
GENETIC VARIABILITY IN
THE FEDERAL THREATENED
MEAD'S MILKWEED,
ASCLEPIAS MEADII TORREY
(ASCLEPIADACEAE), AS
DETERMINED BY ALLOZYME
ELECTROPHORESIS¹

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ABSTRACT

Most populations of the federal threatened Mead's milkweed, *Asclepias meadii* Torr. (Asclepiadaceae), occur primarily in prairie haymeadows in Kansas and Missouri, where annual summer mowing prevents seed production. Exceptions are large populations in fire-managed habitats at the Rockefeller Prairie, a former Kansas haymeadow, and at Weimer Hill, a glade complex in southeastern Missouri. This perennial rhizomatous species is self-incompatible. The few remaining small populations in Illinois, Iowa, and northern Missouri persist vegetatively but no longer produce seeds and are vulnerable to stochastic extinction processes. Allozyme electrophoresis was used to measure the amount and distribution of genetic variation in *A. meadii* and to provide guidance for its recovery and restoration. Samples were obtained from 19 populations encompassing the extant range of the species in Kansas, Missouri, Iowa, and Illinois. *Asclepias meadii* was genetically variable for most of the 12 loci examined, with a mean of 1.53 alleles per locus, 40.8% polymorphic loci, and observed heterozygosity of 0.158. These values are comparable to published values for other milkweed species. More than half of the total 42 alleles were rare, with 15 alleles unique to single populations. About 74% of the genetic variation in *A. meadii* occurs within populations ($F_{ST} = 0.261$), and analyses do not provide conclusive evidence for a geographic pattern in genetic variation among populations. The two fire-managed populations had more genotypes and fewer ramets per genet than the haymeadows. For the latter, inhibition of sexual reproduction may have resulted in clonal spread and attrition of genotypes, thus exacerbating the effects of sexual incompatibility and inbreeding. These factors suggest that multiple propagule sources would maximize genetic diversity for recovering depauperate populations or for restoring new populations; however, sampling from a few larger, genetically diverse populations would provide much of the species' genetic diversity. Such sources would include the large fire-managed populations. Empirical data are needed to determine the population-genetic consequences of long-distance crosses and introductions that are apparently needed to restore viable populations of this species in the eastern part of its range.

The restoration of declining species enters a realm of conservation biology in which environmental, demographic, and genetic factors limit the survival and growth of populations (Shaffer, 1981; Wilcox & Murphy, 1985; Gilpin & Soulé, 1986). When once widespread self-incompatible or outcrossing plant species have reduced population sizes, forced inbreeding can result in either total

reproductive failure or lower reproductive fitness with increased homozygosity and lower evolutionary potential (Menges, 1991; Schaal et al., 1991; Weller, 1994). Thus, restored populations of plants must be large enough to withstand loss from environmental or demographic events, and populations of either self-incompatible or outcrossing species must also contain sufficient numbers of genetically

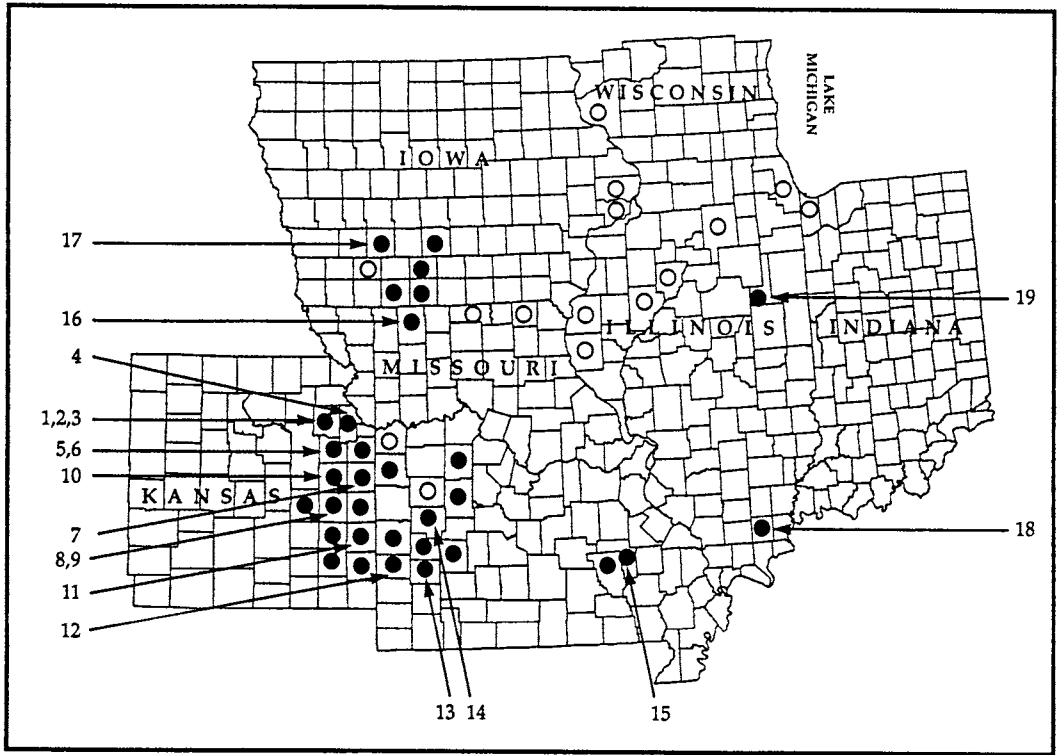
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Site Number, Name & State

