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A STUDY OF SPATIAL FEATURES OF CLONES IN A POPULATION OF BRACKEN FERN, PTERIDIUM AQUILINUM (DENNSTAEDTIACEAE)[1]

JAMES C. PARKS[2] AND CHARLES R. WERTH

Department of Biology, Millersville University, Millersville, Pennsylvania 17551; and Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409

Allozymes were used to study the spatial attributes of clones (genets) comprising a population of *Pteridium aquilinum* (L.) Kuhn var. *latiusculum* (Desv.) Underw. ex Heller (bracken fern) in the Appalachian Mountains of Virginia. Ramets (individual leaves) were sampled at intervals of 165 m (or less in some cases) and genotyped for six polymorphic isozyme loci to produce a map depicting the spatial patterning of genets. Forty-five distinct genotypes were detected, 14 of which were sampled more than once, five of these more than four times. Genotype proportions at all loci except *Pgm-1* conformed to Hardy-Weinberg expectations. Estimation of allele frequencies in the population used a "round-robin" approach that removed any upward bias for rare alleles that distinguish genets. Based on these allele frequencies, the probability that each genotype could arise independently and be sampled was calculated. Some genotypes represented by widely separated ramets had very low probabilities of re-encounter, documenting fragmentation of widespread genets. Coarse-scale mapping indicated a population consisting of many small genets and a few very large ones (up to 1,015 m across). The larger genets tended to be irregular in shape, fragmented, and overlapping. Fine scale mapping of individual fronds in spatially discrete patches of ramets revealed extensive intergrowth of genets, indicating that *P. aquilinum* exhibits a "guerrilla-type" clonal morphology.

Clonal plants present special problems for analyses of populations because single genetic individuals (genets) may comprise numerous morphological units (ramets) that appear distinct (Silander, 1985a; Maddox et al., 1989). Determinations of population characteristics such as population size, recruitment, mortality, levels of polymorphism, and conformance to Hardy-Weinberg equilibrium all are contingent upon observations on genetically distinct individuals. Thus, it is important to discriminate among genets in clone-forming species, allowing ramets belonging to each clone to be recognized and the spatial configurations of the clones to be visualized (Cook, 1983).

For certain slow-growing perennials, information on clonal extent may be obtained through excavations (Edwards, 1984; Reinartz and Popp, 1987). For a majority of species, however, genet recognition is facilitated by molecular markers such as isozymes (Huenneke, 1985; Sheffield, Wolf, and Haufler, 1989; Bayer, 1990; Aspinwall and Christian, 1992; Parker and Hamrick, 1992), DNA fingerprints (Nybom and Schaal, 1990; Turner et al., 1990), or RAPD markers (Smith, Bruhn, and Anderson, 1992). In fact, molecular techniques represent the only reasonable approach for confident assignment of ramets to genets in rapidly expanding and fragmenting clonal plants.

The bracken fern, *Pteridium aquilinum* (L.) Kuhn, is an aggressive, often weedy species that spreads via rhizomes. Because of bracken's importance as a weed it has become the most intensively studied pteridophyte (Smith and Taylor, 1986). The rhizome system of bracken comprises a three-tiered hierarchy, with thick, deep rhizomes connected to thinner, shallower frond-bearing rhizomes by rhizomes of intermediate thickness (Conway, 1957). Growth rates of as much as 2.1 m in one season have been reported (Fletcher and Kirkwood, 1979). Although spore-releasing fronds are frequently produced, sexual reproduction of bracken, as evidenced by juvenile sporophytes, is seldom observed in nature (Page, 1976; Wolf, Haufler, and Sheffield, 1988). Thus, bracken populations are maintained principally by long-lived and rapidly spreading genets.

Various studies have addressed the clonal structure of populations in bracken. Oinonen's (1967) attempts to determine the size of *P. aquilinum* genets based on morphological features and tracing of rhizomes resulted in underestimates of clonal size (Sheffield, Wolf, and Haufler, 1989). Although morphological differences among genets in a *Pteridium* population proved inadequate for reliable recognition, recent studies successfully used multilocus allozyme genotypes to establish genet identity. These studies document the great variation in bracken clone size (Sheffield, Wolf, and Haufler, 1989), indicate that *P. aquilinum* is an outcrossing, functionally diploid species maintaining moderate levels of genetic polymorphism (Wolf, Haufler, and Sheffield, 1987, 1988), and implicate high levels of gene flow that minimize among-population divergence (Wolf, Sheffield, and Haufler, 1991).

The present study also used allozymes to determine in finer detail some spatial features of genets in a southern Appalachian population of *P. aquilinum* var. *latiusculum* (Desv.) Underw. ex Heller. In particular, we sought to determine the nature of interfaces between genets by examining the genetic composition of dense patches of ramets and to evaluate the extent of genet fragmentation by sampling in two dimensions across a densely colonized open area and a more sparsely occupied adjacent forest.

