Biological Journal of the Linnean Society (1996), *58:* 357–369. With 4 figures

Population genetic variation in rare and endangered *Iliamna* (Malvaceae) in Virginia

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Received 28 March 1995, accepted for publication 30 August 1995

Random amplified polymorphic DNA (RAPD) markers were used as input for an analysis of molecular variance (AMOVA), homogeneity of molecular variance analysis (HOMOVA), and cluster analysis to describe the population genetic structure of *Iliamna corei*, a federally endangered plant located only in Virginia, and *I. remota*, a rare plant in Virginia, Indiana, and Illinois. The analysis was performed to help clarify the taxonomic relationship between the two closely related species. We analysed four clones in the only known population of *I. corei*, breeding stock derived from seeds originating from the population site, and three *I. remota* populations in Virginia. Eighty-five percent of screened primers revealed DNA polymorphisms in *Iliamna*. Ninety-nine informative markers were generated using seven primers. No significant statistical differences (at $P = 0.05$) in RAPD variation was found between species (24% of variance) using the AMOVA procedure. However, within species/among populations (31% of the variance) and within populations (45% of the variance) there were significant differences ($P < 0.002$). An unweighted paired group method using arithmetic averages (UPGMA) cluster analysis showed the federally endangered *I. corei* population to be genetically distinct from the apparently recently introduced (in Virginia: ~ 100 ybp) *I. remota*. The lack of significant differences from the AMOVA and the high number shared bands between *I. corei* and *I. remota* suggest that *I. corei* may be more appropriately classified as a subspecies of *I. remota*. *Iliamna corei* plants in the natural population were genetically similar to one another while the *I. corei* breeding stock plants and *I. remota* plants were genetically relatively diverse.

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ADDITIONAL KEY WORDS: — RAPD – random amplified polymorphic DNA – conservation genetics – plant taxonomy – mallow – AMOVA - analysis of molecular variance – endangered species.

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INTRODUCTION

Iliamna is a North American genus of the Malvaceae containing seven or eight species. Most of the species are indigenous west of the Mississippi River. However, two species are found exclusively in the east: *I. corei* and *I. remota.* They have showy, insect-pollinated, perfect flowers, and are self-incompatible (T. Wieboldt and J. Randall, unpublished data). *Iliamna corei* (Sherff) Sherff, the Peters Mountain mallow, is a federally endangered plant species that exists naturally in a single population, on Peters Mountain, Giles County, Virginia, USA) (Fig. 1). This population consists of four clumps, each comprising a clone (Stewart & Porter, 1995). The Peters Mountain site is a sandstone cliff (1000 m elevation) above the New River. Seeds were unearthed from the site and germinated. The resulting plants were used to establish a breeding population on the campus of Virginia Polytechnic Institute and State University, Blacksburg, Virginia. *Iliamna remota* Greene, the Kankakee mallow, is a rare species that was first collected on Altorf Island in the Kankakee River, Illinois (Strausbaugh & Core, 1932). The species apparently has spread eastward along railroad lines to Virginia (Porter & Wieboldt, 1991). In contrast with the mountain-dwelling *I. corei*, the Virginia *I. remota* populations are located near the James River, not unlike the *I. remota* habitat in Illinois.

Since the discovery of *I. corei* (Strausbaugh & Core, 1932), the proper taxonomic placement of the taxon has been in question. Strausbaugh & Core (1932) placed *I.*

