeast Iowa. Thirteen sites are in the Baraboo Hills of Sauk County, Wisconsin, and five in outlying areas (fig. 2). All three species are found at most, but not all sites (*Cryptotaenia*, 17 sites; *Osmorhiza*, 16 sites; *Sanicula*, 14 sites). The vegetation at most sites is relatively undisturbed, southern mesic or dry-mesic forest (Curtis 1959). The Baraboo Hills and Kickapoo River sites are part of large, contiguous blocks of forest in which direct interpopulation gene flow can potentially occur.

The sampling design includes four hierarchical levels: geographic *regions* (65–375 km between population subdivisions), *drainages* within regions (2.5–20 km apart), *populations* within drainages (0.5–7.0 km apart), and *subpopulations* within populations (5–100 m apart). The four outlying regions are represented by one or two populations each. In Sauk County, four different drainages containing two to four populations each were sampled (fig. 2, table 1). Populations are further subdivided into varying numbers of sub-

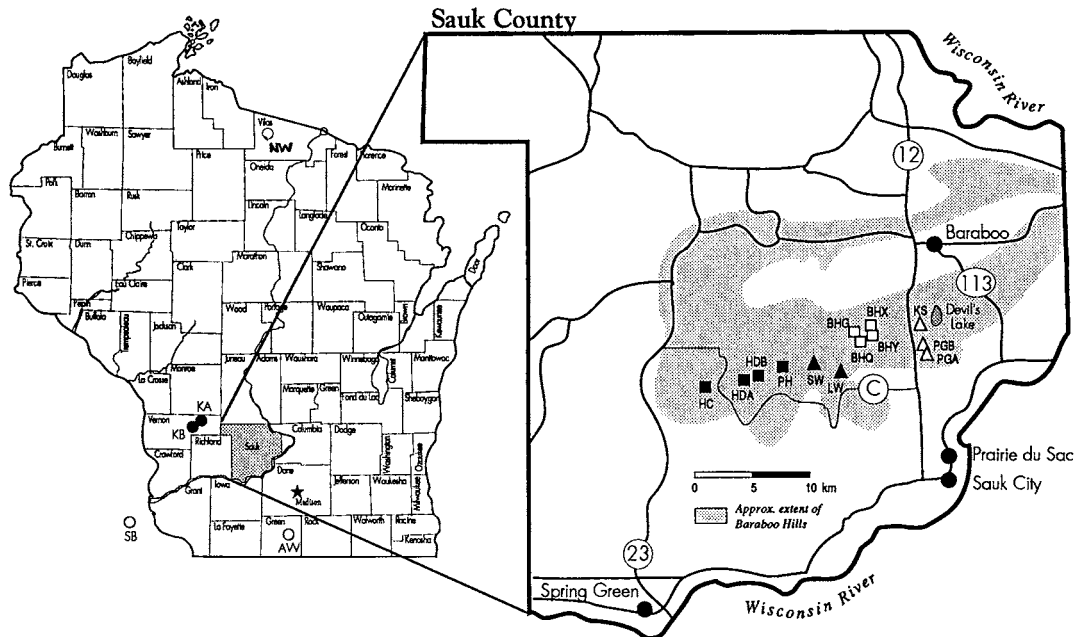


FIG. 2. Locations of study populations in Wisconsin and Iowa. Populations from the same drainage in the Sauk County, Wisconsin region are indicated by the same symbols.

populations (table 1) as follows. In most populations, groups of individuals were sampled at 5- to 15-m intervals along a 50-m transect. In some very low density populations, plants were sampled as encountered along 100- to 200-m transects, or from scattered, distinct groups. Subpopulation boundaries coincide with those of naturally occurring groups of plants except in dense, continuous populations in which five to six plants were sampled every 5 m, and two such points are combined to form a subpopulation sample. Subpopulations encompass areas ranging from approximately 50 m² to 250 m². Subpopulation size in these transect sampled sites ranges from 3–28 ($\bar{x} \pm 1$ SD; *Cryptotaenia*, 13.9 \pm 6.6; *Osmorhiza*, 12.8 \pm 4.0; *Sanicula*, 10.7 \pm 4.6) plants. At two sites used for a study of microspatial genetic structure, 158–435 individuals of each species were sampled from mapped locations on a grid. The sampled area covers 30 m \times 60 m at site BHG (70 m \times 80 m for *Cryptotaenia*) and 10 m \times 20 m at site HDB. At BHG, the sampled area is divided into 15 m \times 15 m quadrats and those quadrats containing plants are used as subpopulations. At HDB, the grid was divided into 5 m \times 10 m quadrats (10 \times 10 for *Osmorhiza*) plus two segments of an adjacent transect sampled earlier. Subpopulation

sample size in these mapped populations ranges from 3–195 (*Cryptotaenia*, 31.2 \pm 40.7; *Osmorhiza*, 25.4 \pm 23.3; *Sanicula*, 35.5 \pm 46.8) plants (table 1).

