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ALLOZYME DIVERGENCE AMONG FIVE DIPLOID SPECIES OF ANTENNARIA (ASTERACEAE: INULEAE) AND THEIR ALLOPOLYPLOID DERIVATIVES[1]

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ABSTRACT

The *Antennaria parlinii* and *A. neodioica* agamic complexes are widely distributed across North America. Morphological data have suggested that these dioecious, perennial, entire-leaved herbs are of multiple hybrid origin from among five sexual diploid species. *Antennaria neglecta, A. plantaginifolia, A. racemosa,* and *A. virginica* are hypothesized to be the diploid progenitors of the *A. neodioica* complex, whereas *A. parlinii* sensu lato is thought to include the genomes of *A. plantaginifolia, A. racemosa,* and *A. solitaria.* An electrophoretic study was initiated to assess the degree of divergence among the five diploid species and to test the hypotheses of the hybrid (allopolyploid) origins of *A. parlinii* and *A. neodioica.* Twenty genetic loci were surveyed in 76 populations of the diploid and polyploid taxa. The diploid species are well defined morphologically, although the genetic basis of differences distinguishing them have not been determined. The species exhibit little divergence at genes specifying soluble enzymes, however each species has unique alleles in highest frequency at one or two genes. Allozymes indicate that gene diversity in the obligately outcrossing diploids occurs primarily within rather than among populations. The shale barren endemic, *A. virginica,* is as genetically diverse as the more edaphically diverse and widespread species. Tetraploid cytotypes of diploid ($2n = 28$) *A. virginica* possess the same allozymes as the diploids and these cytotypes appear to be of autopolyploid (non-hybrid) origin. Enzyme electrophoresis is concordant with morphological data in suggesting that *A. neodioica* contains the genomes of *A. neglecta, A. virginica, A. plantaginifolia, A. racemosa* and perhaps *A. solitaria* whereas the latter three species are the progenitors of *A. parlinii.*

THE *Antennaria parlinii* sensu lato and *A. neodioica* sensu lato polyploid agamic complexes are two polymorphic taxa occurring in North America. *Antennaria parlinii* occurs in the deciduous forests of the eastern U.S. and adjacent Canada, while *A. neodioica* is found across North America between 40° and 60° latitude, primarily north of the terminal margin of the Wisconsin glaciation. *Antennaria parlinii,* which was recently circumscribed as consisting as two subspecies, *A. parlinii* Fern. ssp. *parlinii* and *A. parlinii* ssp. *fallax* (Greene) Bayer and Stebbins, has sexual and agamospermous populations, each with a distinct geographic distribution (Bayer and Stebbins, 1983). Phenetic analysis of the sexual diploids *A. plantaginifolia* (L.) Richardson, *A. racemosa* Hook., and *A. solitaria* Rydb. has suggested them as the probable progenitors of *A. parlinii* (Bayer 1985b). Similar analyses of morphological features indicated that *A. neodioica* (Bayer, 1985a) is of multiple hybrid origin with the genomes of *A. neglecta* Greene, *A. plantaginifolia, A. racemosa,* and *A. virginica* Stebbins the probable diploid species involved. Bayer and Stebbins (1982) and Bayer (1984) recognized four variable subspecies of *A. neodioica*: *A. neodioica* Greene ssp. *canadensis* (Greene) Bayer and Stebbins; *A. neodioica* ssp. *howellii* (Greene) Bayer; *A. neodioica* ssp. *neodioica*; and *A. neodioica* ssp. *petaloidea* (Fern.) Bayer and Stebbins. Unlike *A. parlinii, A. neodioica* is totally agamospermous and therefore composed almost entirely of pistillate clones. *Antennaria neodioica* consists of tetraploids ($2n = 56$) and hexaploids ($2n = 84$), while *A. parlinii* has tetraploid, pentaploid ($2n = 70$), hexaploid, and octoploid ($2n = 112$) cytotypes (Bayer and Stebbins, 1981; Bayer, 1984).

The most widely distributed diploid species is *A. neglecta,* which occurs in prairies and

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TABLE 1. *Population designations, number of plants examined per population (in parentheses), locality data, and voucher number for populations of* Antennaria. *Agamospermous populations are indicated by an asterisk. Subspecies identifications for members of* A. neodioica *s.l. are given by the first three letters of the subspecific epithet (followed by their chromosome number) in parentheses after the voucher number. Collection numbers are those of the senior author; vouchers are at OS.*

A. neglecta Greene ($2n = 28$)—U.S.: 1(17), IL: Vermillion Co., *KP-10,* 2(20), IN: Bartholomew Co., *HV-216,* 3(14), Brown Co., *MZ-218,* 4(18), Dearborn Co., *DB-219,* 5(18), OH: Delaware Co., *BPN-56,* 6(17), Fairfield Co., *CT-06.*

A. plantaginifolia (L.) Richardson ($2n = 28$)—U.S.: 7(17), KY: Johnson Co., *SK-206,* 8(10), Lawrence Co., *CK-207,* 9(21), Lawrence Co., *COW-278,* 10(20), Magoffin Co., *PV-282,* 11(28), Magoffin Co., *SV-279,* 12(18), Magoffin Co., *MA-280,* 13(21), Morgan Co., *GC-281,* 14(20), VA: Bath Co., *HS-293,* 15(18), Bath Co,. *BA-294,* 16(17), WV: Greenbrier Co., *GB-291,* 17(19), Raleigh Co., *SS-289,* 18(18), Summers Co., *SU-290.*

