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Patterns of Isozyme Variation in Western North American *Antennaria* (Asteraceae: Inuleae). I. Sexual Species of sect. *Dioicae*

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ABSTRACT. *Antennaria* is a genus of dioecious, perennial herbs, widely distributed throughout temperate and arctic North America and Eurasia. Four sexually reproducing, primarily diploid species ($2n = 28$) in section *Dioicae* from western North America were investigated for genetic differentiation at 19 putative isozymes. *Antennaria corymbosa, A. marginata, A. microphylla,* and *A. rosulata* are morphologically distinct, but display only moderate divergence at gene loci specifying soluble enzymes. Some correlations exist between genetic distances and geographic patterns within the species examined. Neighboring populations tend to be more similar electrophoretically than geographically distant or marginal ones. The inferred mode of speciation, based on morphology and biochemical genetics, is one in which rapid evolution (adaptive radiation) has occurred, leading to morphological divergence, but only conservative amounts of divergence at the 19 isozymes surveyed.

Western North America is the center of diversity for *Antennaria* Gaertner, a genus of dioecious, perennial herbs. *Antennaria* consists of about 20 sexually reproducing species, which are primarily diploid ($2n = 28$) and five, morphologically variable, polyploid agamic complexes, *A. alpina* (L.) Gaertner, *A. neodioica* Greene, *A. parlinii* Fern., *A. parvifolia* Nutt., and *A. rosea* Greene (Bayer 1987a). These complexes have euploid races ranging from triploid to decaploid (Bayer and Stebbins 1987).

Antennaria corymbosa E. Nelson, *A. marginata* Greene, *A. microphylla* Rydb., and *A. rosulata* Rydb. are four sexually reproducing, primarily diploid, species that are members of section *Dioicae* (Bayer 1987a; Bayer and Stebbins 1987). These species probably represent the sexual progenitors of the *A. rosea* polyploid agamic complex (Bayer 1987a; Bayer and Stebbins 1987). Two of the species, *A. marginata* and *A. rosulata,* occur in the southern Rockies (fig. 1). *Antennaria corymbosa* occurs in the northern and southern Rockies with disjunct populations in the Sierra Nevada of California. The most widely distributed species of the four is *A. microphylla,* which occurs from the southern Rockies and Great Basin, north to the Northwest Territories and east to Hudson Bay. The four sexual species usually grow as allopatric populations and only *A. corymbosa*/*A. microphylla* and *A. microphylla*/*A. rosulata* are known to be locally sympatric (Bayer 1987a: pers. obs.). They are sympatric with *A. rosea* over much of its range in the southern half of the western North American cordillera (fig. 1; Bayer and Stebbins 1987).

Additionally, these species have apparently undergone niche diversification because each species is associated with a distinct community (Bayer, unpubl. obs.). *Antennaria corymbosa* occurs in subalpine/alpine mesic *Salix* thickets, whereas *A. microphylla* is found at steppe to lower montane elevations usually in mesic sagebrush flats adjacent to rivers primarily in the northern Rockies (fig. 1). *Antennaria marginata* and *A. rosulata* occur at montane elevations in the southern Rockies (fig. 1) and *A. marginata* is associated with dense stands of *Pinus ponderosa* and *Pseudotsuga menziesii,* whereas *A. rosulata* occurs in open *Pinus ponderosa*/*Artemisia* savanna parkland.

Numerical taxonomic studies (Bayer 1987b) have demonstrated that the four species have diverged from one another morphologically and they are morphologically distinct. This is the typical situation in *Antennaria,* in that the sexual species are usually morphologically distinct from each other with no intergradation (Bayer 1985a, 1985b). *Antennaria marginata* has adaxially glabrous leaves, which distinguish it from the other three species (Bayer 1987b). The heads of *A. rosulata* are sessile and the flowering stalks are monocephalous (sometimes bicephalous), differentiating it from *A. corymbosa* and *A. microphylla,* which have tall, polycephalous, flow-