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[2] Author for correspondence (FAX: 717-872-3985).

As a corequisite for evaluating clonal extents, an approach was developed to estimate confidence levels for genet identity based on allozymes.

MATERIALS AND METHODS

Study site—The study area (Fig. 1) is in the vicinity of the upper portion of Doe Creek Ravine, near Mountain Lake Biological Station, Pembroke, Virginia in the ridge and valley province of the Appalachian Mountains. The greater portion of the area is on the WNW-facing slope of Salt Pond Mountain, which reaches 1,328 m (4,363 feet) altitude on the SE edge of the study site; a smaller portion is on the S-facing slope of adjacent Beanfield Mountain. The change in relief from the SW side of the site is ca. 390 m (1,100 ft). The lower portion of the slope is cloaked in mixed mesophytic forest which intergrades with an oak (*Quercus* spp.), maple (*Acer* spp.) forest upslope, and a rocky sandstone summit dominated by heaths (Ericaceae). The trees in the mixed mesophytic forest may exceed 60 cm diameter at breast height (DBH), but the oaks upslope rarely reach 30 cm DBH. Most of the area has been logged at least once, a portion most recently in the 1930s. A few charred stumps and old burn scars on tree trunks evidence fire within the last 75 years, but no signs of recent fires were found on the site.

Two electrical transmission line clearings, supporting luxuriant bracken growth, were included in the study area. A smaller line, built in the 1920s, services The Mountain Lake Hotel at the summit and was the site of intensive sampling. Seven poles, spaced about 100 m apart, were used as landmarks in this area to mark 100-m × 30-m quadrats numbered I–VII. A wider clearing for a high voltage line was designated as the southern edge of the study area. Two paved roads pass through and intersect within the study site: Route 700, originally a pre-Civil War wagon road, and Route 613, a more recent road that was unpaved until the mid-1970s.

Robust fronds of *P. aquilinum* were found in the powerline swaths in discrete, dense clusters (patches) that varied in average width from 0.1 to 30 m. In contrast, fronds in the open oak forest were widely scattered and typically smaller. Despite diligent searching, no *P. aquilinum* was found in the dense mixed mesophytic forest NW and SE of the small powerline or S of the large transmission line. Bracken was also excluded by human activity from a sizable area between Routes 700 and 613 near their intersection. Bracken extended downslope in both powerlines well below the point where it ceased to occur in the flanking mesic forest, which was apparently unsuitable for bracken growth. However, in the smaller powerline there occurred a 130-m gap with no bracken that included all of quadrat IV (see Fig. 1).

Field sampling—Fronds were chosen for sampling (246 in all) using varied strategies for different scales of study. Initial sampling was carried out in 1989 on 34 patches of bracken within the smaller powerline swath to obtain preliminary insights into the number and spatial distribution of genets. Patches were defined as clusters of ramets for which no ramet was greater than 2 m distant from a patch member. To determine whether each patch consisted of a single genet (cf. Wolf, Haufler, and Sheffield, 1988), one to four fronds, depending on the size of the patch, were taken from each patch margin.

To evaluate fine scale spatial relationships between genets, two patches from powerline quadrat II, known from the preliminary survey to be genetically heterogeneous, were chosen for detailed analysis. The position of every leaf in these patches was recorded (99 in the larger patch and 20 in the smaller), and allozyme genotypes were determined for a majority of these (79) to determine genet assignment. The relative position of each ramet was then plotted on a map (Fig. 2).

In 1990 a more extensive two-dimensional sampling strategy was employed to estimate the size and distribution of genets in the wooded area surrounding the powerline and to maximize the number of genotypes available for calculating allele frequencies. After an initial transect with a 15-m sampling interval yielded only a single genotype, the sampling interval was increased by an order of magnitude. Samples were taken from the frond nearest to transect points at 165-m intervals along compass bearings. Parallel transects, oriented along elevational contours, were separated also by 165 m, resulting in a two-dimensional grid of sample points. The 165-m interval is approximately one-half the sample interval used by Wolf, Sheffield, and Haufler (1991). The somewhat rugged terrain resulted in some irregularity in placing of sample points, especially in the N and E parts of the study area. Each frond sampled was tagged, its position noted (along with permanent landmarks such as powerline poles and trees), and a small portion of leaf tissue removed for electrophoresis. Samples were placed into plastic bags, transported to the laboratory in an insulated container, and kept refrigerated until processing the next day. Subsequently, the position of each sample was plotted (by genotype number) on a map (Fig. 1).