Leaf material was collected for isozyme analysis at the different sites from 1987 to 1989. Because these are perennial species, sample genotype frequencies within populations should not change greatly over this period. To confirm this expectation, different individuals were sampled from two sites (BHG and HDB) in repeated years. At these two sites, no significant differences in allele or genotype frequencies are observed between years; thus data from individuals and populations sampled in different years are analyzed together without regard to year of collection.

Seed Adherence

We estimated the *relative* adherence of different fruits to dispersers by comparing the length of time they remained attached to mammal fur. We used museum skins of deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), and squirrel (*Sciurus carolinensis*), which represent three common, potential dispersal agents in the study area. Fruits were gently rubbed onto the dorsal surface of the skin with several passes of a wooden tongue depressor. Twenty fruits were initially

TABLE 1. Hierarchical structure of sampling sites, abbreviations used in subsequent maps and figures, and sample sizes for each species. N_i , total number of individuals sampled. N_s , number of subpopulations used in the hierarchical F -statistics analysis.

Site	Abbreviations	<i>Cryptotaenia</i>		<i>Osmorhiza</i>		<i>Sanicula</i>	
		N_i	N_s	N_i	N_s	N_i	N_s
Region 1: Sauk Co., Wisc.							
Drainage 1: Devil's Lake							
Pine Glen (A)	PGA	39	3	36	3	0	0
Pine Glen (B)	PGB	36	2	40	3	0	0
Koshawago Springs	KS	36	3	39	4	13	1
Drainage 2: Baxter's Hollow							
East Plateau (X)	BHX	69	4	51	4	42	4
East Valley (Y)	BHY	41	1	27	2	26	1
West Plateau (G)	BHG	158	9	264	8	172	5
West Valley (Q)	BHQ	36	2	34	2	33	3
Drainage 3: Pan Hollow							
Leopold Woods	LW	40	2	37	3	40	4
Scott Woods	SW	35	3	0	0	36	3
Drainage 4: West Baraboo Hills							
Pine Hollow	PH	33	2	36	2	34	3
Hemlock Draw (A)	HDA	33	3	33	3	31	3
Hemlock Draw (B)	HDB	435	10	258	4	360	10
Honey Creek	HC	36	3	36	3	36	3
Region 2: Green Co., Wisc. (South)							
Abraham's Woods	AW	43	4	54	4	0	0
Region 3: Vernon Co., Wisc. (West)							
Wildcat Mtn. (A)	KA	36	4	0	0	36	4
Hay Valley (B)	KB	8	1	41	3	36	4
Region 4: Vilas Co., Wisc. (North)							
Northern Wisconsin	NW	0	0	45	3	0	0
Region 5: Clayton Co., Ia. (far West)							
Steele's Branch	SB	35	3	29	3	33	4

applied in each replicate using deer and raccoon skins, and 10 fruits were applied on the squirrel skin. The specimen was then inverted, causing some seeds that did not adhere to the fur to drop off (zero shakes). The number of remaining seeds dislodged after 1, 3, 5, 10, and 20 shakes of the study skin were recorded. Shakes were standardized by holding the specimen by the anterior end and dropping the posterior end approximately 12 inches to a table edge. Ten replicates were made per each fruit and mammal species. Differences between plant species in the proportion of fruits remaining attached to mammal skins were tested with ANCOVA using the number of shakes as the covariate. *Cryptotaenia* seeds did not adhere at all; therefore, only differences between *Osmorhiza* and *Sanicula* were tested. Significant heterogeneity of slopes existed among the three mammal species, such that comparisons between fruits were made for each disperser

separately. Differences between plant species adjusted for the number of shakes were tested using the model: $(\ln) \text{ proportion adherence} = \text{constant} + \text{fruit species (categorical)} + \text{number of standard shakes (continuous)}$. Analyses were run using the multivariate general linear hypothesis (MGLH) module of Systat 5.0 (Wilkinson 1989).

Electrophoresis

Leaf samples ranged from 50 to 500 mg fresh weight, and except for small seedlings, sampling was nondestructive. Immediately after collection, samples were placed in labeled plastic bags and kept on ice or in a 5°C refrigerator until processed, usually within 24 h. Electrophoresis was performed using crude leaf homogenate ground with an ice-cold mortar and pestle. The grinding buffer was a slight modification of that described by Wendel and Parks (1982) and Marty et al. (1984). Samples kept on ice after grinding

TABLE 2. Single locus estimates of hierarchical F -statistics for *Cryptotaenia canadensis*, *Osmorhiza claytonii*, and *Sanicula odorata*. For each locus, the number of individuals sampled, number of alleles scored, and the mean frequency per population of the most common allele are given. F -statistics estimated are genetic variance among individuals in the total sample (\hat{F}_{it}), inbreeding within subpopulations (\hat{f}_{is}), and differentiation among subpopulations within populations ($\hat{\theta}_s$), among populations within drainages ($\hat{\theta}_p$), and among drainages within the total ($\hat{\theta}_d$). Populations from outlying regions are included as separate drainages in the analysis.