1 Rockefeller, KS	6 Jack's, KS	11 Hinton Creek, KS	16 Helton, MO
2 Dog Leg, KS	7 Osawatomie, KS	12 Cook Meadow, MO	17 Woodside, IA
3 French Creek, KS	8 Garnett, KS	13 Niawathe, MO	18 Saline, IL
4 High, KS	9 Sunset, KS	14 Wah-kon-tah, MO	19 Ford, IL
5 Colyer, KS	10 Fowler Hill, KS	15 Weimer Hill, MO	

Figure 1. Distribution by county of *Asclepias meadii*. Closed circles are counties with extant populations; open circles are counties from which populations have been extirpated. Allozyme study-site locations are numbered corresponding to Table 1.

different individuals to allow reproduction, avoid inbreeding, and have evolutionary potential (Les et al., 1991; DeMauro, 1993, 1994).

Understanding and resolving genetic problems is an important need in the conservation and recovery of declining plant species (Asins & Carbone, 1987; Barrett & Kohn, 1991; Falk & Holsinger, 1991; Amos & Hoelzel, 1992; Fenster & Dudash, 1994). This need is exemplified for the federal threatened (Harrison, 1988) Mead's milkweed, *Asclepias meadii* Torr. (Asclepiadaceae). This species is an obligate outcrossing, long-lived, late-successional perennial that is restricted to virgin tallgrass prairies, prairie haymeadows, and glades (Betz, 1989). The plant is pollinated by small bumblebees

(*Bombus*) and miner bees (*Anthophora*) and, like all milkweeds, its pollen is dispersed in pollinia and its seeds are wind-dispersed from follicles (Betz & Lamp, 1992; Betz et al., 1994). Plants have an underground rootstock that produces multiple ramets, and from which rhizomes up to 1 m long have been observed in the field and in potted plants (M. L. Bowles, pers. obs.).

Asclepias meadii formerly ranged from Kansas eastward through Missouri and Illinois to northwest Indiana, and north into southern Iowa and northwest Wisconsin (Fig. 1). The origin of *A. meadii* is speculative. Gleason (1922) indicated that it was derived from the more eastern *A. amplexicaulis* Sm., and Fernald (1925) suggested it survived gla-

ciation in the driftless area of Wisconsin and adjacent states. Woodson (1954) and Noamesi and Illitis (1957) proposed a more likely post-glacial migration from the Ozark uplands similar to that proposed for *Silene regia*, a prairie species whose highest genetic diversity is in the Missouri Ozarks (Dolan, 1994).

When *A. meadii* was discovered in 1843, it may not have been rare. Owing to habitat destruction and fragmentation, this species is presently known from a total of just 31 counties in parts of its former range in Illinois, Iowa, Missouri, and Kansas, the latter of which contains the largest number of populations. In Iowa, small populations, each with relatively few plants, are found in prairie remnants in four counties. Of the two known Illinois "populations," one is represented by only a single plant from a railroad prairie in Ford Co. The other occurs in the Shawnee Hills, Saline Co. This population comprises four colonies fragmented by forest encroachment among four small glades, one of which is isolated from the others by more than a kilometer. The largest colony has 17 ramets, and the others have only a few ramets. In the southwestern and west-central part of Missouri, *A. meadii* occurs in small populations within remnant prairies. It is also locally abundant in large glades of the St. Francis mountain system of the Ozark Plateau in Iron and Reynolds Cos., Missouri.

Although *A. meadii* is locally abundant in Kansas and Missouri, most suitable habitats were converted to native prairie haymeadows nearly a century ago (Fitch & Hall, 1978). This cultural management practice removes developing seed pods (follicles), thereby eliminating pollen-mediated gene flow and sexual reproduction (McGregor, 1977; Betz, 1989). In such populations, reproduction is limited to vegetative spread of rhizomes. One exception is Kansas University's Rockefeller Prairie, Jefferson Co., Kansas, which was converted from annual haymowing to a two- or three-year burning rotation in about 1956 (Fitch & Kettle, 1988). Because *A. meadii* is a long-lived perennial (Betz, 1989), it can survive decades of haymowing and reproductive failure, and may increase ramet (but not genet) numbers by rhizomatous spread. In a seven-year demographic study of 140 ramets in unmowed railroad prairies, Betz (1989) annually removed all mature seed pods and found a 9.3% decline in ramet numbers, suggesting that some sexual reproduction is required for long-term maintenance of natural populations. This life-history strategy correlates with low levels of follicle production reported for most milkweeds (Wyatt, 1976; Wyatt & Broyles, 1994). For example, al-

though 77% of 140 ramets studied by Betz (1989) flowered annually, less than six follicles matured annually.

The glade populations of *A. meadii* in the Ozark Mountains of Missouri have escaped conversion to agriculture and haymowing but, as in Illinois, appear to be fragmented by forest encroachment resulting from fire suppression (Guyette & McGinnes, 1982; Guyette & Cutter, 1991; Ladd, 1991). The greatest concentration of these plants occurs in Iron Co. at Weimer Hill and in adjacent Reynolds Co. on Proffit Mountain, both of which are fire-managed natural areas. The Weimer Hill population contains 100 or more plants distributed among numerous glade openings (each less than 1 ha in area) that occur along a kilometer of bluff line habitat.

