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VERY LOW GENETIC HETEROZYGOSITIES IN SEXUAL AND AGAMOSPERMOUS POPULATIONS OF EUPATORIUM ALTISSIMUM (ASTERACEAE)[1]

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An electrophoretic study revealed very low genetic heterozygosities for both sexual and agamospermous populations of *Eupatorium altissimum* in eastern North America. Low heterozygosity or gene diversity ($H_T = 0.03$) can be explained by the small sizes of the extant sexual populations, estimated to be on the order of 10^4. There was no substantial difference in observed heterozygosity between sexual and agamospermous races. This result shows that a higher level of heterozygosity is not a prerequisite for the evolution of agamospermous races from the sexual ancestor.

Apomixis by seed (agamospermy) or spore (agamospory) has been documented for numerous taxa belonging to various groups of angiosperms and pteridophytes. Several amphi-agamic complexes were the subjects of extensive studies until the 1950s due to their taxonomical and cytological complexities. These classical works are reviewed by Gustafsson (1946–1947), Stebbins (1950), Nygren (1954), and more recently by Grant (1981) and Nogler (1984). These authors concluded that those apomictic taxa examined genetically are all highly heterozygous. Recent electrophoretic studies of some amphi-agamic plant groups also reported that agamospermous taxa have higher heterozygosities than their sexual relatives (Bayer and Crawford, 1986; Watano and Iwatsuki, 1988; Bayer, 1989a; see also Vrijenhoek, 1990 for evidence from parthenogenetic animals). These agamospermous races are, however, probably allopolyploids that maintain a high level of fixed heterozygosity even in the sexual state (Roose and Gottlieb, 1976). There have been limited data comparing heterozygosities of sexual and agamospermous races within a single species (Bayer, 1989b; Vrijenhoek, 1990), and it is still uncertain whether agamospermous races in general have higher heterozygosity than their sexual ancestors.

It has been theoretically postulated that apomictic mutants can easily invade sexual populations because they do not produce males or there is twofold parent-offspring relatedness (see Williams, 1975; Maynard Smith, 1978, 1984). From this theoretical perspective, higher heterozygosity is not a prerequisite for the evolution of apomictic races from a sexual ancestor. Thus it is of particular interest to compare heterozygosities of sexual and agamospermous races within a single species.

Among the 23 species of North American *Eupatorium* L., both sexual and agamospermous races have been reported for nine species. In these species, sexual and agamospermous races are indistinguishable in gross morphology. These species provide a good opportunity to examine heterozygosities of sexual and agamospermous populations within a single species. As the first attempt to compare heterozygosities between sexual and agamospermous populations of these nine species, we examined allozyme variation in *Eupatorium altissimum* L.

MATERIALS AND METHODS

Sexual *Eupatorium altissimum* is an outcrossing (self-incompatible) perennial without vegetative reproduction. This species occurs as sexual diploid plants with normal pollen and triploid plants that have no pollen, or less frequently, malformed abortive pollen grains; the latter produce fertile seeds and thus are regarded as agamospermous (Sullivan, 1976).

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TABLE 1. *Collection codes and localities of sampled populations of* Eupatorium altissimum

Collection codes	Localities
Sexual populations	
45	US 65S, 10 miles S of JCT 60, Christian Co., MO
46	US 65S, 13 miles S of JCT 60, Christian Co., MO
47	Rt. 248, 3 miles N of JCT 65, Taney Co., MO
48	US 65S, 8 miles N of Bear Creek Spring, near Burlington, Boone Co., AR
49	US 62E, 8 miles W of Yellville, Marion Co., AR
50	US 62E, 3 miles W of Yellville, Marion Co., AR
51	Rt. 5S, 3.8 miles S of Mountain Home, Baxter Co., AR
53	Rt. 5S, 2.7 miles S of White River, Stone Co., AR
Agamospermous populations	
55	Rt. 14S, 17 miles NW of Batesville, Stone Co., AR
57	IS 55N, 1 mile S of St. Mary, Ste. Genevieve Co., MO
58	IS 70E, 1 mile W of Altamont, Effingram Co., IL
59	IS 70E, 2 miles E of JCT 231, Putnam Co., IN
OH	Campus of Ohio State University, Columbus, OH