Locus	Sample size	No. alleles	Mean allele frequency	F -statistics				
				\hat{F}_{it}	\hat{f}_{is}	$\hat{\theta}_s$	$\hat{\theta}_p$	$\hat{\theta}_d$
A. <i>Cryptotaenia canadensis</i>								
<i>Fe-1</i>	1137	3	.766	.565	.261	.068	.194	.217
<i>Idh-2</i>	1149	2	.664	.778	.439	.158	.163	.440
<i>Lap-1</i>	1071	3	.620	.726	.447	.096	.067	.413
<i>Mdh-2</i>	1149	2	.506	.713	.429	.139	.107	.346
<i>Pgi-2</i>	840	3	.980	.671	.521	.165	-.040	.210
<i>Pgm-1</i>	1149	2	.989	.968	.904	.665	-.029	.027
<i>Pgm-2</i>	1143	3	.602	.705	.486	.256	.168	.075
<i>Pgm-3</i>	1148	3	.992	.921	.782	.681	-.120	-.200
<i>Tpi-3</i>	1116	3	.928	.937	.923	.038	.057	.105
B. <i>Osmorhiza claytonii</i>								
<i>Est-1</i>	915	2	.869	.955	.915	.051	.199	.297
<i>Idh-1</i>	939	3	.859	.941	.884	.108	.064	.392
<i>Me-1</i>	931	2	.968	.922	.909	.093	.003	.055
<i>Pgm-2</i>	926	3	.902	.977	.967	.185	.057	.058
<i>Tpi-2</i>	928	2	.891	.908	.889	.051	.086	.049
C. <i>Sanicula odorata</i>								
<i>Aat-2</i>	912	2	.837	.381	.207	.089	.007	.138
<i>Aco-1</i>	921	2	.963	.400	.306	.029	.009	.102
<i>Aco-2</i>	920	2	.978	.131	.062	.017	.029	.029
<i>Dia-1</i>	926	2	.978	.206	.105	-.010	.034	.091
<i>Dia-2</i>	927	2	.986	.557	.540	.001	.027	.008
<i>Est-1</i>	895	3	.878	.671	.581	.064	.078	.089
<i>Pep-3</i>	895	3	.912	.317	.275	.023	.013	.022
<i>Pgi-1</i>	928	3	.864	.326	.088	.031	.037	.208
<i>Pgm-1</i>	681	2	.915	.913	.787	.102	.552	-.020
<i>Pgm-2</i>	928	3	.935	.817	.733	.118	.067	.167
<i>6pg-3</i>	668	2	.987	.529	.506	-.005	.015	.037

then stored at -80°C retained good activity for ≥ 2 yr for most enzymes. We prepared 11.2% or 12.0% w/v starch gels using the microwave technique of Marty et al. (1984). Homogenate was absorbed into 3×14 mm, Whatman #3 filter paper wicks. Gels were run in a 5°C refrigerator at ≤ 50 mA and 150 V for 4–6 h.

We used four gel buffer systems to resolve the different enzymes studied in each species: (1) AC, Morpholine-citrate pH 6.7 (modified from Clayton and Tretiak 1972, and Marty et al. 1984); (2) S4, Tris-citrate pH 7.5 (#4 of Soltis et al. 1983); (3) S6, NaOH-boric Acid pH 8.6 electrode/Tris-citrate pH 7.8 gel (#6 of Soltis et al. 1983); and (4) S11, Histidine-citrate pH 7.0 (#11 of Soltis et al. 1983). Stain recipes are those found in Marty et al. (1984) except LAP (Soltis et al. 1983), and TPI (Stuber et al. 1988). Optimal buffer \times enzyme combinations for each species are reported in Williams (1991).

A total of 15 enzyme systems coding for 31 putative loci were scored for *Cryptotaenia*, 14 systems coding for 24 loci were scored for *Osmorhiza*, and 16 systems coding for 35 loci were scored for *Sanicula* (Williams 1991). Twelve polymorphic loci were resolved for *Cryptotaenia*, 7 for *Osmorhiza*, and 13 for *Sanicula*. All polymorphic loci were used for estimating pairwise genetic distances. Only those loci with mean frequencies of the most common allele < 0.95 overall, or a frequency of < 0.95 in at least one population, were used for estimating F -statistics (9 loci for *Cryptotaenia*, 5 for *Osmorhiza*, 11 for *Sanicula*; table 2). Complete allele frequency data are reported in Williams (1991).

Analyses of Genetic Structure

We calculated several pairwise genetic distance measures between proportions for all polymorphic loci (Nei 1972, 1978; Rogers 1972; Ca-

valli-Sforza and Edwards 1967; Edwards 1971) using the BIOSYS-1 program (Swofford and Selander 1981). All measures produced similar groupings of populations using unweighted pair-group arithmetic averaging (UPGMA) clustering (Sneath and Sokal 1973). Results presented here use the geometric (chord) genetic distance of Edwards (1971), because of its large range of observed values and similarity to Wright's F_{st} (Hartl and Clark 1989).

We calculated average pairwise genetic distances between populations for three levels of spatial organization: (1) within drainages, (2) between drainages within geographic regions, and (3) between regions. Statistical comparisons of average genetic distance estimates both within hierarchical levels between species, and between hierarchical levels within species employed bootstrapped confidence intervals (CIs) (Efron 1982) calculated from all pairwise distances at a given level of the hierarchy. Ninety-five percent CIs (one-tailed) were calculated as the central 90% of observations from the distribution of 2500 bootstrapped estimates. Two estimates are regarded as statistically different at the $P < 0.05$ significance level if their one-tailed 95% CIs do not overlap.