A. racemosa Hook. ($2n = 28$)—U.S.: 19(20), MT: Carbon Co., *BTP-214,* 20(20), Granite Co., *M-350,* 21(18), Granite Co., *M-330,* 22(17), Ravalli Co., *M-336,* 23(17), Ravalli Co., *M-337,* 24(20), WY: Park Co., *FC-213.*

A. solitaria Rydb. ($2n = 28$)—U.S.: 25 (11), KY: Bath Co., *MBS-44,* 26(11), Magoffin Co., *SV-279,* 27(18), VA: Smyth Co., *GS-238,* 28(21), WV: Boone Co., *MD-286.*

A. virginica Stebb. ($2n = 28$)—U.S.: 29 (23), OH: Columbiana Co., *AV-78,* 30(17), WV: Hampshire Co., *RA-250,* 31(18), Pendleton Co., *AV-298,* 32(18), Pendleton Co., *BR-109.*

A. virginica Stebb. ($2n = 56$)—U.S.: 33(18), WV: Grant Co., *GR-110,* 34(10), Hampshire Co., *HR-110,* 35(17), Hampshire Co., *PV-300,* 36(20), Hardy Co., *HY-299.*

A. neodioica s.l. ($2n = 56$, 84, or 140)—CANADA: 37(20), Ont: Frontenac Co., *MS-247,* (PET, 84)*, 38(3), Peterborough Co., *BF-87,* (PET, 84)*, 39(3), Peterborough Co., *KL-182,* (CAN, 84)*, 40(24), Peterborough Co., *BO-220,* (CAN, PET, 84)*. U.S.: 41(3), OH: Delaware Co., *ALC-53,* (NEO, 56)*, 42(10), Fairfield Co., *CR-82,* (NEO, PET, 84)*, 43(5), Guernsey Co., *CM-19,* (NEO, 84)*, 44(1), Jefferson Co., *AN-79,* (CAN, 84)*, 45(3), MT: Meagher Co., *8109,* (NEO, 84)*, 46(15), Ravalli Co., *M-348,* (HOW, 140)*, *M-349,* (PET, 84)*, 47(2), NY: Erie Co., *AFG-61,* (PET, 84)*, 48(3), Erie Co., *EA-58,* (NEO, 56)*, 49(3), Erie Co., *EA-63,* (CAN, 84)*, 50(6), Erie Co., *AFG-62,* (PET, 84)*, 51(18), PA: Greene Co., *RO-301,* (NEO, 56)*, 52(5), VA: Highland Co., *MO-295,* (NEO, PET, 84)*, 53(6), WV: Hampshire Co., *HR-162-A,* (PET, 84)*, 54(4), Pendleton Co., *ON-111,* (NEO, 56)*, 55(9), Randolph Co., *EK-252,* (NEO, 84)*.

A. parlinii s.l. ($2n = 84$)—U.S.: 56(28), IN: Brown Co., *NA-217**, 57(7), Brown Co., *MZ-218**, 58(11), KY: Bath Co., *MB-41,* 59(17), Greenup Co., *KE-277,* 60(17), Greenup Co., *GR-275,* 61(21), Greenup Co., *GR-276,* 62(10), Lawrence Co., *CK-207,* 63(18), OH: Fairfield Co., *PS-202**, 64(19), Fairfield Co., *DA-12,* 65(35), Fairfield Co., *BK-88,* 66(18), Hocking Co., *SC-192,* 67(17), Hocking Co., *BC-198,* 68(13), Perry Co., *MX-68,* 69(17), Vinton Co., *NP-29,* 70(18), PA: Centre Co., *CE-255,* 71(19), VA: Allegheny Co., *VA-292,* 72(5), Highland Co., *MO-296,* 73(19), WV: Boone Co., *BN-287,* 74(18), Lincoln Co., *HM-285,* 75(17), Pendleton Co., *FR-297,* 76(18), Raleigh Co., *BK-288.*

pastures from New England, SW to Oklahoma, NW to South Dakota, and north to the Northwest Territories of Canada. *Antennaria plantaginifolia* occurs in dry forest margins in the Appalachian region and New England with disjunct populations in the driftless area of Wisconsin and the upper Mississippi watershed. Moist forest slopes of the Appalachian Region south of the terminal margin of the Wisconsin glaciation are the habitat of *A. solitaria. Antennaria racemosa* is found in the dry coniferous forests of British Columbia, Alberta, south to Washington, Oregon, northern California, Montana, Idaho, and Wyoming. The shale barren endemic *A. virginica* has the most narrow distribution of the five, occurring in West Va., western Va., western Md., extreme south-central Pa., and a few localities in extreme eastern Oh. All species are strictly diploid ($2n = 28$) except *A. virginica* which has some tetraploid ($2n = 56$) populations (Bayer, 1984a).

The present study employed enzyme electrophoresis in order to: 1) determine genetic variation within the five diploid species of *Antennaria,* 2) measure genetic divergence among the diploid species; and 3) ascertain whether the hypothesis of the hybrid origin of the *Antennaria parlinii* and *A. neodioica* agamic complexes could be confirmed.