As with most milkweeds, *A. meadii* appears to be self-incompatible, as selfed garden plants do not produce follicles (M. L. Bowles, pers. obs.). Incompatibility in *Asclepias* appears to involve a late-acting ovarian system, and is not thoroughly understood (Seavey & Bawa, 1986; Shannon & Wyatt, 1986; Broyles & Wyatt, 1991; Kahn & Morse, 1991; Karron, 1991; Broyles & Wyatt, 1993a; Wyatt & Broyles, 1994). This life-history characteristic, combined with low effective population sizes (in some cases so low as to prevent sexual reproduction), impacts directly upon federal recovery planning to recover or restore sustainable populations of *A. meadii* (M. L. Bowles, pers. obs.). Furthermore, it is critical to assess the amount of genetic variation among populations to determine how genetic differentiation should be managed in recovery or restoration operations. This information may be especially important for restorations, where limited habitat size may require metapopulation management in which translocation of genetic material is required to maintain a high level of genetic diversity among populations (Bowles et al., 1998).

The purpose of this research was to determine the annual and distribution of genetic variation within and among *A. meadii* populations, and to compare the effects of management (burning versus mowing) on this variation. Allozymes provide a relatively rapid means to assess neutral genetic variation within and among populations of rare, endangered and/or threatened plant species (Waller et al., 1987; Lesica et al., 1988; Nickrent & Wiens, 1989; Billington, 1991; Hickey et al., 1991; Schaal et al., 1991; Godt & Hamrick, 1996a, 1996b), and multilocus genotypic diversity within populations (Sipes & Wolf, 1997). Detection of genetic diversity by DNA-based methods such as RAPDs (random amplified polymorphic DNA) is gaining use, especially when allozyme variability is low (e.g., Rieseberg et

Table 1. Mead's milkweed study sites sampled for allozymes (see Fig. 1 for site locations). Population sizes obtained from Kansas and Missouri Natural Heritage Program census data or M. L. Bowles (pers. obs.).

Population number	Population name	State	County	Habitat or management	Population size (approx. no. indiv.)
1.	Rockefeller	Kansas	Jefferson	former haymeadow ^a	200
2.	Dog Leg	Kansas	Jefferson	former haymeadow	< 20
3.	French Creek	Kansas	Jefferson	haymeadow	< 25
4.	High	Kansas	Leavenworth	haymeadow	< 25
5.	Colyer	Kansas	Douglas	haymeadow	< 25
6.	Jack's	Kansas	Douglas	haymeadow	> 300
7.	Osawatomie	Kansas	Miami	roadside	< 10
8.	Garnett	Kansas	Anderson	haymeadow	> 100
9.	Sunset	Kansas	Anderson	haymeadow	> 100
10.	Fowler Hill	Kansas	Franklin	haymeadow	< 25
11.	Hinton Creek	Kansas	Bourbon	former haymeadow	> 400
12.	Cook Meadow	Missouri	Barton	rotation ^b	< 25
13.	Niawathe	Missouri	Dade	rotation ^c	< 300
14.	Wah-kon-tah	Missouri	St. Clair	rotation ^c	< 20
15.	Weimer Hill	Missouri	Iron	glades ^d	100
16.	Helton	Missouri	Harrison	preserve/burned	< 5
17.	Woodside	Iowa	Adair	haymeadow (mowed late)	< 30
18.	Saline	Illinois	Saline	glades	< 40
19.	Ford	Illinois	Ford	railroad prairie	< 5

^a Burned every 2 or 3 years since 1956.

^b Former haymeadow, unmowed when milkweeds flower since ca. 1990.

^c Burn/hay/rest since ca. 1980.

^d Metapopulation managed by burning.

al., 1989; Rieseberg & Gerber, 1995). However, when the resolution of this genetic variation is fine-scale, such as genotypic variation within populations, allozyme variation may provide a more conservative system that is useful across a broader range of genetic diversity within and among populations (Swensen et al., 1995). Allozymes have already proven useful in population-level studies of relatively common (Broyles & Wyatt, 1990, 1991, 1993b; Foré & Guttman, 1996) as well as rare milkweeds (Edwards & Wyatt, 1994).

MATERIALS AND METHODS

POPULATION SAMPLING

Leaf samples were collected in 1992 from individual ramets of *Asclepias meadii* from 19 populations throughout the extant range of the species; supplemental collections were made from the Weimer Hill study site in 1993 (Table 1, Fig. 1). When possible, at least 20 individuals were sampled from each population. However, this number was sometimes not achieved because of the limited number of individuals located during this study. This was despite previous censuses of larger populations at some large sites, and no doubt was due to difficul-

ties in locating small vegetative plants when flowering individuals were absent (Alexander et al., 1997). Sampling was minimally destructive, and consisted of removing one or two leaves from the lower nodes of the stem. Samples were individually numbered and labeled by population name, sealed in a plastic bag, and stored on ice until returned to the lab. Samples that were not extracted immediately were quickly frozen with liquid nitrogen, then maintained at -75°C until extraction.

ISOZYME ELECTROPHORESIS

Enzymes were extracted by using a Polytron homogenizer (Brinkman Industries, Westbury, NY) to grind the leaf tissue in ca. 2.0 ml of "microbuffer" pH 7.5 (Werth, 1985) supplemented with 5% (w/vol) polyvinylpyrrolidone (MW 40,000) and 0.1% 2-mercaptoethanol. For extremely small samples, hand-held glass homogenizers were used with varying amounts of extraction buffer (less than 1.0 ml). Samples were kept on ice throughout the extraction process. Cellular debris was pelleted via centrifugation at 10,000 rpm for 10 minutes, and the supernatant was poured into a microcentrifuge tube and stored at -75°C .