Hierarchical F -statistics (Wright 1951) were calculated following the methods of Weir and Cockerham (1984) and Weir (1990). This method facilitates the calculation of multilocus estimates and their variances using a jackknife procedure. F -statistics were calculated for genetic variance among individuals in the total sample (\hat{F}_{it}), inbreeding within subpopulations (\hat{f}_{is}), and differentiation among subpopulations within populations ($\hat{\theta}_s$), among populations within drainages, ($\hat{\theta}_p$), and among drainages within the total ($\hat{\theta}_a$), as outlined in the Appendix. Negative estimates of θ can occur because the variance components from which F -statistics are calculated are in fact covariances and hence not constrained to be positive. Small negative values may result from bias in the estimator and should be regarded as not significantly different from zero. Negative estimates may also arise because genes are more similar between than within population subdivisions (a negative intraclass correlation) (Weir 1990). As with average genetic distances, statistical differences in multilocus estimates of F -statistics were tested as nonoverlap of one-tailed 95% CIs (two-tailed 90% CIs for \hat{F}_{it} and \hat{f}_{is}). Each bootstrap estimate was generated by drawing with replacement from the set

of variance components used for each single locus estimate. The central 90% of the distribution of 1500 multilocus bootstrap estimates was used to define the upper and lower bounds of the CIs. Hierarchical F -statistics were estimated using the complete data set in which populations from outlying regions are treated as separate drainages (i.e., there were nine total drainages, four from Sauk County and five from outlying regions). F -statistics were also estimated for just the 13 populations in four drainages from Sauk County to be used to estimate rates of gene flow, Nm .

Estimates of effective rates of gene flow among population subdivisions, Nm , were calculated using the formula,

$$Nm = (1/\theta - 1)/4$$

(Wright 1931). The effects of differences in sample size among population subdivisions on estimates of θ are accounted for by the method of Weir and Cockerham (1984), and hence Nm can be estimated directly from $\hat{\theta}$ (Slatkin 1985). Nm was estimated for each of the three levels of population subdivision used in the F -statistics analysis from $\hat{\theta}_s$, $\hat{\theta}_p$, and $\hat{\theta}_a$, respectively. Standard errors of Nm were estimated using a jackknife procedure. Confidence intervals around each Nm estimate were calculated by bootstrapping 1500 times over the variance components of single locus estimates of each F -statistic and then calculating Nm from each bootstrapped multilocus estimate of θ .

RESULTS

Seed Adherence

Marked differences exist in adherence of fruits to mammal skins and among the three mammal species tested (fig. 3). Fruits of *Sanicula* adhere more firmly than those of *Osmorhiza* when adjusted for the number of "shakes" as the covariate (deer, $F = 11.9$, $P = 0.001$; raccoon, $F = 99.3$, $P < 0.001$; squirrel, $F = 56.5$, $P < 0.001$; ANCOVA). The fruits of *Cryptotaenia* do not adhere at all. The initial rate of seed loss, over the interval 0–1 shakes, is much steeper for *Osmorhiza* than *Sanicula*, suggesting that a large proportion of attached *Osmorhiza* fruits are lost over a relatively short time or distance in natural situations. In both *Osmorhiza* and *Sanicula*, there is a long tail to the distribution of the number of fruits remaining attached indicating the potential for long-distance dispersal.

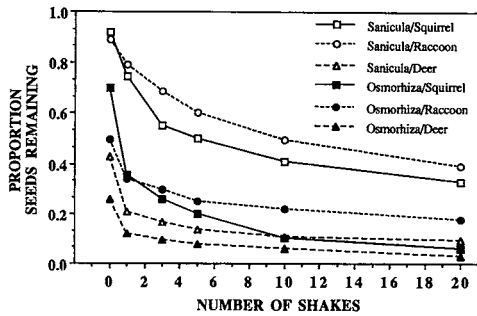


FIG. 3. Relative adherence abilities of *Sanicula* and *Osmorhiza* fruits to the fur of different mammal species. The proportion of fruits remaining attached to a mammal skin following 0, 1, 3, 5, 10, and 20 "shakes" are shown. Each point represents the mean of 10 replicates.

Genetic Distance

Within each species, average genetic distance between populations increases from within drainages to between drainages. These differences are significant at $P < 0.05$ as indicated by the nonoverlap of the one-tailed 95% confidence intervals around each estimate (fig. 4). Populations from outlying regions are not, on average, significantly more genetically dissimilar than populations between drainages within a region for *Cryptotaenia* and *Sanicula*. Genetic distance between populations increases significantly at each level of the spatial hierarchy in *Osmorhiza*.