MATERIALS AND METHODS—Portions of clones (ramets) for isozyme studies were gathered from populations in the United States and adjacent Canada (Table 1). Plants were collected from a total of 76 populations: *A. neglecta* (6); *A. plantaginifolia* (12); *A. racemosa* (6); *A. solitaria* (4); *A. virginica* (4 diploid and 4 tetraploid); *A. neodioica* (19); and *A. parlinii* (21) (Table 1). Chromosome counts were determined for all populations employing the methods of Bayer (1984a). Clones from the populations were planted in flats and placed in the greenhouse until they became established and developed floral buds. The flats were placed in a cold room (1 C ± 0.5 C) under lights for a period of two to three months; individual flats were then moved back to the greenhouse or placed in a growth chamber until the plants flowered (ca. two weeks). Young flowering heads were used as sources of enzymes because they gave strong, clear banding patterns; young

leaves were used when flowering heads were not available.

The extracting buffer was 0.1 M tris-HCl, pH 7.5, 4 mM 2-mercaptoethanol, 1 mM EDTA (tetrasodium salt), 10 mM KCl, and 10 mM $MgCl_2$ (Gottlieb, 1981a). About 20 mg of PVP were added to each sample at the time of grinding. The buffer was made up in 20% sucrose to obtain the necessary density for loading into wells in the acrylamide gel. The extracts were centrifuged and the supernatant either pipetted into wells in the acrylamide or soaked onto filter paper wicks for insertion into 12.25% starch gels. Two buffer systems were employed for starch gels. Phosphoglucose isomerase (PGI), leucine amino peptidase (LAP), and triose phosphatae isomerase (TPI) were resolved using a gel buffer of nine parts tris-citrate (0.05 M tris, 0.007 M citric acid·H_2O, pH 8.3) and one part lithium-borate (0.038 M lithium hydroxide, 0.188 M boric acid, pH 8.3). The electrode buffer consisted only of lithium-borate. Shikimate dehydrogenase (SKDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGDH), and acid phosphatase (ACP) were resolved on a system with 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, Stuber, and Goodman, 1981). An acrylamide system described by Maurer and Allen (1972) was employed for phosphoglucomutase (PGM), glutamate dehydrogenase (GDH), and glyceraldehyde-3-phosphate dehydrogenase [(NADP) G3PDH]. The buffer for the separating gel (5.5% acrylamide) was 0.38 M tris—HCl, pH 8.9 and the buffer for the spacer gel (3.0% acrylamide) was 0.08 M tris—HCl, pH 6.9. The electrode buffer was 0.05 M tris-0.38 M glycine, pH 8.3. A second acrylamide system (Maurer and Allen, 1972) was employed to resolve the isozymes PGI-1 and -2 and TPI-1 and -2. This used a separating gel buffer (8.0% acrylamide) of 0.67 M imidazole brought to pH 7.8 with HCl, a spacer gel (3.0% acrylamide) of 0.07 M imidazole adjusted to pH 5.9 with HCl, and an electrode buffer of 0.096 M arginine—0.007 M imidazole, pH 7.3. Gels were stained according to the procedures of Soltis et al. (1983).

Intact chloroplasts were isolated from leaves employing the methods of Gastony and Darrow (1983). The final chloroplast pellet was resuspended in the extracting buffer described previously. Pollen was soaked in the extracting buffer, wtih the enzymes in the leachate identified as the cytosolic isozymes (Weeden and Gottlieb, 1980a).

The locus specifying the most anodally migrating form of each enzyme was designated 1, the next 2, and so on. Likewise, the most anodally migrating allozymes were designated as A, the next as B, and so on. Standard genetic identity and distance statistics were calculated utilizing the methodology of Nei (1972). The methods of Nei (1973) were also employed to compute gene diversity statistics. Proportion of polymorphic loci, mean number of alleles per locus, and observed and expected proportion of heterozygous loci were calculated for each population of the diploid species. A chi-square test was employed to determine whether observed values differed from those expected under Hardy-Weinberg equilibrium; both the 5% and 1% significance levels were used. All statistics were calculated using the GENESTAT program written by Richard Whitkus and executed at the Instructional Research Computer Center of The Ohio State University. The genetic distance matrix was used to construct a phenogram by the unweighted pair-group method using arithmetic averages (UPGMA; Sneath and Sokal, 1973). The cluster analysis was generated by the TAXON subroutine of the NT-SYS program of Rohlf, Kishpaugh, and Kirk (1974).

RESULTS—Locality data, chromosome numbers, maximum number of individuals surveyed and voucher numbers for each population are presented in Table 1. Allelic frequencies for populations of the sexual diploids (including one tetraploid population of *A. neodioica* ssp. *neodioica*) are available from the senior author. Hereafter diploid and tetraploid populations of *A. virginica,* will be referred to only as *A. virginica.* Allelic frequencies for most populations of *A. neodioica* and *A. parlinii* could not be obtained because of the high ploidy levels of the plants.

Ten enzyme systems coded by 20 genetic loci were surveyed. They are as follows: ACP-1, GDH, (NADP) G3PDH-1, (NADP) G3PDH-2, LAP-1, MDH-1, -2, -3, -4, PGI-1, PGI-2, PGI-3, PGM-1, PGM-2, SKDH-1, SOD-1, SOD-2, and TPI-1, TPI-2, TPI-3. The 6-phosphogluconate dehydrogenase (6PGDH) was scored for most runs, but could not be readily interpreted because of overlap of isozymes. Additional isozymes for ACP, LAP, and SKDH were occasionally visualized in the gels, but were not included in the analyses because of their sporadic occurrence.