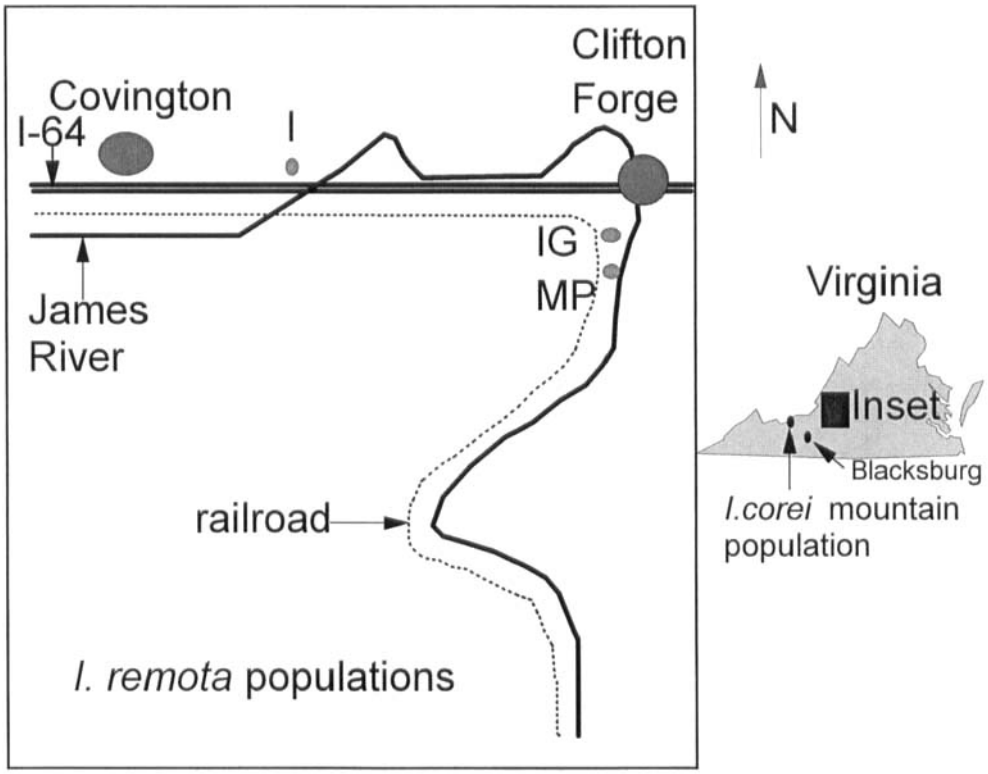

Figure 1. The sites sampled for mallow DNA in Virginia, USA. See Table 3 for population abbreviations.

corei in the same species as *I. remota* (*Phymosia remota* (Greene) Britton) on the basis of similar morphological features. Sherff (1946) revised the classification such that the Kankakee mallow was recognized as *I. remota* var. *typica* and the Peters Mountain mallow was renamed *I. remota* var. *corei* Sherff. The basis for this split was a difference in leaf morphology (the Kankakee mallow leaf typically has a broadly triangular terminal lobe subtended by obtuse sinuses, while the Peters Mountain mallow leaf has an oblong terminal lobe with sharp sinuses), leaf size (Kankakee mallow is larger than Peters Mountain mallow), and plant height (Kankakee mallow, 1.0–1.7 m; Peters Mountain mallow, 0.6–0.9 m). Three years later Sherff (1949) further split the taxa into two species, *I. remota* Greene and *I. corei* (Sherff) Sherff, on the additional characters of differing corolla colors (*I. corei* has a deeper hue), and floral odor (*I. remota* is scented whereas *I. corei* is not). Therefore, the division of *I. remota* and *I. corei* was based upon very few different morphological characters observed on plants growing in ecologically diverse natural habitats. It is conceivable that the characters used by Sherff in separation of the species are plastic. Plastic traits are defined as those that vary in response to spatial (edaphic and geographic), and temporal heterogeneity, and are known to confound taxonomic classifications (Schlichting, 1986). For example, we have noticed that plants in the breeding population are taller than those on Peters Mountain. Likewise, Bounds (1992) reported that there are statistically significant *in situ* morphological differences between the population of *I. corei* and *I. remota* populations in Virginia. In addition, Strausbaugh & Core (1932) reported the height of *I. corei* on the mountain site approached 2 m, although in recent years, the stems have been shorter. Common garden studies would be appropriate to control for differences among *Iliamna* sites. However, to our knowledge, no data stemming from common garden comparisons between *I. corei* and *I. remota* have ever been published, although Sherff (1949) and Scott (1973) reported growing them together in such a situation. An alternative to phenotype analysis to delineate taxa is to perform genetic analysis, which is, by nature, not affected by environment. However, there has been limited work using genetic markers in *Iliamna*. Bounds (1988) reported apparent polymorphisms in five isozymes of *I. corei* and *I. remota* with interclonal variation found in *I. corei*. In a preliminary random amplified polymorphic DNA (RAPD) study, two primers were used to distinguish clones (Stewart & Porter, 1995). No formal population-level analysis has been published to date.

Methods have recently been developed to utilize RAPD profiling (Welsh & McClelland, 1990; Williams *et al.*, 1990) in formal population genetic analyses (Lynch & Milligan, 1994; Stewart & Excoffier, 1996). Although numerous DNA polymorphisms may easily by revealed using RAPDs, the markers are primarily dominant, i.e. homozygotes are indistinguishable from heterozygotes (Tinker, Fortin & Mather, 1993; Williams *et al.*, 1993). Stewart & Excoffier (1996) have modified the analysis of molecular variance (AMOVA) technique (Excoffier, Smouse & Quattro, 1992), adapting it for use with dominant markers. In short, they used estimated amounts of autogamy (selfing frequency from 0 to 1.0) to estimate the average genotype frequencies from the phenotypic (RAPD) data. These estimates were then used in the AMOVA. The AMOVA partitions variance to hierarchical levels, e.g. among and within populations, and tests for significance at these predefined levels, similar to an analysis of variance (ANOVA).

