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Species and Evolution in Asexually Reproducing Independent Fern Gametophytes

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ABSTRACT. Vegetative reproduction by the gametophyte generation has allowed a number of fern species to persist beyond their normal geographic range. In at least two of these, *Trichomanes* and *Vittaria* in the eastern United States, the sporophyte stage of the life cycle has been eliminated completely, perhaps for ten million years or more. Despite this long period of reliance on vegetative reproduction, genetic diversity in the Appalachian *Vittaria* gametophytes, as measured by starch gel enzyme electrophoresis, remains comparable to that of sexual plants, although it is largely partitioned into monomorphic populations. Widely separated populations are not particularly divergent; the taxon as a whole appears equally as cohesive as sexual species, suggesting that factors other than gene flow are responsible for long term maintenance of species integrity. The Appalachian *Vittaria* gametophytes and similarly derived taxa merit recognition as distinct species.

Sexually reproducing pteridophytes alternate a diploid, spore-producing plant, the sporophyte, with a haploid, gamete-producing plant, the gametophyte. In most taxa, the gametophyte generation is short-lived and inconspicuous relative to the sporophyte plants, which are generally perennial. Life spans of fern gametophytes in the wild are generally less than one year, primarily due to erosion of their substratum (Peck 1980; Cousens 1988). However, in three families of homosporous ferns, the Vittariaceae, Hymenophyllaceae, and Grammitidaceae, a mechanism has evolved for perpetuating the gametophyte generation indefinitely without intervention of the sporophyte generation. This mechanism allowing independence of the gametophyte generation is the production of vegetative propagules called gemmae (Farrar 1967, 1974; Stokey 1940, 1948; Stokey and Atkinson 1958).

Does vegetative reproduction allow the gametophyte generations of these plants to exist and evolve as autonomous organisms? The fern families having gemmiferous gametophytes are primarily inhabitants of wet tropical forests. Although dense populations (clones ?) of gametophytes of these families are often observed in tropical habitats, there is little evidence to suggest that their evolutionary processes in these habitats are out of the ordinary. It is in temperate habitats that the potential for autonomous existence of gemmiferous gametophytes is realized. This is because gametophytes of some tropical ferns can tolerate lower temperatures and greater desiccation than can their sporophyte counterparts and thereby flourish in latitudes where the sporophytes cannot survive (Farrar 1978).

A brief accounting of this phenomenon in the eastern United States illustrates the success of gemmiferous gametophytes in temperate habitats. In Macon County, North Carolina, in a "rock house" behind a waterfall on the Blue Ridge Escarpment is the only site in the continental United States of any species of the Grammitidaceae. Here *Grammitis nimbata* (Jenm.) Proctor exists as occasional juvenile sporophytes, apparently sexually produced, and a population (clone ?) of gametophytes reproducing vegetatively via gemmae (Farrar 1967). Because sporophytes never attain spore production, we can assume that the population is maintained by asexual reproduction of the gametophytes.

In similar habitats in Pickens County, South Carolina, are the only occurrences of sporophytes of *Hymenophyllum tunbrigense* (L.) Smith. Gametophytes of *Hymenophyllum* are common in surrounding counties, including the Macon County site of *Grammitis*, but they never produce sporophytes (Wagner et al. 1970). *Trichomanes* (Hymenophyllaceae) is represented in south temperate United States by two species, *T. boschianum* Sturm ex Bosch. and *T. petersii* A. Gray, both of which have rare occurrences of sporophytes in moderated habitats in rock houses and narrow canyons from Arkansas to North Carolina. Gametophytes of *Trichomanes* occur abundantly in these habitats and northward, extending to central Indiana and north-