For electrophoresis, the extract was absorbed onto 6×19 mm Whatman #3 filter paper wicks and loaded into 13% starch gels (Starch Art, Smithville, Texas) for typical horizontal starch gel electrophoresis (Wendel & Weeden, 1989). Ten enzyme systems representing 12 putative loci were assayed using three gel electrode buffer systems. Alcohol dehydrogenase (Adh-2), isocitrate dehydrogenase (Idh-1), glutamate dehydrogenase (Gdh-2), and shikimate dehydrogenase (Skdh) were resolved using the Tris-citrate pH 7.5 buffer (Soltis et al., 1983). Aspartate aminotransferase (Aat-2, -3), glucose phosphate isomerase (Gpi-2), malic enzyme (Me-1), menadione reductase (Mnr), phosphoglucomutase (Pgm-1), and triose phosphate isomerase (Tpi-1, -2) were resolved using a lithium hydroxide buffer (Ridgeway et al., 1970). Finally, malate dehydrogenase (Mdh-2) and 6-phosphogluconate dehydrogenase (6-Pgd) were resolved with a pH 6.0 histidine citrate (Olmstead, 1989). Enzyme-staining protocols were essentially as reported in Soltis and Soltis (1989). Shikimate dehydrogenase showed activity but was not resolved for all populations; hence it was excluded from analysis. Menadione reductase (Mnr-2) showed high levels of variability within and between populations, but resolution was inadequate; hence this system was also excluded from analysis.

GEL SCORING AND ANALYSIS

Gel banding patterns were recorded photographically and genotypes were inferred based upon knowledge of enzyme subunit composition and the number of loci per enzyme system commonly seen in other plants (Gottlieb & Weeden, 1981; Weeden & Wendel, 1989). Enzyme patterns previously documented for other species of *Asclepias* were also consulted. Allelic isozymes were measured and recorded as relative mobilities using the most common allele as the standard (relative mobility of 100). When more than one locus appeared for an enzyme system, the most anodal one was designated "locus one."

Relative mobility numbers were converted to alphabetic genotypes prior to entry into BIOSYS-1 (Swofford & Selander, 1981). This program was used to calculate genetic variability measures by population, such as mean sample size per locus (N), mean number of alleles per locus (A), percentage of polymorphic loci (P), direct-count heterozygosity (H_c), expected heterozygosity given Hardy-Weinberg equilibrium (H_e), and chord and arc genetic distances between populations (Cavalli-Sforza & Edwards, 1967). To analyze partitioning of genetic

variability within and among populations, the fixation index (Wright, 1978) was used. Because of its small sample size, the subdivided Saline Co., Illinois, population was analyzed as a single population, as well as the larger fragmented population at Weimer Hill, Missouri. We also used hierarchical F -statistics (Wright, 1978) to analyze genetic variation within and among seven subdivided populations sampled at Weimer Hill. This allowed comparison to hierarchical analysis of patch subdivision of *Asclepias verticillata* L. made at a similar landscape scale in southern Ohio (Foré & Guttman, 1996). The PCORD (McCune, 1993) software program was used to analyze population samples by Principal Component Analysis (PCA), using allele frequencies in a variance-covariance matrix, and to calculate allele diversity (H') for each population using the Shannon diversity index where $H' = -\sum p_i \log p_i$, where p_i = allele frequency at each locus.

Because *Asclepias meadii* has the potential to form more than one aerial shoot (ramet) from its rhizome system, the true number of genets per population could not be determined at the time of sampling. Following analyses of the electrophoretic data, it was determined that for some populations, individual ramets represented the same genet. This was determined by (1) the presence of identical multiple-locus genotypes from the isozyme analyses, (2) identical RAPD patterns on some collections (D. Hayworth and B. Schaal, pers. comm.), and (3) ramet proximity obtained from field maps. Such ramet genotypes were then "collapsed" into a single genotype and, for analysis purposes, assumed to be parts of the same genet. This process results in a conservative estimate of the actual number of genets because different genets could have the same multiple-locus genotype. To assess the relative abundance of ramets and genets within each population, the percentage of all ramets associated with each genotype was determined, and their mean and standard error calculated for each population. The average percent ramets/genet is also calculated as the inverse of the number of genotypes multiplied by 100.

RESULTS

Twelve polymorphic gene loci were identified corresponding to 10 enzyme systems for *Asclepias meadii* (data matrix available upon request). Although no monomorphic loci were found, two of the polymorphic loci (Adh-2 and Me-1) had one allele that was essentially fixed and a second allele that was scored only once. There was an average of 1.5 alleles per locus (Table 2), with 42 alleles occurring

Table 2. Genetic diversity statistics for Mead's milkweed populations: $P(\%)$ = percent polymorphic loci; A_p = number of alleles per polymorphic locus; H' = Shannon diversity index; H_o = observed heterozygosity; H_e = expected heterozygosity.