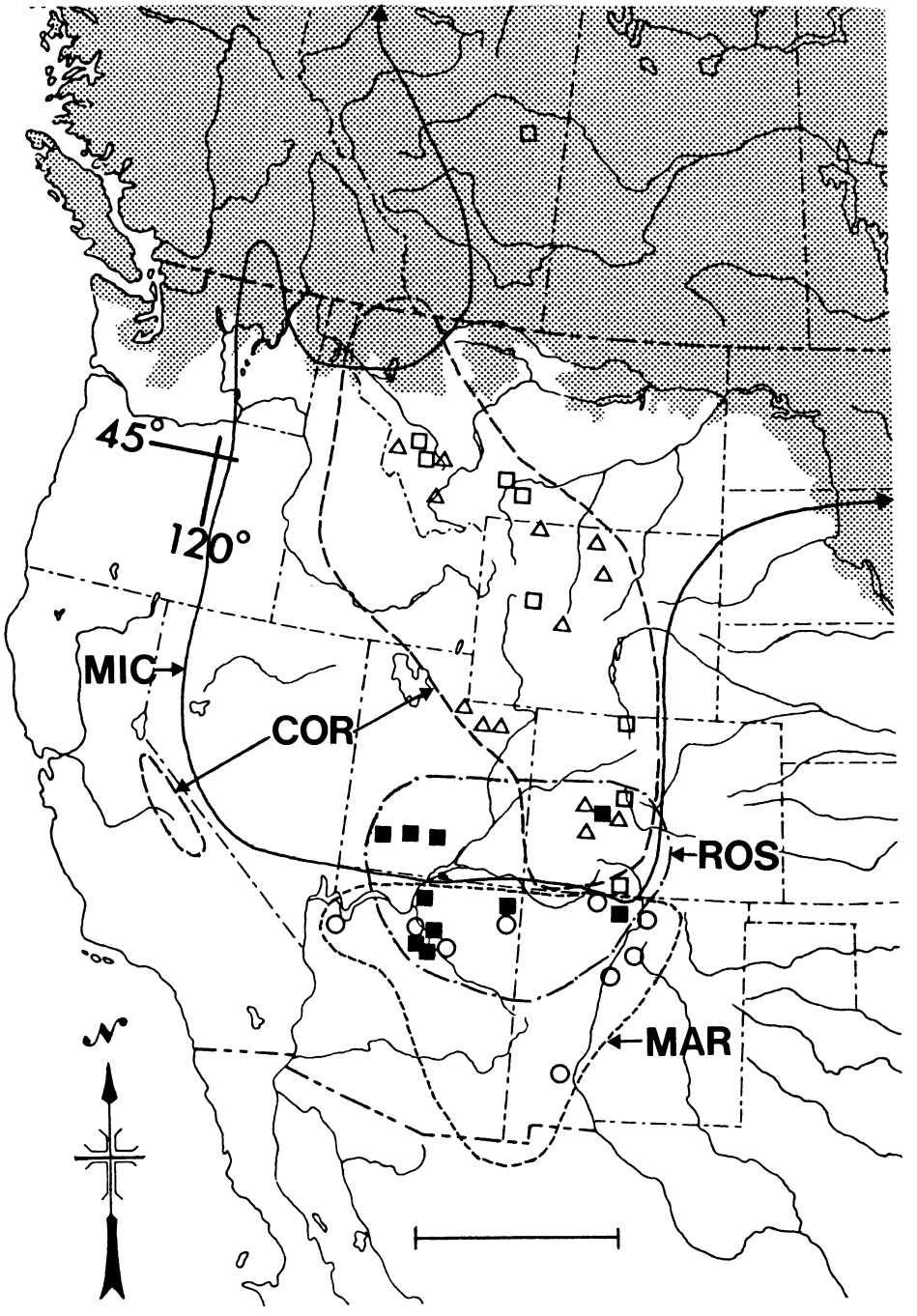

FIG. 1. Ranges of *Antennaria corymbosa*, *A. marginata*, *A. microphylla*, and *A. rosulata* and positions of 41 populations. The margins of the known ranges of the species are labeled with the first three letters of their specific epithets. The entire range of *A. microphylla* is not shown, but extends east to Minnesota and Hudson Bay and north to southern Northwest Territories and northeastern British Columbia. Stippling indicates maximum extent of Wisconsinan glaciation. Individual populations are labeled as follows: open triangle, *A. corymbosa*; open circle, *A. marginata*; open square, *A. microphylla*; and solid square, *A. rosulata*. Bar equals 500 km.

TABLE 1. Locality data for 41 populations of *Antennaria corymbosa, A. marginata, A. microphylla,* and *A. rosulata,* with species names, state/province, county, and collection numbers. Population designations, referred to in the text, are in parentheses following the collection numbers. Latitude, longitude, and elevation (meters) above sea level are given parenthetically after each population designation. All populations have been determined as diploid ($2n = 28$), except those marked by an asterisk, which are tetraploid ($2n = 56$). Voucher specimens are deposited at ALTA.

A. corymbosa E. Nelson. U.S.A. **Colorado:** Gunnison Co., *Bayer and Lebedyk CO-512* (C1), (38°54′, 106°57′, 2743 m); *Bayer and Lebedyk CO-516* (C2), (38°50′, 106°24′, 3352 m); *Bayer and Lebedyk CO-509* (C3), (39°01′, 107°04′, 3230 m). **Montana:** Beaverhead Co., *Bayer and Lebedyk MT-508* (C4), (45°22′, 112°54′, 2499 m); Deerlodge Co., *Bayer and Lebedyk MT-513* (C5), (46°04′, 113°16′, 2499 m); Ravalli Co., *Bayer et al. MT-606* (C6), (46°09′, 114°28′, 2286 m). **Utah:** Duchesne Co., *Bayer et al. UT-619* (C7), (40°35′, 110°50′, 3124 m); Summit Co., *Bayer et al. UT-617* (C8), (40°41′, 110°53′, 3194 m). **Wyoming:** Big Horn Co., *Bayer et al. WY-635* (C9), (44°17′, 107°09′, 2895 m); Park Co., *Bayer and Lebedyk WY-515* (C10), (44°56′, 109°32′, 2926 m); Sheridan Co., *Bayer and Lebedyk WY-518* (C11), (44°46′, 107°45′, 2926 m); *A. corymbosa* (black-phyllaried form). **Utah:** Duchesne Co., *Bayer et al. UT-620* (C12), (40°35′, 110°50′, 3124 m). **Wyoming:** Fremont Co., *Bayer and Lebedyk WY-504* (C13), (42°38′, 108°55′, 3048 m).

A. marginata Greene. U.S.A. **Arizona:** Apache Co., *Bayer and Lebedyk AZ-500* (M1), (36°30′, 109°10′, 2438 m); Coconino Co., *Bayer et al. AZ-610* (M2), (34°54′, 111°28′, 2249 m); *Bayer et al. AZ-607* (M3), (34°54′, 111°40′, 2012 m); Mohave Co., *Bayer et al. AZ-705* (M4), (35°10′, 113°55′, 1975 m). **New Mexico:** Rio Arriba Co., *Bayer and Lebedyk NM-510* (M5), (36°53′, 107°00′, 2286 m); Sandoval Co., *Bayer and Lebedyk NM-500* (M6), (35°14′, 106°24′, 2286 m); Sante Fe Co., *Bayer et al. NM-411** (M7), (35°43′, 105°50′, 2591 m); Sierra Co., *Bayer et al. NM-700* (M8), (32°54′, 107°46′, 2438 m); Taos Co., *Bayer et al. NM-704* (M9), (36°23′, 105°28′, 2353 m).

A. microphylla Rydb. CANADA. **Alberta:** Beaver Co., *Bayer and Kohli AB-705* (MI1), (53°00′, 111°32′, 701 m); U.S.A. **Colorado:** Conejos Co., *Bayer et al. CO-700* (MI2), (37°08′, 106°25′, 2688 m); Jackson Co., *Bayer and Lebedyk CO-526* (MI3), (40°33′, 106°24′, 2484 m); Lake Co., *Bayer and Lebedyk CO-525* (MI4), (39°08′, 106°19′, 2896 m). **Montana:** Deerlodge Co., *Bayer and Lebedyk MT-512* (MI5), (46°04′, 113°16′, 2499 m); Granite Co., *Bayer and Lebedyk MT-528* (MI6), (46°16′, 113°20′, 1524 m); Park Co., *Bayer and Lebedyk MT-504* (MI7), (46°10′, 110°44′, 1615 m); Sweetgrass Co., *Bayer et al. MT-637* (MI8), (45°24′, 110°12′, 1798 m). **Wyoming:** Sublette Co., *Bayer et al. WY-600* (MI9), (43°15′, 109°45′, 2320 m).