The number of isozymes for GDH, MDH (NADP) G3PDH and PGM is the same as normally found in diploid plants (Gottlieb, 1982). Three isozymes were detected for PGI

TABLE 2. *Genetic variation within populations of species of* Antennaria *including percent of loci polymorphic (frequency of most common allele less than 0.99), average number of alleles per locus, and observed and expected proportion of loci heterozygous. One asterisk indicates significant difference at the 5% level, two asterisks denote significance at the 1% level. Population designations same as in Table 1*

	Proportion of loci polymorphic	Average number of alleles/locus	Observed proportion of loci heterozygous	Expected proportion of loci heterozygous
A. neglecta				
1.	0.250	1.25 ± 0.433	0.127	0.090
2.	0.308	1.38 ± 0.624	0.181**	0.095
3.	0.143	1.14 ± 0.350	0.087	0.054
4.	0.143	1.15 ± 0.360	0.094**	0.035
5.	0.150	1.20 ± 0.510	0.035	0.038
6.	0.200	1.20 ± 0.040	0.095	0.062
A. plantaginifolia				
7.	0.250	1.28 ± 0.450	0.050	0.035
8.	0.267	1.40 ± 0.710	0.020	0.037
9.	0.177	1.29 ± 0.670	0.023	0.027
10.	0.250	1.33 ± 0.590	0.045	0.038
11.	0.300	1.35 ± 0.570	0.066	0.049
12.	0.300	1.50 ± 0.810	0.068	0.079
13.	0.438	1.56 ± 0.700	0.059	0.061
14.	0.250	1.35 ± 0.650	0.053	0.031
15.	0.250	1.40 ± 0.800	0.059	0.062
16.	0.200	1.20 ± 0.400	0.048	0.050
17.	0.150	1.20 ± 0.510	0.072	0.056
18.	0.167	1.22 ± 0.530	0.051	0.052
A. racemosa				
19.	0.214	1.29 ± 0.590	0.051	0.071
20.	0.222	1.22 ± 0.420	0.017**	0.061
21.	0.200	1.30 ± 0.690	0.036	0.044
22.	0.167	1.28 ± 0.650	0.020	0.032
23.	0.200	1.33 ± 0.870	0.056**	0.015
24.	0.177	1.24 ± 0.550	0.050*	0.074
A. solitaria				
25.	0.188	1.25 ± 0.560	0.074	0.059
26.	0.125	1.13 ± 0.330	0.023	0.011
27.	0.143	1.14 ± 0.350	0.044	0.050
28.	0.300	1.35 ± 0.570	0.087	0.109
A. virginica				
29.	0.177	1.18 ± 0.380	0.059	0.067
30.	0.250	1.25 ± 0.430	0.153*	0.101
31.	0.350	1.50 ± 0.920	0.112	0.113
32.	0.235	1.24 ± 0.420	0.047	0.043
33.	0.150	1.20 ± 0.510	0.094*	0.056
34.	0.067	1.07 ± 0.250	0.040	0.023
35.	0.350	1.35 ± 0.480	0.165*	0.115
36.	0.250	1.35 ± 0.730	0.073	0.068
A. neodioica s.l.				
37, 40, 51				
Average			0.137	
A. parlinii s.l.				
56–76				
Average			0.128	

and TPI whereas most diploid plants have only two (Gottlieb, 1982). For (NADP) G3PDH, PGI, PGM and TPI, one isozyme is normally present in the cytosol and the other in the plastids (Gastony and Darrow, 1983; Gottlieb, 1982; Weeden and Gottlieb, 1980a, b). Leaching pollen in the extracting buffer for a limited period of time removes the cytosolic isozymes whereas isolated chloroplasts may be lysed to release their isozymes (Gastony and Darrow,

TABLE 3. *Gene diversity statistics for five species of* Antennaria. *H_T total gene diversity within a species, H_S = diversity within populations, D_{ST} = gene diversity between populations, and G_{ST} = degree of differentiation between populations*

Taxon	H_T	H_S	D_{ST}	G_{ST}
A. neglecta	0.098	0.073	0.025	0.255
A. plantaginifolia	0.066	0.059	0.007	0.106
A. racemosa	0.080	0.061	0.019	0.238
A. solitaria	0.098	0.082	0.016	0.163
A. virginica	0.107	0.087	0.020	0.187
All taxa	0.136	0.071	0.065	0.478

1983; Weeden and Gottlieb, 1980a, b). Soaked pollen gave (NADP) G3PDH-1, PGI-3, PGM-2 and TPI-3, indicating these are cytosolic isozymes. Isolated chloroplasts, upon lysing, produced (NADP) G3PDH-2, PGI-1, PGI-2, PGM-1, TPI-1, and TPI-2. This suggests that the one additional isozyme for PGI and TPI occurs in the plastids. Although no genetic analyses could be performed due to lack of variation with both enzymes, the same three-banded pattern (interpreted as two isozymic homodimers and an interlocus heterodimer) present in parental plants always occurred in progeny. The extra isozymes are interpreted as the result of duplications of the genes coding the plastid forms of PGI and TPI. Duplication of the gene specifying plastid TPI has been reported for *Clarkia* (Pichersky and Gottlieb, 1983).

Unbalanced heterozygotes, synthesized by crossing *A. parlinii* (6*x*) and *A. solitaria* (2*x*) were used as a standards to compare heterozygotes in tetraploid populations of *A. virginica* and *A. neodioica* ssp. *neodioica*. Proportionate (2:2-dosage) and disproportionate (3:1-dosage) heterozygotes are readily discernible in tetraploid individuals. In hexaploid cytotypes of *A. parlinii* and *A. neodioica*, it was impossible to distinguish the 5:1 from the 4:2 dosage types with any degree of confidence.