Population	Samples	Genotypes	Mean % ramet/ genet \pm s.d.	$P(\%)$	A_p	H'	H_o	H_e
1. Rockefeller	21	15	6.66 \pm 2.4	58.3	1.8	2.80	0.194	0.201
2. Dog Leg	7	4	25.00 \pm 13.7	41.7	1.5	2.74	0.204	0.175
3. French Creek	9	3	33.33 \pm 29.4	33.3	1.3	2.70	0.065	0.074
4. High Prairie	3	2	50.00 \pm 23.6	8.3	1.1	2.52	0.028	0.028
5. Colyer	12	9	11.11 \pm 4.2	50.0	1.5	2.73	0.181	0.167
6. Jack's	18	5	20.00 \pm 26.2	41.7	1.6	2.68	0.175	0.126
7. Osawatomie	3	2	50.00 \pm 23.6	33.3	1.6	2.77	0.194	0.206
8. Garnett	21	8	12.50 \pm 1.9	50.0	1.8	2.68	0.139	0.140
9. Sunset	10	9	11.11 \pm 3.3	75.0	2.1	2.79	0.218	0.232
10. Fowler Hill	4	1	100	16.7	1.2	2.60	0.167	0.095
11. Hinton Creek	15	9	11.11 \pm 4.7	58.3	1.7	2.75	0.172	0.179
12. Cook Meadow	16	2	50.00 \pm 17.7	25.0	1.3	2.68	0.135	0.135
13. Mawathe	22	8	12.50 \pm 10.8	50.0	1.8	2.74	0.141	0.157
14. Wah-kon-ah	8	6	16.67 \pm 6.45	58.3	1.8	2.80	0.206	0.213
15. Weimer Hill	48	27	3.70 \pm 2.5	75.0	1.8	2.72	0.121	0.147
16. Helton	2	1	100	25.0	1.3	2.66	0.250	0.167
17. Woodside	7	6	16.67 \pm 5.8	33.3	1.4	2.70	0.167	0.149
18. Saline	10	5	20.00 \pm 10.0	25.0	1.4	2.65	0.092	0.104
19. Ford	1	1	100	16.7	1.2	2.60	0.167	0.167
Mean \pm s.d.				40.8 \pm 19.2	1.5 \pm 0.3	2.70 \pm 0.07	0.159 \pm 0.054	0.151 \pm 0.050

across all 12 loci. Ten alleles at ten different loci were abundant, averaging over 90% frequency among all populations. There were 25 rare alleles (less than 10% frequency), representing all but the Mdh-2 locus. Rare alleles were distributed widely among populations. Only two sites, High Prairie and Fowler Hill, did not have any of the 25 rare alleles but these sites had sample sizes of only three and four, respectively. Eleven populations had one or more of 15 alleles that were unique to single populations. The Saline Co. population had the highest number, representing the Got-2, Pgm-1, and Tpi-1 loci. None of the other three disjunct populations in eastern Illinois, eastern Missouri, or Iowa had unique alleles.

The mean percentage of polymorphic loci across all populations was 40.8%, with 0.159 observed and 0.151 expected mean heterozygosity (Table 2). Percent polymorphic loci was positively correlated with sample size ($r^2 = 0.432$, $P = 0.0022$) and reached 75% at Weimer Hill ($n = 48$) and Sunset haymeadow ($n = 10$). The lowest values (16.7%) occurred in sites with single plants, but Saline Co. ($n = 10$) had only 25% polymorphic loci. Observed and expected heterozygosity were not correlated with either sample size ($r^2 = 0.026$, $P = 0.507$; $r^2 = 0.0046$, $P = 0.783$, respectively) or number of genotypes ($r^2 = 0.0002$, $P = 0.954$; $r^2 = 0.0664$, $P = 0.287$, respectively). Among all *A. meadii* populations, 123 multiple-locus genotypes were identified among the 237 "collapsed" samples, with 79 (64.2%) restricted to single populations (Table 2). Genotypes and sample size were positively correlated ($r^2 = 0.8016$, $P < 0.0001$), with the highest numbers at Weimer Hill (27 genotypes) and Rockefeller (15 genotypes). The mean allele diversity (H') was 2.70, and values ranged from 2.52 at High Prairie to 2.80 at the Rockefeller and Wa-kon-tah prairies (Table 2). These values were not correlated with sample size ($r^2 = 0.092$, $P = 0.206$). Although the highest H' (2.80) occurred at Rockefeller Prairie, five haymeadows had higher H' values than the fire-managed Weimer Hill ($H' = 2.72$).

Results of genetic-distance analyses (data not shown) indicated that all populations clustered at a chord distance (Cavalli-Sforza & Edwards, 1967) of 0.32 or less, and that clustering did not conform to expectations based on geographic proximity. The greatest interpopulational distances also involved those populations with the smallest sample sizes, and hence these relationships may be artifactual. There was also no clear geographic pattern among populations with PCA of allozyme frequencies (results not shown). The first PCA axis accounted for 35.18% of the variation and was most highly cor-

related (i.e., factor loadings > 0.4) with variation in two alleles at the pgm-1 locus. The second axis accounted for 57.6% cumulative variation and was most highly correlated with variation in one allele at the pgi-2 locus. The third axis accounted for 77.9% cumulative variation and was also highly correlated with variation in one allele at the pgi-2 locus. Three additional axes brought cumulative variation to 94.9%, with the strongest correlation in four alleles at the gdh-2 and got-3 loci.

Wright's fixation index provides information on the degree of fixation of individuals relative to their specific population (F_{is}) and to all populations (F_{IT}), and the differentiation among all populations relative to complete fixation (F_{ST}). An F_{ST} value of 0.0 indicates that all variance resides within populations whereas a value of 1.0 shows that all variance is between populations (i.e., no alleles are shared among populations). The F_{ST} value for *A. meadii* was 0.261, which shows that about 74% of the genetic variation sampled resides within any one population. Hierarchical F -statistical analysis of the subdivided Weimer Hill provided information on the proportion of variance explained by the interactions of subpopulations to the total population. An F_{VI} value of 0.355 for subpopulations to total indicates that about 65% of the variance in the Weimer Hill population of *A. meadii* occurs within its subpopulations.

