VERY LOW GENETIC HETEROZYGOStIES IN SEXUAL AND AGAMOSPEROUS POPULATIONS OF EUPATORIUM ALTISSIMUM (ASTERACEAE)

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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 ($H_e = 0.03$) can be explained by the small sizes of the extant sexual populations, estimated to be on the order of $10^2$. 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 alloployploids that maintain a high level of fixed heterozygosity even in the sexual state (Roos 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).
Table 1. Collection codes and localities of sampled populations of Eupatorium altissimum

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

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 Oddyzykoski 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 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
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 re-

**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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele frequencies in sexual population</th>
<th>AR</th>
<th>MO</th>
<th>IL</th>
<th>IN</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC1</td>
<td>a (0.048), b (0.952)</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>AC2</td>
<td>a (0.993), b (0.007)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>IDH1</td>
<td>a (1.000)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>IDH2</td>
<td>a (1.000)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>IDH3</td>
<td>a (0.996), b (0.004)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>PG1</td>
<td>a (1.000)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>PG2</td>
<td>a (1.000)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>PG3</td>
<td>a (0.993), b (0.007)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>PG4</td>
<td>a (1.000)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>TPI1</td>
<td>a (0.052), b (0.907), c (0.041)</td>
<td>b/c</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td></td>
</tr>
<tr>
<td>TPI2</td>
<td>a (1.000)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean genetic identities for all pairwise comparisons of populations</td>
<td>0.998</td>
</tr>
<tr>
<td>Range of genetic identities for all populations</td>
<td>0.994–1.000</td>
</tr>
<tr>
<td>Total gene diversity ($H_T$)</td>
<td>0.031*</td>
</tr>
<tr>
<td>Population-level gene diversity ($H_S$)</td>
<td>0.030*</td>
</tr>
<tr>
<td>Gene diversity between populations ($D_{2T}$)</td>
<td>0.065*</td>
</tr>
<tr>
<td>Differentiation among populations ($G_{ST}$)</td>
<td>0.049*</td>
</tr>
</tbody>
</table>

* Calculated using 13 loci.

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

<table>
<thead>
<tr>
<th>Populations</th>
<th>Sample size</th>
<th>Observed heterozygosity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>18</td>
<td>0.07</td>
</tr>
<tr>
<td>46</td>
<td>16</td>
<td>0.06</td>
</tr>
<tr>
<td>47</td>
<td>81</td>
<td>0.07</td>
</tr>
<tr>
<td>48</td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td>49</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>50</td>
<td>66</td>
<td>0.07</td>
</tr>
<tr>
<td>51</td>
<td>47</td>
<td>0.02</td>
</tr>
<tr>
<td>53</td>
<td>10</td>
<td>0.14</td>
</tr>
</tbody>
</table>

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 |

* Calculated using 13 loci.
At all 13 loci examined, all four agamosper- mous races had the same allele that was pre-
dominant 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 hetero-
zygosity in sexual and agamospermous pop-
ulations suggest nonhybrid origins of the aga-
mospermous 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 repro-
duction 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 hybrid-
ization between sexual and apomictic plants
will produce apomictic hybrids. This process
is recently documented in several fern genera
(Gastony and Gottlieb, 1985; Gastony, 1988;
Wataho and Iwatsuki, 1988; Suzuki and Iwatsu-
suki, 1990) and in angiosperms including Eupatoria (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 re-
combination (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 aga-
mospermous races are of relatively recent or-
igin.

Another interesting finding in this study is
the very low genetic heterozygosity in the sex-
ual populations of E. altissimum. It has been
considered that several recessive genes should
be brought together for the origin of agamo-
spermy, and thus the sexual ancestors of aga-
mosperms 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 sex-
ual 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.
alpissimum. This result may suggest that apo-
micts 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 aga-
mospermous races. Available evidence is ad-
vantageous to the latter hypothesis as stated
below.

Because diploid E. altissimum is a self-in-
compatible, predominantly outcrossing spe-
cies, the low level of genetic heterozygosity can
be attributed to reduced population size in the
extant population and/or a population bottle-
neck in the past (Nei, Maruyama, and Chak-
raborty, 1975). Under the neutral theory, ex-
pected heterozygosity (H) in equilibrium with
mutation and genetic drift is:

\[ H = \frac{4N_e \nu}{(4N_e \nu + 1)} \]

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

\[ (N_e \nu)_{est} = \frac{H_T}{4(1 - H_T)}. \]

Because \( H_T \) is 0.031 in sexual E. altissimum,
\( N_e \nu \) is estimated to be \( 8 \times 10^{-3} \). This gives
an estimate of \( N_e \) as \( 6 \times 10^4 \) assuming \( \nu = 1.3 \times 10^{-7} \) (for an estimate of electrophoretically de-
tectable neutral mutation rate see Kimura and
populations of E. altissimum occur very spo-
radically around the Missouri-Arkansas bor-
dor. 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 ap-
proximately with the estimate of effective pop-
ulation size. Thus the reduced genetic diversity
in sexual E. altissimum can be explained with-
out assuming a population bottleneck in the
past.

Among the four agamospermous races stud-
ied three had alleles found only in low fre-
quencies 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 or-
igin of these agamospermous races. Because
the agamospermous race in higher plants is
advantageous not only in enhanced parent-off-
spring relatedness, but also in ensured fertility
(Bierzychudek, 1987; Yahara, 1990), the sex-
ual race could survive only in extreme habitats
within a small area after the origin and sub-