Ten isozymes were found to be monomorphic for all the species surveyed: (NADP) G3PDH-1, MDH-1, MDH-2, MDH-3, MDH-4, PGI-1, PGI-2, SOD-2, and TPI-1 and TPI-2. Percent of loci polymorphic, proportion of loci heterozygous, average number of alleles per locus and observed and expected proportion of loci heterozygous (with significant differences indicated) are given in Table 2 for populations of the five diploid taxa. The mean observed proportion of heterozygous loci are shown for populations of the polyploid taxa (Table 2).

TABLE 4. *Mean genetic identities and ranges of identities for pairwise comparisons of populations within and among five diploid species of* Antennaria. *Also included is one tetraploid population of* A. neodioica *(see text for further discussion)*

	Mean	Range
Within species		
A. neglecta	0.967	0.923–0.996
A. plantaginifolia	0.994	0.980–0.999
A. racemosa	0.976	0.951–0.998
A. solitaria	0.983	0.964–0.995
A. virginica	0.976	0.948–0.993
Among species		
A. neglecta ×		
A. plantaginifolia	0.930	0.890–0.955
A. racemosa	0.899	0.820–0.950
A. solitaria	0.882	0.816–0.923
A. virginica	0.876	0.813–0.925
A. neodioica	0.877	0.831–0.892
A. plantaginifolia ×		
A. racemosa	0.945	0.913–0.979
A solitaria	0.873	0.825–0.913
A. virginica	0.892	0.828–0.927
A. neodioica	0.915	0.881–0.931
A. racemosa ×		
A. solitaria	0.883	0.840–0.916
A. virginica	0.869	0.841–0.923
A. neodioica	0.927	0.903–0.948
A. solitaria ×		
A. virginica	0.873	0.832–0.913
A. neodioica	0.892	0.885–0.902
A. virginica ×		
A. neodioica	0.941	0.909–0.973

Total gene diversity (H_T), gene diversity within (H_S), and between (D_{ST}) populations, and degree of genetic differentiation between populations (G_{ST}) for each diploid taxon are given in Table 3. These statistics are presented as a mean including both variant and invariant loci.

Mean genetic identities for pair-wise comparisons of populations within and among species are given in Table 4. Only the diploid species and the tetraploid *A. neodioica* are shown because allelic frequencies could not be determined for plants with higher ploidy levels. A phenogram constructed from a matrix of genetic distances from pair-wise comparisons of populations of all species is presented in Fig. 1.

Those 10 genes monomorphic in the diploids are also monomorphic in the polyploids. Several loci polymorphic in the diploid species (*Acp-1, Gdh,* (NADP) *G-3-Pdh-2, Pgm-1, Pgm-2* and *Skdh-1*) share the same common alleles, but may possess different ones in low frequen-