A. rosulata Rydb. U.S.A. **Arizona:** Apache Co., *Bayer and Lebedyk AZ-501* (R1), (36°20′, 109°10′, 2743 m); Coconino Co., *Bayer et al. AZ-600* (R2), (35°17′, 111°38′, 2457 m); *Bayer et al. AZ-608* (R3), (34°59′, 111°28′, 2249 m); *Bayer et al. AZ-612* (R4), (36°23′, 112°07′, 2682 m); *Bayer et al. AZ-603* (R5), (35°05′, 111°44′, 2069 m). **Colorado:** Gunnison Co., *Bayer and Lebedyk CO-514* (R6), (38°49′, 106°50′, 2438 m). **New Mexico:** Rio Arriba Co., *Bayer and Lebedyk NM-505* (R7), (36°39′, 106°02′, 2621 m); **Utah:** Garfield Co., *Bayer et al. UT-602* (R8), (37°45′, 111°56′, 2530 m); *Bayer et al. UT-601* (R9), (37°33′, 112°37′, 2536 m); Iron Co., *Bayer et al. UT-600* (R10), (37°34′, 112°48′, 2962 m).

ering stalks (Bayer 1987b). *Antennaria corymbosa* has linear-oblanceolate basal leaves and phyllaries that are white with a distinct black spot at their base, whereas *A. microphylla* has cuneate-spatulate leaves and phyllaries that are white with light green bases.

The logical extension of my analysis is to assess the degree of genetic differentiation among the four species at loci encoding soluble enzymes. In addition, genetic diversity within and among populations of the species and geographic patterns of isozyme variation are evaluated. The xenogamous breeding system of dioecious *Antennaria* is evaluated based on mean fixation indices derived from the electrophoretic data.

MATERIALS AND METHODS

Locality data for the 41 populations used in this study are presented in table 1 along with the elevation of the site and chromosome numbers of individuals. All populations are diploid ($2n = 28$), except one tetraploid population of *A. marginata* (*NM-411;* table 1). The margins of the ranges of each of the species are shown in figure 1 along with the position of each of the 41 sites in relation to the ranges. The number of populations and individuals, respectively, examined for each species was: *A. corymbosa* (13, 186), *A. marginata* (9, 145), *A. microphylla* (9, 152), and *A. rosulata* (10, 159). Populations were selected from throughout the entire range of each

species. Generally, ramets were removed from plants in the field because seeds are only available for a limited time before they disperse. When ripe seeds were accessible, they were collected from 30 or more clones and treated as a bulk sample. In most cases individual clones are spatially distinct, but seeds or ramets were collected from individuals a few meters apart to insure that different clones were being sampled. Seedlings or ramets collected from the field were grown in The University of Alberta Phytotron until they were suitable for electrophoresis.

Methodologies are similar to those used in a previous study of *Antennaria* (Bayer and Crawford 1986). Fresh pieces of actively growing leaf tissue or flower heads were used for electrophoresis. Materials were ground in ice-cold Tris-HCl extraction buffer: 0.1 M Tris-HCl, pH 7.5, 4.0 mM 2-mercaptoethanol, 1.0 mM EDTA (disodium salt), 10.0 mM KCl, and 10.0 mM $MgCl_2$ (Gottlieb 1981b). About 20 mg of Polyvinylpolypyrrolidone (Sigma P6755) was added to each sample at the time of grinding. Samples were stored at 4.0°C, allowed to soak overnight and centrifuged the next morning. Supernatant was soaked onto filter paper wicks before being loaded into 12.5% starch gels. General protein (GP), glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), superoxide dismutase (SOD), and triose-phosphate isomerase (TPI) were resolved on a system composed of a gel buffer consisting of 1 part 0.038 M lithium hydroxide·H_2O–0.188 M boric acid (pH 8.3), and 9 parts 0.045 M Tris–0.007 citric acid (pH 8.4) (Soltis et al. 1983), with the electrode buffer consisting only of the lithium borate constituent. Acid phosphatase (ACP), malate dehydrogenase [(NAD) MDH], phosphoglucomutase (PGM), and shikimic acid dehydrogenase (SKDH) were visualized on a system consisting of a gel buffer of 0.016 M L-histidine (free base) and 0.002 M citric acid·H_2O (pH 6.5), and an electrode buffer of 0.065 M L-histidine (free base) 0.007 M citric acid·H_2O (pH 6.5) (Cardy et al. 1981). Procedures used for assaying of all enzymes followed protocols of Soltis et al. (1983), except for general protein (GP) in which the gel was stained with a solution containing 5 parts methanol, 5 parts water, 1 part acetic acid, and 100 mg/100 ml Brilliant Blue G stain. After incubation for 30 min at 37.0°C the gel was destained with a solution of the same composition excluding the stain component.

Pollen leachates and chloroplast extracts, useful in determining the subcellular location of isozymes, were prepared according to procedures of Bayer and Crawford (1986), Gastony and Darrow (1983), and Weeden and Gottlieb (1980a, 1980b). These extracts were assayed for PGI, PGM, and TPI on a continuous cellulose acetate gel system using a 0.024 M Tris–0.19 M glycine, pH 8.4 buffer system (Bayer et al. 1987). Gels were electrophoresed at 200 V (constant) for 25 min at 4.0°C and stained using agarose overlays according to Soltis et al. (1983), but using one half quantities of all ingredients.

The locus specifying the most anodally migrating isozyme was designated as 1, the next 2, and so on. In a similar manner, the most anodal allozyme at a given gene was labeled A, etc.