The objectives of our study were threefold: (1) identify population-level genetic markers in *I. corei* and *I. remota*; (2) provide baseline data on the genetic variability of

I. corei and *I. remota* and (3) clarify the taxonomic relationship between *I. remota* and *I. corei*. Our approach was to use RAPD markers in a population genetic analysis. This represents the first use of the AMOVA for RAPDs to analyse an obligately outcrossing plant species, although Stewart & Excoffier (1996) used it for *Vaccinium macrocarpon* (Ericaceae), a selfing species, and Huff, Peakall & Smouse (1993) used an earlier version of AMOVA, which had not been modified for RAPD data, to analyze obligate outcrossing *Buchloe dactyloides* (Poaceae). Our approach was to assume that each surveyed population of *Iliamna* (not including the one *I. rivularis* sample we included for comparative purposes) were members of a panmictic metapopulation. The AMOVA, a permutational statistics package, tests for population substructuring and will indicate whether significant molecular differences exist within and among local breeding populations. More importantly, the AMOVA will also indicate whether or not significant molecular differences exist between *I. remota* and *I. corei*. The null hypothesis tested is that there is no subdivision at the population and species levels. The associated permutational homogeneity of variance procedure (HOMOVA) tests whether population genetic heterogeneity is significantly different among populations. This procedure is of particular interest in rare species and conservation biology as it tests whether some populations are genetically more depauperate than others.

MATERIAL AND METHODS

Sampling strategy

The *I. corei* (PM) population (Peters Mountain and Garden) was discussed above, and the clonal structure is presented in more detail in Stewart & Porter (1995). We sampled the three sites in Virginia where *I. remota* could be found in June, 1992 (Fig. 1). The largest population was the Mallow Preserve population (MP), located adjacent to a railroad line, and protected by The Nature Conservancy. The population consisted of randomly-spaced clumps of plants, assumed to be single clones. We randomly sampled plants along two transects. Located a few kilometers down-track was the small Iron Gate population (IG), located between a railroad track and road, and across the street from a large factory. The population consisted of only four clumps of plants, all of which were sampled, but only three of which yielded products in the RAPD analysis. This population appeared to be regularly mowed as part of road maintenance. The third population was located alongside Interstate 64 (I). It differed from the other two populations in that it was located farther from the railroad line, and consisted of a single 10 m long, 3 m wide oblong clump of plants. We samples every fifth stem along a transect that ran through the length of the clump. A fourth *I. remota* population was located in Bedford County, Virginia near the James River. However, this population could not be sampled in 1992 because of flooding. For comparative purposes, we also included a DNA sample of *I. rivularis* from Cache County, Utah.

RAPD profiling and statistical analyses

We used fresh leaf tip samples as a source of DNA. DNA extraction, a rapid

miniprep, and RAPD profiling methods are described elsewhere (Stewart & Via, 1993). The raw data for all analyses was a band presence (1)/absence (0) rectangular matrix taken from the composite RAPD profile of each sample (Table 1). Missing data (?) were not analyzed by the AMOVA. The composite profiles were generated from the suite of primers we used in the RAPD reactions: OPA2 (5' TGCCGAGCTG), OPA3 (5' AGTCAGCCAC), OPA13 (5' CAGCACCCAC), OPA16 (5' AGCCAGCGAA), OPB10 (5' CGTCTGGGAC), CA 947 (5' CCAACCACCC), GT 947 (5' GGGTTGGTG). OP primers are from Operon Technologies (Alameda, California), and CA and GT primers were designed by Dr Douglas Rhoades (University of Arkansas-Fayetteville). Genetic distances were estimated using euclidean squared distances as discussed in Huff *et al.* (1993) and Stewart & Excoffier (1996) (Table 2). We used a non-parametric AMOVA procedure originally described in Excoffier *et al.* (1992) as modified by Stewart & Excoffier (1996) to describe population structuring and variability among populations. The associated HOMOVA was used to test for significant molecular variance homogeneity among populations. The *I. rivularis* sample was excluded from these analyses. In the AMOVA and HOMOVA, we used a hierarchical nested analysis with individuals gathered into populations which were, in turn, gathered into species (Fig. 2). We also performed an unweighted paired group method using arithmetic averages (UPGMA) cluster analysis to produce a dendrogram as a visual aid (Rohlf, 1988). We used the Mantel test to test for goodness-of-fit between a cophenetic (ultrametric) matrix, which was derived from the UPGMA dendrogram and the genetic distance matrix (Mantel, 1967; Rohlf, 1988). This matrix comparison approach is described further in Stewart & Nilsen (1995).