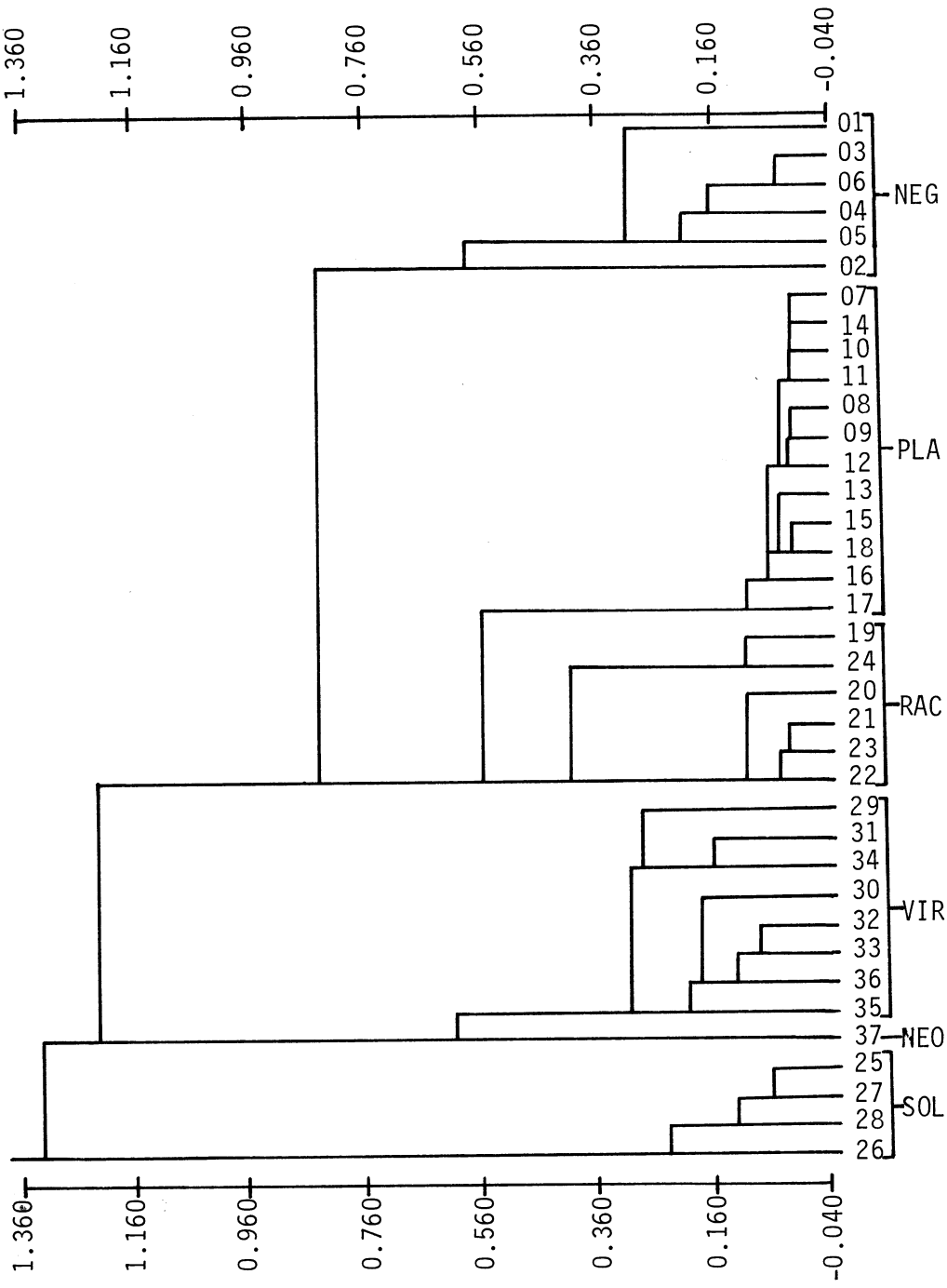

Fig. 1. Distance phenogram (UPGMA) composed of *A. neglecta* (populations 1–6), *A. plantaginifolia* (populations 7–18), *A. racemosa* (populations 19–24), *A. solitaria* (populations 29–36), *A. virginica* (populations 29–36), and *A. neodioica* ssp. *neodioica* (population 51). Population designations are those given in Table 1.

cies. The two most diverse loci in the diploids are *Lap-1* and *Pgi-3*, with each species having different alleles in high frequency, or unique high frequency alleles, at one or both of the genes.

Antennaria neglecta has no unique high frequency allele at either *Lap-1* or *Pgi-3* (Fig. 2). It does contain *Lap-1*^{h}, *Lap-1*^{i}, and *Pgi-3*^{d} as low frequency, unique alleles. These three alleles, however, were not detected in the poly-