These agamospermous plants occur widely in eastern North America, but sexual diploids are restricted to the Ozark Mountains lying in southern Missouri and northern Arkansas, and to a small disjunct area in central Tennessee. We sampled 259 individuals from eight populations of the Ozark Mountains. Examination of pollen fertility indicated that all samples are sexual, and chromosome counts confirmed them as diploids ($2n = 20$). These samples cover a large part of the range of diploid *E. altissimum* in the Ozarks. We also sampled 144 individuals from five agamospermous populations (Table 1; see Table 4 for sample size of each population). Chromosome counts confirmed them as triploids ($2n = 30$), and they lacked pollen.

Plants bearing flower buds and young, green leaves were collected in the field, transported to the laboratory on ice, and stored in a refrigerator until used for electrophoretic analysis. For electrophoresis, flower buds and a small piece of leaf material of individual plants were ground in 1.0 ml of cold extraction buffer as described by Odryzykoski and Gottlieb (1984) and modified by Yahara et al. (1989). Enzymes were resolved in 12% starch gels using the three buffer systems described by Yahara et al. (1989). System I resolved phosphoglucoisomerase (PGI) and triosephosphate isomerase (TPI), system II resolved alcohol dehydrogenase (ADH) and phosphoglucomutase (PGM), and system III was employed to resolve aconitase (ACN), isocitrate dehydrogenase (IDH), and shikimate dehydrogenase (SKDH). Staining schedules followed Soltis et al. (1983).

Examination of gels stained for ACN, IDH, PGI, PGM, and TPI provided reliable genetic data for 13 isozyme loci. Many diploid species of North American *Eupatorium* show extensive duplications for genes encoding IDH, PGI, and PGM (Yahara et al., 1989). In sexual *E. altissimum,* three isozymes were detected for these enzyme species. Based on these data, Nei's statistics of genetic identity (Nei, 1972) and gene diversity (Nei, 1973) were calculated for sexual diploid populations, and mean heterozygosities (mean of observed heterozygosity over all loci examined) were calculated for sexual and agamospermous populations. Gene diversity was calculated using all 13 loci for comparison with theoretical expectations, and using polymorphic loci to compare with the data reviewed by Hamrick and Godt (1990). The enzymes ADH and SKDH were not interpreted genetically due to overlapping expression of different loci, and thus were not included in the calculation.

RESULTS

Of the 13 loci scored, six were polymorphic in sexual populations (Table 2). At each of these six loci, a common allele occurs in a mean frequency higher than 0.90 (Table 2). Mean and ranges of genetic identities for pair-wise comparisons of populations and gene diversity (expected heterozygosity) statistics for sexual populations of *E. altissimum* are given in Table 3. The data show that eight populations are genetically very homogeneous; there is no marked differentiation among populations, and gene diversity (heterozygosity) is very low. Mean H_T for previously published data for predominantly outcrossing, animal-pollinated species is 0.310 (Hamrick and Godt, 1990), and is 4.6 times higher than in *E. altissimum.*

For agamospermous plants, four genotypes were recognized using 13 enzyme loci, and all populations were monoclonal (Table 2). Plants from Indiana and Ohio were identical. Three genotypes from the agamospermous plants (other than the Illinois type) exhibited one or two rare alleles from the sexual populations in the heterozygous condition. Mean observed

TABLE 2. *Mean allele frequencies at 13 enzyme loci in sexual* E. altissimum *and corresponding electrophoretic phenotypes in agamospermous* E. altissimum. *Mean allele frequencies for eight populations are given in parentheses*

