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Investigations into the Evolutionary History of the Polyploid Complex *Antennaria neodioica* (*Asteraceae: Inuleae*)[1]

By

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Abstract: The *Antennaria neodioica* polyploid agamic complex is a polymorphic species occurring across North America mainly north of the terminal margin of the Wisconsin glacier. This taxonomically difficult group has recently been treated as consisting of the four subspecies *A. neodioica* subsp. *canadensis*, subsp. *howellii*, subsp. *neodioica*, and subsp. *petaloidea*. The *A. neodioica* agamic complex has been considered of hybrid origin with several sexual diploid species constituting its parentage. Crosses were made among five sexual diploid species of *Antennaria*, morphologically similar to *A. neodioica* s.l., in an attempt to discover its origins. Representative specimens of the five diploid species, *A. neodioica* s.l., and the synthetic interspecific F_1 hybrids were subjected to various analyses including PCA, cluster (UPGMA), and discriminant analyses. Results suggest that the *A. neodioica* complex is of multiple hybrid origin involving the four diploid species *A. neglecta, A. plantaginifolia, A. racemosa*, and *A. virginica*. Because *A. neodioica* is the result of diverse origin it is more desirable to consider the agamic complex as a separate, distinct species from its sexual diploid relatives. Several morphological characters in the diploid species were determined to be polygenically inherited.

The *Antennaria neodioica* polyploid agamic complex is widely distributed across North America between 40° and 60° north latitude. BAYER & STEBBINS (1982) circumscribed *A. neodioica* GREENE of the eastern United States as consisting of the three subspecies *A. neodioica* subsp. *canadensis*

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[1] Investigations into the Evolutionary History of the Polyploid Complexes in *Antennaria (Asteraceae: Inuleae)*. I.

(GREENE) BAYER & STEBBINS, subsp. *neodioica,* and subsp. *petaloidea* (FERN.) BAYER & STEBBINS. More recently, *A. howellii* GREENE of western North America has been included as a fourth subspecies (BAYER 1984 a). The polymorphic *A. neodioica* s. l. is characterized by its relatively large single-nerved basal leaves and primarily white-tipped involucral bracts. STEBBINS (1932) demonstrated that pistillate members of the *A. neodioica* complex reproduce agamospermously (diplospory followed by diploid parthenogenesis). Rare staminate plants were found to have highly irregular meiosis and were likely to be sterile (STEBBINS 1932). Because these rare staminate clones of *A. neodioica* are sterile, their presence is reproductively inconsequential and the species as a whole reproduces asexually. The species consists of both tetraploids (2 n = 56) and hexaploids (2 n = 84) (BAYER & STEBBINS 1981, BAYER 1984 a). The *A. neodioica* agamic complex can be considered to be in the early mature stage of development (terminology of GRANT 1981), because the sexual diploids are still abundant, the genus as a whole contains approximately 26 sexual diploids (BAYER 1984 a).

In many cases agamospermous members of agamic complexes have arisen through hybridization among several sexual diploid species (GRANT 1981). Diploid *Antennaria neglecta* is found in prairies and pastures from Maine, southwest to Oklahoma, northwest to the Canadian Northwest Territories, and southeast to Quebec. The deciduous forest of the Appalachian Region, Piedmont, and Atlantic Coastal Plain from Georgia to Maine is the range of diploid *A. plantaginifolia.* Another diploid, *Antennaria racemosa,* occurs in dry coniferous montane forests from British Columbia and Alberta south to South Dakota, Wyoming, Idaho, and California. The shale barren endemic, *A. virginica,* occurs in a restricted area of Maryland, Pennsylvania, West Virginia, Virginia, and at one locality in Ohio. In this paper I will test the hypothesis that the *A. neodioica* complex is the product of hybridization among four predominantly diploid (2 n = 28) species: *A. neglecta* GREENE, *A. plantaginifolia* (L.) RICHARDSON, *A. racemosa* HOOK., and *A. virginica* STEBBINS.

The purposes of this paper are (1) to determine via phenetic methods if the polyploid *A. neodioica* complex is distinct from its presumed diploid progenitors; (2) to investigate whether the five partially sympatric, sexual diploids are morphologically distinct from one another; (3) to compare synthetic interspecific hybrids among the diploids with the *A. neodioica* as a means of testing whether the latter is of multiple hybrid or single origin; (4) to employ the results from phenetics and pollen viability of interspecific hybrids as a means of inferring relationships among the sexual diploids; and (5) to ascertain the mode of inheritance of several morphological characters.

Materials and Methods

Field observations were made throughout the ranges of the taxa in the Spring of 1978 – 1984. Over 400 populations of *Antennaria* were collected and subsequently cultivated in the greenhouse at OSU. Interspecific hybrids were obtained as outlined in BAYER & STEBBINS (1982). Several backcrosses were also attempted to determine how the phenotype of the B_1 differed morphologically from that of the F_1 hybrids. While *A. virginica* exists as both sexual diploids and sexual tetraploids (BAYER 1984 a), only sexual diploids were used in the crossing experiments. Pollen viability was ascertained by the use of Alexander's differential staining method (ALEXANDER 1980). Three hundred grains/individual were scored for viability.

Specimens were examined from CAN, CM, GH, MO, MONTU, NDG, NY, OS, PAC, RM, SDU, US, VPI, WIS, and WVA for morphological studies. Herbarium specimens of field collections and the artificial interspecific hybrids are on deposit at OS.

Initially, 38 characters (16 vegetative and 22 reproductive; Tab. 1) were measured on 274 OTUs (operational taxonomic units) to construct a basic data matrix for phenetic analysis. The basic data matrix can be found in BAYER (1984 b, appendix) or can be obtained from the author. Principal components analysis (PCA), cluster analysis, and correlation coefficients were generated using the NT-SYS package of ROHLF & al. (1974). The basic data matrix was standardized by the STAND subroutine of NT-SYS. The correlation and distance matrices were computed using the SIMINTVL subroutine. Clusters analysis (UPGMA) was produced by the TAXON subroutine and PCA was computed by the FACTOR subroutine of NT-SYS. The BMDP package of DIXON (1981) was used to compute simple univariate statistics and perform stepwise discriminant analysis. PCA, cluster analysis, and discriminant analysis were used to compare the diploids, hybrids, and polyploid complex. In addition to PCA, cluster analysis, and discriminant analysis, correlation coefficients (transformed into a mean similarity matrix) using average values of characters for each taxon were employed to compare the natural taxa to each other and to artificial interspecific hybrids between the diploids. A discriminant analysis (based on characters that best discriminate among taxa) was used to place the artificially synthesized hybrids in their most probable group of inclusion. Computations were carried out at the Instructional Research Computer Center of The Ohio State University.