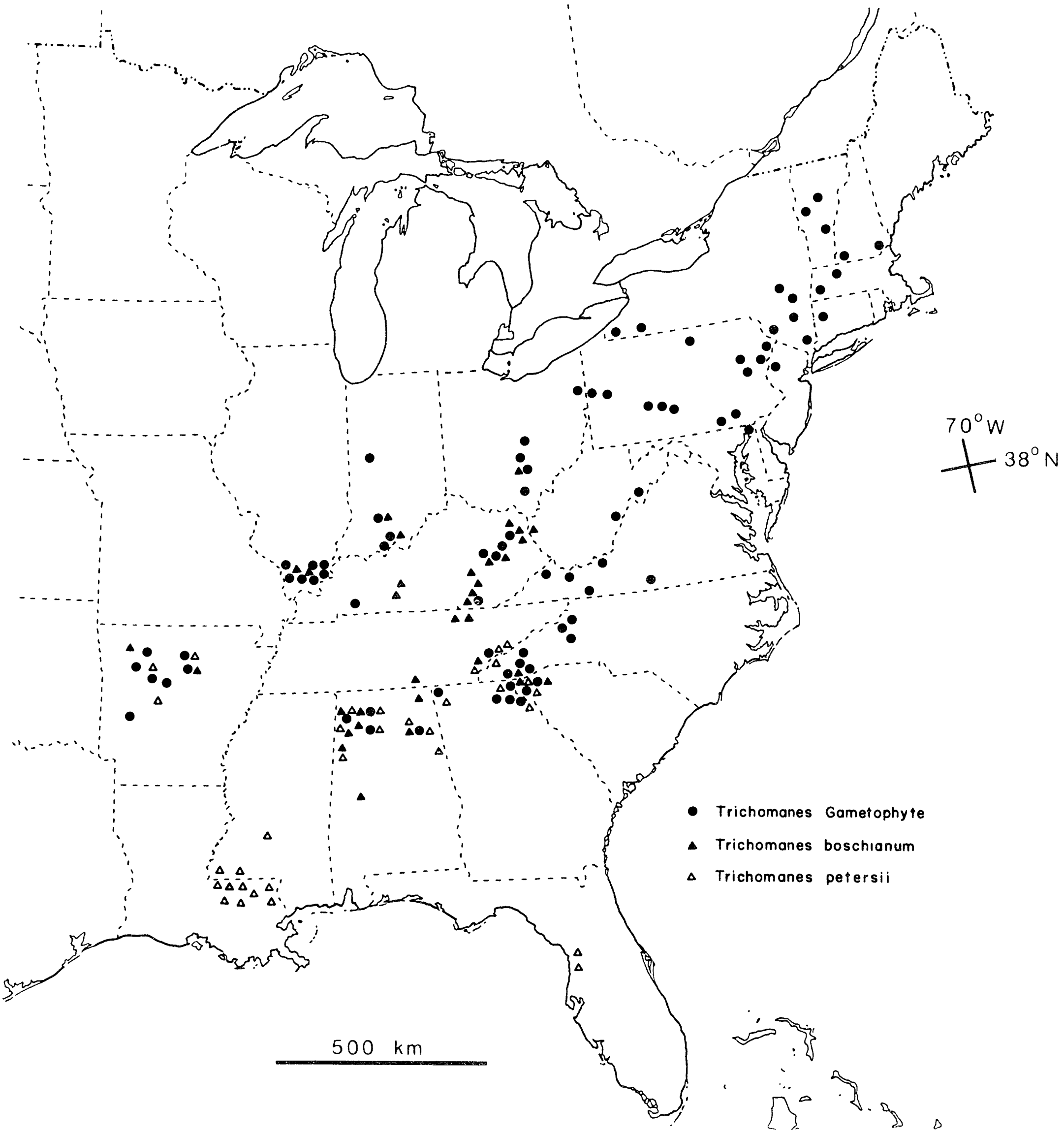


FIG. 1. The distribution of *Trichomanes* in the eastern United States. Each symbol represents a county in which the taxon has been recorded.

ern Vermont, nearly 1000 km beyond any sporophyte occurrences (fig. 1). The most dramatic separation of gametophytes from sporophytes is in the Vittariaceae. Gametophytes of *Vittaria* occur commonly in canyon habitats from western Kentucky, northern Alabama, and northern Georgia in the south to Indiana, New York, and Pennsylvania in the north (fig. 2). Sporophytes of this genus are limited to peninsular Florida (Farrar 1985).

The independent gametophytes described above have several features in common. They are found in habitats that are environmentally moderated, but in which freezing and desiccation do occur. They exist as extensive populations or clones consisting of thousands of in-

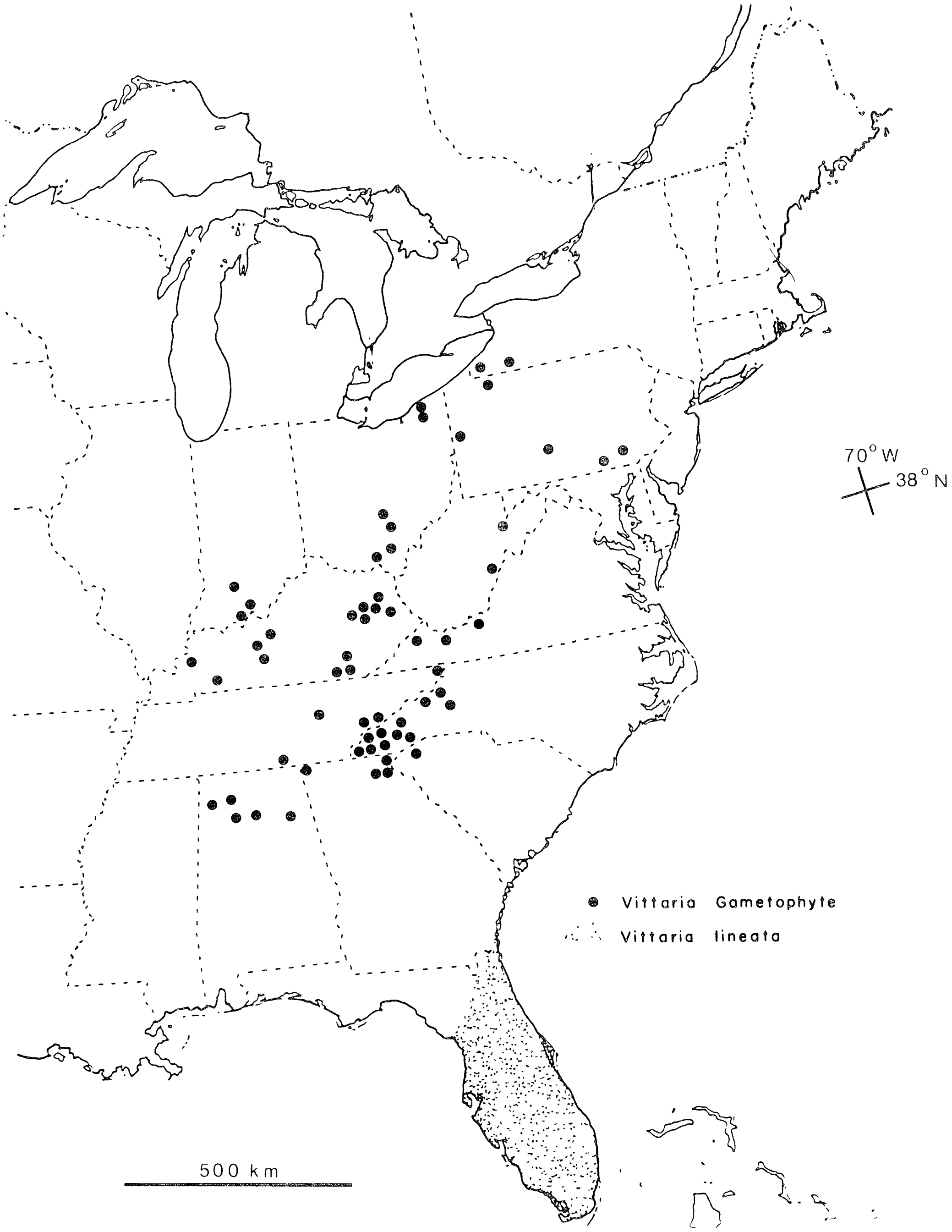


FIG. 2. The distribution of *Vittaria* in the eastern United States. Each dot represents a county in which independent gametophytes have been recorded. The shaded area of Florida and southeasternmost Georgia is the approximate distribution of *Vittaria lineata* J. E. Smith.

dividuals (except *Grammitis*) dominating their habitats. Although they produce apparently functional gametangia, they never produce sporophytes (except rarely in *Grammitis*). They reproduce vegetatively through copius production of gemmae.

What is the origin of the independent gametophytes? One possibility is that they are recently, and perhaps continuously, initiated from spores transported by wind from existing sporophyte populations. Recent introduction is a probable explanation for *Grammitis nimbata* as it occurs in only one site and has retained the ability to produce sporophytes. This could also be the case for *Hymenophyllum tunbrigense*, although it does not account for the inability of independent gametophytes of *Hymenophyllum* to produce sporophytes.