Genetic variation was evaluated using mean number of alleles per locus (A) (including monomorphic loci), proportion of loci polymorphic (P), observed and expected average heterozygosities (H_{obs} and H_{exp}, respectively), and the fixation index (F_T). The observed and expected average heterozygosities were compared using Chi-square tests to determine if the natural populations deviated from Hardy-Weinberg equilibrium expectations. For a discussion of the calculation of these statistics consult Hartl (1980). Gene diversity statistics, and standard genetic distances and identities were obtained utilizing the methods of Nei (1972, 1973). Total gene diversity (H_T), intrapopulational gene diversity (H_S), interpopulational gene diversity (D_{ST}), and the coefficient of gene differentiation (G_{ST}) are related by the equations $H_T = H_S + D_{ST}$ and $G_{ST} = D_{ST}/H_T$ (Nei 1973). Nei's statistics were calculated by the GENESTAT program (Whitkus 1985) executed on the Amdahl computer at the University of Alberta. Matrices of genetic distances were used to generate phenograms by the unweighted pair-group method (UPGMA; Sneath and Sokal 1973) using the SAHN subroutine of the NTSYS-pc (Rohlf 1987) program.

Chromosome numbers were obtained for the populations using techniques outlined previously (Bayer 1984; Bayer and Stebbins 1987).

Results

The 10 enzyme systems resolved are interpreted as being encoded by 19 putative loci. The interpretation of the genetic basis of the enzyme phenotypes has been elucidated through controlled crosses performed on other *Antennaria* species (Bayer and Crawford 1986) and through inferences based on segregation patterns observed in plants from natural populations. The 19 isozymes (and their allozymes) are: ACP-1-(A to C), GDH-1-(A and B), GP-1-(A), LAP-1-(A to F), LAP-2-(A to E), MDH-1-(A), MDH-2-(A), MDH-3-(A), MDH-4-(A), PGI-1-(A), PGI-2-(A), PGI-3-(A to E), PGM-1-(A and B), SKDH-1-(A to C), SOD-1-(A), SOD-2-(A), TPI-1-(A and B), TPI-2-(A), and TPI-3-(A to F). Isozymes not scored in a previous study (Bayer and Crawford 1986) were GP-1 and LAP-2. PGM-2 was resolved, but complex phenotypes, possibly due to gene duplication, make determination of the genotypes impossible at present. At least two additional unresolved isozymes of ACP were visualized on most gels.

Several enzyme systems are known to have isozymes which are localized either in the cytosol or the chloroplasts (Gottlieb 1982). Pollen leachate and extracts from lysed isolated chloroplasts are useful in determining the subcellular location of each of the isozymes (Bayer and Crawford 1986; Gastony and Darrow 1983; Weeden and Gottlieb 1980a, 1980b). Pollen leachate contains cytosolically localized isozymes alone, and not the isozymes situated in the chloroplasts. Pollen leachates contained PGI-3, PGM-2, and TPI-3 and extracts from lysed chloroplasts PGI-1, PGI-2, PGM-1, TPI-1, and TPI-2.

The number of isozymes present for each enzyme system is a feature that is highly conserved among diploid plants (Gottlieb 1982). A full discussion of the genetic interpretation of the enzyme phenotypes in *Antennaria* can be found in Bayer and Crawford (1986). The number of isozymes (in parentheses) present for GDH (1), MDH (4), PGM (2), SOD (2), SKDH (1), ACP (3 total; with 2 unresolved) are the same number as those reported in most other diploid plants (Crawford 1983; Gottlieb 1981a, 1982).

As was noted by Bayer and Crawford (1986), there is apparently a gene duplication for PGI and TPI. In most diploid plants, there are two isozymes for these enzymes, one located in the cytosol and one in the chloroplasts (Gottlieb 1982). In *Antennaria* there are apparently three isozymes, the duplicated locus being in the chloroplast form of the enzymes in both PGI and TPI. The putative duplications occur in all investigated species of the genus. Genetic analysis of the PGI duplication has not been possible due to lack of allelic variation at these loci; the enzyme phenotypes being a consistent 3-banded pattern (i.e., fixed heterozygotes) and interpreted as two isozymic homodimers and one interlocus heterodimer. Recently, an allelic variant has been found at *Tpi-2* in several populations of *A. corymbosa*. Populations are monomorphic, being either $Tpi\text{-}1^a/Tpi\text{-}2^a$ or $Tpi\text{-}1^a/Tpi\text{-}2^b$. Interpopulational crosses are underway to verify the genetic interpretation of the putative TPI duplication. The relatively large number of putative duplications in *Antennaria* (i.e., three; PGI, PGM, and TPI) is probably indicative of ancient polyploidy in diploid *Antennaria*. The diploid number ($2n = 28$) is high for a diploid species, and although no lower numbers are known in *Antennaria*, $2n = 14$ diploids are known in the closely related genus *Gnaphalium* L. (Bayer 1987a; Bayer and Stebbins 1987). The diploid *Antennaria* are undoubtedly paleopolyploids that function as diploids (Bayer 1987a).

Segregation at polymorphic loci, as well as isozyme phenotypes observed in artificial interspecific hybrids, provide evidence for determining the subunit composition of several enzymes. These are in agreement with previous studies (Bayer and Crawford 1986; Gottlieb 1981a): ACP (dimeric), GDH (tetrameric), LAP (monomeric), PGI (dimeric), PGM (monomeric), SKDH (monomeric), TPI (dimeric).

Gp-1, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Mdh-4*, *Pgi-1*, *Pgi-2*, *Sod-1*, *Sod-2*, and *Tpi-2* were monomorphic in all 41 populations, but the other loci were polymorphic in populations of some or all of the species. A table of allelic frequencies is available from the author on request. The values of A range from 1.00 (all loci monomorphic) to an average of 1.63 with a mean of 1.26 for all species (table 2). The proportion of loci polymorphic (P) ranged from 0.0 to 0.41 with a mean

TABLE 2. Genetic variation in 41 populations of *Antennaria* sec. *Dioicae*. Included are: mean number of alleles per locus (A); proportion of polymorphic loci, where the frequency of the most common allele is less than 0.99 (P); observed average heterozygosity [$H_{(obs)}$]; expected average heterozygosity [$H_{(exp)}$]; and mean fixation index (F_T). Populations designations are given in table 1. * = significantly different at the 5% level; ** = significantly different at the 1% level.