Initially five diploid species of *Antennaria*; i.e., *A. neglecta, A. plantaginifolia, A. racemosa, A. solitaria,* and *A. virginica,* were chosen for analysis with the *A. neodioica* complex because they were partially sympatric with and morphologically closest, based on superficial appearance, to members of that complex. All taxa occur within the eastern half of North America except the diploid *A. racemosa* and polyploid *A. neodioica* GREENE subsp. *Howellii* (GREENE) BAYER, which are found in the Rocky Mountains of western North America (for details of the ranges see BAYER & STEBBINS, 1982 and BAYER, 1984). *Antennaria virginica* consists of diploids and sexual autotetraploids (BAYER 1984a), but the two can not be separated consistently on the basis of any known morphological character including multivariate differences. In this paper both diploids and their sexual autotetraploid derivatives were included in the analysis and will hereafter be referred to under the inclusive term *A. virginica.*

Several data matrices were used in the study: (1) a 202 OTU matrix consisting of the four diploid species (40 OTUs of *A. neglecta,* 48 of *A. plantaginifolia,* 34 of *A. racemosa,* and 40 of *A. virginica*) plus a fifth diploid, *A. solitaria* RYDB. (40 OTUs), (2) a 39 OTU matrix utilizing the interspecific hybrids (30 OTUs) plus average

Table 1. List of 38 characters and characters states used in the numerical analysis of *Antennaria*. Numbers or letters following each character indicate the scale used or the qualitative state measurements

Basal rosette characters: 1. Length of entire basal leaf, mm. 2. Length of the petiole, mm. 3. Maximum width of the basal leaves, mm. 4. Length, along the mid-vein, from leaf tip to maximum width, mm. 5. Shape of the anterior margin, i.e. length from tip to widest point in the leaf, mm. 6. Number of principle veins in the leaf. 7. Presence of a crinkled margin on the leaf, 0.0 = absent, 1.0 = present. 8. Adaxial leaf surface pubescence, 0.0 = glabrous, 0.1 = glabrous-villous, 0.2 = villous, 0.3 = pilose, 0.4 = lanate, 0.5 = tomentose, 1.0 = canescent. 9. Number of leaves per basal rosette. Stolon characters: 10. Number of leaves per stolon. 11. Length of the largest leaf, mm. 12. Width of the largest leaf, mm. 13. Length of the smallest leaf, mm. 14. Width of the smallest leaf, mm. 15. Stolon length, mm. 16. Number of stolons per basal rosette.

Staminate capitulescence characters: 33. Height of the involucre, mm. 34. Number of heads per capitulescence. 35. Phyllary length, mm. 36. Phyllary width, mm. 37. Corolla length, mm. 38. Pappus length, mm.

Cauline (flowering) stem characters: 17. Flowering stem height, mm. 18. Number of leaf nodes per cauline stem. 19. Width of the longest leaf, mm. 20. Length of the longest leaf, mm. 21. Width of the shortest leaf, mm. 22. Length of the shortest leaf, mm. 23. Presence of a scarious flag-like structure on upper leaves, 0.0 = absent, 1.0 = present.
Pistillate capitulescence characters: 24. Height of the involucre, mm. 25. Number of heads per capitulescence. 26. Phyllary length, mm. 27. Phyllary width, mm. 28. Corolla length, mm. 29. Pappus length, mm. 30. Achene length, mm. 31. Phyllary colors, 1.0 = green base, white tips, 2.0 = green base, purple or brown middle, white tips, 3.0 = brown or purple base, white tips, and 4.0 = green base, light brown tips. 32. Number of florets per head.

values for the nine natural taxa, (3) a 274 OTU matrix consisting of representative specimens of the four diploid species of *Antennaria* (as above), the four subspecies of *A. neodioica* (20 OTUs of each), interspecific hybrids among the diploid taxa (30 OTUs), and putative natural hybrids (2 OTUs), (4) a 170 OTU matrix (consisting of 20 OTUs of each of the diploids, 30 OTUs of the interspecific hybrids, and 15 of each of the subspecies of *A. neodioica*), basically the same as the 274 OTU matrix, but with 104 OTUs eliminated as an aid in displaying the output. Matrices 1, 2, and 4 are basically subsets of matrix number 3. The 104 OTUs eliminated from matrix 4 were those that had the highest correlation with the other OTUs based on an initial phenogram computed from matrix 3. Two 3-dimensional plots of the first three principal components for matrices 1 and 3 were constructed. A discriminant analysis was performed using matrix number 3. Matrix number 4 was used to construct a phenogram by the unweighted pair-group method using arithmetic averages (UPGMA; SNEATH & SOKAL 1973). Pearson product-moment correlation coefficients (SNEATH & SOKAL 1973) were used to compare OTUs of matrix num-

ber 2. *Antennaria solitaria* was eliminated from matrices 3 and 4 as were the artificial interspecific hybrids between *A. solitaria* and the other four diploid species because they more closely resemble members of the *A. Parlinii* s.l. (BAYER & STEBBINS 1982) polyploid complex which is to be the subject of another paper.

Results

Phenetics of the Diploid Species. The five diploids were subjected to a PCA (Fig. 1) to ascertain the morphological distinctness of these taxa. The first three principal components account for 62.03% of the variation. The first eight components have eigenvalues greater than one, indicating the characters are not highly correlated. Component one emphasizes vegetative characters, while component two accentuates reproductive features. The third component has high loadings for both types of character. The five diploid species appear distinct from one another. The apparent overlap of *A. neglecta* and *A. plantaginifolia* due primarily to the orientation from which Fig. 1 is drawn in 3-dimensional space. Indeed, close inspection shows that on the plane of factor 1 versus factor 2 these species are well separated (Fig. 1). Two very similar species based on superficial morphology, *A. neglecta* and *A. virginica,* are distinct and are separated primarily on the basis of reproductive characters along component 2 (Fig. 1). The same is true for the species pair of *A. plantaginifolia − A. solitaria. Antennaria racemosa* is separated from *A. plantaginifolia* on the basis of both vegetative and reproductive characters, and while it appears very distinct due to its racemose arrangement of capitula, this analysis reveals its close morphological similarity to *A. plantaginifolia.*

The similarity matrix (correlation coefficients) among all taxa (Tab. 2) reveals that *A. plantaginifolia* is most similar to *A. racemosa* and vice-versa. *Antennaria neglecta* most closely resembles the polyploid *A. neodioica* subsp. *canadensis,* while *A. virginica* is more like subsp. *neodioica* indicating the probable predominance of these parental diploid genomes in their respective polyploid derivatives. The fifth diploid, *A. solitaria,* is most similar to diploid *A. neglecta.* While these two taxa differ in their basal leaves and habitat preferences, they both have long lash-like stolons, a flag-like structure in the upper cauline leaves[1], and heads that are alike with respect to a number of features (esp. phyllary color and shape). All four subspecies of *A. neodioica* are most similar to each other (Tab. 2)