I have presented several reasons for discounting a recent origin for the independent gametophytes of *Vittaria* and *Trichomanes* (Farrar 1978, 1985). One of these is the general non-production of sporophytes by the independent gametophytes. This holds true even when they are cultured under the same conditions in which gametophytes of tropical species of these genera do produce sporophytes. Gametophytes of the independent *Vittaria* are also distinct morphologically from all known tropical American species, especially in the size and shape of their gemmae and in the pattern in which the gemmae are produced (Farrar 1978).

The most compelling evidence in support of an ancient origin of the independent *Vittaria* and *Trichomanes* gametophytes is from enzyme electrophoresis (Farrar 1985). Allelic differences between the independent gametophytes and the most probable sporophyte sources (nearest or most common species) are so great as to make untenable any hypothesis of recent origin from those species. The alternative hypothesis is that the independent gametophytes of *Trichomanes* and *Vittaria* in eastern temperate North America are of ancient origin and their sporophyte producing ancestors are no longer identifiable and possibly no longer exist. Supporting this hypothesis is the geographic distribution of genotypes in *Vittaria* as assayed by enzyme electrophoresis and reported in this paper.

An ancient development of the independence of the gametophytes discussed above could have arisen via two routes. They may have originated as spores carried northward from tropical sources. (But in *Vittaria* there are currently no tropical American species that sufficiently meet morphological and genetic criteria to be considered as source species.) Alternatively, the independent gametophytes may have originated from species possessing normal alternation of generations that lived in the eastern United States when climatic conditions were suitable for sporophyte growth. The last widespread occurrence of subtropical vegetation in eastern North America was in the late Miocene (Braun 1937; Cain 1943; Fenneman 1938). If the species were reduced to gametophytes only at the close of that epoch, they have been maintained by vegetative reproduction for 10 million years or more.

The hypothesis of ancient origin is examined here in the context of the patterns of genetic diversity in independent *Vittaria* gametophytes as revealed by enzyme electrophoresis. From these data an assessment is made of evolution and the organization of genetic diversity in a species reproducing solely by vegetative reproduction. If these *Vittaria* and *Trichomanes* species have existed as independent gametophytes over a million years or more, they present an exceptional opportunity for examination of long term evolution in the absence of sexual reproduction (see Mishler and Budd, this symposium, for an overview of species concepts and evolution in asexual organisms).

MATERIALS AND METHODS

Gametophytes of *Vittaria* were collected from 92 populations in 26 sites. A site is defined as a more-or-less continuous set of rock cliffs usually extending 100 meters or less. Each site is isolated from other sites by discontinuity of suitable habitat. Populations are defined as discrete and more-or-less continuous stands of plants usually one to five meters in length and usually less than one meter in width. Plant densities within populations are from 10 to 20 plants per square centimeter. Samples consisted of several hundred individuals selected from throughout the populations. Samples were field stored in glass vials on ice until they could be stored in a refrigerator at 5°C. Plants from about one quarter of the collections were grown in culture on natural substrate, *Osmunda* fiber, or mineral nutrient agar. Cultured plants yielded isozyme patterns identical to those from the wild.

To obtain conveniently sufficient enzyme activity, samples for electrophoresis consisted of a combined extract of about 10 individuals from the collection. Multiple tests were made on most collections. If results indicated the possibility of more than one genotype in the sample, individual gametophytes were tested to resolve this possibility. Phosphoglucose isomerase was clearly resolved in all collections, but many early collections not placed in culture were lost before buffer systems sufficient for resolution of other enzymes were developed.

Starch gel electrophoresis was performed on crude whole-gametophyte extracts. Approximately 25 mg of tissue were placed in a cold porcelain spot plate and ground in 0.05 ml extraction buffer using the end of a cold glass test tube. The extraction buffer consisted of 75 mM Na-phosphate, pH 7.5, containing 0.5% BSA, 5% (w/v) sucrose, 10% (w/v) polyvinylpolpyrrolidone, 14 mM mercaptoethanol, 100 mM ascorbic acid, 10 mM dithioerythrotol, and 10 mM diethyldithiocarbamate. Extracts were frozen at −70°C until electrophoresis. Enzymes were separated in 12.8% (w/v) starch gels. The following gel and electrode buffers were found to yield scorable enzyme patterns. For triose-phosphate isomerase (TPI), electrode buffer: 0.19 M boric acid adjusted to pH 8.3 with lithium hydroxide; gel buffer: 1 part electrode buffer to 9 parts 52 mM Tris—8 mM citrate, adjusted to pH 8.3 with citric acid. For phosphoglucose isomerase (PGI), catalase (CAT), and aspartate aminotransferase (AAT), electrode buffer: 76 mM Tris—5 mM citrate, pH 8.6; gel buffer: 0.3 M boric acid titrated to pH 8.0 with NaOH. For malate dehydrogenase (MDH), phosphoglucomutase (PGM), aconitate hydratase (ACO), shikimate dehydrogenase (SKD), and isocitrate dehydrogenase (IDH), electrode buffer: 65 mM L-histidine—19 mM citrate, pH 6.5; gel buffer: 1:6 dilution of the electrode buffer. Electrophoresis was allowed to proceed for 5 hours for all gels, using between 13 and 16 watts constant power. Enzymes were visualized using staining methods detailed in Wendel and Weeden (1988). Allele and locus numbers are inferred from migration patterns and from comparison to results reported for sexual fern taxa.