Detection of genotypes—Starch-gel electrophoresis was used to determine allozyme genotypes for nine enzyme systems coded by 12 scorable loci. Homogenates were prepared using the pH 7.5 "microbuffer" of Werth (1985) containing 5% polyvinylpyrollidone (mw 40,000) and 1% mercaptoethanol. These were loaded onto 12% starch gels and electrophoresed. Malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), isocitrate dehydrogenase (IDH), and shikimate dehydrogenase (SKDH) were resolved on the morpholine-citrate buffer system described in Werth (1991). Phosphoglucomutase (PGM) and glucose-phosphate isomerase (GPI) were resolved on buffer system 6 of Soltis et al. (1983). Hexokinase (HK), leucine aminopeptidase (LAP), and aspartate amino transferase (AAT) were resolved on the lithium-hydroxide system (Werth, 1985). Staining schedules were similar to those of Soltis et al. (1983) but employed the "zymecicle" methodology of Werth (1990). Isozyme band patterns corresponded closely to those previously illustrated (Wolf, Haufler, and Sheffield, 1987; Wolf, Sheffield, and Haufler, 1991) and were readily interpreted as diploid genotypes at individual loci (see Wolf, Haufler, and Sheffield, 1987 for discussion of the ploidy of bracken). Allozymes, and the alleles encoding them, were designated by numbers with the lowest number representing the most anodal migration. Allele identity was controlled by running individuals with known genotypes on each gel.

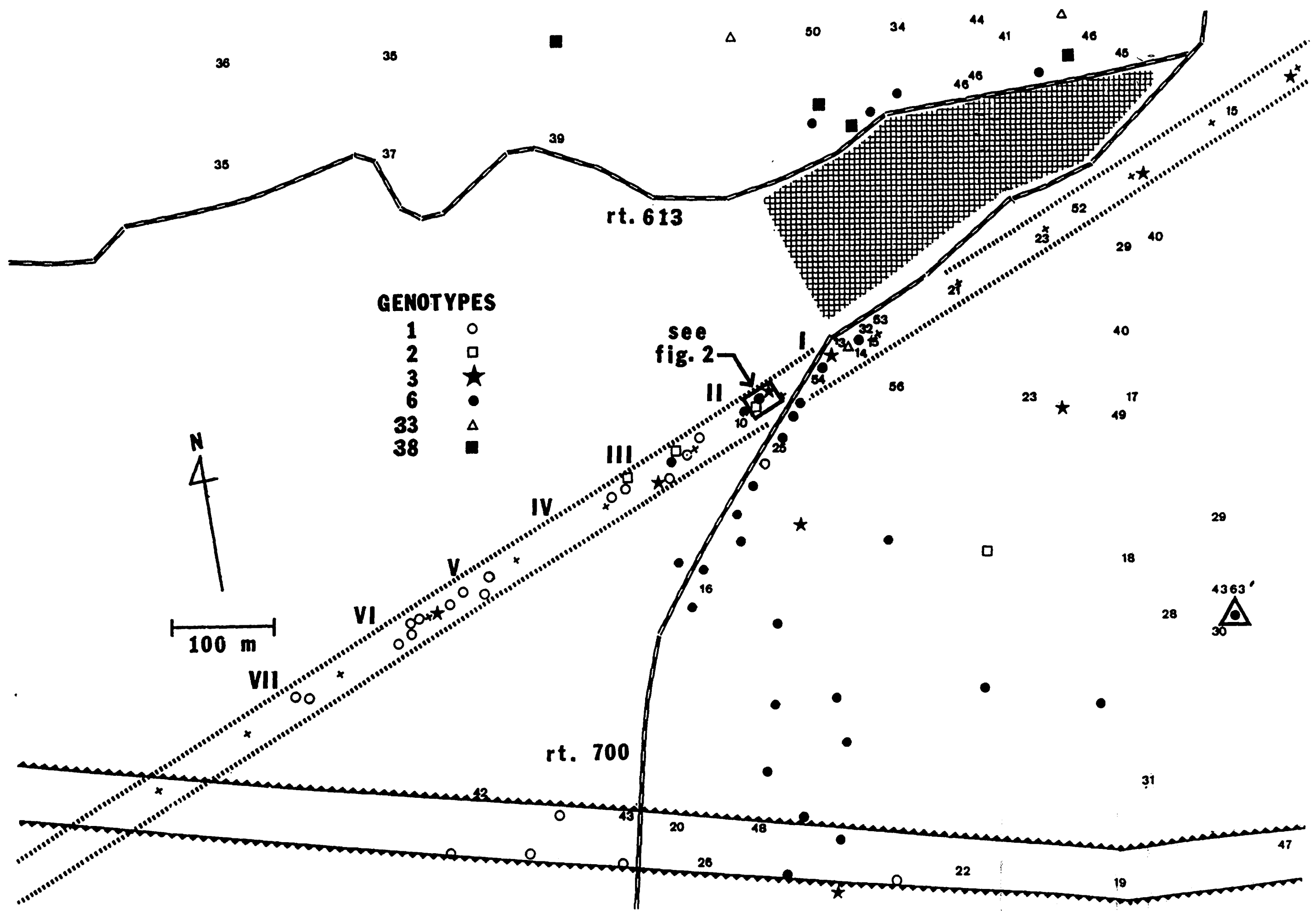

Fig. 1. Spatial distribution of genotypes in the Doe Creek, Virginia population of *Pteridium aquilinum* as based on coarse scale sampling. Genotypes occurring for fewer than four samples are indicated by genotype numbers (see Table 2). Those occurring for four or more samples are indicated by symbols. Narrow and wide electric transmission lines are delimited by dotted and scalloped lines, respectively. The study area is wooded except for roads, powerline clearings, and the shaded portion of the area between the two roads. Bracken is absent or exceedingly rare in areas with no sample points. Poles in small powerline clearing, indicated by "+," mark quadrat boundaries. Quadrats cited in text are indicated with Roman numerals.