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[1] The flag-like structure is a flat or curled scarious tip at the end of the upper cauline leaves below the aggregate of heads and excluding the bracteal leaves in the corymb or raceme. This character is accorded a great deal of taxonomic importance and is used as a key character in separating several taxa.
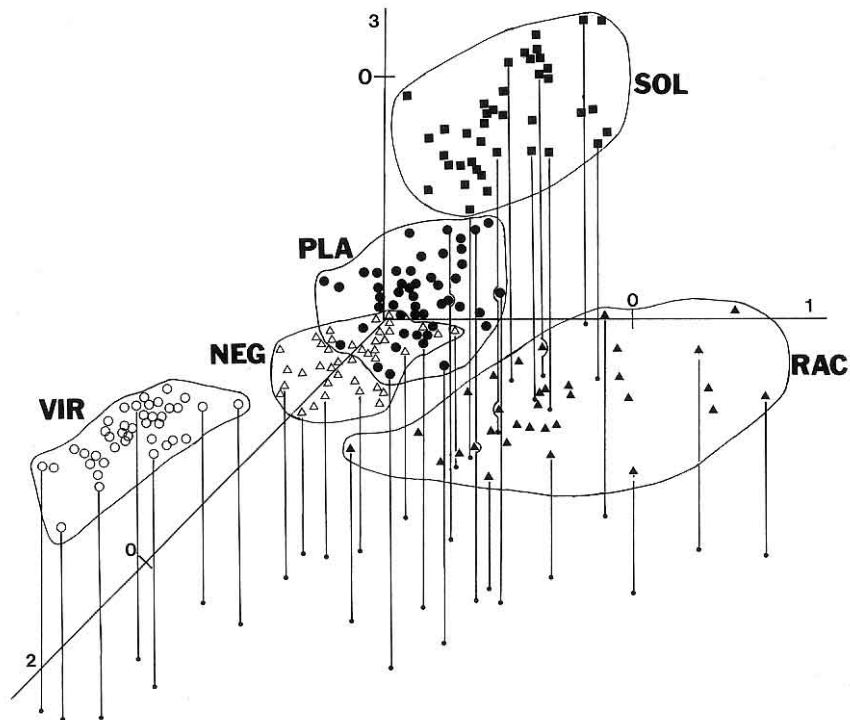
Fig. 1. PCA composed of 202 OTUs including five diploid taxa of *Antennaria*. Group outlines are indicated by a line. △ *A. neglecta*, ● *A. plantaginifolia*, ▲ *A. racemosa*, ■ *A. solitaria*, and ○ *A. virginica*

Table 2. Mean similarity matrix (correlation coefficients) of nine taxa of *Antennaria* with each other. Taxa are labelled with the first three letters of the specific or subspecific epithet

	NEG	PLA	RAC	SOL	VIR	CAN	HOW	NEO	PET
NEG	1.000								
PLA	−0.411	1.000							
RAC	−0.233	0.559	1.000						
SOL	0.418	−0.100	−0.154	1.000					
VIR	−0.091	0.125	−0.157	−0.311	1.000				
CAN	0.619	−0.330	−0.123	0.064	−0.125	1.000			
HOW	0.383	−0.237	0.256	0.047	−0.302	0.590	1.000		
NEO	−0.116	0.398	0.220	−0.522	0.394	0.332	0.259	1.000	
PET	0.323	0.059	0.102	−0.095	−0.036	0.595	0.473	0.609	1.000

except for subsp. *canadensis* which is closest to the diploid *A. neglecta* from which it may be derived.

Interfertility of the Diploid Species. Interfertility of the diploids can be used as a measure of relationship. BAYER & STEBBINS (1982) reported that the diploid species of *Antennaria* are reproductively isolated because low numbers of seeds were set in interspecific as compared with conspecific
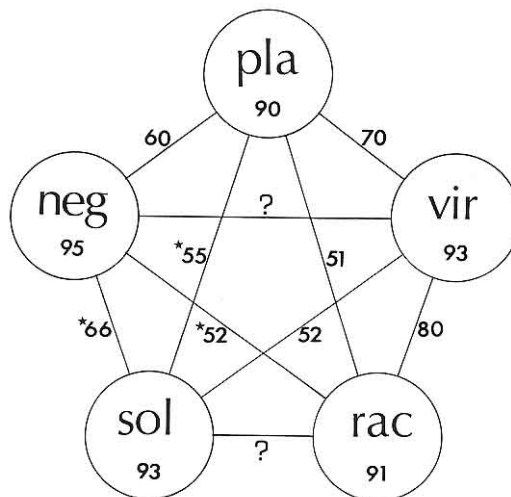

Fig. 2. Percent average pollen stainability of diploid species (5 of each) of *Antennaria* and their interspecific hybrids. Taxa are labelled with the first three letters of the specific epithet. ? = in two cases staminate individuals were not obtained, i.e. only pistillate individuals were obtained. Standard deviations were less than 10.0%, except in three cases (labelled with a star) in which they were less than 20.0%

crosses. Hybrids among species isolated by habitat differences (i.e. *A. neglecta* and *A. solitaria*) displayed higher seed set, indicating that post-mating reproductive isolating mechanisms are not strongly developed. Several hybrids between *A. neglecta* and *A. virginica,* were weak, had small, reddish, deformed leaves, and eventually died before flowering. Pollen stainabilities of the hybrids were determined, but only 27% of 55 interspecific hybrids were staminate, thus limiting the available data. Fig. 2 presents the pollen stainabilities for these hybrids and five of each of the five diploid species. The average stainabilities of all hybrids range from 51.0% to 80.0% while individual cross stainabilities range from 40.5% to 85.3% (Fig. 2). Stainable pollen in the naturally occurring diploids is almost always above 85.0%.
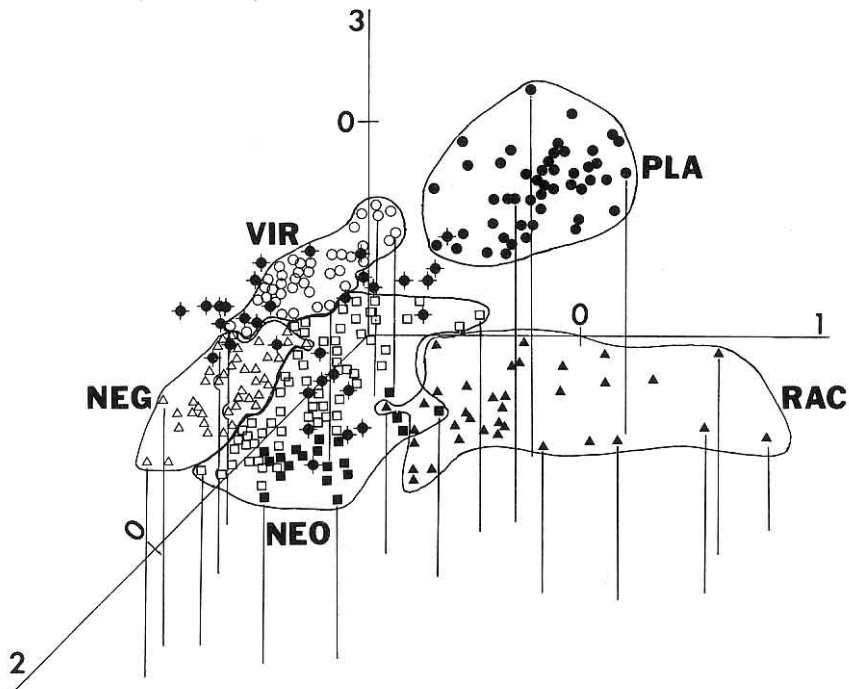
R. J. BAYER:

Fig. 3. PCA composed of 274 OTUs including four diploid *Antennaria* species, interspecific hybrids, and the polyploid *A. neodioica* s.l. Group outlines are indicated by a line. △ *A. neglecta*, ● *A. plantaginifolia*, ▲ *A. racemosa*, ○ *A. virginica*, ✦ interspecific hybrids, □ *A. neodioica* subsp. *canadensis*, subsp. *neodioica*, subsp. *petaloidea*, and ■ *A. neodioica* subsp. *Howellii*. Parentages of interspecific hybrids are given in Fig. 4

Phenetics of the Diploids, Hybrids, and Polyploids. The first three factors of PCA of four diploids, their interspecific hybrids, and *A. neodioica* s.l. accounted for 54.43% of the variance (Fig. 3). The first ten factors have eigenvalues greater than 1.0 indicating that the characters are not highly correlated. The high loadings for the first three factors were similar to those given for the first PCA analysis. Inspection of the PCA (Fig. 3) shows that the four diploids surround the *A. neodioica* polyploid complex. Also, *A. neglecta* and *A. virginica*, which were well separated in the first PCA (Fig. 1), are drawn much closer together in this PCA (Fig. 3) which may be attributable to the hybrid nature of the complex. The interspecific hybrids, for the most part, fall within the *A. neodioica* complex. *Antennaria neodioica* subsp. *Howellii*, which is diagrammatically represented as being separate from the remainder of *A. neodioica* s.l. (Fig. 3), is most similar to the diploid *A. racemosa*, with which it is largely

sympatric (BAYER 1984 a). Although the remaining three subspecies of *A. neodioica* s. l. are not depicted separately in the figure, they do arrange within definite areas of the *A. neodioica* grouping. *Antennaria neodioica* subsp. *canadensis* is aligned most closely to *A. neglecta;* subsp. *neodioica* closest to *A. virginica;* and subsp. *petaloidea* is more or less associated with *A. neglecta* and *A. plantaginifolia* (Fig. 3).

Discriminant analysis has been shown to be useful in the identification of hybrids, but only when hybrids of known parentage are included in the analysis (NEFF & SMITH 1979). Posterior probabilities of group member-ship, in which the highest probability indicates to which group each of the artificial hybrids may belong, are presented in Tab. 4. Characters which best discriminate are pappus length, presence or absence of a scarious flag-like appendage on the upper cauline leaves, number of primary veins in the basal leaves, involucre height, and leaf pubescence. Missing data are not allowed in the discriminant analysis, thus staminate hybrids could not be analyzed because the members of the *A. neodioica* complex lack staminate plants. The results are displayed pictorially in Fig. 4; only the group outlines from PCA are shown along with the interspecific hybrids positioned according to PCA. Probable group of inclusion of OTUs, as indicated by discriminant analysis, is shown by an arrow (Fig. 4). These results demonstrate that many artificially synthesized hybrids are most similar to members of the *A. neodioica* complex. In cases where the hybrids were not placed in the *A. neodioica* complex it was always associated most closely with one of its parents.

Another measure of similarity useful in assessing the relationship of hybrids to natural taxa is a matrix of correlation coefficients (JENSEN & ESHBAUGH 1976 a, b). As previously mentioned, averages for all characters of each of the natural taxa were used to compare the natural taxa with the artificially synthesized hybrids. The results (Tab. 3) were similar to those obtained for the discriminant analysis, in which the majority of hybrids were most similar to either the polyploids or one of their parents. Discriminant analysis, PCA (Fig. 4), and mean similarity matrices (Tabs. 2 and 3) demonstrate that hybrids having *A. solitaria* as one of their parents (e.g. HY-11, 13, 21, 24, 25, 26, 27, 30, 31, and 34) do not closely resemble members of the *A. neodioica* complex, thus supporting the view that *A. solitaria* is not involved in the parentage of the complex. *Antennaria neglecta × A. racemosa* and *A. virginica × A. racemosa* hybrids most closely resemble members of the *A. neodioica* complex. The table of correlation coefficients (Tab. 3) also shows that other hybrid com-binations, while most resembling their parents, have second and third highest correlations within members of the *A. neodioica* complex e.g. HY-10 (*A. neglecta × A. virginica*) or HY-18 (*A. plantaginifolia × A. virginica*).

152 R. J. Bayer:

Table 3. Mean similarity matrix of taxa of *Antennaria* with thirty artifical hybrid subspecific epithet. Thirty artifical hybrids and their parentage are presented ** second highest, and * third highest similarity between each hybrid and the taxa resembling one parent, [3] hybrids resembling the poly

	NEG	PLA	RAC
NEG × VIR (HY09)[1]	0.174**	− 0.348	− 0.449
NEG × SOL (HY11)[1]	0.311**	− 0.426	− 0.593
NEG × SOL (HY21)[1]	0.497***	− 0.567	− 0.540
NEG × SOL (HY24)[1]	0.240**	− 0.89	− 0.138
NEG × SOL (HY26)[1]	0.111***	− 0.526	− 0.564
NEG × SOL (HY29)[1]	0.133**	− 0.278	− 0.481
NEG × SOL (HY30)[1]	0.549**	− 0.253	− 0.246
NEG × SOL (HY31)[1]	0.487***	− 0.394	− 0.407
NEG × SOL (HY34)[1]	0.427**	− 0.447	− 0.514
PLA × VIR (HY18)[1]	− 0.149	0.072*	− 0.261
PLA × VIR (HY19)[1]	− 0.330	0.126**	− 0.047
VIR × SOL (HY25)[1]	− 0.176	− 0.285	− 0.524
VIR × SOL (HY27)[1]	0.135**	− 0.546	− 0.559
NEG × RAC (HY14)[2]	− 0.322	0.023	0.0221**
NEG × RAC (HY15)[2]	− 0.075	− 0.092	0.299***
NEG × RAC (HY16)[2]	− 0.277	− 0.173	0.190***
NEG × RAC (HY20)[2]	− 0.140	− 0.102	0.188***
NEG × RAC (HY28)[2]	− 0.256	− 0.097	0.178***
NEG × RAC (HY32)[2]	− 0.283	− 0.002	0.053**
NEG × VIR (HY10)[2]	− 0.167	− 0.108	− 0.375
NEG × VIR (HY35)[2]	− 0.102	− 0.337	− 0.636
PLA × NEG (HY17)[2]	− 0.480	0.582***	0.302**
PLA × NEG (HY22)[2]	− 0.442	0.569***	0.338**
PLA × NEG (HY23)[2]	− 0.422	0.453**	0.459***
VIR × SOL (HY13)[2]	− 0.036	− 0.528	− 0.572
NEG × RAC (HY12)[3]	− 0.215	− 0.161	0.051
NEG × RAC (HY33)[3]	− 0.232	− 0.068	0.000
VIR × RAC (HY43)[3]	0.095	− 0.105	0.312
VIR × RAC (HY44)[3]	− 0.069	− 0.194	0.136
VIR × RAC (HY45)[3]	0.287	− 0.187	0.286