RESULTS AND DISCUSSION

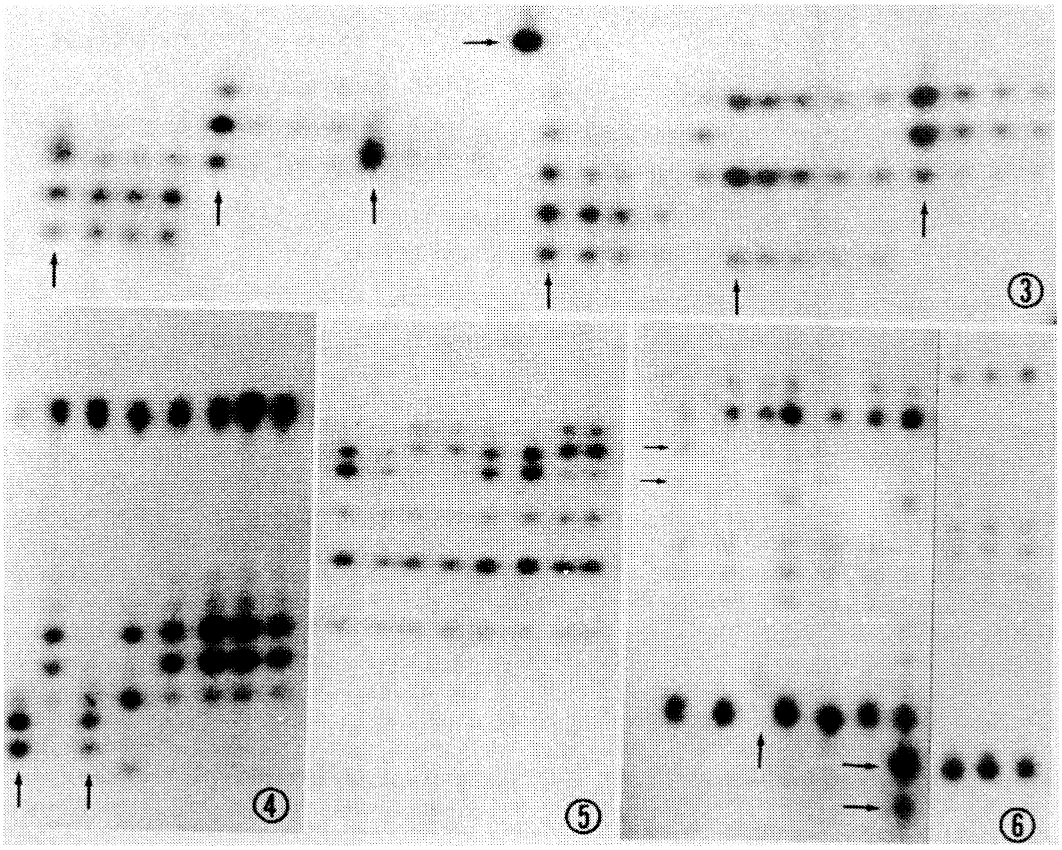
Allozyme Analysis. Nine enzymes yielded banding patterns scorable for allele frequencies and/or for calculation of other measures of genetic diversity. From these, a total of 20 alleles encoded by 12 loci were identified. Percent polymorphism of loci scored was 41.7.

The most extensive isozyme data are for PGI. Of the two loci resolved, the one coding for the most anodal protein, PGI-1, is invariant. In the position of PGI-2, individual plants show one of five three-banded patterns (figs. 3, 4). Of 92 populations assayed, none showed a single sharp PGI-2 band indicative of homozygosity at a single locus.

Two possible interpretations of the PGI-2 patterns are suggested. The first is that two loci, with two and three alleles respectively, encode the observed proteins. However, gene duplication at Pgi-2 has not been observed in other species of the genus, and this explanation does not account easily for the observed assortment of alleles. The second alternative is a single locus with five alleles. The genotypes observed are consistent with a single locus, considering the diploid chromosome number recorded for these plants (Gastony 1977), but the absence of homozygotes under this hypothesis must be explained.

The observed patterns could be explained by assuming an interspecific hybrid origin of this species from parental diploid species with distinct Pgi-2 alleles. This origin accounts for the "missing" Pgi-2 homozygotes (and two of the four possible Mdh homozygotes). It also provides a possible cause for the inability of gametophytes to produce sporophytes. It is not uncommon in ferns for sexually sterile interspecific hybrids to produce some viable diploid spores (Morzenti 1962; Gastony 1986), which can germinate and grow into gametophytes. Such gametophytes are usually incapable of producing sporophytes, except occasionally by apogamy, which has been observed on two occasions in cultures of the independent *Vittaria* gametophytes (Caponetti et al. 1982; A. G. Stokey, pers. comm.). On the other hand, gemmiferous reproduction would allow such gametophytes to persist indefinitely.

In light of the above considerations, the single locus interpretation of Pgi-2 is adopted in



FIGS. 3–6. Electrophoretic banding patterns of the Appalachian *Vittaria* gametophytes. In all figures the origin is at the bottom. 3. Banding patterns for PGI-2. Six populations are represented by lanes 1–4, 5–8, 9–12, 14–18, 19–23, and 24–27. In each set the first lane (vertical arrows) is a combined sample of about 10 individual plants, the following lanes are individual plants. Lanes 14–18 represent a polymorphic population. Lane 13 (horizontal arrow) is *Vittaria lineata*. 4. Banding patterns for PGI. Lanes 1 and 3 (arrows) show the Pgi-2, 2/4 genotype, which is not present in figure 3 (compare with 2/5 genotype in fig. 3, lanes 14–18, relative to 1/5 genotype in fig. 3, lane 19, and fig 4, lane 4). 5. Banding patterns for TPI showing a three-banded pattern at TPI-1 typical of many ferns, but with variation as to whether the first or third band is weak in intensity. 6. Banding patterns for MDH. Lane 1 (small horizontal arrows) shows a slow allele and heterodimer for MDH-1; lane 7 (large horizontal arrows) shows a slow allele and heterodimer for MDH-3. (The displacement of MDH-3 in lane 3 (vertical arrow) is due to preparation artifact.) Lanes 8–10 are *Vittaria lineata*.

this paper. Although the alternatives described modify calculations of allele frequencies, they have little effect on calculations of polymorphism and none on genotype distributions. Regardless of interpretation, the basic conclusions of this study are unchanged.

Triose-phosphate isomerase (TPI) shows a six-banded pattern. The anodal three bands (TPI-1) are a pattern commonly observed in ferns and have been alternatively interpreted as resulting from a single gene duplication or from post-translational modification of protein coded by a single gene (Gastony 1988). In most ferns the top (most anodal) band is weak relative to the lower two. In the *Vittaria* gametophytes, variation is observed. In some populations the top two bands are strong and the lowest band is weak (fig. 5). The simplest interpretation of TPI-1, followed here, is that these represent allelic variations of a single gene. The

TABLE 1. Distribution of genotype polymorphism among populations and sites of the Appalachian *Vittaria* gametophytes. [a] Four sites are represented by only one population. [b] Seven sites are represented by only one population.

	% polymorphic		
	Populations	Sites	Populations of polymorphic sites
Scored for PGI only	4.3 (4/92)	15.4 (4/26)[a]	19.0 (4/21)
Scored for all loci	14.7 (5/34)	33.3 (5/15)[b]	26.3 (5/19)

lower (cathodal) three TPI bands were invariant.