Population designations	A	P	$H_{(obs)}$	$H_{(exp)}$	F_T
A. corymbosa					
C1	1.40 ± 0.737	0.27	0.068	0.040	−0.450
C2	1.00 ± 0.000	0.00	0.000	0.000	—
C3	1.38 ± 0.650	0.27	0.180	0.101*	−0.782
C4	1.41 ± 0.793	0.23	0.076	0.083	+0.840
C5	1.46 ± 0.776	0.31	0.163	0.126	−0.294
C6	1.63 ± 0.806	0.41	0.108	0.126	+0.143
C7	1.27 ± 0.460	0.27	0.088	0.070	−0.257
C8	1.07 ± 0.258	0.07	0.013	0.012	−0.083
C9	1.47 ± 0.743	0.33	0.128	0.110	−0.164
C10	1.50 ± 0.855	0.29	0.052	0.121	−0.058
C11	1.00 ± 0.000	0.00	0.000	0.000	—
C12	1.44 ± 0.892	0.25	0.060	0.073	+0.178
C13	1.33 ± 0.516	0.33	0.330	0.167	−0.976
averages	1.34 ± 0.198	0.23	0.097	0.079	−0.173
(±s.d.)		(±0.13)	(±0.09)	(±0.053)	(±0.488)
A. marginata					
M1	1.08 ± 0.278	0.08	0.021	0.019	−0.105
M2	1.18 ± 0.529	0.12	0.063	0.050	−0.260
M3	1.15 ± 0.376	0.14	0.143	0.063**	−1.270
M4	1.38 ± 0.806	0.24	0.109	0.100	−0.090
M5	1.07 ± 0.258	0.07	0.067	0.033**	−1.030
M6	1.47 ± 0.834	0.27	0.156	0.102**	−0.525
M7	1.53 ± 0.800	0.35	0.082	—	—
M8	1.13 ± 0.352	0.13	0.010	0.034*	+0.706
M9	1.31 ± 0.602	0.25	0.108	0.070*	−0.543
averages	1.26 ± 0.172	0.18	0.084	0.059	−0.329
(±s.d.)		(±0.097)	(±0.05)	(±0.035)	(±0.615)
A. microphylla					
MI1	1.07 ± 0.258	0.07	0.049	0.015**	−2.267
MI2	1.13 ± 0.352	0.13	0.083	0.026**	−2.192
MI3	1.06 ± 0.250	0.06	0.031	0.023	−0.348
MI4	1.15 ± 0.376	0.15	0.045	0.032	−0.406
MI5	1.15 ± 0.376	0.15	0.154	0.077	−1.000
MI6	1.00 ± 0.000	0.00	0.000	0.000	—
MI7	1.19 ± 0.544	0.13	0.029	0.025	−0.160
MI8	1.11 ± 0.323	0.11	0.095	0.056**	−0.696
MI9	1.13 ± 0.342	0.13	0.043	0.033	−0.303
averages	1.11 ± 0.058	0.10	0.059	0.032	−0.922
(±s.d.)		(±0.05)	(±0.046)	(±0.023)	(±0.848)
A. rosulata					
R1	1.31 ± 0.480	0.23	0.077	0.066	−0.167
R2	1.25 ± 0.447	0.25	0.095	0.064*	−0.484
R3	1.28 ± 0.461	0.29	0.120	0.101	−0.188
R4	1.40 ± 1.060	0.20	0.100	0.068*	−0.470
R5	1.29 ± 0.588	0.24	0.089	0.058*	−0.534
R6	1.14 ± 0.534	0.07	0.071	0.045	−0.578
R7	1.24 ± 0.562	0.18	0.073	0.060	−0.217

TABLE 2. Continued.

Population designations	A	P	$H_{(obs)}$	$H_{(exp)}$	F_T
R8	1.31 ± 0.602	0.25	0.101	0.084	−0.202
R9	1.35 ± 0.702	0.24	0.124	0.095	−0.305
R10	1.29 ± 0.588	0.23	0.147	0.102*	−0.441
averages	1.29 ± 0.069	0.22	0.100	0.074	−0.359
(±s.d.)		(±0.06)	(±0.025)	(±0.02)	(±0.159)
grand averages	1.26 ± 0.163	0.19	0.087	0.065	−0.413
(±s.d.)		(±0.103)	(±0.061)	(±0.04)	(±0.604)

of 0.087 (table 2). The observed and expected heterozygosities (H_{obs} and H_{exp}, respectively; table 2) in most cases were not significantly different from Hardy-Weinberg expectations. H_{obs} ranged from 0.00 to 0.167 with an average of 0.065 (table 2). Values for the mean fixation index (F_T), useful in determining the degree of inbreeding in populations, are mostly less than 0.000, with an average of −0.143 (table 2). A few populations (C4, M7, and M8; table 2) have values of F_T that are greater than 0.2, indicating some functional inbreeding is occurring in certain populations.

The gene diversity statistics (Nei 1973) are presented in table 3 for individual and pooled species and loci. Nei's measures of genetic distance (D) and identity (I) (Nei 1972) are presented for interspecific (table 4) and intraspecific (table 5) pair-wise comparisons. Individual genetic distances and identities for all the pairwise comparisons of 41 populations is too large to present here, but a phenogram based on the matrix of genetic distances summarizes these data (fig. 2). A phenogram derived from a cluster analysis (UPGMA) of the genetic distance matrix in table 4 is presented as figure 3.

DISCUSSION

Results from this study reveal several salient aspects of the population genetics of *Antennaria* and these can be compared with results from other studies. With regard to population structure, *Antennaria* compares favorably with other outcrossing (dioecious), perennial herbs. Generally, amounts of genetic divergence among and within the species is low and this is revealed by genetic statistics (tables 2–5). The average number of alleles per locus for all 41 populations is 1.26 (table 2), similar to averages from other plants with life-history traits paralleling *Antennaria* (Hamrick et al. 1979). Percent loci polymorphic is 19.0% (table 2), which is slightly lower than values given from other genera (Hamrick et al. 1979). Mean heterozygosity values (for all species $H_{obs} = 0.87$; table 2) are lower than those for other outcrossing, sexual, long-lived perennials (Hamrick et al. 1979), although most of the taxa used for comparison were conifers. The reason for deviations from Hardy-Weinberg equilibrium expectations between H_{obs} and H_{exp} in some populations is unclear. Compared to other species of *Antennaria* the values of A, P, and H_{obs} obtained here are remarkably similar [1.28, 22.0%, 0.68 for A, P, and H_{obs}, respectively; averages for *A. neglecta* Greene, *A. plantaginifolia* (L.) Richards., *A. racemosa* Hook., *A. solitaria* Rydb., and *A. virginica* Stebb.; Bayer and Crawford 1986].