The cluster analysis (Fig. 5) has a cophenetic correlation coefficient of 0.794, indicating that the phenogram is an adequate representation of the original resemblance matrix. The number of OTUs was reduced to aid in the visual display of the results. The phenogram shows that each diploid (except *A. racemosa*) clusters in an uninterrupted group and that the subspecies of *A. neodioica* are interspersed among the diploids, although not in continuous groups (Fig. 5). The cluster analysis agrees with results of other analyses in showing a close morphological similarity between *A. racemosa* and *A. plantaginifolia* (cf. Figs. 3 and 5). Not surprisingly, some of the subsp. *Howellii* segregates cluster within *A. racemosa,* with which

combinations (HY −). Taxa are labelled with the first three letters of the specific or along with their mean similarities to the naturally occurring taxa. *** highest, are indicated. [1] hybrids closely resembling both parents, [2] hybrids closely ploids (CAN, HOW, NEO, PET), but lnot either parent

SOL	VIR	CAN	HOW	NEO	PET
0.012	0.446	− 0.012	− 0.085	0.072*	0.050
0.375***	− 0.015	0.186*	− 0.292	− 0.366	− 0.144
0.452**	− 0.024	0.103*	− 0.254	− 0.471	− 0.196
0.386***	− 0.143	− 0.105	− 0.318	− 0.422	− 0.232
− 0.027**	− 0.040	− 0.059	− 0.243	− 0.354	− 0.402
0.304***	0.085	− 0.209	− 0.329	− 0.469	− 0.592
0.585***	− 0.224	− 0.120	− 0.028	− 0.434	− 0.118
0.383**	− 0.042	0.006	− 0.193	− 0.388	− 0.243
0.478***	− 0.328	0.079	− 0.379	− 0.568	− 0.275
− 0.198	0.704***	− 0.233	− 0.325	0.168**	− 0.047
− 0.246	0.477***	− 0.356	− 0.402	0.071*	− 0.234
− 0.008**	0.435***	− 0.336	− 0.436	− 0.146	− 0.335
0.028	0.619***	− 0.200	− 0.130	− 0.292	− 0.344
− 0.533	0.018	0.042	0.147*	0.326***	0.076
− 0.358	− 0.241	0.066*	0.240**	− 0.105	− 0.031
− 0.555	− 0.176	0.004	0.151**	− 0.023	− 0.111
− 0.209	− 0.434	0.005	0.039	− 0.170	0.070**
− 0.113	− 0.415	− 0.083	− 0.032**	− 0.331	− 0.168
− 0.168	− 0.261	0.080***	− 0.015	− 0.043	− 0.042
− 0.300	0.531***	− 0.279	− 0.361	0.174**	− 0.283
− 0.205	0.444***	− 0.130	− 0.378	− 0.002**	− 0.220
− 0.103	− 0.137	− 0.522	− 0.258	0.014*	− 0.265
− 0.367	0.019	− 0.317	− 0.140	0.260*	0.050
− 0.258	− 0.152	− 0.297	− 0.039	0.151*	0.086
− 0.042	0.691***	− 0.281	− 0.313	− 0.285	− 0.381
− 0.146	− 0.364	0.257**	0.314***	0.171	0.162
− 0.241	− 0.331	0.241**	0.397***	0.204*	0.153
− 0.288	0.096	0.460	0.708***	0.571**	0.470*
− 0.294	0.059	0.204*	0.356***	0.191	0.270*
− 0.089	− 0.068	0.544**	0.645***	0.316	0.523*

they are often sympatric. The hybrids cluster either within the polyploids or near one of their diploid parents, in agreement with previous analyses. A single, field collected, specimen (Fig. 5; HY-08) interpreted as being a possible natural hybrid between *A. plantaginifolia* and *A. neglecta* is most similar to *A. plantaginifolia*. Additionally, a specimen of subsp. *neodioica* (Fig. 5; HY-06) looks superficially like a robust *A. virginica*, and clusters among *A. neodioica* subsp. *neodioica*, further indicating the close relationship of subsp. *neodioica* to *A. virginica*.

　　　Visual inspection of the basal leaves of the diploids (*A. neglecta, A. plantaginifolia, A. racemosa,* and *A. virginica*), their interspecific hybrids,

11*

Table 4. Table of posterior probabilities derived from a discriminant analysis. Twwnty-six artificial interspecific hybrids of *Antennaria* (HY−) and their parentage along with the posterior probability to which of nine naturally occurring taxa of *Antennaria* they should belong. Taxa are labelled with the first three letters of the specific or subspecific epithet. [1] hybrids most closely resembling one of their parents, [2] hybrids most closely resembling one of the subspecies of *A. neodioica*

	NEG	PLA	RAC	SOL	VIR	CAN	HOW	NEO	PET
NEG × SOL (HY11)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY21)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY24)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY26)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY29)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY30)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY31)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × SOL (HY34)[1]	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NEG × VIR (HY10)[1]	0.000	0.000	0.000	0.000	0.922	0.000	0.000	0.076	0.002
PLA × NEG (HY17)[1]	0.000	0.843	0.000	0.000	0.005	0.000	0.000	0.043	0.109
PLA × NEG (HY22)[1]	0.000	0.801	0.000	0.000	0.144	0.000	0.000	0.049	0.005
PLA × NEG (HY23)[1]	0.000	0.901	0.000	0.000	0.073	0.000	0.000	0.014	0.012
PLA × VIR (HY18)[1]	0.000	0.000	0.000	0.000	0.965	0.000	0.000	0.032	0.003
PLA × VIR (HY19)[1]	0.000	0.000	0.000	0.000	0.997	0.000	0.000	0.003	0.000
VIR × RAC (HY45)[1]	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
VIR × SOL (HY25)[1]	0.000	0.000	0.000	0.000	0.882	0.000	0.000	0.114	0.004
NEG × RAC (HY12)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.379	0.132	0.489
NEG × RAC (HY14)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.521	0.432
NEG × RAC (HY16)[2]	0.000	0.000	0.260	0.000	0.000	0.740	0.000	0.000	0.000
NEG × RAC (HY20)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.025	0.973
NEG × RAC (HY28)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.001	0.994
NEG × RAC (HY32)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.020	0.949
NEG × RAC (HY33)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.338	0.242	0.420
NEG × VIR (HY35)[2]	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.457	0.527
VIR × RAC (HY43)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.880	0.075	0.045
VIR × RAC (HY44)[2]	0.000	0.000	0.000	0.000	0.000	0.000	0.980	0.011	0.009

and the naturally occurring polyploids (*A. neodioica* s. l.) further reveals the resemblance of the interspecific hybrids to the naturally occurring polyploids (Fig. 6). Likewise with respect to number, size, and arrangement of heads the interspecific hybrids closely resemble the naturally occurring polyploids (Fig. 6).