Malate dehydrogenase (MDH) typically yields a six-banded pattern, probably representing three loci (fig. 6). MDH-1 is invariant for a single band except for one population that displayed a three-banded heterozygous pattern at this locus. MDH-3 is usually single-banded but occasionally displays a three-banded heterozygous pattern. MDH-2 stained too lightly to permit accurate scoring. Phosphoglucomutase (PGM) and catalase (CAT) yielded patterns indicative of two loci. In each case the fast (anodal) locus was too faint to permit scoring and the slow locus was invariant. Isocitrate dehydrogenase (IDH), aconitase (ACO), and shikimate dehydrogenase (SKD) yielded invariant patterns, presumably coded by a single locus. Aspartate aminotransferase (AAT) yielded either a single band or a three-banded pattern, indicative of polymorphism, but banding was insufficiently sharp to permit determination of allele frequency or genotype distribution patterns.

Genotypic Analysis. The proportion of polymorphic loci in the independent *Vittaria* gametophytes, 41.7%, is not appreciably lower than the mean of 51% reported for 19 outcrossing angiosperm species (Gottleib 1981). It is also comparable to the amount of polymorphism (mean of 33%) listed by Pleasants and Wendel (1989) for 12 primarily clonally reproducing vascular plants. It is significantly higher than the mean polymorphism of 18% reported for 21 self-pollinating species of angiosperms (Gottleib 1981). Clearly, loss of genetic diversity within the species is not an immediate consequence of reliance on vegetative reproduction. On the other hand, the distribution of diversity among populations and sites is affected.

Considering the genotypes of PGI alone, only four of 92 populations sampled contained more than a single genotype (table 1), despite the variability of this locus. Furthermore, 22 of the 26 sites supporting these populations contained only one genotype. Fewer populations could be analyzed for genotypes based on all polymorphic loci, and most of these were from polymorphic sites. Still, on this basis, 85% of populations were monomorphic. Five populations were polymorphic for PGI, three were polymorphic for TPI, and two were polymorphic for both PGI and TPI. No populations were polymorphic for MDH. The average polymorphism per population (1.7%) is much lower than that for outcrossing angiosperms (37%) and comparable to that for self-pollinating angiosperms (4.4%) (Gottleib 1981). It seems probable that most of the stands treated as populations in this study are in fact single clones.

The most extensive and continuous occurrences of habitat suitable to growth of the *Vittaria* gametophytes is along the northeast-southwest axis of the Appalachian Plateau region, northwest of the Appalachian Mountain chain (fig. 2). Here, massive-bedded sandstones cemented with iron oxides form extensive high-walled canyons providing moderated habitats supporting large populations of gametophytes. Sites sampled in Alabama and Ohio are at the southern and northern ends of this central range of this species. Sites to the west, east, and north are in areas where suitable habitats are less extensive and more isolated from one another.

Considering genotypes of PGI, four of five genotypes are present in Ohio. Two genotypes are present in Alabama and North Carolina, only one in each of the other areas sampled (fig. 7). The only polymorphic populations and sites detected are in Ohio and Alabama. Areas outside Ohio and Alabama contain only the genotypes that are most common, as defined either on the basis of all populations or on the basis of populations from polymorphic sites. Considering

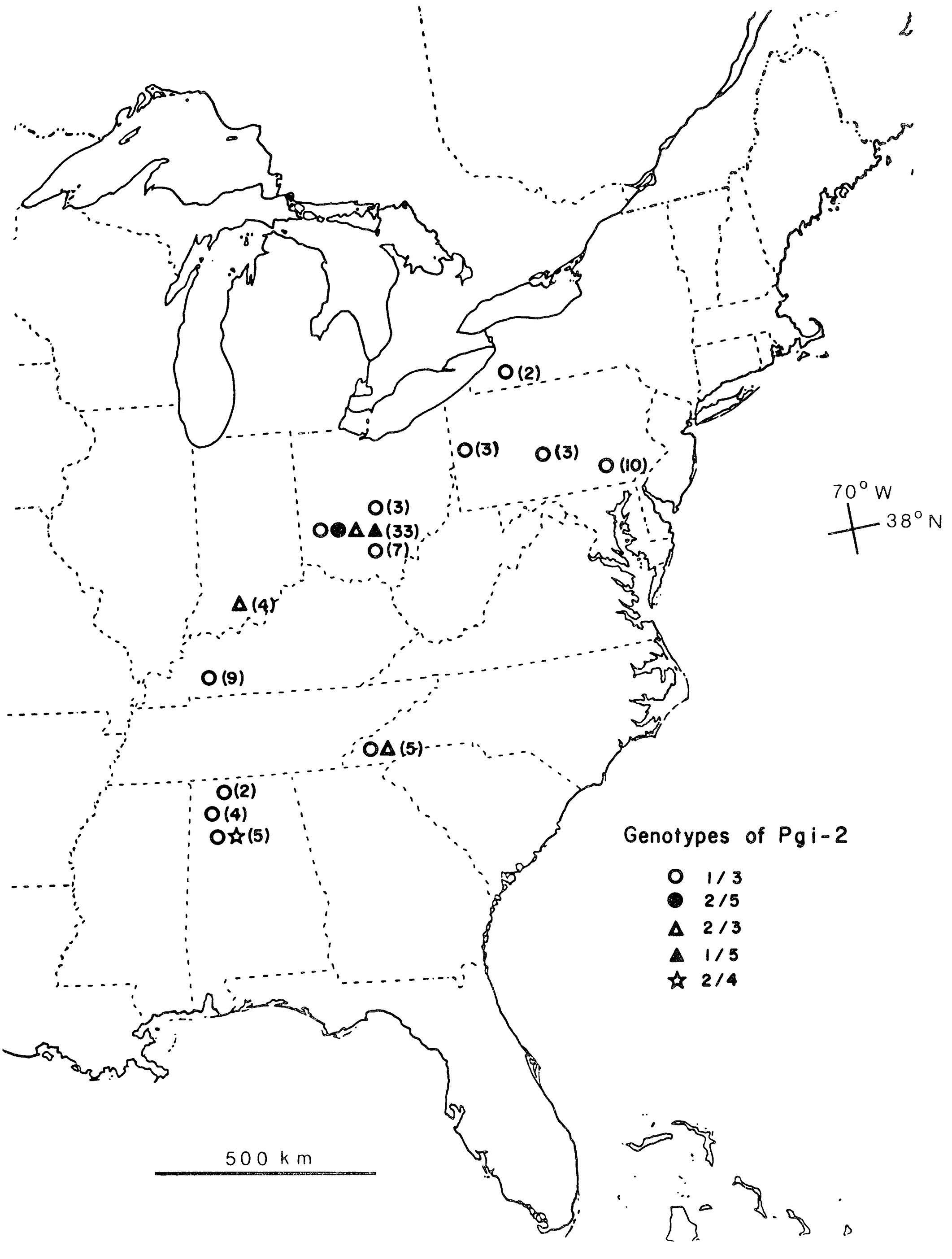


FIG. 7. The distribution of genotypes of Pgi-2 among sampled populations of the Appalachian *Vittaria* gametophytes in the eastern United States. The numbers of populations represented by each symbol or series of symbols is indicated in parentheses.