The coefficient of inbreeding (F_T) is useful for determining the degree of inbreeding or outcrossing in populations. Negative values of F_T indicate predominantly outcrossing, while positive values indicate inbreeding and 0.00 is indicative of random mating. The values for *Antennaria* populations in this study are primarily negative indicating a high degree of outcrossing, as would be expected in a dioecious plant. Several populations have highly negative values because observed numbers of heterozygotes greatly exceed those expected. The reasons for the excess number of heterozygotes are speculative at this time, but they could be the result of 1) higher survivorship of heterozygotes and/or 2) agamospermous seed production (currently unknown in diploid *Antennaria*, but common in polyploid taxa) by heterozygous individuals. Excess heterozygotes are common in

TABLE 3. Nei's genetic diversity statistics for individual species and section *Dioicae* (i.e., all species). Presented are gene diversities for individual polymorphic loci and pooled loci. Only species displaying polymorphism at a given locus are represented; monomorphic loci have gene diversity statistics values of 0.000. H_T = total gene diversity within a taxon, H_S = gene diversity within populations of a taxon, D_{ST} = gene diversity between populations within a taxon, G_{ST} = coefficient of gene differentiation.

Loci	Species	H_T	H_S	D_{ST}	G_{ST}
Acp-1	*A. corymbosa*	0.322	0.236	0.086	0.268
	A. marginata	0.157	0.129	0.028	0.180
	A. microphylla	0.007	0.056	0.000	0.000
	A. rosulata	0.315	0.256	0.059	0.187
	All species	0.502	0.178	0.324	0.646
Gdh-1	*A. corymbosa*	0.408	0.240	0.168	0.412
Lap-1	*A. corymbosa*	0.543	0.239	0.304	0.559
	A. marginata	0.567	0.352	0.215	0.379
	A. microphylla	0.273	0.057	0.216	0.789
	A. rosulata	0.119	0.098	0.021	0.172
	All species	0.605	0.189	0.416	0.688
Lap-2	*A. corymbosa*	0.190	0.168	0.022	0.115
	A. marginata	0.407	0.270	0.137	0.336
	A. microphylla	0.316	0.206	0.110	0.348
	A. rosulata	0.063	0.048	0.015	0.229
	All species	0.542	0.148	0.394	0.726
Pgi-3	*A. corymbosa*	0.308	0.219	0.089	0.288
	A. marginata	0.058	0.058	0.000	0.000
	A. rosulata	0.337	0.297	0.040	0.119
	All species	0.510	0.154	0.356	0.698
Skdh-1	*A. corymbosa*	0.106	0.007	0.008	0.537
	A. marginata	0.445	0.343	0.102	0.229
	A. microphylla	0.254	0.197	0.057	0.226
	A. rosulata	0.050	0.060	0.000	0.000
	All species	0.235	0.147	0.088	0.375
Tpi-1	*A. corymbosa*	0.353	0.040	0.313	0.885
Tpi-3	*A. corymbosa*	0.436	0.337	0.099	0.228
	A. marginata	0.215	0.155	0.060	0.281
	A. microphylla	0.332	0.183	0.149	0.449
	A. rosulata	0.549	0.477	0.072	0.132
	All species	0.412	0.297	0.115	0.279
All loci	*A. corymbosa*	0.136	0.078	0.058	0.173
	A. marginata	0.097	0.068	0.029	0.074
	A. microphylla	0.062	0.037	0.028	0.095
	A. rosulata	0.075	0.064	0.011	0.044
	All species	0.171	0.061	0.110	0.262

populations of agamospermous *A. rosea* (Bayer, unpubl. data). A few populations have positive values indicating some inbreeding is taking place. One explanation for the positive values is that the populations are substructured as a consequence of functional inbreeding, i.e., crossing among neighbors.

Gene diversity statistics for *Antennaria* indicate that *Acp-1*, *Lap-1*, *Lap-2*, and *Pgi-3*, are the most genetically diverse loci. The four species each have different particular loci that are most diverse (table 2). Gene diversities within populations (H_S) are greater in all four species than are diversities between populations (D_{ST}). This is reflected in the values of G_{ST} (if all loci are considered, table 2), because the relatively low values of G_{ST} indicate that a greater proportion of the genetic diversity is due to within population differences rather than between. If total gene diversities (H_T) within species are consid-