RESULTS

RAPD profiling

The primary focus of this paper is to delineate the taxonomic of *I. corei* and *I. remota* to one another using RAPD markers in a population genetics framework. RAPDs have been used before in the population genetics of rare and endangered species, although analyses have typically been qualitative and not quantitative (see Discussion). The primary attractive factors of RAPDs in conservation studies are the ease of methodologies, abundant polymorphisms, and the small amount of tissue needed for analysis. Indeed, over-collecting by botanists is cited as being an important factor in *I. corei*'s decline on Peters Mountain (Porter & Wieboldt, 1991). In the case of *I. corei*, only four plants were in existence in nature in 1992, so it was not desirable to sacrifice much tissue. Our methodologies required only a fraction of one leaf per plant.

In order to determine whether RAPD profiling could be used to characterize genetic variation among *Iliamna* populations, 42 different primers were used to screen four random *Iliamna* samples. Eighty-five per cent (36) of these revealed polymorphisms. Seven primers used for the analysis were selected based on the following criteria. The primers had to: (1) reveal polymorphisms, (2) consistently produce strong (brightly staining) amplification products, (3) produce uniform, reproducible markers between replicate PCRs, (4) be insensitive to DNA template concentrations varying from 1 ng/μL to 100 ng/μL (McClelland & Welsh, 1994). Furthermore, we

TABLE 1. RAPD band states for *Iliamna corei* and *I. remota*. 1=presence; 0=absence, ?=missing data or unscorable band. Following are abbreviations: riv=*Iliamna rivularis*, Cache Co., Utah; pmX=*I. corei*, Peters Mountain, VA.; pm Ax, Bx, Cx, Dx=breeding *I. corei*, Virginia Polytechnic Institute and State University experimental garden; igX=Iron Gate *I. remota*, Alleghany Co., VA., mpX=Kankakee mallow preserve *I. remota*, Glen Wilton, Botetourt Co., VA.; iX=*I. remota* along a roadbank on Interstate 64 between Covington and Clifton Forge, VA.

	Primer						
Sample	OPA16	OPB10	OPA2	OPA3	OPA13	CA	GT
riv	00010?110010101001	11001101100100110	0100101011101111	110111111	01000000101	01100110010111011	10011101001
pm1	00011111000011111	01010101010001000	1000010010001101	100111111	11110011111	00010101001011001	00111110011
pm2	000111110001111101	01010000010001001	1000010010101101	100111111	11110111111	00000100001011001	00111010011
pm3	001111110101111111	01010111010001001	1000010010101101	100111111	11110111111	00000101001011001	01111110011
pm4	001110110101111111	01010111010001000	1000010011101101	100111111	11110011111	00000101101011001	01111010011
pmA1	001111110011111111	01010000000001000	1000110010101011	100111111	11100011111	00000101001011001	10101101011
pmA2	001111110101111111	01010000000001001	1000000011101111	100111111	11110011111	00010101001011001	00111001011
pmB1	001111110110111101	01010011010001101	1000010011101001	100111111	11110011111	10000101001011001	11111001011
pmB2	001110110111111111	01010011000001101	1000100010001111	100111111	11111011111	10010101101011001	11111101011
pmC1	101111110111111111	01010001010001000	1000100010001111	100111111	11111011111	10000100000001001	01111001011
pmC2	001111110110111111	01010011?0001001	1000010011101101	100111111	11110011111	00001010010100011	11111101011
pmC3	001111110111111111	01010000000001100	1000101010101101	100111111	11110111111	10000100001011001	11111001001
pmD3	001111110111111111	01011001010001000	1000100010001101	100111111	11110011111	10010101001010001	11111001011
pmD4	00?111110110111111	01010011010001100	1000110000101101	100111111	11110011111	10010101001011001	11111001011
pmD6a	001111110101111111	01010011010001000	1000100010001101	100111111	11111011111	00100101001001001	00010101001
pmD6b	001111110101111111	01010000000001000	1000100011101101	100111111	11110011111	10010101001011001	11111001011
mp1	001111110011111??01	01010011000017?001	1100100011101101	100111111	00010011111	10000100001011001	11111101001
mp2	001111110000111??01	01010010000101?000	1100110011101101	100111111	10010011111	00001110001010001	11111101001
mp3	100111111001111101	01010011011011101	1100100011101101	100111111	00010011111	10000100001010100	00111111001
mp4	101111110001111101	01010001011011001	1100110010111101	100111110	00011011111	10100100011010001	11111101001
mp6	010111110001111101	01010011011011001	1100110000111101	100111111	10011011111	10100100001010001	11111101001
mp7	001111110001111101	01010011011011001	1101010111001101	100111111	10011011111	10001010001010101	11111101001
mp8	001111110001000101	01010011011011100	1100110100001101	100111111	10010011111	00000110001010001	11111101000
mp9	001111110001011001	01010101011011001	1100110011101101	100111111	10010011111	00000100001010101	01111101101
mp0	001110111001101101	01010001011011001	1100110100111001	110111111	10011011111	00100100011011001	11111101101
ig1	001110110101111101	01010011011011001	1100110101111101	110111111	10010111111	10100100001010001	10111101001
ig3	001110110101111101	01010010000011001	1100110101111111	111111111	10010011111	11010110001010001	10111101101
ig4	000111110001111101	01010011011011001	1100110110101101	110111111	10010011111	10001110001010000	11111101001
i1	001111110101100101	01100000110111100	1110110011101111	100111111	10010011111	10000100001010100	11111101101
i2	001111110101111101	01100111110111100	1110110011101111	100111111	10010011111	10000100001010100	11111101101
i4	001111110101111101	01100001110111100	1110110011101111	100111111	10010011111	10000100001010100	11111101101
i5	001111110101111101	01100000110111100	1110110011101101	100111111	10010011111	10000100001010100	11111101101
i6	001111110101100101	01100010110111100	1110110011101111	100111111	10010011111	10000100001010100	11111101101
i7	001111110101100101	01100000110111100	1110110011101111	100111111	10010011111	10000100001010100	11111101101
i8	001111110101101101	01100010110111100	1110110011101111	100111111	10010011111	10000100001010100	11111101101