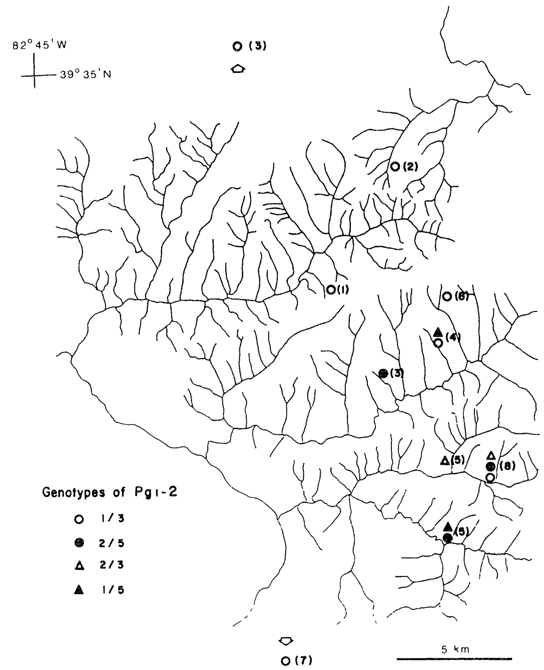


FIG. 8. The distribution of genotypes of Pgi-2 among sampled populations of the Appalachian *Vittaria* gametophytes in Hocking County, Ohio, and nearby sites (indicated by arrows). The number of populations represented by each symbol or series of symbols is indicated in parentheses. Arrows indicate sites 8 km north and 40 km south of the mapped area.

Ohio sites alone (fig. 8), we find that genotypes other than the most common are found only in two adjacent watersheds whereas outlying sites contain only the common genotype. The rarest of the Ohio alleles occurs only in the two watersheds mentioned. Though the total number of samples in Ohio was greater than elsewhere, comparable numbers of populations per site and the distribution of genotypes among Ohio sites suggest that absence of rare genotypes in outlying sites is not due to insufficient sampling, and that this is a pattern likely to be repeated along the central axis of distribution, perhaps with the addition of other rare alleles, as detected in Alabama.

Genotypic analysis based on all scorable loci yielded 10 distinct multilocus genotypes (fig. 9). Of these, only the three most common were found outside Ohio and Alabama (fig. 10). Three genotypes were unique to Ohio and four were unique to Alabama. These are attributable to one allele (Pgi-2, allele 5) found only in Ohio, and two alleles (Pgi-2, allele 4; Mdh-1, allele 2) found only in Alabama. Though based on fewer populations (34) and sites (15), these results parallel those based on PGI alone. It is especially significant that, whereas variation among sites in Ohio and Alabama is very high (each new site sampled is likely to contain a different genotype or set of genotypes), sites in other areas do not show a continuation of this pattern, but only repeat the common genotypes.

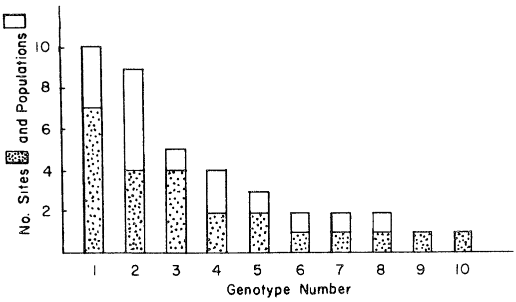


FIG 9. The frequency of 10 multilocus genotypes among sampled populations of the Appalachian *Vittaria* gametophytes. Genotypes are based on four polymorphic loci: Pgi-2, Tpi-1, Mdh-1, and Mdh-2.

Origin of the Appalachian Vittaria. The distribution of genotypes observed is consistent with an hypothesis of restricted migration from a center of diversity. It is not consistent with any hypothesis of recent introduction via long distance transport of spores or other propagules. Depauperization of genetic variability in the outlying populations could be explained by severe bottlenecking, which would tend to fix the most common genotypes. However, there are no obvious causes for differential severity of bottlenecking among sites. All are highly moderated habitats of similar nature, there is no consistent pattern of non-variable sites being further west or north, and none is closer to the Pleistocene glacial terminus than the Ohio sites. All Ohio sites are in the same geological substrate and within a 40 km radius, yet the most peripheral sites here show the same fixation of genotypes as those in distant sites.

Although local reproduction by gemmae is abundant, it is clear that dispersal of the vegetative propagules is greatly restricted, as most