The type of management regime in effect at each population is shown in Table 1. Fire-managed populations contained more genotypes and a greater proportion of ramets with different genotypes than haymeadows (Tables 1, 2). For example, in thirteen haymeadows, the average percentage of all ramets for each genotype ranged from 11.11% (at three sites) to 100%, with an average of 30.98%. In contrast, the average percentage of ramets per genotype was 6.66% and 3.70% in the fire-managed Rockefeller and Weimer Hill prairies, respectively. Although these average values are positively correlated with sample size ($r^2 = 0.3464$, $P = 0.008$), they are meaningful when they represent total population samples. For those populations with ten or more samples, a plot of the ramet/genet ratio and the number of genotypes graphically illustrates the effects of burning versus mowing (Fig. 2). The burned Rockefeller and Weimer Hill sites had high numbers of genotypes and low ramet/genet percentages, whereas the mowed haymeadows had higher mean ramet/genet percentages and lower numbers of genotypes.

DISCUSSION

Given that several other species of *Asclepias* have been examined using allozymes, it is informative to

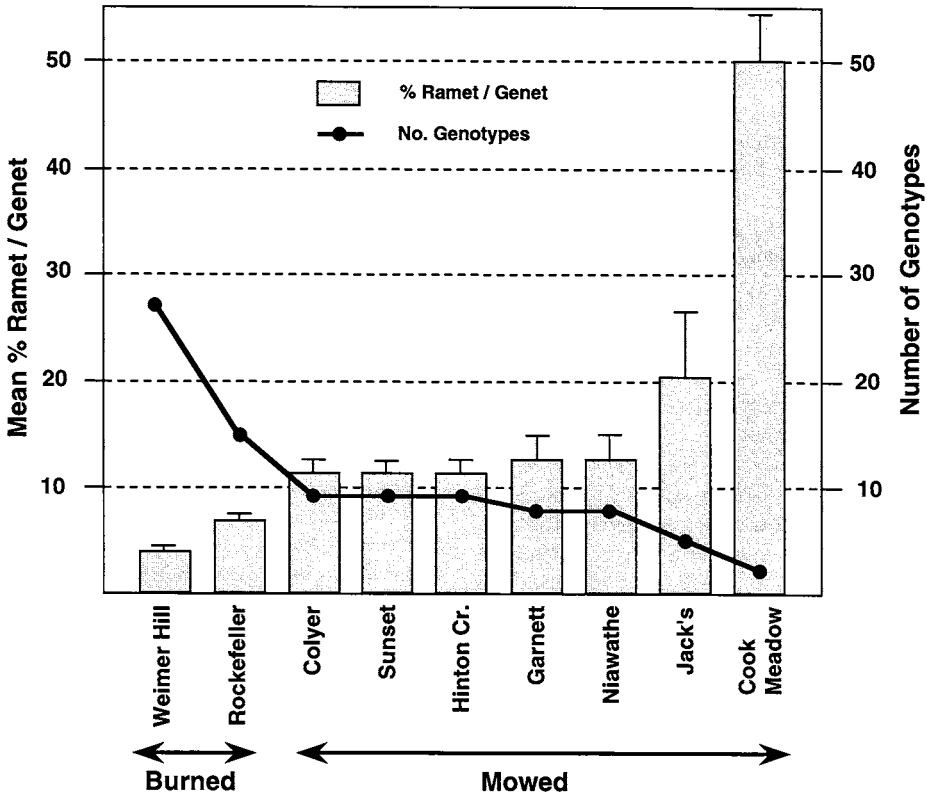


Figure 2. Effect of management methods on genetic structure of Mead's milkweed populations. Only those populations with allozyme sample sizes greater than or equal to 12 are shown. See text and Table 2 for calculations of mean percent ramets per genet and multiple-locus genotypes.

place the *A. meadii* results in context by comparing genetic diversity statistics among these species (Table 3). Although the objectives and methodologies differed somewhat in each study, similar patterns or trends are apparent. The mean number of alleles per locus and percentage of polymorphic loci for *Asclepias texana* Heller, a rare species, and *A. perennis* Walt., a widespread sister species, are remarkably similar to each other and to those of *A. meadii*. Heterozygosity levels in *A. meadii* were not as high as in *A. exaltata* L. or *A. verticillata*, two widespread North American species, but higher than in *A. texana* and *A. perennis*. These data indicate that genetic diversity in milkweeds may be less affected by effective population size and number than in other plants, likely because of their obligate-outcrossing mode of reproduction (see below). Despite severe reductions in population size, *A. meadii* has retained a comparatively high level of genetic diversity, thus making it an excellent candidate for restoration activities. Because levels of heterozygosity and mean allele diversity were not correlated with sample size or number of genotypes,

these estimates are likely accurate reflections of the actual genetic diversity in *A. meadii* populations. In contrast, the percentage of polymorphic loci (P) was significantly correlated with sample size, possibly because sample size exceeded 20 plants for only 4 of the 19 populations studied. However, samples for many of the fragmented populations in Iowa, Illinois, and northern Missouri often represented the entire population, and thus these results are meaningful.