Inheritance of Morphological Characters in *Antennaria*. The interspecific hybrids synthesized during this study provide an opportunity to study the inheritance of characters, some of which are of taxonomic importance. The first of the characters to be considered is pubescence of the adaxial leaf surfaces. *Antennaria racemosa* is glabrous while the remaining diploids are pubescent. In crosses involving *A. racemosa* with the other
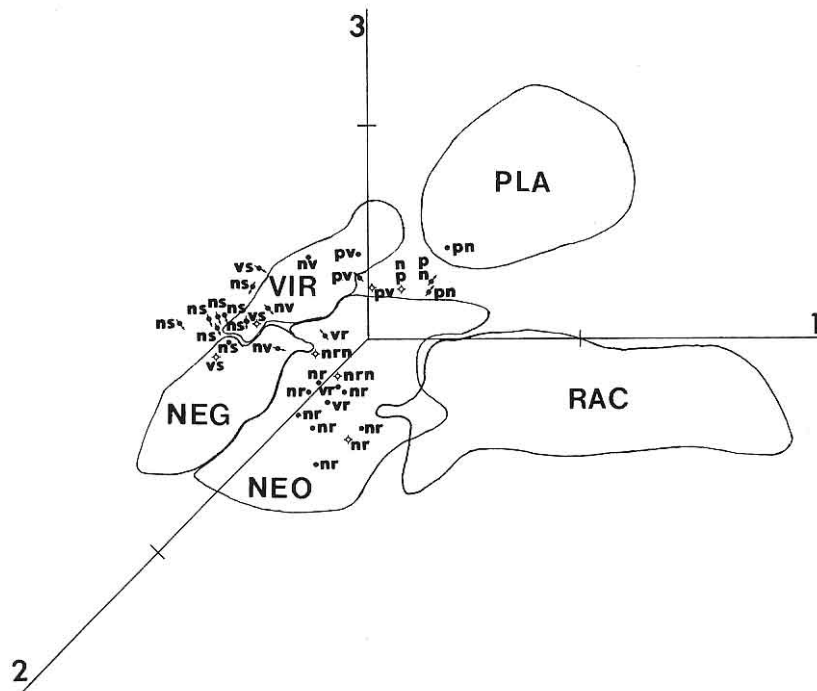
Fig. 4. PCA group outlines with interspecific hybrid OTUs. Letters next to the OTUs refer to their parentage and is given by the first letter of the specific epithet of the parental taxa (i.e. n = *A. neglecta*, p = *A. plantaginifolia*, r = *A. racemosa*, s = *A. solitaria*, v = *A. virginica*, and vs = *A. virginica* × *A. solitaria*). Backcross segregates (*A. neglecta* × *A. racemosa*) × *A. neglecta* are indicated by "nrn". ● OTUs subjected to a discriminant analysis that most probably belong to the group in which they are enclosed in PCA ordination space. ◈ Outlying OTUs subjected to discriminant analysis with arrow pointing toward most probably group of inclusion (cf. Tab. 4). ⊖ Staminate OTUs that could not be included in the discriminant analysis

four diploids, the hybrids (total of 26 individuals observed) range from entirely glabrous to very sparingly pubescent. This suggests that while leaf pubescence may be controlled by more than one gene, factors for glabrity are dominant to those for pubescence. Two other characters unique to *A. racemosa*, namely presence of a citronella-like odor in the leaves and purple glands on the upper cauline stem, are inherited in the same manner as leaf pubescence. These two characters are hard to quantify, but their presence appears to be dominant to lack of the odor and absence of glands. *Antennaria racemosa* has an open racemose arrangement of
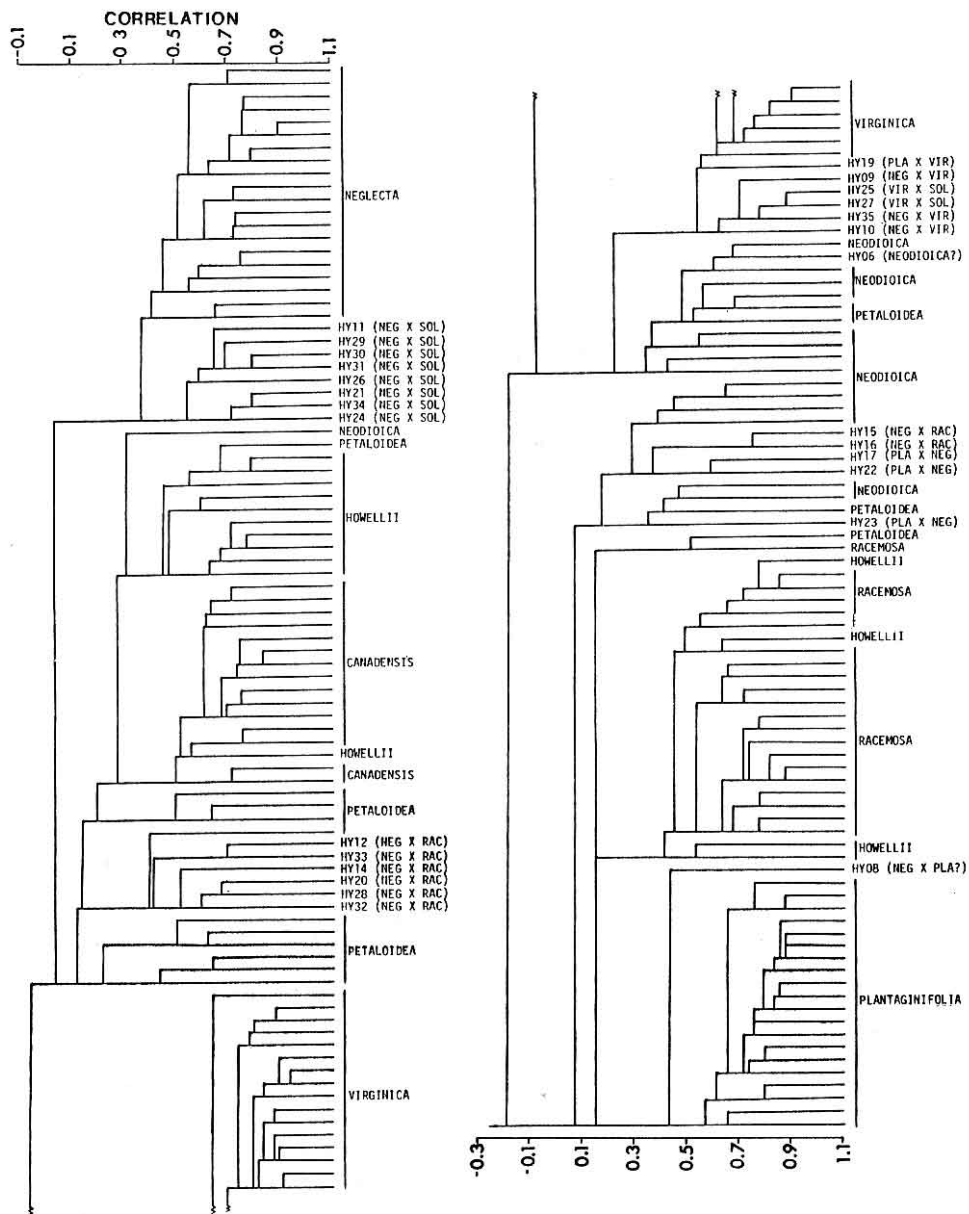
Fig. 5. Correlation phenogram (UPGMA) composed of 170 OTUs including four diploid species of *Antennaria*, interspecific hybrids, and *A. neodioica* s. l. Taxa are labelled with specific or subspecific epithets. Parentage of hybrids is given in parentheses and are denoted by the first three letters of the parental specific epithets. Two anomalous specimens are HY-06, *A. neodioica* subsp. *neodioica* which superficially resembles a robust specimen of *A. virginica;* HY-08 is a probable natural hybrid between *A. neglecta* and *A. plantaginifolia.* Cophenetic correlation coefficient is 0.794
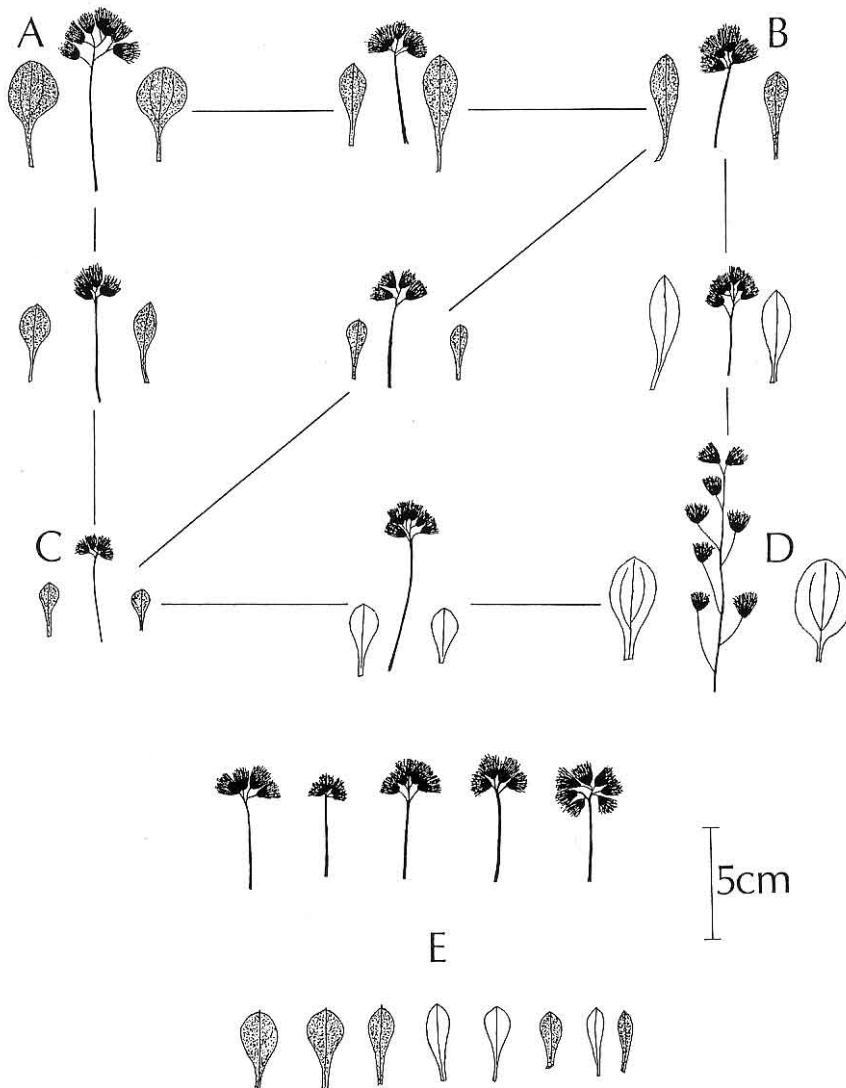
Fig. 6. Line drawings of capitulescences and basal leaves of representative specimens among four diploid species of *Antennaria,* their interspecific hybrids, and 5 individuals of *A. neodioica* s.l. Adaxial leaf pubescence is indicated by stippling. Variation among taxa is apparent with respect to leaf shape and pubescence, number of veins in basal leaves, and number, size, and arrangements of heads in the capitulescence. *A A. plantaginifolia, B A. neglecta, C A. virginica, D A. racemosa,* and *E A. neodioica* (all subspecies). Bar = 5 cm