TABLE 2. Pairwise euclidean squared distances between samples. See Table 1 for abbreviations.

Sample																																		
riv	0																																	
pm1	43	0																																
pm2	45	10	0																															
pm3	48	9	9	0																														
corei4	47	11	13	6	0																													
pmA1	39	16	16	17	19	0																												
pmA2	43	14	12	13	13	12	0																											
pmB1	43	18	16	13	13	16	14	0																										
pmB2	44	20	24	17	17	16	14	14	0																									
pmC1	44	19	19	18	18	17	15	17	13	0																								
pmC2	39	23	23	18	20	22	20	15	22	20	0																							
pmC3	39	22	16	17	19	14	14	14	14	13	22	0																						
pmD3	44	15	19	16	16	15	13	13	11	10	20	11	0																					
pmD4	43	14	18	14	14	15	15	7	11	14	18	11	8	0																				
pmD6a	43	16	20	15	17	18	16	20	16	13	23	18	15	17	0																			
pmD6b	43	16	16	15	13	12	6	12	12	13	20	8	7	9	16	0																		
mp1	36	27	23	20	22	21	19	15	17	20	21	15	18	18	21	15	0																	
mp2	38	25	21	22	22	19	21	21	25	26	19	19	22	22	23	17	12	0																
mp3	43	26	22	25	27	30	26	24	28	27	31	26	27	26	26	26	15	17	0															
mp4	43	28	24	25	29	26	28	24	26	23	27	24	23	25	24	24	15	15	16	0														
mp6	45	26	22	23	27	26	28	22	24	25	27	24	23	20	22	24	15	13	16	8	0													
mp7	79	28	26	25	27	30	28	22	26	27	21	28	25	27	26	26	17	11	18	16	14	0												
mp8	44	27	27	26	28	27	31	25	27	28	28	25	24	22	25	27	19	11	21	19	15	15	0											
mp9	42	23	21	20	22	25	23	23	29	26	26	25	24	26	25	23	15	11	15	15	15	15	16	0										
mp0	43	31	27	28	30	27	31	27	29	30	32	29	30	28	27	29	22	20	25	13	13	21	18	18	0									
ig1	43	30	24	23	25	28	26	22	26	29	27	24	25	23	24	24	15	15	18	14	10	16	17	17	16	0								
ig3	43	33	29	30	30	27	23	27	25	32	29	27	28	26	31	23	18	16	25	23	19	21	22	22	20	11	0							
ig4	43	26	22	23	27	26	28	22	26	27	23	24	23	22	26	24	15	9	14	14	10	10	13	15	17	12	17	0						
i1	41	34	30	31	31	28	28	26	30	29	33	24	27	27	32	24	20	18	22	24	24	24	21	19	27	24	23	22	0					
i2	40	31	31	26	26	29	29	23	27	28	30	25	26	24	29	25	18	18	19	23	21	21	22	16	28	21	22	19	5	0				
i4	41	31	28	29	29	26	26	24	28	27	31	22	25	25	30	22	19	17	20	22	22	22	23	17	27	22	21	20	2	3	0			
i5	42	31	27	28	28	27	27	23	29	28	30	21	24	24	29	21	18	16	19	21	21	21	22	16	26	21	22	19	3	4	1	0		
i6	42	35	31	30	30	29	29	25	29	30	32	25	28	26	31	25	19	17	21	25	23	23	20	20	28	23	22	21	1	4	3	4	0	
i7	41	34	30	31	31	28	28	26	30	29	33	24	27	27	32	24	20	18	22	24	24	24	21	19	27	24	23	22	0	5	2	3	1	0
i8	41	34	30	29	29	28	28	24	28	29	31	24	27	25	30	24	19	17	20	24	22	22	21	19	27	22	21	20	2	3	2	3	1	2	0