Significant differences exist ($P < 0.05$) among species in average pairwise genetic distance at each level of the hierarchy as indicated by nonoverlap of one-tailed 95% confidence intervals (CIs) (fig. 4). Within drainages, *Cryptotaenia* populations are significantly more differentiated than *Sanicula* populations, whereas *Osmorhiza* is intermediate but not significantly different from the other species. Between drainages, *Cryptotaenia* has significantly larger average genetic distances among populations than either of the other species, which do not differ significantly from one another. Between regions, average genetic distance varies significantly among all species, with *Cryptotaenia* > *Osmorhiza* > *Sanicula*.

Populations do not form clusters based on pairwise genetic distances as predicted by geographic proximity alone (fig. 5). In general, populations sharing a drainage form fairly well-defined clusters, but outlying populations (outside Sauk County region) are not genetically distinct. Few novel alleles or large shifts in gene frequencies are found in the outlying populations. Also,

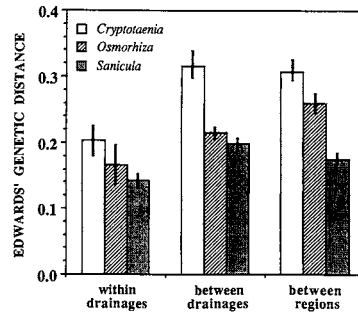


FIG. 4. Average pairwise genetic distance at three spatial scales: (1) between populations within drainages, (2) between populations in different drainages within a region, and (3) between populations in different regions. Error bars are bootstrapped one-tailed 95% confidence intervals (CIs) of the mean. Nonoverlap of CIs indicates means differ significantly at $P < 0.05$.

no strong correlation exists between genetic and geographic distances among the Sauk County populations (Williams 1991).

Hierarchical F-Statistics

Considerable variation exists among single locus estimates of each F -statistic. More than half of all estimates fall above or below the bounds of the 95% CIs of the multilocus estimates. However, the standard errors of the multilocus estimates are relatively small (tables 2, 3). Single locus estimates that vary most widely from the mean are those with low frequencies of the less common allele(s) or with alleles occurring in only one or a few populations (Williams 1991). Such variation in single locus estimates have a relatively small effect on the multilocus estimates of each F -statistic. Jackknife multilocus estimates, sequentially omitting each locus, vary only slightly (< 0.06 absolute) from the mean estimate in all cases. However, this deviation can amount to up to 50% of the mean for some very small (< 0.10) estimates of θ .

Significant differences appear among species in multilocus estimates of each F -statistic (tables 3, 4). The species-specific patterns of differentiation among population subdivisions are completely consistent with predictions based on their relative seed-dispersal abilities. At each level of the spatial hierarchy, the rank order of species' estimates are the reverse of predicted dispersal ability, *Cryptotaenia* > *Osmorhiza* > *Sanicula*. The only exception to this pattern is differentiation among Sauk County populations, where *Osmorhiza* has a somewhat higher estimate of

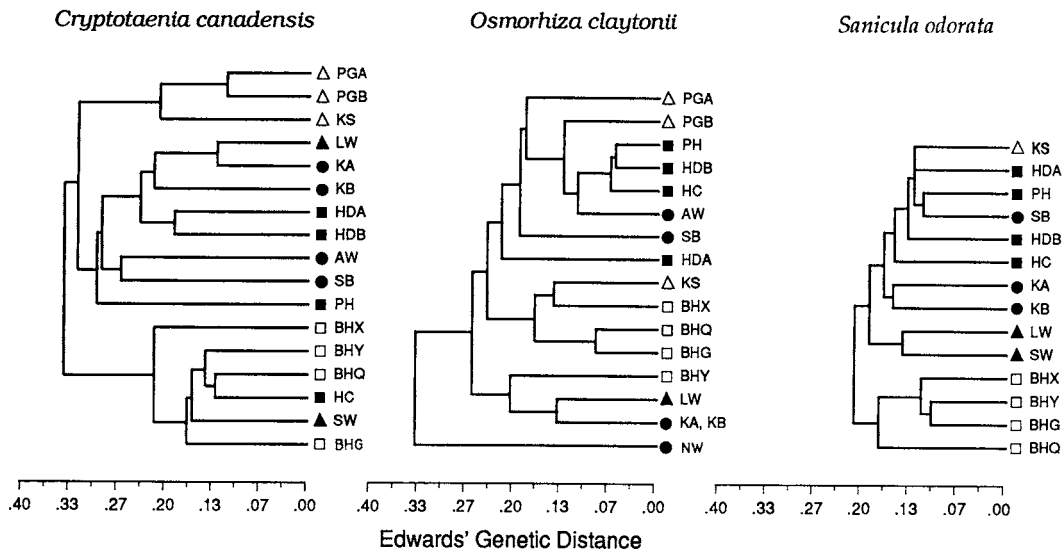


FIG. 5. UPGMA cluster diagrams of pairwise genetic distances (Edwards 1971) among populations of *Cryptotaenia*, *Osmorhiza*, and *Sanicula*. Symbols and population abbreviations correspond to those in figure 2.