capitula, while the other four diploid species have a cymose or solitary (in *A. solitaria*) arrangement. In the hybrids involving *A. racemosa* and the other diploids (total of 26 observed) the arrangement is usually cymose or cymose with a tendency toward being racemose in the lower heads (see Fig. 6). This indicates, that as with number of heads, the arrangement of heads is controlled polygenically. The racemose head arrangement is apparently recessive to the cymose or solitary arrangement of heads in the capitulescence.

A similar pattern of inheritance was observed for number of heads per capitulescence. When the monocephalous *A. solitaria* is crossed to polycephalous species, the resultant hybrids (total of 28 observed) have an intermediate number of heads per capitulescence, but many hybrids have one or two heads. Head number is probably a polygenic character with alleles for monocephaly being dominant to those for polycephaly. Number of veins in the basal leaves is another character which is likely to be polygenic, but displays dominance.

Antennaria neglecta and *A. virginica* both have single-nerved basal leaves, while the other diploids have 3–5 nerves in the basal leaves (Fig. 6). When *A. neglecta* or *A. virginica* are crossed with *A. plantaginifolia, A. racemosa,* or *A. solitaria* the resultant hybrids (total of 45 observed) usually have a single nerve or occasionally a single nerve and two small lateral ones (Fig. 6). Thus number of nerves in the basal leaves is a polygenic character with the single-nerved condition being dominant to the many nerved condition. In most hybrids the stolon length is intermediate between that of the parents. However, in crosses between *A. neglecta* and *A. solitaria* (total of 19 observed), both having very long, lash-like stolons, the hybrids have stolons that surpass both parents in length. This suggests that transgressive segregation has occurred.

Presence or absence of the scarious flag-like appendage on the upper cauline leaves displays discontinuous variation (a character accorded taxonomic significance throughout the entire genus). *Antennaria neglecta* and *A. solitaria* possess the flag while the other three diploid species lack it. The flag is apparently a single gene recessive trait because all hybrids (total of 21 observed) involving *A. neglecta* or *A. solitaria* with one of the other three diploids lack the flag. When *A. neglecta* and *A. solitaria* are crossed the flag is expressed in the hybrids (total of 19 observed). It will be interesting to see whether the feature is expressed in F_2 hybrids and in what proportions.

Crosses between pubescent-leaved *A. neglecta* and glabrous-leaved *A. racemosa* produced an F_1 that was glabrous like *A. racemosa,* but had leaves shaped more like *A. neglecta.* When one of these F_1s (HY-33) was backcrossed to *A. neglecta* the morphology was altered such that the B_1 hybrids were now very similar to *A. neglecta,* the recurrent parent. Two of

these B_1 hybrids (labelled "nrn", Fig. 4) are included in the PCA analysis and their location in the ordination space is most easily discerned in Fig. 4.