Sample	riv	pm 1	pm 2	pm 3	pm 4	pm A1	pm A2	pm B1	pm B2	pm C1	pm C2	pm C3	pm D3	pm D4	pm D6a	pm D6b	mp 1	mp 2	mp 3	mp 4	mp 6	mp 7	mp 8	mp 9	mp 0	ig1	ig2	ig4	i1	i2	i4	i5	i6	i7	i8

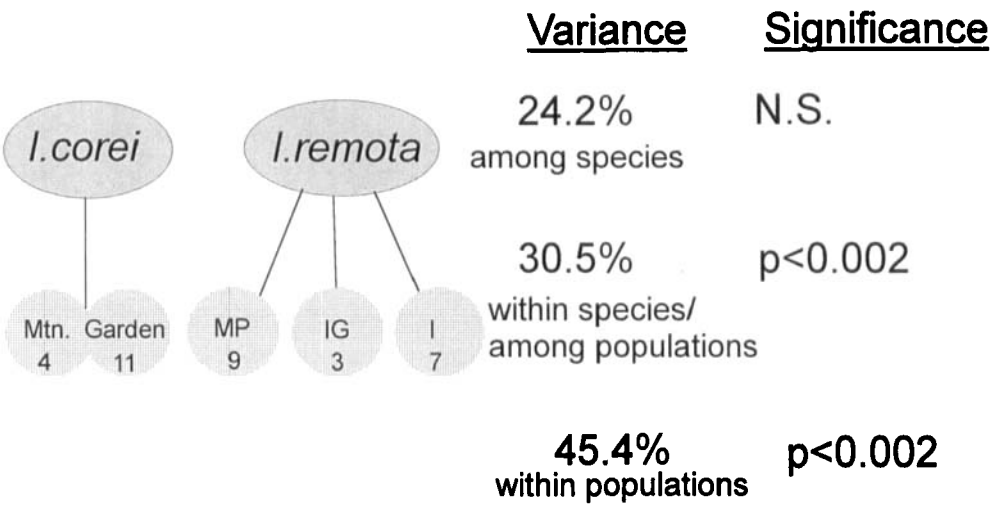

Figure 2. The nested experimental design for the analysis of molecular variance (AMOVA). The results of partitioning of variance by the AMOVA are shown. Molecular variances within populations and within species/among populations are significant ($P < 0.002$). The molecular variance between species is not significant $P = 0.27$). See Table 3 for population abbreviations.

only scored reproducible fragments (shared fragments between replicate RAPD reactions) that were in the middle molecular weight range (see Penner *et al.*, 1993; Stewart & Porter, in press). Ninety-nine informative markers were generated for analyses. A representative gel showing amplification products using one primer is shown in Figure 3.

Statistical analyses

The nested AMOVA was used to test the null hypothesis that no genetic subdivision exists among populations or among species. The global analysis showed the molecular variation within and among the four populations tested was significant ($\Phi_{SC} = 0.43$, $\Phi_{ST} = 0.51$, $P < 0.002$), where Φ_{SC} and Φ_{ST} are F-statistic analogues (Excoffier *et al.*, 1992). The very high Φ_{ST} indicates extreme population subdivision (Wright, 1978). This amount of population subdivision is very high compared to the

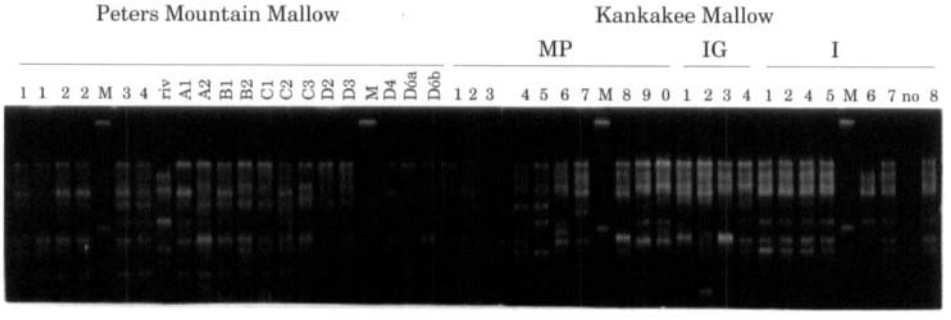

Figure 3. Typical RAPD gel (0.8% synergel/0.8% agarose composite) stained with ethidium bromide using primer OPA2. See Table 1 for sample abbreviations.

TABLE 3. Pairwise Φ_{ST} between populations. pm = *I. corei* mountain and garden population mp = *I. remota* mallow preserve population; ig = *I. remota* Iron Gate population; i = *I. remota* Interstate 64 population.