		Alleles found in agamospermous population				
Locus	Allele frequencies in sexual population	AR	MO	IL	IN	OH
Aco-1	a (0.048), b (0.952)	b	b	b	b	b
Aco-2	a (0.993), b (0.007)	a	a	a	a	a
Idh-1	a (1.000)	a	a	a	a	a
Idh-2	a (1.000)	a	a	a	a	a
Idh-3	a (0.996), b (0.004)	a	a	a	a	a
Pgi-1	a (1.000)	a	a	a	a	a
Pgi-2	a (1.000)	a	a	a	a	a
Pgi-3	a (0.993), b (0.007)	a	a	a	a/b	a/b
Pgm-1	a (1.000)	a	a	a	a	a
Pgm-2	a (1.000)	a	a	a	a	a
Pgm-3	a (0.946), b (0.054)	a	a/b	a	a	a
Tpi-1	a (0.052), b (0.907), c (0.041)	b/c	a/b	b	b	b
Tpi-2	a (1.000)	a	a	a	a	a
Frequency of corresponding genotype in diploid		0.07	0.02	0.52	0.01	0.01

heterozygosity of agamospermous populations ranged from 0.00 to 0.15 with a mean of 0.08 (Table 4). Mean observed heterozygosities calculated for eight sexual populations ranged from 0.01 to 0.14 with a mean of 0.07, and the difference from agamospermous populations was not significant by Mann-Whitney U test. This statistical result and complete homozygosity in the Illinois type indicates that there is no substantial difference in heterozygosity between sexual and agamospermous populations of *E. altissimum.*

DISCUSSION

Agamospermous plants have been considered to be advantageous because they maintain hybridity or high heterozygosity without the breaking up of advantageous gene combinations by recombination. From a theoretical perspective, however, asexual reproduction itself has a twofold advantage over sexual reproduction because it does not produce males and there is enhanced parent-offspring relatedness (Williams, 1975; Maynard Smith, 1978, 1984). There is evidence supporting this theoretical advantage of agamospermous plants (Michaels and Bazzaz, 1986; Bierzychudek, 1989, 1990; Yahara, 1990). In *E. altissimum,* there was no substantial difference in heterozygosity between sexual and agamospermous races. This result clearly shows that a higher level of heterozygosity is not a prerequisite to the evolution of agamospermous races and is consistent with the above theoretical perspective.

TABLE 3. *Genetic identities and gene diversity statistics for sexual populations of* Eupatorium altissimum

Mean genetic identities for all pairwise comparisons of populations	0.998
Range of genetic identities for all populations	0.994–1.000
Total gene diversity (H_T)	0.031[a] 0.067[b]
Population-level gene diversity (H_S)	0.030[a] 0.065[b]
Gene diversity between populations (D_{ST})	0.001[a]
Differentiation among populations (G_{ST})	0.049[a]

[a] Calculated using 13 loci.

[b] Using six polymorphic loci.

TABLE 4. *Observed heterozygosities in sexual populations and observed heterozygosities in agamospermous populations of* Eupatorium altissimum

Populations	Sample size	Observed heterozygosity[a]
Sexual		
45	18	0.07
46	16	0.06
47	81	0.07
48	14	0.06
49	10	0.03
50	66	0.07
51	47	0.02
53	10	0.14
Mean for sexual populations		0.07
Agamospermous		
55 (AR)	24	0.08
57 (MO)	24	0.15
58 (IL)	24	0.00
59 (IN)	24	0.08
OH	48	0.08
Mean for agamospermous populations		0.08

[a] Mean for 13 loci.

At all 13 loci examined, all four agamospermous races had the same allele that was predominant in the sexual populations. Four other alleles found in agamospermous races were found in low frequencies in sexual populations. This result and the equivalent level of heterozygosity in sexual and agamospermous populations suggest nonhybrid origins of the agamospermous races. Morphological similarity between the sexual and agamospermous races supports this view. Recently, Gastony and Gottlieb (1985) and Gastony (1988) suggested electrophoretically that agamospermous races of a few fern species are of autopolyploid origin. Although association between asexual reproduction and hybridity has been documented in plants (see Richards, 1986 for a most recent review) and also in many parthenogenetic animals (White, 1978; Vrijenhoek, 1990), our result shows that this is not the rule. This association can be secondary because hybridization between sexual and apomictic plants will produce apomictic hybrids. This process is recently documented in several fern genera (Gastony and Gottlieb, 1985; Gastony, 1988; Watano and Iwatsuki, 1988; Suzuki and Iwatsuki, 1990) and in angiosperms including *Eupatorium* (Yahara, 1990).