The partitioning of genetic variation in *A. meadii*, as measured by F -statistical analyses, showed that the greater proportion of genetic variation (74%) is within populations. However, this species partitions more than two to three times as much variation between populations as compared to *A. texana*, *A. perennis*, or *A. exaltata* (Table 3). Similarly, the hierarchical partitioning of 65% of the genetic diversity within population subdivisions at Weimer Hill was much less than the 97% found within patches of *A. verticillata* (Foré & Guttman, 1996). These comparisons indicate that *A. meadii* maintains more than a moderate amount (26%) of genetic variation

Table 3. Comparison of milkweed genetic-diversity statistics based on allozyme studies: A_p = number of alleles per polymorphic locus; % unique alleles = percentage of alleles restricted to single populations; $P(\%)$ = percent polymorphic loci, H_n = observed heterozygosity, H_e = expected heterozygosity, F_{ST} (also reported as G_{ST}) = proportion of genetic variation among populations.

<i>Asclepias</i> species	No. pops.	Total samples	No. loci	A_p	Total alleles	% unique alleles	$P(\%)$	H_n	H_e	F_{ST}	Reference
<i>A. meadii</i>	19	237	12	1.53	42	35.7	40.8	0.159	0.151	0.261	this paper
<i>A. texana</i>	11	357	16	1.51	48	8.3	36.4	0.055	0.061	0.068	Edwards & Wyatt (1994)
<i>A. perennis</i>	18	942	16	1.57	58	24.1	41.3	0.055	0.061	0.082	Edwards & Wyatt (1994)
<i>A. exaltata</i>	18	846	16	2.36	75	14.7	64.5	0.202	0.182	0.093	Broyles & Wyatt (1993a)
<i>A. verticillata</i>	9	459	6	2.17	19	10.5	75.5 ^a	0.197	0.214	0.033 ^b	Foré & Guttman (1996)

^a Only polymorphic enzyme systems (of 20 examined) were used in this analysis; P would be lower if loci were considered monomorphic that had the most common allele at a frequency of 0.99 or greater.

^b Based on F_{ST} (patch-total) hierarchical analysis (Wright, 1978).

within its populations. However, this variation is only slightly higher than the expected level (20%) for widespread, outcrossing species reported by Hamrick and Godt (1990) and follows from the life-history characteristics of milkweeds in general (i.e., wind-dispersed seeds and durable pollinia carried by insects). Thus, any natural *A. meadii* population that meets a minimum size might be expected to comprise much of the genetic diversity occurring across the range of the species, but many genotypes may be distributed among populations. Despite the modern rarity of *A. meadii*, its populations maintain higher levels of genetic variation than naturally rare or endemic colonizing plants such as *Pedicularis furbishiae* S. Wats. (Waller et al., 1987), *Howellia aquatilis* A. Gray (Lesica et al., 1988), and *Trifolium stoloniferum* Eaton (Hickey et al., 1991). The outcrossing breeding system (Schoen & Brown, 1991) and occasional pollen- or seed-mediated gene flow among populations (Wyatt & Broyles, 1994), along with the great longevity (Betz, 1989) and increased survival of heterotic individuals (Schaal & Levin, 1976; Mitton & Pierce, 1980; Ledig, 1986), may contribute to the maintenance of genetic diversity within fragmented populations of *A. meadii*.

Although generally considered neutral, allozyme frequencies may be associated with different soil characteristics (Heywood & Levin, 1985). The lack of (1) large genetic distances among populations and (2) a geographic pattern to genetic variation is somewhat surprising because *A. meadii* is widely distributed over areas where soil conditions range from acid and nutrient-poor in the south to calcareous and nutrient-rich in the north (Bowles et al., 1998). However, our samples did not adequately cover the northeastern part of the former range of this species. *Asclepias meadii* populations in unglaciated Missouri or Kansas could represent points of origin for all *A. meadii* populations. Given their geographic proximity and habitat similarity, glade populations in southern Illinois also might be most closely related to those in Iron Co., Missouri. However, allozymes provide little positive information to address biogeographic hypotheses relating to pre-glaciation refugia, lineages and migration routes, founder events, and current distribution patterns for *A. meadii*.

Burning and mowing management practices appear to have different effects on the genetic structure of *A. meadii* populations, with high numbers of genotypes and low ramet/genet percentages in burned sites, and fewer genotypes with higher ramet/genet percentages in haymeadows. Although these results may correlate with sample size, sim-

ilar ramet/genet percentages were found with RAPDs (D. Hayworth & B. Schaal, pers. comm.), and higher ramet densities with lower percentages of flowering ramets also occur in haymeadows (Bowles et al., 1998). This evidence suggests that haymowing, which prevents sexual reproduction, promotes clonal spread of certain genotypes but attrition of others. Although sexual reproduction is arrested by the mechanical removal of flowering stems, if such "pruning" does not remove all photosynthetic tissues from ramets, or misses smaller ramets, vegetative growth may continue throughout the growing season. If above-ground tissue is lost, it is also possible that mowing may stimulate lateral buds to form new shoots, thereby increasing rhizome branching. New growth may also be enhanced by reallocation of resources that might have gone toward sexual reproduction (Bowles et al., 1998). The net effect would be an increase in the number of vegetative shoots (ramets) that subsequently arise from the underground root system. Over time, stochastic factors would result in the successive loss of genotypes that do not spread vegetatively, since they are prevented from undergoing sexual reproduction.