Populations	pm	mp	ig	i
pm	0			
mp	0.37	0		
ig	0.43	0.08	0	
i	0.62	0.54	0.74	0

mean analogous G_{ST} values of other endemics (0.248), long-lived herbaceous perennials (0.213), and animal-mediated outcrossers (0.197) (Hamrick & Godt, 1989). We located local differences by performing all possible pairwise comparisons using the same procedure. The Φ_{ST} values between populations showed that the two closest *I. remota* populations (IG and MP) were not significantly different from one another at the $P = 0.05$ level ($\Phi_{ST} = 0.08$) (Table 3). This suggests that the smaller IG population possibly originated from the larger MP population at some date following the initial dispersal of *I. remota* to Virginia. In addition, Φ_{ST} between PM and *I. remota* populations (0.47) was about the same as the overall average Φ_{ST} (0.51) but was less than that for population I and the other populations (0.63) (Table 3).

The HOMOVA tests whether all populations are equally variable (homogeneous variances). The global HOMOVA analysis shows that variance heterogeneities differed among populations ($P < 0.002$). We located local differences of variance by performing all possible pairwise comparisons using the same procedure. Pairwise comparisons at the 0.05 level revealed that the variance of PM (7.12) was not different than MP (7.0) but was significantly greater than that of IG (4.4) and I (1.1). The very low variance of I indicates that this population is nearly monomorphic and is the product of the founding of very few individuals, or is simply one clone varying by somatic mutation. Although there is appreciable genetic variation within both species, it is striking that the variance of the presumably much older PM population is not higher than the young large *I. remota* population (MP). Hence, the lack of genetic diversity may explain some of the rarity of *I. corei*. However, the lack of extensive RAPD variation of *I. remota* populations may be explained by recent founding events.

The UPGMA dendrogram depicts the genomic relatedness of individuals to each other based on RAPD markers (Fig. 4). Discreet populations (PM, I) each form a cluster, and IG and MP individuals intermingle within a single cluster. In addition, the dendrogram provides a very good fit to the triangular euclidean distance matrix ($P < 0.001$; $r = 0.93$). Thus, the cluster analysis corroborates the AMOVA by showing three discreet populations (PM, I, IG-MP) from the sample taken. At the species level, *I. remota* and *I. corei* each form clusters, but the AMOVA indicates that the genetic differences are not statistically significant.

DISCUSSION

RAPDs have proven to be useful markers in conservation genetics. They have been shown to be roughly equivalent to conventional allozyme markers in measuring genetic diversity (e.g. Liu & Furnier, 1993). That is, when polymorphisms are

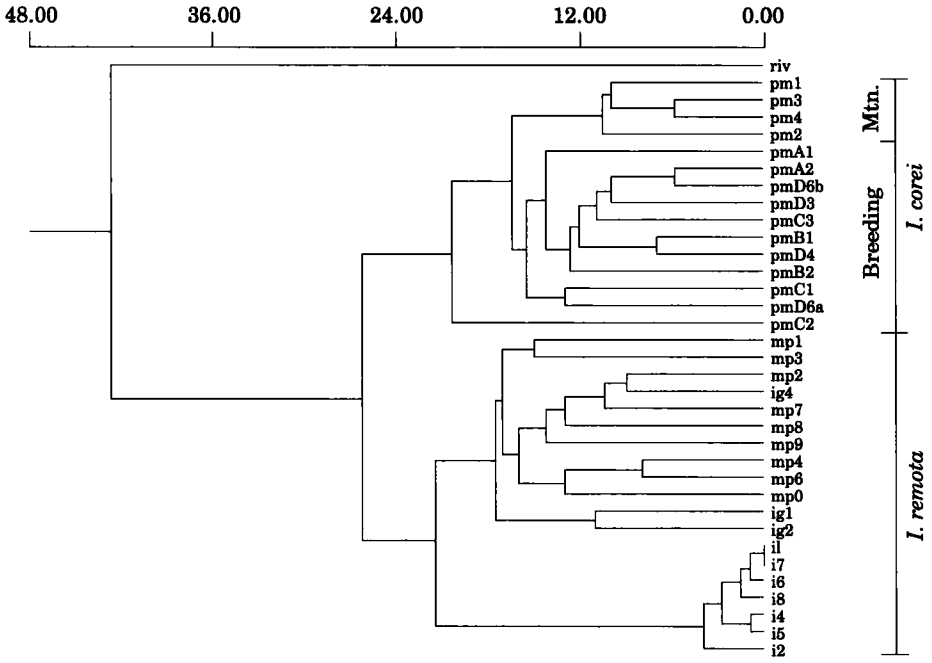

Figure 4. A single UPGMA tree constructed from 99 RAPD characters. Branch lengths indicate relative RAPD similarity (euclidean squared distances are on axis). See Table 1 for sample abbreviations.

observed in both systems, the relative genetic variation among individuals and populations is similar. Where low genetic divergence is evident, RAPDs reveal polymorphisms when allozymes do not (Brauner, Crawford & Stuessy, 1992; also see Stewart & Excoffier, 1996).