RESULTS

Population genetic analysis—Sampling for the nine enzymes assayed yielded six consistently scorable polymorphic loci that could be used for genet discrimination at the study site: *Hk, Lap, Mdh-2, Pgm-1, Pgm-2, 6-Pgd-2*. Not all enzymes previously examined in *P. aquilinum* were included in the present study; therefore, calculations of levels of polymorphism or of genetic identities with other populations would be inappropriate. Many of the polymorphisms encountered in the present study appear comparable to allele arrays reported for a New Hampshire population of *P. aquilinum* (Wolf, Sheffield, and Haufler, 1991), but no attempt has yet been made to compare allele identities between the two regions.

Our sampling of the bracken population resulted in detection of 45 different multilocus isozyme genotypes, each presumably representing at least one separate genet (barring mutation). These were numbered consecutively as encountered, although resolution of some initial uncertainties led to some gaps in the numbering sequence. As expected, separate ramets often possessed identical genotypes. To evaluate whether these actually represented the same genet, an approach was developed by CRW to estimate the probability that identical genotypes could result from independent formation of zygotes.

This approach is conditioned on the following assumptions: 1) each different genotype encountered results directly from a zygote rather than alteration of an original genotype through mutation (while this assumption may not be entirely valid, it is approximately so, as mutation rates for individual loci are small [Mukai and Cockerham, 1977]); 2) mating is random; and 3) genotypes at separate loci are independent, i.e., loci assort independently and there is no recombinational disequilibrium among haplotypes. Adherence to assumptions 2 and 3 (violations may occur in clonal populations—see Cook, 1983) can be tested using allozyme data. For example, the assumption of random mating is justified in that the present bracken population is in Hardy-Weinberg equilibrium (see below). Given these assumptions, the probability that a zygote acquires a given diploid genotype, designated

TABLE 1. *Estimates of allele frequencies at six polymorphic loci in the Doe Creek area population of* P. aquilinum. *Round-robin estimate is based on subsampling as described in text. Unadjusted estimate is based on frequencies of 45 distinct genotypes (see Table 2)*

Locus	Allele	Frequency estimate	
		Round-robin	Unadjusted
Hk	1	0.167	0.189
	2	0.795	0.767
	3	0.038	0.044
Lap	1	0.190	0.211
	2	0.798	0.778
	3	0.012	0.011
Mdh-2[a]	0	0.011	0.011
	1	0.967	0.967
	2	0.022	0.022
Pgm-1[b]	2	0.279	0.289
	3	0.529	0.489
	4	0.162	0.200
	5	0.029	0.022
Pgm-2	1	0.128	0.111
	2	0.385	0.367
	3	0.487	0.522
6-Pgd-2	1	0.526	0.500
	2	0.051	0.045
	3	0.423	0.455

[a] Allele "0," anodal to allele "1," was discovered after allele "1" had been designated.

[b] Allele "1" was found in initial screening for polymorphisms that included samples outside the study area, but was never encountered within the study area.

p_{gen}, is calculated as the product of locus genotype probabilities, thus

$$p_{gen} = \left(\prod p_i\right)2^h \qquad (1)$$

where p_i is the frequency in the population of each allele (two per locus) represented in the genotype and h is the number of loci that are heterozygous.

However, estimation of allele frequencies, which involves tallying allozyme genotypes of individuals, is problematic in that these genotypes have been used to determine the separateness of individuals. Circular reasoning emerges when only individuals with different genotypes are entered into the data pool, with the probable result of overestimating the frequency of rare alleles that would mark different genets. This difficulty was circumvented by a subsampling approach. To estimate allele frequencies at a given locus, all *other* loci (i.e., excluding the locus in question) were used to discern individuals and thus define a subsample of the 45 distinct genotypes. From groups that had the same genotype for the subset of loci used, only the first encountered was included in the data pool. Genotypes of individuals comprising the subsample were then used to calculate allele frequencies for the locus in question. This procedure was repeated for each locus in a "round-robin" fashion.