The consequences of mowing on the functional dynamics of milkweed populations are apparent. Because of the self-incompatibility of *Asclepias meadii*, its populations may be sensitive to minimum numbers and spatial patterns of genotypes within populations. Even though populations have high levels of heterozygosity, they could still have limited reproductive capacity if they have low numbers of genotypes. A greater percentage of similar genotypes in haymeadows would inflate population numbers, while effective population size (N_e) remained relatively small. Even if large numbers of genotypes are present in haymeadows, the potential for crossing among genetically identical individuals and for consequent reproductive failure is increased in these populations because most local movement of pollinators will be within clones (Pleasants, 1991). Finally, repeated mowing prevents sexual reproduction in haymeadows, regardless of their genetic structure.

The maintenance of most genetic diversity within populations has important implications for restoration and management of *Asclepias meadii*. This structuring of genetic diversity may be selectively advantageous by maximizing the number of different compatibility types, thereby avoiding inbreeding among related individuals. Although plants with different multiple-locus allozyme genotypes are genetically distinct, they may still share identical alleles at a compatibility locus, thus preventing sex-

ual reproduction (Les et al., 1991). Such genetic processes may limit reproduction in extremely small *A. meadii* populations, such as those in Saline Co., Illinois. Demographic factors such as physical isolation of population subdivisions and non-synchronous flowering of compatible plants may prevent pollen transfer among these populations. Although five different genotypes were detected among the four Saline Co. populations (Table 2), such genetic, demographic, and stochastic factors are apparently preventing sexual reproduction, since it has not occurred in this population. Finally, reproduction in small milkweed populations may also be limited by lack of appropriate pollinators, even if sexually compatible plants are present. Such factors may be in operation in the subdivided Weimer Hill population, where hierarchical F -statistics found a lower percentage of heterozygosity among population subdivisions (65%) than that found among all milkweed populations (74%). However, the Weimer Hill population regularly produces viable seeds via natural pollination (Bowles et al., 1998).

Without intervention, small, fragmented, clonal populations of *Asclepias meadii*, such as those in the eastern part of the range or possibly those in small haymeadows, appear to have relatively low opportunities for sexual reproduction and therefore high extinction probabilities. Because of the high proportion of rare or unique alleles within populations, a large reservoir of genetic variation would be lost with each extinction. To achieve greater viability and evolutionary potential in small populations, genotype diversity should be maximized through either pollen flow or the introduction of additional, genetically different plants. An argument that local genotypes will be lost through genetic swamping is not relevant, since they would almost certainly be lost otherwise through attrition and lack of sexual reproduction, and once genes are introduced, local habitat selection can act on novel combinations. The same consideration applies to the restoration of new milkweed populations, requiring the establishment of many genetically different individuals to maximize reproduction, evolutionary potential, and high population growth rates. The questions then arise: How many genotypes must be introduced to restore populational viability, and what seed sources should be used? Approximately 30 allozyme genotypes were detected at Weimer Hill, yet it is not presently clear that this represents the minimum number that should be used for restoration purposes. If the primary recovery objective for *A. meadii* is to maximize levels of genetic diversity and numbers of genotypes with-

in populations, then replicating the diversity found in natural viable populations is a logical paradigm. If, as suggested earlier, there has been little selection for genetic change across the range of *A. meadii*, populations in unglaciated Missouri or Kansas would thus be suitable sources of genetic material for population restoration in any part of this species' range. Multiple populations also might be used to collectively maintain higher levels of genetic diversity by management as a metapopulation through periodic transfer of pollen or seed among sites to maintain high levels of diversity and avoid negative effects of inbreeding.

Another concern is that long-distance crossing for population recovery could potentially disrupt locally co-adapted gene complexes and cause outbreeding depression in naturally evolving populations. However, heterosis resulting from such crosses might outweigh any deleterious consequences (Fenster & Dudash, 1994), and such complexes might be regularly broken in outcrossing species with large neighborhoods. Although outbreeding depression may occur in milkweed species (Wyatt, 1976), no evidence has been found for optimal outcrossing because of their usually large neighborhood sizes (Broyles & Wyatt, 1991; Wyatt & Broyles, 1994). No differences were seen in seed germination percentages among natural and geographically distant crosses of *A. meadii* (Bowles et al., 1998). This study also showed that seedlings from distant crosses were competitively superior to seedlings derived from natural crosses, suggesting that heterosis may counteract negative effects of outbreeding. Clearly, these experiments must be extended to the field to ascertain long-term fitness components relevant to restoration efforts.

CONCLUSIONS

Conservation efforts aimed at increasing population size and stability of *Asclepias meadii* can be augmented with genetic data. Most genetic variation in *A. meadii* is contained within populations, and genetic analyses do not provide conclusive evidence for geographic patterning of genetic variation among *A. meadii* populations. Regardless of genetic differences that may occur among populations, the high level of allozyme diversity within extant populations indicates that restoration should attempt to maximize genetic diversity. If different genotypes are correlated with the outcrossing breeding system of milkweeds, then the number of genetically different individuals in restorations must also be maximized. The fragmented eastern populations of *A. meadii* are apparently too small

to allow population recovery or restoration from their in situ genotypes, and hence supplemental propagule sources must be identified among the remaining Missouri and Kansas populations for effective restoration. This selection process should balance the maintenance of potential genetic differences across the range of the species against the need for maximizing genetic diversity in restorations. Experimentation is needed to evaluate the genetic consequences of population restoration of *A. meadii*, especially the effects of long-distance crossing among geographically different populations and the long-distance movement of genotypes.

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