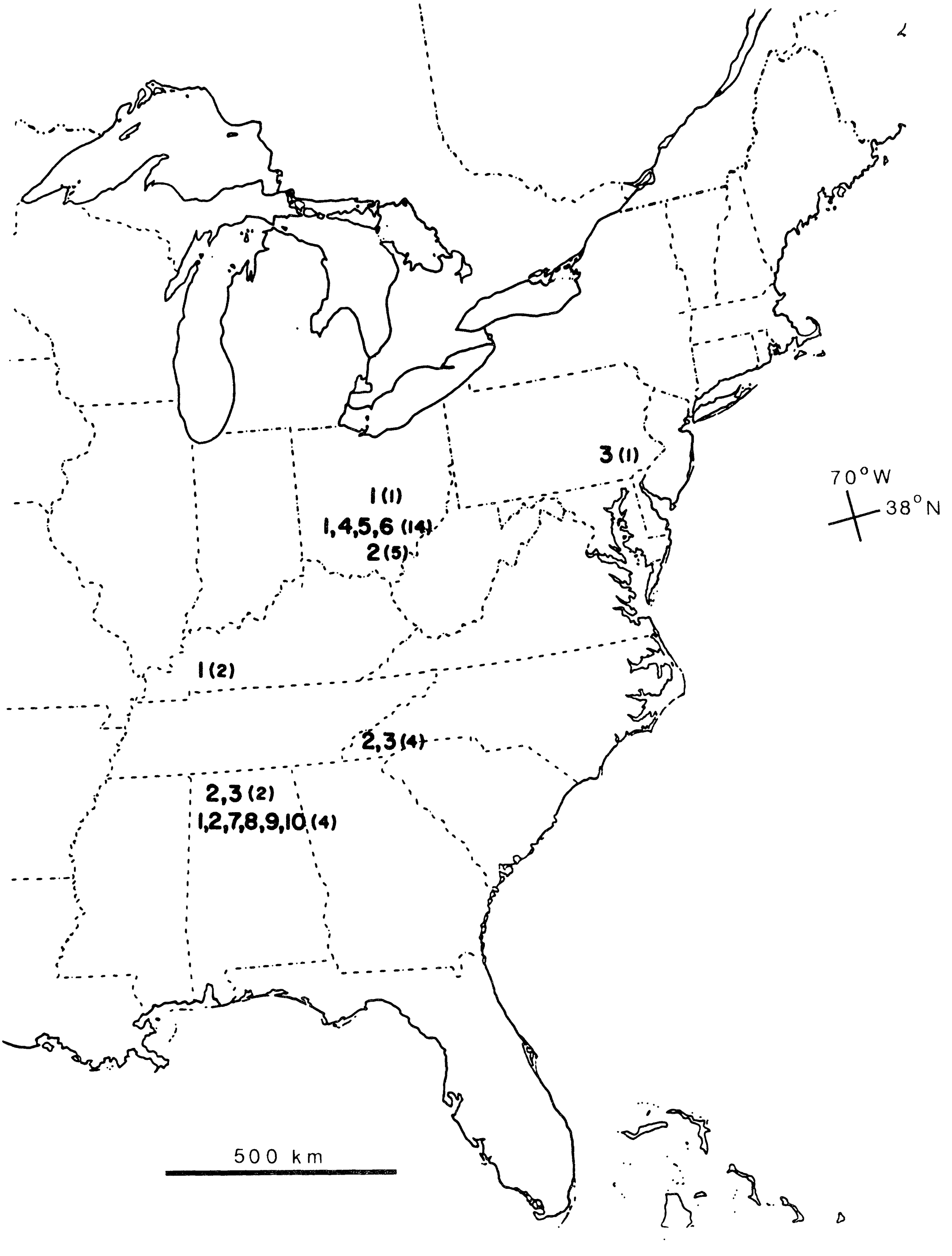


FIG. 10. The distribution of multilocus genotypes among sampled populations of the Appalachian *Vittaria* gametophytes in the eastern United States. Each number represents a distinct genotype. The number of populations represented by each genotype or series of genotypes is indicated in parentheses.

sites are monomorphic, and within polymorphic sites, most populations are monomorphic. This is not surprising, as the size of the propagules (0.2 to 1.0 mm) is too large to be easily dispersed by wind. Other evidence of restricted dispersal are the absence of the species from many apparently suitable habitats in the midst of populated habitats, the absence of plants from man-disturbed sites (road cuts, tunnels, etc.), and the restriction of the species to sites south of the maximum extent of Pleistocene glaciation or at most a few kilometers (<100) within glaciated territory (Cusick 1983; Parks and Farrar 1984). The latter is made more striking by the distribution of *Trichomanes* gametophytes, which co-occupy most *Vittaria* sites, but disregard the glacial boundary and extend northward nearly into Canada (fig. 1).

How long have Appalachian *Vittaria* plants persisted as independent gametophytes? At some time they must have originated from spores. Appalachian gametophytes possess morphological characteristics distinctive of *Euvittaria*, a small subgenus represented in tropical America by three common species. None of these species has sufficient isozymic similarity to the Appalachian plants to be considered conspecific (Farrar 1985). It is highly probable then that sporophytes of the Appalachian *Vittaria* no longer exist anywhere. Most likely they were eliminated by a cooling climate late in the Tertiary period. A minimum age for their disappearance is the time of Illinoian glaciation, for had they survived maximum glaciation of the Pleistocene, they would surely have tolerated subsequent climates. As sporophytes of other *Vittaria* species do not tolerate freezing, a much earlier date, perhaps the close of the Miocene, is likely.

The limited dispersal capacity of gametophytes suggests that their current distribution may reflect an earlier distribution of sporophytes, prior to the elimination of this stage of the life cycle. Alternatively, sporophytes may have been limited to the Ohio to Alabama Appalachian Plateau region with outlying gametophyte populations founded by long distance spore dispersal. The latter provides some explanation for predominance of the most common genotypes in outlying populations coupled with some site to site variation.

Another alternative, recognizing the possibilities of an origin through interspecific hybridization, is that sporophytes of this taxon never existed in large numbers. Rather they were generated sporadically over the range of one or both of the parental species and produced only sexually sterile gametophytes. Subsequent spread of the gametophytes would have been via vegetative propagules, again accounting for the dominant pattern of genotype fixation.

Evolution in Independent Gametophytes. How much evolution has occurred in the independent gametophytes since their sole reliance on vegetative reproduction, and what is their potential for continued evolution? Certainly they are adapted to their temperate habitats relative to tropical congeners, but this adaptation may have occurred while sporophytes were still present. One adaptation that has probably been enhanced through selection on the gametophyte stage is the failure to produce sporophytes. Individuals, and clones from those individuals, not producing sporophytes would be favored over those expending resources in production of sporophytes that could not survive.

Morphological variations among populations exist, in general robustness, in amount of gemma production, and especially in the number of cells per gemma (Farrar 1978). The first two types of variation seem to correlate with habitat suitability and are not maintained in culture. Differences in gemma form are maintained in culture and probably have a genetic basis, however there are no clear distributional patterns of variant types.

There is scant evidence to suggest that long-isolated populations have generated genetic novelty. Two alleles are unique to sites in Alabama. These may have arisen by selection of new mutations, but their occurrence in an area of high genetic diversity suggests that they could also have been retained from the original species' diversity. On the whole, it appears that isolated populations have not proceeded toward genetic divergence other than by fixation of different genotypes.

On the other hand, there is evidence that despite the absence of sexual reproduction, there has been little decay of genetic variation from the species as a whole. Although individual sites and populations are frequently monomorphic, polymorphism for the species is comparable to that of sexual, outcrossing species. The partitioning of variation into monomorphic populations is not unexpected for terrestrial plants relying on asexual reproduction and is in fact found to a similar degree in self-pollinated flowering plants.