Application of this round-robin method to the present bracken data set resulted in exclusion of six individuals for purposes of calculating *Hk-2* allele frequencies, three for *Lap-2,* none for *Mdh-2,* 11 for *Pgm-1,* six for *Pgm-2,* and five for *6-Pgd-2.* Allele frequencies determined by the round-robin method are very similar to those obtained

TABLE 2. *Forty-five distinct multilocus genotypes of* Pteridium aquilinum *encountered in the study area and their estimated probability of re-encounter*

Genotype number	Genotype at six enzyme loci						No. of ramets	Probability of genotype occurrence	Probability of second encounter
	Hk-2	Lap-2	Mdh-2	Pgm-1	Pgm-2	6-Pgd-2			
1	12	12	12	23	22	11	54	0.00004	0.00186
2	22	22	11	22	33	11	36	0.00192	0.08294
3	23	22	11	33	13	13	10	0.00056	0.02483
6	22	22	11	33	23	11	49	0.01093	0.39007
10	22	22	11	24	33	11	1	0.00223	0.09568
13	22	12	11	44	33	11	3	0.00031	0.01379
14	22	23	11	33	22	33	2	0.00008	0.00377
15	22	12	11	23	33	33	5	0.00224	0.09619
16	12	22	11	33	13	11	1	0.00153	0.06642
17	22	22	11	44	33	12	1	0.00013	0.00564
18	22	22	11	33	22	33	1	0.00279	0.11827
19	22	22	01	23	23	11	1	0.00026	0.01173
20	22	22	12	23	22	11	1	0.00021	0.00929
21	12	22	11	33	22	33	1	0.00117	0.05147
22	11	22	11	33	23	33	1	0.00031	0.01395
23	12	22	11	44	33	12	2	0.00005	0.00237
25	12	22	11	33	33	11	1	0.00290	0.12264
26	12	12	11	33	23	11	1	0.00219	0.09378
28	12	22	11	23	33	13	1	0.00493	0.19925
29	22	12	11	22	33	33	2	0.00059	0.02630
30	12	22	11	24	23	13	1	0.00238	0.10188
31	12	22	11	33	23	13	1	0.00738	0.28358
32	22	22	11	22	23	11	1	0.00304	0.12801
33	22	22	11	33	33	12	4	0.00134	0.05856
34	22	22	11	33	22	13	1	0.00695	0.26926
35	22	22	11	44	22	13	2	0.00065	0.02890
36	22	22	11	33	33	33	1	0.00447	0.18255
37	22	22	11	24	22	13	1	0.00224	0.09615
38	12	22	11	44	22	13	4	0.00027	0.01224
39	22	22	11	24	33	33	1	0.00144	0.06294
40	22	22	11	35	13	23	2	0.00006	0.00279
41	22	12	11	34	13	13	1	0.00170	0.07388
42	12	11	11	33	23	13	1	0.00042	0.01866
43	22	22	11	22	12	33	1	0.00052	0.02298
44	22	22	11	22	23	13	1	0.00489	0.19790
45	23	11	11	33	11	13	1	0.00001	0.00019
46	23	11	11	35	13	13	3	0.00001	0.00016
47	12	12	11	22	33	11	1	0.00038	0.01716
48	12	11	11	22	12	33	1	0.00001	0.00055
49	22	22	11	44	23	13	1	0.00165	0.07154
50	22	22	11	33	23	13	1	0.01757	0.54972
52	22	12	11	22	22	13	1	0.00092	0.04058
53	12	12	11	33	33	—	1	0.00600	0.23714
54	12	12	11	23	33	33	1	0.00094	0.04157
56	23	12	11	34	13	13	1	0.00016	0.00730

if all genotypes are used indiscriminately (Table 1). Because of the substantial genetic diversity of *P. aquilinum,* especially in North American populations (Wolf, Sheffield, and Haufler, 1991), there were relatively few duplicate genotypes even when one of the loci was removed from consideration. In clonal species possessing lower levels of genetic diversity it is likely that the calculated frequency of rare alleles (that would allow discovery of separate genets) would be exaggerated upward if the round-robin approach is not used.

Values for p_{gen} (genotype probabilities), based on the adjusted allele frequencies and using equation 1, are <0.05 in all cases with most <0.01 (Table 2). These p_{gen} values represent the probability that two consecutive ramet samples that belong to different genets would by chance have the same genotype. As these values are small for all ge-

notypes, clusters of two or more closely spaced ramets of identical genotype are assumed to belong to the same genet.

Different considerations apply to distantly spaced ramets with identical genotypes. The probability that a given pair (or larger number) of remote genetically identical ramets are separate genets is equivalent to the chance of finding a second individual with that genotype, and therefore must take into account both the p_{gen} and the number of "trials," i.e., separate genets for which the genotype was determined. This probability may be calculated using the binomial expression

$$\sum_{x=n}^{G} \frac{G!}{x!(G - x)!}(p_{gen})^x(1 - p_{gen})^{G-x} \qquad (2)$$

where G is the number of separate genets genotyped and n is the number of separated fragments with identical genotype to some previously encountered ramet. This expression sums probabilities of re-encountering n through G samples of identical genotype. The probability for a second encounter only, i.e., n = 1, designated p_{se} may be more simply calculated as

$$p_{se} = 1 - (1 - p_{gen})^G \qquad (3)$$

i.e., the probability the genotype is *not* encountered subtracted from unity. Although G cannot be known with certainty because of circular reasoning, in the present case it can be approximated closely as simply the number of distinct genotypes obtained, i.e., 45. For clonal species possessing much less genetic diversity, the number of distinct genotypes would represent a substantial underestimate of the number of separate genets actually sampled, as a fair number of genets would possess identical isozyme genotypes.