Discussion

It was JUEL (1900) who first realized that *Antennaria* agamospecies were probably of hybrid origin. He came to this conclusion after examining rare staminate clones of the primarily agamospermous *A. alpina* (L.) GAERTNER, which he found to be sterile. Because he knew that many hybrids were sterile, he hypothesized that *A. alpina* was the result of hybridization between two diploid species *A. dioica* (L.) GAERTNER and *A. monocephala* DC. STEBBINS (1932) discussed the origin of several of the agamospermous species of *Antennaria* occurring in the eastern United States and suggested that *A. petaloidea* (= *A. neodioica* subsp. *petaloidea*) could have resulted from hybridization between *A. neglecta* and *A. plantaginifolia*. *Antennaria virginica* was likewise added to the list of possible diploid progenitors of the *A. neodioica* complex by STEBBINS (1935). The crossability and interfertility data from this study support previous conclusions (BAYER & STEBBINS 1982) that the diploid species are isolated by both spatial and reproductive isolating mechanisms (in the terminology of LEVIN 1978). Some species such as *A. neglecta* and *A. solitaria* are isolated ecologically, while others such as *A. plantaginifolia* and *A. neglecta* occasionally occur sympatrically BAYER & STEBBINS 1982) but are apparently isolated by cross incompatability mechanisms and hybrid sterility. The interspecific hybrids display meiotic irregularities (BAYER 1984 a) and reproductive isolation among these taxa may be due to one or more inversions or translocations causing hybrid sterility. Despite all of these isolating mechanisms, several putative hybrids were discovered in the field (BAYER & STEBBINS 1982). West Virginia is apparently an area where hybridization among all four of the eastern diploids is common. Numerous populations with mixed numbers of diploids and putative hybrids were observed (BAYER & STEBBINS 1982) in this area. The Black Hills of South Dakota is also a potential area of hybridization because this is where *A. neglecta* and *A. racemosa* are sympatric (BAYER & STEBBINS unpubl. obs.).

The close similarity of some of the segregates of the *A. neodioica* complex, diploid *A. neglecta*, *A. racemosa*, *A. plantaginifolia*, and especially *A. virginica* (Fig. 7) is demonstrated by cluster analysis (Fig. 5) and PCA (Figs. 3 and 4). PCA has been shown to be useful in the identification of hybrid swarms of mixed genetic origin (WHIFFIN 1973, JENSEN & ESHBAUGH 1976 a). The high variability in the *A. neodioica* complex (Fig. 3) could be the result of extensive backcrossing following

initial F_1 hybrid formation, as has been demonstrated for other groups (Neff & Smith 1979). The PCA suggests that the genome of *A. neodioica*, while consisting mainly of genes from *A. neglecta* and *A. virginica* also contains genes from *A. racemosa* and, to a lesser extent, *A. plantaginifolia* (Figs. 3 and 7). Discriminant analysis (Tab. 4) and correlation coefficients (Tabs. 2 and 3) show the close similarity of the interspecific hybrids to members of the *A. neodioica* complex. Studies on backcross hybrids demonstrate how a single backcross can make a significant difference in
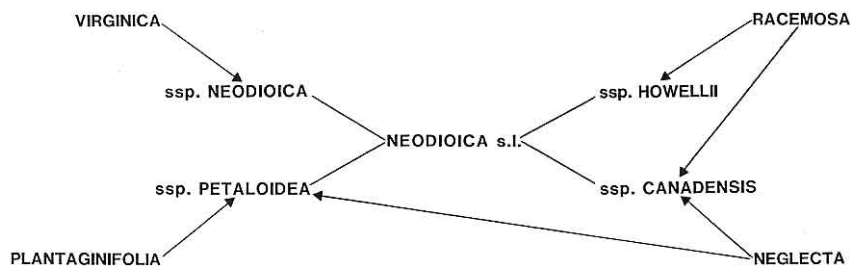

Fig. 7. Proposed relationship of four diploid species of *Antennaria* (*A. neglecta, A. plantaginifolia, A. racemosa,* and *A. virginica*) to the *A. neodioica* agamic complex. *Antennaria neodioica* is composed of four subspecies, *A. neodioica* subsp. *canadensis,* subsp. *howellii,* subsp. *neodioica,* and subsp. *petaloidea.* For detailed explanations consult the text

the morphologies between the F_1 and B_1 (Fig. 4). Unfortunately, polyploid derivatives of the interspecific diploid hybrids were not obtained although several attempts were made to polyploidize them by the use of colchicine. It is uncertain to what degree polyploidy would affect the morphology of the hybrids, but evidence from other species indicates that the morphology would not be significantly changed. For example, four diploid species of *Antennaria,* namely *A. aromatica* Evert, *A. media* Greene, *A. umbrinella* Rydb., and *A. virginica* have both diploid and tetraploid cytotypes that are indistinguishable morphologically (Bayer 1984 a).

Antennaria neglecta, A. plantaginifolia, A. racemosa, and *A. virginica* are the most likely progenitors of the *A. neodioica* complex (Fig. 7). *Antennaria neodioica* subsp. *neodioica* apparently arose somewhere in the area of West Virginia, where it is sympatric with its closest diploid relative, *A. virginica* (Bayer 1984 a). In areas closest to *A. virginica, A. neodioica* subsp. *neodioica* occurs as a tetraploid, but on the edges of its range (i.e. Montana or New England) it is hexaploid (Bayer 1984 a). In natural

systems the backcrossing of F_1s to their parents could easily have produced all the phenotypes that are present in the *A. neodioica* complex. Therefore, introgression has probably been an important evolutionary mechanism in the evolution of the polyploid complexes in *Antennaria*. It is thought that continued introgression is more important than initial interspecific hybridization alone in the formation of polyploid complexes (STEBBINS 1950).

These findings support the contention of BAYER & STEBBINS (1982) that members of the polyploid complex, which were called *A. neodioica,* should be recognized as a distinct species from the sexual diploid *A. neglecta.* CRONQUIST's intuitive classification (1945) recognized these probable allopolyploid derivatives as varieties of *A. neglecta.* The polyploids are no doubt of varied hybrid origin involving several diploids (Fig. 7), a compilospecies in the terminology of HARLAN & DeWET (1963). The evolutionary history of the group seems best reflected by the classification of BAYER & STEBBINS (1982).

Most of the characters studied displayed continuous variation. There were no apparent reciprocal differences in the inheritance of characters in the interspecific hybrids, indicating that maternal inheritance is not a factor in the characters studied. Most of the characters are no doubt polygenic, i.e. under the control of several genes with small additive effects. The mode of inheritance of such characters requires several generations (usually up to at least F_4) in order to ascertain how many genes are involved in the determination of the characters (BACHMANN & al. 1982). Unfortunately, partial sterility in the F_1s and slow growth prohibit detailed analysis at this time. The flag character is the only one studied that appears to be under single gene control (complete dominance).

The glabrous adaxial leaf surfaces present in *A. neodioica* subsp. *canadensis* and subsp. *Howellii* may be due to genes contributed by *A. racemosa,* because the other diploids lack this feature (Fig. 7). All the polyploid subspecies have a cymose arrangement of heads; occasionally the racemose arrangement is displayed by some individuals of subsp. *Howellii,* suggesting it is more closely related to diploid *A. racemosa* than the other diploid species. Genes for single-nerved basal leaves, present in the subspecies of *A. neodioica,* were apparently contributed by *A. neglecta* and *A. virginica* (Fig. 7). *Antennaria neodioica* subsp. *canadensis* is the only subspecies of *A. neodioica* to express the flag character, and this trait is probably due to genes contributed by *A. neglecta,* its apparent closest diploid progenitor (Fig. 7). Thus the four subspecies of *A. neodioica* that are recognized by BAYER & STEBBINS (1982) and BAYER (1984 a) may be the result of the predominance of the genomes of one or two diploids in their genetic constitution (Fig. 7).

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