θ_p than *Cryptotaenia*. In both Sauk County and the larger sample of populations, *Cryptotaenia* has a significantly larger value of θ_s than *Sanicula*, whereas *Osmorhiza* has an intermediate val-

ue of $\hat{\theta}_s$ with a confidence interval overlapping those of both other species. Although there is no statistically significant variation among species in differentiation at the population level, $\hat{\theta}_p$, they again occur in the predicted rank order, except in the Sauk County sample as noted above. *Cryptotaenia* has significantly greater genetic differentiation among drainages, $\hat{\theta}_d$, than *Sanicula*. *Osmorhiza* is again intermediate and has a con-

TABLE 3. Multilocus estimates of hierarchical *F*-statistics, their jackknifed standard errors (in parentheses) and the upper and lower bounds of their bootstrapped confidence intervals [in brackets] for the total samples of populations. Loci used for multilocus estimates are the same as those in table 2. Estimates that differ significantly between species (at $P < 0.05$ in one-tailed test for $\hat{\theta}_s$, $\hat{\theta}_p$, and $\hat{\theta}_d$; or $P < 0.10$ in two-tailed test for \hat{F}_{it} , and \hat{f}_{is}) are indicated by different superscripted letters.

<i>F</i> -statistic	<i>Cryptotaenia canadensis</i>	<i>Osmorhiza claytonii</i>	<i>Sanicula odorata</i>
\hat{F}_{it}	.720 ^a (± .041) [.789-.662]	.947 ^b (± .008) [.958-.929]	.483 ^c (± .082) [.612-.373]
\hat{f}_{is}	.480 ^a (± .084) [.630-.376]	.912 ^b (± .011) [.933-.891]	.335 ^a (± .103) [.492-.206]
$\hat{\theta}_s$.155 ^a (± .038) [.224-.104]	.088 ^{ab} (± .029) [.139-.058]	.052 ^b (± .012) [.070-.033]
$\hat{\theta}_p$.124 ^a (± .025) [.155-.080]	.122 ^a (± .058) [.171-.050]	.077 ^a (± .042) [.158-.024]
$\hat{\theta}_d$.273 ^a (± .059) [.355-.173]	.250 ^{ab} (± .064) [.319-.055]	.113 ^b (± .032) [.153-.063]

TABLE 4. Multilocus estimates of hierarchical *F*-statistics and their error statistics for the subset of populations from Sauk County, Wisconsin. Error statistics and loci used for multilocus estimates are as in Table 3.

<i>F</i> -statistic	Species		
	<i>Cryptotaenia canadensis</i>	<i>Osmorhiza claytonii</i>	<i>Sanicula odorata</i>
\hat{F}_{it}	.737 ^a (± .039) [.807-.685]	.947 ^b (± .010) [.955-.925]	.488 ^c (± .083) [.618-.371]
\hat{f}_{is}	.504 ^a (± .089) [.660-.392]	.916 ^b (± .006) [.931-.906]	.314 ^a (± .109) [.474-.179]
$\hat{\theta}_s$.148 ^a (± .047) [.228-.085]	.080 ^{ab} (± .031) [.140-.051]	.051 ^b (± .013) [.073-.031]
$\hat{\theta}_p$.145 ^a (± .029) [.183-.094]	.166 ^a (± .076) [.217-.063]	.091 ^a (± .051) [.186-.024]
$\hat{\theta}_d$.273 ^a (± .063) [.361-.169]	.179 ^a (± .105) [.236-.034]	.136 ^a (± .039) [.182-.071]

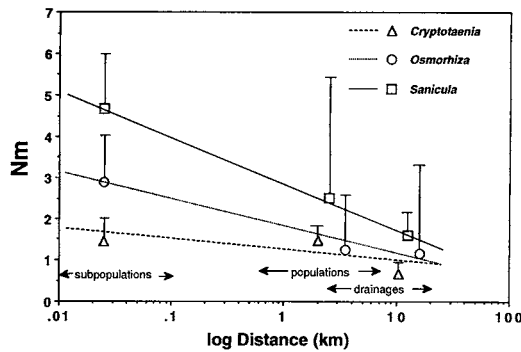


FIG. 6. Estimates of effective rates of gene flow, Nm , among population subdivisions in Sauk County, Wisconsin at three hierarchical spatial scales. Nm was calculated from hierarchical estimates of θ using the formula $(1/\theta - 1)/4$. Error bars are jackknifed standard errors of the estimate.

fidence interval overlapping those of the other species in the total population sample (table 3). Although the rank order among species is the same, differences in estimates of θ_d are not statistically significant in the Sauk County analysis (table 4). Moreover, wide variation exists among species for estimates of F_{it} ($Osmorhiza > Cryptotaenia > Sanicula$) and f_{is} ($Osmorhiza > Cryptotaenia = Sanicula$).

The relative degree of genetic differentiation among spatial levels *within* species shows the expected increase at greater geographic scales (tables 3, 4). In both *Osmorhiza* and *Sanicula*, differentiation increases monotonically from subpopulations to populations to drainages. In *Cryptotaenia*, there is somewhat greater differentiation among subpopulations within populations than among populations within drainages. However, in no case is this pattern statistically significant at $P < 0.05$ except between the levels of populations and drainages in *Cryptotaenia* in the total population sample (table 3).