Values of p_{se} (based on equation 3, with G = 45) show considerable variation among genotypes (Table 2). Those genotypes with $p_{se} < 0.05$ (slightly more than half) may be assumed with greater than 95% confidence to comprise a single genet. Those genotypes represented by remote ramets but with $p_{se} > 0.05$ (discussed below) cannot be assumed to comprise a single genet.

The array of 45 genotypes was tested with chi-square analysis for conformance to Hardy-Weinberg proportions using the BIOSYS computer program (Swofford and Selander, 1981). Individual genotype data were entered into the input file, so that the allele frequencies used to compute expected proportions were based on all 45 genotypes, rather than on round-robin subsamples as described above. Five of the loci conformed to Hardy-Weinberg expectations, consistent with previous findings on *P. aquilinum* (Wolf, Sheffield, and Haufler, 1991). The single locus that did not conform, *Pgm-1,* showed a highly significant deficit of heterozygotes ($P < 0.001$). This result might reflect population substructuring, i.e., Wahlund effect (Wahlund, 1928). This explanation would not be consistent with the complete lack of population substructuring found in *P. aquilinum* var. *aquilinum* over a huge area in Britain (Wolf, Sheffield, and Haufler, 1991).

Spatial features of clones—Mapping of genotyped ramet samples (Fig. 1) indicated that the bracken population consists of a few very large genets and numerous smaller

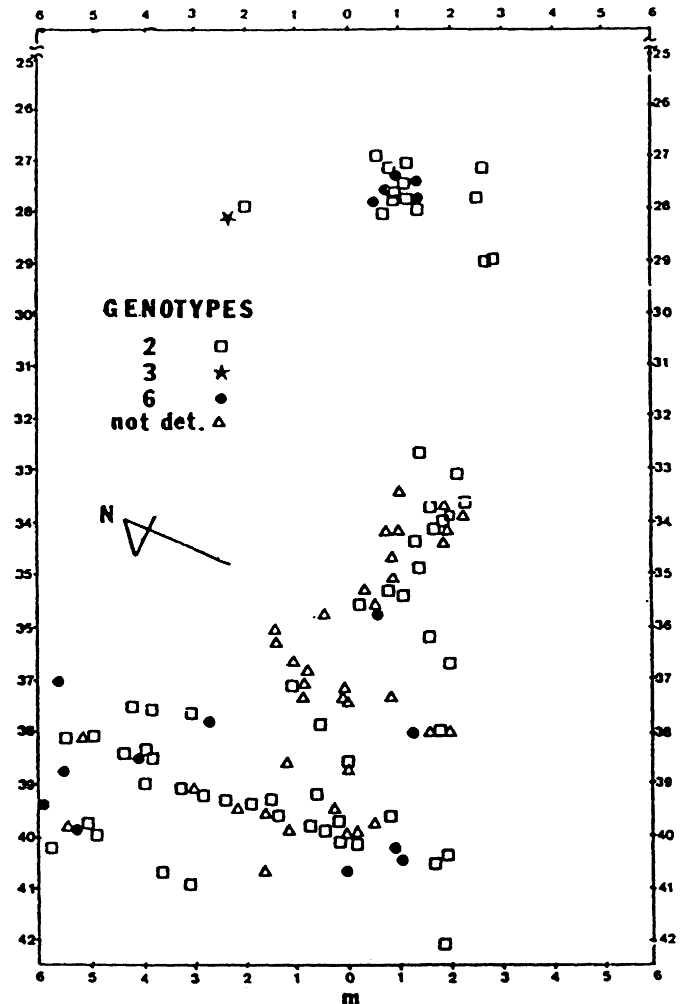

Fig. 2. Fine scale spatial distribution of genotypes comprising two patches of *Pteridium aquilinum* in the Doe Creek, Virginia population (small powerline quadrat II). All ramets are plotted, those not genotyped indicated by triangles.

ones. Of the 45 genotypes detected, 14 were sampled more than once (Table 2), 11 of these during coarse scale sampling. Seven were represented by four or more samples (genotypes 1, 2, 3, 6, 15, 33, and 38). Ramets assignable to genets 1, 2, and 6 are somewhat overrepresented among the samples because portions of these clones occurred within the intensive sampling area; nevertheless, they were also represented repeatedly in the extensive sampling area.