It is considered that apomicts are likely to become more heterozygous than their sexual progenitors over time, because apomicts should accumulate mutations in the absence of recombination (see Lokki, 1976a, b; Vrijenhoek, 1990 and references therein). In this process, apomicts are expected to gain unique alleles which are not found in their sexual progenitors. In *E. altissimum,* however, all alleles of the agamospermous races are also found in sexual populations. This fact suggests that the agamospermous races are of relatively recent origin.

Another interesting finding in this study is the very low genetic heterozygosity in the sexual populations of *E. altissimum.* It has been considered that several recessive genes should be brought together for the origin of agamospermy, and thus the sexual ancestors of agamosperms are likely to be highly outcrossing (Powers, 1945; Asker, 1980; Marshall and Brown, 1981). In this hypothesis, the recessive genes for agamospermy are assumed to be maintained in low frequencies in ancestral sexual populations, and thus the sexual ancestors are expected to be highly heterozygous. The result of this study, however, revealed very low heterozygosity in the sexual populations of *E. altissimum.* This result may suggest that apomicts can arise from sexual stocks with very low heterozygosity. Alternatively, the sexual populations of *E. altissimum* were more highly heterozygous at the time of origin of the agamospermous races. Available evidence is advantageous to the latter hypothesis as stated below.

Because diploid *E. altissimum* is a self-incompatible, predominantly outcrossing species, the low level of genetic heterozygosity can be attributed to reduced population size in the extant population and/or a population bottleneck in the past (Nei, Maruyama, and Chakraborty, 1975). Under the neutral theory, expected heterozygosity (H) in equilibrium with mutation and genetic drift is:

$$H = 4N_ev/(4N_ev + 1),$$

where N_e is effective population size and v is mutation rate. This equation gives an estimate of N_ev using estimated expected heterozygosity or total gene diversity (H_T) as:

$$(N_ev)\mathrm{est} = H_T/4(1 - H_T).$$

Because H_T is 0.031 in sexual *E. altissimum,* N_ev is estimated to be 8×10^{-3}. This gives an estimate of N_e as 6×10^4 assuming $v = 1.3 \times 10^{-7}$ (for an estimate of electrophoretically detectable neutral mutation rate see Kimura and Ohta, 1971; Kimura, 1983; Nei, 1987). Sexual populations of *E. altissimum* occur very sporadically around the Missouri-Arkansas border. We observed a few large populations with more than 100 individuals, and the number of these large sexual populations in the Ozark Plateau is also estimated to be several hundred. Thus the estimate of the actual population size (i.e., total number of reproductive individuals) is on the order of 10,000, which agrees approximately with the estimate of effective population size. Thus the reduced genetic diversity in sexual *E. altissimum* can be explained without assuming a population bottleneck in the past.

Among the four agamospermous races studied three had alleles found only in low frequencies in the sexual populations. Expected frequencies of the genotypes corresponding to these agamospermous races are quite low (0.07–0.01) in the extant sexual population (see Table 2), and it is doubtful that these originated from the extant sexual population. This fact may suggest that the sexual race ranged more widely and was more heterozygous at the time of origin of these agamospermous races. Because the agamospermous race in higher plants is advantageous not only in enhanced parent-offspring relatedness, but also in ensured fertility (Bierzychudek, 1987; Yahara, 1990), the sexual race could survive only in extreme habitats within a small area after the origin and subsequent colonization by the agamospermous races of habitats formerly occupied by the sexual race. This process could decrease genetic diversity in the sexual populations.

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