Gene Flow

Differences among the three species' multilocus estimates of effective gene flow, Nm , (fig. 6) further support predictions based on their relative seed dispersal abilities. In the sample of 13 Sauk County populations, rates of gene flow decline twofold to threefold over the range of distances sampled. As expected, the highest rates of gene flow are among subpopulations (< 100 m apart) and the lowest gene flow among drainages

(2.5–20 km apart) in each species. Within species, estimates of Nm are not significantly different ($P < 0.05$, nonoverlap of 95% CIs) among spatial scales, although estimates differ at $P < 0.10$ between populations and drainages in *Cryptotaenia*, and subpopulations and drainages in *Sanicula*. The species maintain their predicted rank order, $Sanicula > Osmorhiza > Cryptotaenia$, at both the smallest and largest scales. Estimates of Nm differed significantly only at $P < 0.05$ between *Sanicula* and *Cryptotaenia* at the subpopulation level. *Sanicula* has the largest rank-order estimate of gene flow at all spatial scales. Standard errors of the estimates are largest in the two animal dispersed species, *Sanicula* and *Osmorhiza*, at all spatial scales.

DISCUSSION

The initial prediction that the morphology of the fruits of these species would be reflected in their dispersal ability was supported by studies of seed adherence. The hooked fruits of *Sanicula* remain attached to mammal fur longer and hence are potentially dispersed farther than the barbed fruits of *Osmorhiza*. The smooth fruits of *Cryptotaenia* do not adhere at all and appear primarily gravity dispersed. Seed and fruit morphology are often used to infer the general mode of dispersal or define dispersal syndromes (van der Pijl 1982). However, morphological adaptations alone cannot be used to predict the dispersal process (Howe and Smallwood 1982). Although the fruits of both *Osmorhiza* and *Sanicula* have obvious morphological structures for attachment to animal dispersers, the process of ectozoochory can be highly stochastic. Many, if not most, ectozoochorous fruits produced are not removed by a dispersal agent but fall near the parent plant (Bullock and Primack 1977; Shmida and Ellner 1983; Sorenson 1986). Thus, even in plants with apparent adaptations for long-distance dispersal, such as *Osmorhiza* and *Sanicula*, the distribution of dispersal distances may still be highly leptokurtic and lead to local genetic differentiation (Levin and Kerster 1974; Levin 1984). Differences also may exist among animal species in the "quality" of dispersal they afford. Differences appear in the adherence of fruits to different types of mammal fur. In general, fruits remain better attached to animals with long, thick underhairs and lacking stiff guard hairs (e.g., raccoon). Other factors besides pelage characteristics, such as the movement patterns, grooming behavior, and size of different animal species will

affect their potential as seed dispersers as well (Agnew and Flux 1970; Lacey 1982; Sorenson 1986).

If differences in seed dispersal among these three ecologically similar species are the primary source of variation influencing gene flow, then the relative amounts of genetic differentiation among population subdivisions should inversely reflect their dispersal abilities (i.e., *Cryptotaenia* > *Osmorhiza* > *Sanicula*). Likewise, if rates of gene flow are a monotonically decreasing function of distance between population subdivisions (Wright 1943, 1946; Kimura and Weiss 1964), then differentiation should increase at greater spatial scales. Two measures of genetic differentiation, average genetic distance, and multi-locus F -statistics, gave concordant results that support both of these predictions.

Variation among species in estimates of the inbreeding coefficient, \hat{f}_{is} , indicates that differences in pollen dispersal may also contribute to variation in rates of gene flow among these species. *Osmorhiza* has significantly higher estimates of \hat{f}_{is} than the other species. This suggests that *Osmorhiza* has a substantially higher level of self-fertilization than the other species. This unexpected difference in three species' mating systems may retard gene flow through pollen in *Osmorhiza* relative to that of *Cryptotaenia* and *Sanicula*, which will lead to a more genetic differentiation among population subdivisions in *Osmorhiza* relative to the other species than would occur because of differences in seed dispersal alone. This may help explain why the amount of differentiation among populations and drainages in *Osmorhiza* is more similar to *Cryptotaenia* than *Sanicula*. More importantly however, the rank order of differentiation among species predicted by seed-dispersal ability was maintained despite the large differences in \hat{f}_{is} , and presumably mating systems, at all spatial scales; *Osmorhiza*, with the highest values of \hat{f}_{is} , still has less genetic differentiation than *Cryptotaenia*. This further supports the assumption that variation in seed dispersal is the primary factor leading to differences in population genetic structure in these species.

Cluster analysis of pairwise genetic distances and correlations of genetic and spatial distance indicate that geographic distance alone does not explain the pairwise migration rates between populations of these species. Long-distance migration may be stochastic, and/or other paths of "connectedness" between populations may bet-

ter reflect gene flow than the linear distance between them. Ritland (1989) found similar patterns of genetic distance among montane populations of *Mimulus caespitosus*. In *Mimulus*, populations along the same streams were genetically very similar, but there was only a weak pairwise relationship between spatial distance and genetic distance at larger spatial scales. Animal dispersers may tend to follow the natural topography, following streambeds or contours. Passively dispersed seeds may be washed downhill. Within drainages, populations in the same stream channel (PGA and PGB, HDA and HDB) or at the top and bottom of a slope (BHG and BHQ, BHX and BHY) tended to be more genetically similar than to populations along another stream or on adjacent ridges. Similar migration patterns along stream drainages have been found in other studies of plants (Waser et al. 1982; Ritland 1989), snails (Selander and Whitam 1983; Arter 1990), and humans (Smouse and Wood 1987).