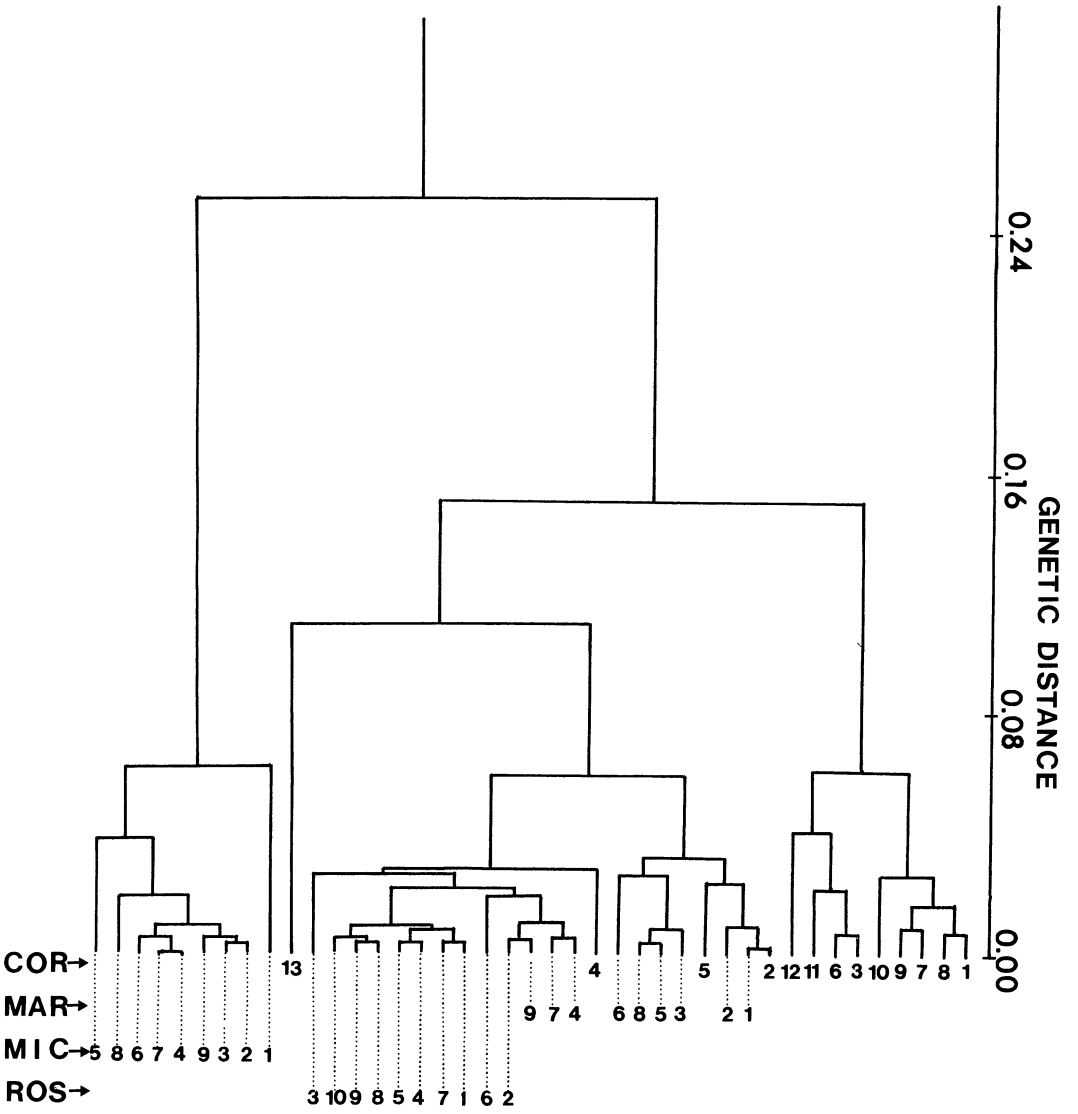

FIG. 2. Distance phenogram (UPGMA) derived from Nei's genetic distances of all pairwise comparisons of 41 populations from among *A. corymbosa, A. marginata, A. microphylla,* and *A. rosulata.* Species abbreviations follow figure 1. Population designations within each species are the same as those given in table 1. Cophenetic correlation coefficient is 0.8659.

ered, they range from $H_T = 0.062$ for *A . microphylla* to $H_T = 0.136$ for *A. corymbosa.* It is of interest that the most widespread species, *A. microphylla,* has the least amount of gene diversity, while the three more geographically restricted ones have higher values of H_T. A comparison of D_{ST} and G_{ST} values (table 2) for the individual species vs. all four at a particular locus indicates that interspecific populational differences are the result of divergence among the species at *Acp-1, Lap-1, Lap-2,* and *Pgi-3.* Most of the interspecific differences are determined by very different frequencies of the same alleles at a locus, not by unique alleles, among the species. Average values of H_T, H_S, and G_{ST} for other outcrossing, dioecious, sexual, insect pollinated, long-lived perennials (see Loveless and Hamrick 1984) are higher than those found in

TABLE 4. Intraspecific mean genetic distances and mean genetic identities for four species of *Antennaria*.

Species	Mean identity (range)	Mean distance (range)
A. corymbosa	0.922 (0.810–0.994)	0.083 (0.006–0.210)
A. marginata	0.973 (0.922–0.997)	0.028 (0.003–0.081)
A. microphylla	0.972 (0.898–1.000)	0.028 (0.000–0.108)
A. rosulata	0.987 (0.965–0.997)	0.013 (0.003–0.036)

all four *Antennaria* species surveyed, except *A. corymbosa*, whose values approach or surpass (G_{ST}) cited values.

Intraspecific interpopulational comparisons show that the average range of identities are 0.922 in *A. corymbosa* to 0.987 in *A. rosulata* indicating a high degree of genetic similarity of populations within the species. These values are similar to intraspecific identities from other taxa (see Crawford 1983; Gottlieb 1981a). Despite high morphological divergence, there is less genetic divergence at isozyme loci as indicated by relatively high values of Nei's genetic identities (I). Recent divergence is indicated by relatively high genetic identity values and low genetic distances (D) for the majority of the interspecific comparisons.

The phenogram resulting from the cluster analysis of a genetic distance matrix (fig. 2) shows the relationships among the 41 populations and four species. High cophenetic correlation coefficients indicate that the phenograms portrayed in figures 2 and 3 are very good representations of the original distance matrices. *Antennaria microphylla* is the most distinct isozymically from the other three species. One population of *A. microphylla* (MI1, from central Alberta) is distinct from the other eight populations of this species from the southern portion of the range (figs. 1, 2; table 1). *Antennaria rosulata* forms a cluster of electrophoretically similar populations (fig. 2), and geographically neighboring sites (e.g., R4 and R5; or R8 and R9) are generally less distant isozymically. *Antennaria marginata* and *A. rosulata* have ranges (fig. 1) that overlap, yet their community associations are different and they have not been observed growing as sympatric populations (Bayer, pers. obs.). Although very different morphologically (Bayer 1987b), *A. marginata* populations are isozymically very similar to *A. rosulata*. *Antennaria marginata* populations form an interrupted cluster. Three populations from the margin of the range of the species (M4, M7, and M9) are most closely connected with the populations of *A. rosulata* than with other populations of *A. marginata*. The close association between these populations of *A. marginata* and *A. rosulata* is difficult to explain, especially because they are so different morphologically and these particular populations of *A. marginata* are outside the margin of the known range of *A. rosulata* (fig. 1). One explanation for this close association might be that it is suggestive of past introgressive hybridization between *A. marginata* and *A. rosulata*; however, there are no morphological indications in support of this. One of these populations, M7, is tetraploid and has highest genetic identity ($I = 0.995$) with a diploid population from Mohave Co., Arizona, M4 (table 1; fig. 2). The tetraploid population of *A. marginata* has no unique alleles, indicating that

TABLE 5. Nei's genetic distances (lower triangle) and genetic identities (upper triangle) for all pairwise comparisons of populations within five taxa of *Antennaria*. *Antennaria corymbosa* (black) are those two atypical morphotypes of *A. corymbosa* with black phyllaries discussed in the text.