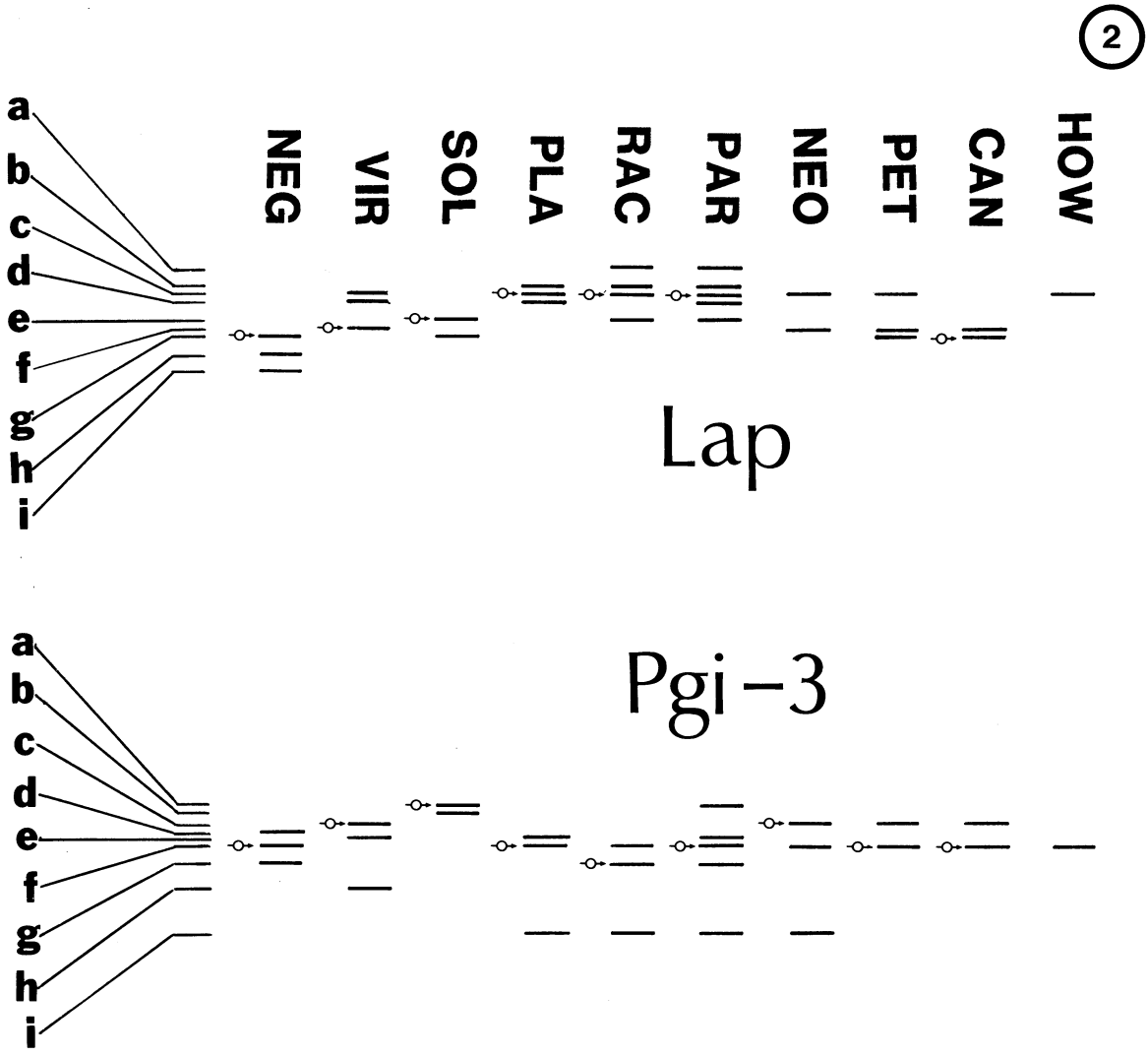

Fig. 2. Diagram showing allozymes of LAP and PGI-3 occurring in diploid and polyploid species of *Antennaria*. Arrow denotes highest frequency alleles for each taxon.

ploids (Fig. 2). *Antennaria plantaginifolia* contains no alleles that are unique to the species nor are high frequencies only in it (Fig. 2). *Antennaria racemosa* exhibits *Lap-1*^{a}, which is unique among the diploids; also, *Pgi-3*^{a} occurs in highest frequency only in this diploid, but is present as a low frequency allele in *A. neglecta*. *Antennaria solitaria* is the only diploid species containing *Lap-1*^{a} as the highest frequency allele, although it does occur in *A. racemosa* (Fig. 2). The former species has *Pgi-3*^{a}, which does not occur in other diploids, in highest frequency (Fig. 2). *Antennaria virginica* contains *Lap-1*^{f} and *Pgi-3*^{c}, both of which are unique for diploid species (Fig. 2). The distribution of the alleles at *Lap-1* and *Pgi-3* in the polyploids will be considered in the DISCUSSION section.

DISCUSSION—*Allozyme diversity within and divergence among the diploid taxa*—The proportion of polymorphic loci within species exhibits large ranges in all species, with a two-fold or greater range present in all taxa except *A. racemosa* (Table 2). In addition, the mean values for all populations of each species are similar. Populations of all species exhibit comparable values for average number of alleles per locus (Table 2). These two measures of genetic variation indicate similar levels of genetic variation in populations of all diploid species. Twenty-eight of the 36 populations of

the diploid species are in Hardy-Weinberg equilibrium (Table 2). Any deviations from expected heterozygosities for populations of a given species are either excesses (in *A. racemosa*) or deficiencies (*A. neglecta* and *A. virginica*). The causes of deviation from expected values in these dioecious species are not known.

When all populations of all taxa are considered (Table 5), the gene diversities within populations ($H_S = 0.071$) and between populations ($D_{ST} = 0.065$) are about equal. The value of G_{ST} (0.478) for all taxa indicates that about 47.8% of the genetic variation in all species as a whole is due to between population gene differences. The most restricted taxon, *A. virginica,* has the greatest amount of gene diversity ($H_T = 0.107$), while one of the most widespread taxa, *A. plantaginifolia,* has the least ($H_T = 0.066$; Table 3). These data demonstrate that the majority of genic diversity in the species resides within (H_S) populations. The greatest amount of between population differentiation is seen in *A. neglecta* ($G_{ST} = 0.255$) and the least amount is between populations of *A. plantaginifolia* ($G_{ST} = 0.106$; i.e., 10.6% of the genetic variation in *A. plantaginifolia* is the result of between population gene differences). The gene diversity in populations of the individual species of *Antennaria* is less than that which has been reported in other outcrossing perennials such as *Coreopsis grandiflora* (Crawford and Smith, 1984), *Pseudotsuga menziesii* (Yeh and O'Malley, 1980), *Pinus contorta* (Yeh and Layton, 1979).

Mean genetic identities for pairwise comparisons of populations within each species range from 0.967 in *A. neglecta* to 0.994 in *A. plantaginifolia* (Table 4), indicating essentially no interpopulational differentiation within species. Similarly high identities have been reported for the vast majority of species examined electrophoretically (Gottlieb, 1977, 1981b; Crawford, 1983). The mean identity for *A. virginica* (0.976) represents the value for four diploid and four tetraploid ($2n = 56$) populations. This high value supports the hypothesis (Bayer and Stebbins, 1981, 1982; Bayer, 1984) that the tetraploid cytotyes are autopolyploid (nonhybrid) derivatives of diploid *A. virginica.* In addition, they both contain the same alleles at all genes, except that one population of tetraploid *A. virginica* contained *Pgi-1*^{h} at a frequency of 0.17. The cytotypes of *A. virginica* cannot be separated with confidence by any known morphological character and also do not segregate in a phenetic analysis (Bayer, 1985a). Our results are similar to those obtained for other genera where suspected autopolyploids were essentially identical genetically to their presumed diploid progenitors (Crawford and Smith, 1984; Epes and Soltis, 1984; Soltis and Bohm, 1984).

Mean genetic identities for populations of different species reveal that *A. neglecta, A. plantaginifolia,* and *A. racemosa* are the most similar to each other, with *A. plantaginifolia* least similar to *A. virginica* and *A. solitaria* (Table 4). Morphologically, *A. plantaginifolia* and *A. racemosa* are most similar (Bayer, 1985a, b), thus there is some concordance between electrophoretic and morphological data. The identities are somewhat higher than reported for other morphologically distinct congeneric species (Gottlieb, 1981a; Crawford, 1983). Phenetic studies demonstrate that these diploid species are diverse morphologically, but electrophoretic data show that they are not nearly as divergent with respect to allozymes. The problem, however, is that little genetic data are available on the morphological features distinguishing the taxa. Preliminary results suggest a relatively simple genetic basis for several diagnostic features such as leaf pubescence, number of veins in leaves and number of heads per capitulescence (Bayer, 1985a, b).