Stochastic rates and patterns of gene flow may also help explain the observed heterogeneity of single-locus estimates of F -statistics. Populations most likely do not receive all alleles at the same rates or from the same locations. Such historical artifacts may persist for long periods if migration rates are small. Somewhat surprisingly, it is in the animal-dispersed species, with the highest rates of gene flow, that the greatest variation in estimates of Nm are found. This suggests that it is occasional long-distance migration that is most important for gene flow, but such migration is very stochastic.

When the relationship between genetic and spatial distance is complicated by stochastic variation in migration rates or nonlinear paths of gene flow, it may be more relevant to examine the average genetic differentiation among population subdivisions at different levels of the spatial hierarchy. This as well as other studies of spatial genetic structure at different scales have shown that genetic differentiation among population subdivisions ($F_{st} = \theta$) tends to increase with increasing spatial distance between sampling sites (Loveless and Hamrick 1984; Ritland and Ganders 1987; Bos et al. 1986; Van Dijk et al. 1988; Heywood 1991). On average, rates of gene flow (and hence genetic differentiation) should be most similar among populations at a given level of spatial subdivision, and less gene flow should occur among more distant populations in increasing hierarchical levels. These are

the assumptions underlying what has been termed the hierarchical island model of gene flow (Slatkin and Voelm 1991). Such a model best explains the patterns of genetic differentiation observed in the three species studied here.

Surveys across a diverse array of plant taxa have not shown a strong relationship between modes of seed dispersal and levels of genetic differentiation among population subdivisions (Loveless and Hamrick 1984; Hamrick and Godt 1990). By comparing three ecologically similar, taxonomically related species we have shown that a reduction in seed dispersal is associated with the predicted increase in genetic differentiation among population subdivisions at spatial scales ranging from a few meters to hundreds of kilometers. The effects of seed dispersal therefore outweigh those of mating system variation or other conflicting ecological and evolutionary factors that may exist among these three species.

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APPENDIX

Computational Formulae for Hierarchical *F*-Statistics

Hierarchical four-level *F*-statistics (Wright 1951) were calculated by the method of Weir and Cockerham (1984) using the formulae given in Weir (1990). This approach is analogous to an ANOVA using allele frequencies and produces unbiased single- and multi-locus estimators of each statistic weighted by allele frequency and different population subdivision sample sizes. Estimates were first computed for each allele, then the appropriate components of variance were summed over alleles for single-locus estimates. Multi-locus estimates were calculated by summing variance components over all alleles at all loci.

Two typographical errors shown in Weir (1990) should be noted in the formulae for calculating four-level hierarchical *F*-statistics. On page 162, the first formula for calculating the sums of squares should read

$$s_i - C = 2 \sum_{i=1}^r u_{i..} (\bar{p}_{Ai..} - \bar{p}_{A..})^2,$$

where $u_{i..}$ replaces the s_1 given. Moreover, the following formula should be

$$s_2 - s_1 = 2 \sum_{i=1}^r \sum_{j=1}^{s_i} u_{ij} (\bar{p}_{Aij} - \bar{p}_{Ai..})^2,$$

in which u_{ij} replaces the t_{ij} given in Weir (1990).

In the hierarchical four-level analysis, the five components of variance V_g , V_i , V_s , V_p , and V_d (variances in genes within individuals, individuals within subpopulations, subpopulations, populations, and drainages respectively) were calculated from their corresponding mean squares and correction terms [calculated per Weir (1990)] as follows

$$V_g = MSG.$$

$$V_i = (MSI - MSG)/2.$$

$$V_s = (MSS - MSI)/2n_{c6}.$$

$$V_p = (MSP - MSI)/2n_{c5}$$

$$- n_{c4}(MSS - MSI)/2n_{c5}n_{c6}.$$

$$V_d = [(MSD - MSI)/2n_{c3}]$$

$$+ [(n_{c2}n_{c4} - n_{c1}n_{c5})(MSS - MSI)/2n_{c3}n_{c5}n_{c6}]$$

$$- [n_{c2}(MSP - MSI)/2n_{c3}n_{c5}].$$

Estimates of the hierarchical F -statistics were then calculated from these variance components as

$$\hat{F}_{it} = \frac{V_i + V_s + V_p + V_d}{V_g + V_i + V_s + V_p + V_d},$$

$$\hat{f}_{is} = \frac{V_i}{V_g + V_i},$$

$$\hat{\theta}_s = \frac{V_s}{V_g + V_i + V_s},$$

$$\hat{\theta}_p = \frac{V_p}{V_g + V_i + V_s + V_p}, \quad \text{and}$$

$$\hat{\theta}_d = \frac{V_d}{V_g + V_i + V_s + V_p + V_d}.$$