The larger genets appear to be irregular in shape, to be fragmented to some degree, and to overlap with other genets. Most of the smaller genets occur within the range of the larger genets. The most expansive genet detected in this study, number 3 ($p_{se} = 0.025$), was encountered as isolated solitary ramet samples in the small powerline, at the edge of the larger powerline, and in the woods between (Fig. 1). This genet included sample points separated by 1,015 m (straight line distance measured on a topographic map between points plotted from field data).

Genet 1 ($p_{gen} = 4.15 \times 10^{-5}$, $p_{se} = 0.0019$), although smaller in maximum extent (500 m), was represented by numerous ramet samples, principally in the lower portions of both powerline clearings where it predominated.

Of 23 ramets sampled from the 12 patches in quadrats V–VII of the small powerline, only one was not genet 1.

Genotype 6 was encountered as two clusters of ramet samples, one occupying an extensive area (maximum extent 500 m) in the woods east of Route 700 and in the smaller powerline, and the other consisting of a linear array of four samples (maximum extent 240 m) in the sapling woods immediately north of Route 613. This genotype, consisting exclusively of common alleles, was one of the most probable, but its p_{gen} value of 0.011 makes it unlikely that either cluster of spatially associated ramets includes more than one genet. Although it is possible that the two clusters comprise a single genet interrupted by the clearing between the two roads, the high probability of second encounter (p_{se} = 0.39) does not allow this to be asserted with statistical confidence.

Additional large genets include numbers 2, 33, and 38 (see Fig. 1 for ramet distribution and Table 2 for p_{se}). The average of the maximum distance between sampled ramets of the ten genets encountered two or more times during coarse scale sampling is 379 m (genotype number 6 was excluded from this calculation because of uncertainty as to how many genets it comprises). This average size is close to the maximum of 390 m previously reported for a bracken genet by Sheffield, Wolf, and Haufler (1989).

The variation in genet size, integrity, and shape resulted in localized differences in the degree to which patches of bracken were heterogeneous. Extensive areas were occupied exclusively, or nearly so, by a single genet (especially numbers 1 and 6, see Fig. 1). Other regions consisted of numerous genets in close proximity, notably the small powerline in the vicinity of its intersection with Route 700. Eight genotypes (numbers 1, 2, 3, 6, 10, 13, 14, and 15) occurred in quadrats I–III, and a number of the spatially distinct patches were found to consist of more than one genet. Two of these heterogeneous patches were thoroughly mapped (see Materials and Methods) to determine the pattern of genet interfaces on a fine scale (Fig. 2). The ramets in these patches were found to be comprised of genotypes 2 and 6 extensively interspersed. Genotype 3 was represented by a single ramet at the west edge of the smaller patch.

DISCUSSION

Clonal organisms vary considerably in the spatial configurations of genets, depending on the means, extent, and rate of their spread. At one extreme are genets that spread extensively, resulting in wide dispersion of ramets that are thereby juxtaposed with different genets ("guerrilla-type" clones), while at the other extreme are those with restricted spread resulting in tighter clustering of ramets that exclude foreign genets ("phalanx-type" clones) (Lovett Doust, 1981; Silander, 1985a). Examples of principally "phalanx-type" clones include *Xanthoxylum americanum* Mill., *Rhus glabra* L., and *Cornus racemosa* Lam. (Reinartz and Popp, 1987), while examples of principally "guerrilla-types" include *Aralia nudicaulis* L. (Edwards, 1984). Spatial analysis of bracken genets in the Doe Creek population (Figs. 1, 2) indicates that their shapes are generally irregular, each is somewhat fragmented, and they often overlap each other spatially. The fine scale study of discrete ramet patches indicates that, in regions where more than one genet is present, patches cannot be assumed to be comprised of single genets, nor are boundaries between genets sharp. Rather, there may be extensive intergrowth among genets in some patches, and some genets may be represented by small, isolated ramet occurrences. These results suggest that *P. aquilinum* in our study area is principally a "guerrilla-type" clone former.

Rapid growth of *P. aquilinum* via rhizomes may result in extensive spread into favorable habitats. Subsequent events such as the intergrowth of genets, fire, and human activities may cause fragmentation and overlap of clones, as was detected by both large and fine scale sampling in our study area. However, some genets may exclusively occupy larger areas, at least for a time, as evidenced by genet 1 in the lower 300 m of the small powerline.

Previous studies indicated a tendency for bracken genets to be smaller and more numerous in the Northeastern United States populations than in British populations (Sheffield, Wolf, and Haufler, 1989). Although certain genets in our study area appear larger than any thus far detected in Britain (Sheffield, Wolf, and Haufler, 1989), the isolated representation of most genotypes sampled suggests a population structure in this southern Appalachian site similar to that in New England. Most clones appear to be restricted in extent, while a few have achieved great dimensions. The encounter of distant ramets of extensive genets in the present study resulted from a two-dimensional sampling strategy employed within a limited area (as suggested by Sheffield, Wolf, and Haufler, 1989). Because of the irregular shape of large genets (Fig. 1), the transect sampling strategy used in previous studies (Sheffield, Wolf, and Haufler, 1989; Wolf, Sheffield, and Haufler, 1991) had limited opportunity to re-encounter dispersed fragments of such genets.