Although there have been great strides in developing molecular techniques in population biology, the development of statistical analyses have lagged behind. In most population-level studies, RAPD data have been treated in a qualitative fashion, and/or been subjected to non-statistical analyses such as cluster analysis (e.g. Castiglione *et al.*, 1993; Hsiao & Rieseberg, 1994; Stewart & Porter, 1995). These approaches are useful in delineating clonal structure and identities within populations, and in determining phylogenetic relationships among taxa. In one example, RAPDs were used successfully in clarifying the taxonomic relationships among varieties of *Ranunculus acriformis* (Ranunculaceae) and allied species (Van Buren *et al.*, 1994). Based upon cladistic analysis and morphological variation, Van Buren *et al.* (1994) elevated *R. acriformis* var. *aestivalis* to *R. aestivalis*. However, for quantitative population-level analyses in which the investigators want information on population genetic substructuring, they have had to use allozyme markers or other codominant markers. In spite of the dominance problem with RAPDs (heterozygotes are indistinguishable from homozygous positives on a gel), some researchers have attempted to use algorithms to describe population structure with RAPDs. For example, Dawson *et al.* (1993) examined highly inbred *Hordeum spontaneum* (Poaceae) populations and assumed high marker homozygosity. They used orthodox statistical approaches based on genotype frequency. However, this assumption is generally not appropriate. Russell *et al.* (1993) used the Shannon diversity index to partition RAPD variability to within and among population components. Although this provides an

estimate for population structure, one cannot test for significant differences. In order to use RAPDs in a quantitative fashion one must correct for dominance. Clark & Lanigan (1993) have presented a method of estimating nucleotide divergence with RAPDs. The primary assumption for their analysis is that populations are in Hardy-Weinberg equilibrium. This is often not a valid assumption, especially in small endangered populations. Gibbs, Prior & Weatherhead (1994) used this approach in examining populations of snakes. Lynch & Milligan (1994) and Stewart & Excoffier (1996) have recently presented methods to analyse RAPD data in quantitative population-level analyses that require no underlying assumptions of genotype distribution. In the latter treatment, methodologies were presented to allow the estimation of allele frequencies on phenotypic RAPD data using non-parametric statistical procedures.

The method presented by Stewart & Excoffier (1996) (AMOVA) was used here to partition the molecular variance into three levels (between species, among populations/within species, and within populations) based on *a priori* taxonomic and geographic criteria and tested whether the null hypothesis should be rejected at the three levels. The null hypothesis, that groups of plants are panmictic, can be rejected if molecular characters among groups are significantly different. The null hypothesis was rejected in the among population and within population levels only. However, the null hypothesis was not rejected at the species level ($P = 0.27$). Therefore, these results offer no evidence in favor of the *I. corei* and *I. remota* split (Sherff, 1949) into separate species. Furthermore, the genetic divergence between *I. corei* and the *I. remota* populations is about the same as for all populations, but less than the differentiation between I and the remaining populations (Table 3). So that PM has more similarity at the DNA level to *I. remota* on average than does population I the other *I. remota* populations.

The UPGMA dendrogram, which has very similar topology to the tree presented in Stewart & Porter (1995), is consistent with the results of the AMOVA with regards to population structure (Fig. 4). However, the AMOVA indicates that the separation of *I. corei* and *I. remota* in the cluster analysis is not significant. These analyses indicate that *I. corei* should perhaps be recognized as a subspecies of *I. remota*. But the taxonomic relationship between the two remains enigmatic. Further study encompassing the other species of *Iliamna* is needed in order to assess the variation throughout the genus, and to properly classify the endangered *I. corei*. To this end we will continue to pursue molecular work including sequencing of the ITS rDNA of all species of *Iliamna* and performing cladistic analyses to further investigate phylogenetic relationships within the genus. Furthermore, we are seeking to elucidate the molecular basis of *I. corei*'s ecological demise on Peters Mountain.

ACKNOWLEDGEMENTS

This work was supported by grants from the Virginia Department of Agriculture and Consumer Services to DMP, and a USDA grant to CNS. Thanks to Tom Wieboldt for encouragement, help with locating field sites, and the *Iliamna rivularis* accession. We appreciate the help of Suzanne Hill and Mary Lipscomb with sample collecting. We also appreciate the comments on the manuscript by Mary Lipscomb, Tom Wieboldt. Thanks also to Carolyn Hightower for assistance in manuscript

preparation. Access to the Peters Mountain site and Mallow Preserve site near Iron Gate was made possible through The Nature Conservancy.

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