The Nature of Asexual Species. Independent fern gametophytes offer insight into the nature of species and our ability to circumscribe them, especially with regard to asexually reproducing organisms. To discuss these issues with regard to the taxa discussed in this paper, it is important to consider separately three aspects: 1) the speciation process producing the asexual taxa; 2) maintenance of the species' integrity in the absence of sexual reproduction; and 3) the potential for continued evolution in the asexual state.

It seems clear that the causal process in the creation of independent gametophytes is their establishment and asexual reproduction in an environment where a normal life cycle (sporophyte production and maturation) cannot be completed. This can occur via transport of spores or gemmae beyond the range of the sporophyte or by climatic change eliminating sporophytes from all or part of an earlier range. Possible interspecific hybridization in the origin of the Appalachian *Vittaria* allows a combination of these modes. If the gametophytes are simply the survivors of an ancestral sporophyte–gametophyte species, their origin would more properly be considered to be by anagenesis rather than by speciation. In any case, it is likely that the independent gametophytes were established from many genetically different propagules and over a considerable period of time. Thus at the initiation of their asexual existence, the independent gametophytes probably contained a broad subset of the genetic variation present in their sexual progenitors.

What happens to the genetic variation in the absence of gene flow? Evidence from the Appalachian *Vittaria* (and other studies, e.g., Pleasants and Wendel 1989) indicates that variation tends to be repackaged into monomorphic populations (clones ?) but is not necessarily depleted with respect to the taxon as a whole. The data from *Vittaria* suggest that a great variety of genotypes have been retained (10 distinct multilocus genotypes were found in 34 populations examined) and that allelic polymorphism is comparable to sexual species. Intuitively these results are not surprising. Given this retention of genetic variation, the more interesting question is why hasn't diversity among the gametophyte populations *increased* in the absence of gene flow to counter effects of selection?

Although differences exist from population to population, plants from throughout the range are still recognizable as this taxon, and for the most part, the same alleles exist throughout the range (and these alleles *are* variable within the genus). In these plants with short generation times (<1 year in culture) and countless individuals, why hasn't somatic mutation generated an abundance of novel genotypes? Why hasn't selection recognized different superior genotypes in different parts of this taxon's geographically large and climatically diverse range? Absence of such differentiation, even after millions of years, strongly suggests that factors other than gene flow are important in maintaining species' integrity and cohesiveness. As pointed out by Mishler (this symposium and 1985; Mishler and Brandon 1987; Mishler and Donoghue 1982) such factors may include developmental canalization and stabilizing selection, which contribute to a genetic inertia not easily altered with or without gene flow.

What is the potential for the independent gametophytes (or other asexual organisms) to spawn new species? As pointed out above, there seems to be no lack of genetic variation on which selection may work, yet the Appalachian *Vittaria* gametophytes show no evidence of further speciation. Information at hand does not allow determination of whether this is due to genetic inertia or to fundamental differences between sexual and asexual organisms in the ability to evolve. On the other hand, the longevity of the Appalachian *Vittaria* argues against an assumption that asexual species are necessarily short-lived evolutionary dead ends.

Species Definition. Should the independent gametophytes be recognized as "species?" What definition of species would appropriately recognize such assemblages of organisms? No evidence suggests that the group of plants comprising the Appalachian *Vittaria* are any less cohesive or less distinct than sexual *Vittaria* taxa currently recognized as species. If all plants of the Appalachian *Vittaria* share a common origin from the same parental species, as suggested by allozyme evidence, then "species" is the most informative term to apply to this evolutionary unit. They exist as an entity recognizable in terms of the characteristics used to recognize sexual species in this genus, so far as this can be done in the absence of sporophytes and measures of reproductive compatibility. Species recognition of the Appalachian *Vittaria* is both useful as a taxonomic handle, and informative as to the evolutionary process causal in their formation. It would not be useful or informative to give species or other formal recognition to each of the individual genotypes among the Appalachian *Vittaria* any more so than it would be to do so among genotypes of sexual species. Neither is it informative to withhold formal recognition of the Appalachian *Vittaria* on the basis of its failure to meet a biological species criterion; i.e., real or potential gene flow.

A species concept is needed that recognizes integrity based on criteria other than, or in addition to, reproductive status. A concept that for the most part satisfactorily accepts species recognition of taxa such as the Appalachian *Vittaria* is the phylogenetic concept of species advocated by Mishler and others (Mishler 1985; Mishler and Brandon 1987; Mishler and Donoghue 1982) where species are monophyletic groups, and species-ranking criteria are "based on the causal agent judged to be most important in producing and maintaining distinct lineages in the group in question" (Mishler and Brandon 1987).

Independent fern gametophytes suggest two additional criteria for a species definition. These have to do with the extent of occurrence of the plants in time and space. It is not difficult to imagine that independent gametophytes of many gemmiferous species are generated sporadically but soon die out. In fact, small populations of two tropical species, *Trichomanes holopterum* Kunze and *Vittaria graminifolia* Kaulf., have been documented in Florida and Louisiana (Farrar and Wagner 1968; Farrar, unpubl. data). These populations are maintained locally by asexual gametophytic reproduction and show no evidence of broader establishment, although the habitat in which they occur is extensive. The North Carolina population of *Grammitis nimbata* is a similar case. Each of the populations discussed here was identifiable to a known species, but had this not been possible, would they have warranted recognition as new species? I think not, because they do not show evidence of permanent establishment in an ecological niche. One can imagine occurrences less permanent than these. I suggest that species recognition be given only when taxa (meeting other species criteria) occur repeatedly in a given habitat or set of habitats over a geologically or climatically circumscribed area. That is, taxa that have attained a degree of predictability in their occurrence in a particular habitat.

Independent fern gametophytes promise additional insights into processes of evolution in asexually reproducing organisms. Continued studies of the Appalachian *Vittaria* and similar studies of the Appalachian *Trichomanes* can further address genetic and morphological differentiation under totally asexual reproduction. Studies currently underway should identify the sister species of the Appalachian *Vittaria* and more accurately compare genetic variability between it and sexual species of *Vittaria* in the American tropics. It will be of particular interest to examine the genetic structure of *Vittaria* gametophyte populations in the tropics in areas where sexual reproduction occurs. It is as yet unclear what the significance of gemmiferous gametophytic reproduction is to the biology of these plants or why it has evolved in three and only three families of ferns.

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