Ellstrand and Roose (1987) compared clonal diversity detected in various studies based on the number of clones (genets) detected divided by the sample size, or proportion distinguishable (PD). The mean value of PD for 21 species surveyed was 0.17. Using data reported by Sheffield, Wolf, and Haufler (1989), the average PD of three British bracken populations is 0.20 and that of four North American populations from New England is 0.65. The present study detected 45 genotypes in a sample of 89 ramets (this excludes ramets sampled in the fine scale study) yielding a PD value of 0.51, similar to that of the New England populations. The high PD values for the North American bracken populations reflect both the wide spacing of samples in these studies and the substantial levels of genetic variability in North American bracken, as evidenced by the high mean heterozygosity ($H = 0.217$) of a New Hampshire population (Wolf, Haufler, and Sheffield, 1988). These apparent differences between North American and British bracken may reflect differing ecological and historical circumstances encountered by these populations. They also may result from genetically based differences in the biology of the two different taxa presently treated as varieties, *P. aquilinum* var. *aquilinum* in Britain and *P. aquilinum* var. *latiusculum* in the Northeastern and Appalachian region of North America.

Age estimates of the larger clones based on growth rate data indicate their considerable antiquity. The rate of rhizome growth in European *P. aquilinum* ranges between 25 and 210 cm per year (Fletcher and Kirkwood, 1979),

averaging 43 cm/yr (Watt, 1954). Growth rates for North American plants have not been determined, but appear to be of a similar magnitude (personal observation). Dividing one-half the maximum distance between ramets by 43 cm (the average annual growth rate) yields an estimated minimum age of 1,180 yr for the largest genet detected by this study (number 3). The estimated minimum ages of other large clones found include #38, 598 yr; #1, 580 yr; #15, 559 yr; #33, 472 yr; and #2, 429 yr. While this method of age estimation can yield only crude approximations, there can be little question that large bracken clones are quite old (Sheffield, Wolf, and Haufler, 1989).

Probably older than the biggest trees in the present forest, the larger clones in the Doe Creek bracken population must have survived and spread in a spatially varied and temporally changing array of environments. At present, the study area is ecologically diverse and gives evidence of past change (old fire scars on trees, abandoned roads, stone fences, etc.). The present position of genets, as partially illuminated by our sampling effort and portrayed in Fig. 1, represents a single frozen frame in a centuries- or millennia-long dynamic existence of the population. It is impossible to infer from the present distribution of a genet's ramets the complex history of events that has resulted in that distribution. Nonetheless, examination of the spatial features of the genets suggests intriguing hypotheses addressable in future studies into the demography and ecology of bracken.

For example, some degree of interclone ecological specialization is suggested by the contrasting microhabitat utilization of clone 1, almost exclusively limited to the open areas of the transmission lines, and genotype 6, which occurs principally in adjacent open forest. Consistent with previous reports (Page, 1976), we observed that *P. aquilinum* fronds are most robust and grow most dense in open, sunny areas, with more scattered growth of smaller fronds occurring in open forests, and virtual absence of bracken from shaded, mesic woods. The powerline clearings provide an apparently optimal bracken habitat that has been extended into the mesic woods which itself seems unsuitable for bracken growth. Whether the predominance of clone 1 in this lower powerline region is fortuitous or attributable to a favored genotype could be tested through long-term reciprocal transplant studies (Silander, 1985b). Long-term observational studies would also be valuable in elucidating dynamic patterns of clone interactions. A hint that competitive interactions exist is provided by clone 3 which, although the most expansive, presently consists of a few widely scattered fragments, suggesting that it may once have been more prevalent and subsequently was displaced by competing clones. It would also be of interest to investigate whether there exist associations between isozyme genotype and biologically important features, such as the correlations between heterozygosity and growth rate reported for varied organisms (Mitton and Grant, 1984) and as suggested by a recent study of a clonal cactus (Parker and Hamrick, 1992).

The substantial genetic diversity observed in this study and others on bracken implicates an important role for sexual recruitment of new genotypes. Success of recruits, introduced by spores, is contingent upon the ecological requirements of the gametophytes. Establishment of bracken sporelings in the forested portion of our study area is unlikely, as this species is known to be a colonizer in which gametophyte establishment occurs principally following fire (Page, 1976). Notably, the portion of the study area that shows the greatest genet diversity (eight genets—small and large) is where Route 700 crosses the small powerline. This spot is one of frequent disturbance by humans, is an interface of forest and open areas, and is near the transition from mixed mesophytic forest downslope and oak-birch forest upslope. This site might provide a nursery for recruits as well as an ecologically diverse place for various genets to proliferate.

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