A cluster analysis (UPGMA) of the interpopulational genetic distance matrix summarizes the data (Fig. 1). The co-phenetic correlation coefficient was 0.883, indicating the phenogram is a reasonably good portrayal of the original distance matrix. Populations of each of the five diploid taxa form distinct groups (Fig. 1). *Antennaria plantaginifolia* and *A. racemosa* are closely associated. *Antennaria neglecta* is also allied to both *A. plantaginifolia* and *A. racemosa* (Fig. 1). The shale barren endemic, *A. virginica* is widely separarted from *A. neglecta* (Fig. 1). Bayer and Stebbins (1982) have recognized the differences between these two taxa with regard to habitat, crossability, distribution, and morphology. These differences are supported by this study and it supports the taxonomic judgement that *A. virginica* [= *A. neglecta* Greene var. *argillicola* (Stebbins) Cronq.] should be retained as a distinct species from *A. neglecta. Antennaria solitaria* is most distant from the remaining four diploid species as is also disclosed by the morphology (Bayer and Stebbins, 1982; Bayer, 1984c).

Gene diversity statistics indicate that the least amount of differentiation occurs among populations of *A. plantaginifolia* ($G_{ST} = 0.106$; Table 3); lack of differentiation is also indicated by the tight linkage of populations of this species in the cluster analysis based on genetic distances (Fig. 1). Two populations of *A. racemosa*

(19, 24) from the southern part of its range are genetically differentiated from the four populations (20, 21, 22, and 23) from more northern localities (Fig. 1). Populations 29, 30, 31, and 32 of *A. virginica* are diploids ($2n = 28$), while 33, 34, 35, and 36 are tetraploid. The two cytotypes are interspersed among each other in a single group (Fig. 1), showing that they are not genetically distinct and suggesting that the tetraploids are of non-hybrid (autopolyploid) origin. Again, the between population genetic distances show that populations of *A. virginica* are as distant from each other as are populations of the widespread species, demonstrating this geographically restricted species maintains as much variability as edaphically widespread species.

Origin of the polyploid agamic complex—The use of enzyme electrophoresis as data for confirming the origin of polyploids has been employed successfully in several groups such as *Tragopogon* (Roose and Gottlieb, 1976), *Stephanomeria* (Gottlieb, 1973), *Coreopsis* (Crawford and Smith, 1984), *Galax* (Epes and Soltis, 1984), *Tolmiea* (Soltis and Bohm, 1984), and several cultivated taxa (see Crawford, 1983, for a review). As was discussed earlier, the *A. parlinii* and *A. neodioica* complexes are considered, on the basis of morphological data, to be of multiple hybrid origin involving three and four diploid species, respectively. One purpose of the present study was to ascertain whether allozymes could provide an independant source of data for documenting genomic constituents of the polyploids.

Morphological data implicates *A. plantaginifolia, A. racemosa,* and *A. solitaria* as the genome donors of *A. parlinii* (Bayer, 1985b), and the allelic data for *Lap-1* and *Pgi-3* provide some support for this hypothesis. For example, *Lap-1*^{a} is unique to *A. racemosa* (among the diploids) and it is also found in *A. parlinii* (Fig. 2). Likewise, *Pgi-3*^{a}, present only in *A. solitaria,* was detected in *A. parlinii* (Fig. 2). The third diploid thought to be present in *A. parlinii, A. plantaginifolia,* contains no diagnostic alleles. It should be mentioned that alleles *Lap-1*^{f} and *Pgi-3*^{c}, unique to *A. virginica,* were not detected in *A. parlinii.* Likewise, morphological data did not indicate the presence of *A. virginica* genome in *A. parlinii.* In summary, electrophoretic data are useful for documenting the genomes of *A. racemosa* and *A. solitaria* in *A. parlinii,* but provide no independent evidence for the presence of the *A. plantaginifolia* genome.

Antennaria neodioica has been viewed as consisting of *A. neglecta, A. plantaginifolia, A. racemosa* and *A. virginica* (Bayer, 1985a). Allozyme data are of limited value for documenting the presumed complex nature of this species. In fact, alleles *Lap-1*^{f} and *Pgi-3*^{c} are the only diploid-specific alleles present in *A. neodioica,* with both of them present in *A. virginica* (Fig. 2). The allele unique to *A. racemosa, Lap-1*^{a}, was not detected in *A. neodioica.* Neither *A. neglecta* nor *A. plantaginifolia* contain diagnostic alleles (Fig. 2). It should be noted that *Pgi-3,* unique to *A. solitaria,* was not detected in *A. neodioica,* and it would not be expected based on morphological data, suggesting the genome of this species is not present in *A. neodioica* (Bayer, 1985a).

The minimal genetic divergence at isozyme genes exhibited by the five diploid species of *Antennaria* limits the value of electrophoretic data for documenting the origin of the two polyploid agamic complexes, *A. parlinii* and *A. neodioica.* Allozymes do provide independent evidence that the genomes of *A. racemosa* and *A. solitaria* are present in *A. parlinii.* The situation is much less clear with *A. neodioica* because only unique alleles of *A. virginica* are found in the polyploid.

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