Species	COR	COR (blk)	MAR	MIC	ROS
A. corymbosa	*****	0.983	0.909	0.841	0.893
A. corymbosa (black)	0.017	*****	0.974	0.832	0.955
A. marginata	0.096	0.027	*****	0.821	0.978
A. microphylla	0.173	0.184	0.197	*****	0.809
A. rosulata	0.113	0.048	0.023	0.212	*****

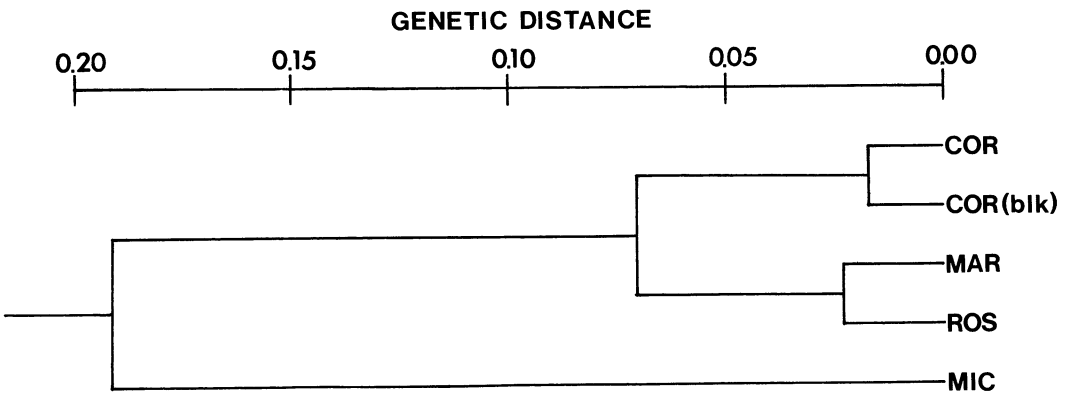

FIG. 3. Distance phenogram (UPGMA) derived from Nei's genetic distances of all pairwise comparisons (table 4) of five taxa of *Antennaria*. *A. corymbosa*, *A. corymbosa* (black phyllaried form, blk), *A. marginata*, *A. microphylla*, and *A. rosulata* are labeled as in figure 1. Cophenetic correlation coefficient is 0.9456.

it is probably of non-hybrid (autopolyploid) origin. *Antennaria corymbosa* populations are unusual; one large group of populations is electrophoretically distinct from populations of the other three species. Two small clusters are evident within this main cluster of *A. corymbosa*, but no geographic pattern is apparent. Populations C2, C4, C5, and C13 are puzzling. Two populations have recently been discovered, one C13 (table 1) from Fremont Co., Wyoming, and the other from the Uinta Mountains, Utah (C12), that are morphologically atypical. *Antennaria corymbosa* characteristically has white phyllaries with a black spot at the base (Bayer 1987b), but plants from these populations have phyllaries that are solid black. The plants are otherwise typical of *A. corymbosa* with respect to morphology and reproductive mode. Possibly such populations have diverged sufficiently, both at genes encoding soluble enzymes and morphologically, to receive taxonomic recognition. The other unusual populations, C2, C4, and C5, are at the margin of the range of *A. corymbosa*. One reason that typical *A. corymbosa* populations are electrophoretically distinct from other species is that they possess an allelic variant at *Tpi-1*, *Tpi-1*^{b}, while all populations of the other species have *Tpi-1*^{a}. The unusual *A. corymbosa* populations, C2, C4, C5, and C13, are all fixed for *Tpi-1*^{a}, which makes them more similar to other species than to other populations of *A. corymbosa*.

When compared to values of genetic identity for other interspecific *Antennaria* comparisons (see Bayer and Crawford 1986), these values for *I* (table 5) are very similar, but when compared to the results of other studies (Crawford 1983), values of *I* are higher than those for other congeneric comparisons. Cluster analysis of genetic distances among the species (table 5), including the black-phyllaried form of *A. corymbosa* as a separate taxon, indicates that the degree of genetic divergence among the species is low. The black-phyllaried form of *A. corymbosa* is almost as distinct from typical *A. corymbosa*, as *A. marginata* is from *A. rosulata*. Taxonomically, it is best to recognize *A. corymbosa*, *A. marginata*, *A. microphylla*, and *A. rosulata* as distinct species because they are morphologically distinct, occur in different plant communities from one another, and are moderately distinct electrophoretically. The black-phyllaried form of *A. corymbosa* needs further study before its taxonomic status can be clarified.

There is perceptible discordance between the degree of morphological and isozymic divergence among sexual diploid species of western North American *Antennaria*. Crawford (1985) provides examples where speciation has occurred with subsequent divergence in morphology, but not in isozymes. Often morphological differences between species are governed by one or a few genes (Gottlieb 1984) and this could be the case in *Antennaria*. Adaptive radiation of insular Compositae genera as Hawaiian *Tetramolopium* (Lowrey and Crawford 1985) and Hawaiian *Bidens* (Helenurm and Ganders 1985), *Dendroseris* (Crawford et al. 1987) illus-

trate a rapid mode of speciation, in which morphological divergence has not been accompanied by a correspondent degree of isozyme divergence. Continental genera such as *Quercus* (Fagaceae) (Manos and Fairbrothers 1987), *Heuchera* (Saxifragaceae) (Soltis 1985), and *Antennaria* (Bayer and Crawford 1986) display a similar pattern. If indeed the morphological differences between *Antennaria* species are governed by few genes then perhaps the discordance between morphological and isozymic divergence may be more apparent than real.

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