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Genetic Variation within and among White Clover Populations from Managed Permanent Pastures of the Northeastern USA

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ABSTRACT

White clover (*Trifolium repens* L.) population dynamics and genetic variation were characterized in populations under continuous grazing management. RAPD (random amplified polymorphic DNA) profiles were developed for individuals sampled from managed permanent pastures on 18 farms in Pennsylvania (PA), New York (NY), and Vermont (VT). Genomic DNA from individual white clover plants (48) sampled over two transects at each location during fall, 1996, was amplified by the PCR (polymerase chain reaction). RAPD profiles of 54 RAPD markers were used to compute genetic distances between pairs of individuals. The data set for each location was evaluated by AMOVA (Analysis of Molecular Variance), which apportioned total variation among individuals within populations, among populations within states (PA, NY, VT), and among state populations (PA, NY, VT). An interpopulation distance matrix of 18 fall-sampled populations was used to calculate a dendrogram. Two groups of 6 and 10 populations were evident, with two populations as single branches. VT populations were in the larger population group, and the PA and NY populations were in both groups. White clover genetic variability in the swards was higher than expected, all populations were significantly different ($P < 0.001$) from each other, and the PA, NY, and VT populations were indistinguishable from each other. Populations from four PA locations also sampled in summer changed significantly two months later in the fall. Rapidly changing white clover diversity may be due to rapid, differential growth of individuals best adapted to seasonal changes in the microenvironment, or to unexpectedly high levels of seedling recruitment.

WHITE CLOVER POPULATIONS in pastures at northern mid-latitudes have been naturally maintained for many decades through seedling recruitment in spring and vegetative propagation throughout summer and fall. In addition, graziers periodically broadcast seed of commercial white clover cultivars in their pastures to ensure production of high quality large leafed plants. However, in actively grazed managed permanent pastures, white clover plant establishment is primarily by root-shoot production at stolon internodes.

Many other factors also influence white clover population structure and dynamics (Grime et al., 1988). This plant species is intolerant of drought, severe frosts, and shade, while it is tolerant of heavy grazing and tramping. Stolons provide a mechanism for colonization of gaps between pasture grass plants. White clover regenerates almost exclusively from rooted stolons and can form large clonal patches, but it colonizes new sites mainly by seedling recruitment. In permanent pastures, seeds germinate primarily in the spring in the temperate, humid northeastern USA from persistent, long-lived seed banks. Seeds can survive ingestion by grazing animals and birds, which can account for movement of seed from its production site. Adaptation to diverse ecological niches is correlated with genetic variation (Grime et al., 1988).

Although white clover spreads primarily by vegetative propagation (Chapman and Anderson, 1987; Turkington et al., 1979), investigators have been unable to ascertain the relative importance of sexual versus vegetative propagation mechanisms. This is because morphological plasticity of the plant masks and alters expression of phenotypic markers in the field and because genetic variability is more like that found between populations with clearly different environmental conditions (Turkington, 1996). Fothergill et al. (1997) reported that within established mixed swards under sheep grazing, seedling recruitment is only one twentieth or less of that observed from a conventional sowing. Even so, white clover is genetically highly variable in pastures. Recruitment from seed reserves in grazed pasture is probably highest in areas where the sward has been disturbed, primarily from hoof prints (Cahn and Harper, 1976a; Turkington et al., 1979).

White clover plants established from natural and machine seeding have high genetic diversity for cyanogenesis (Jones, 1972), Rhizobium strain specificity (Mytton, 1975), leaf markings (Brewbaker, 1955; Carnahan et al., 1955; Corkhill, 1971), and self-incompatibility alleles (Williams, 1931, cited in Turkington et al., 1979). Genetic coefficients for variation of 11 plant characters ranged from 12 to 38% for 150 genotypic clones of white clover (Lee et al., 1993). Polymorphic leaf marks in naturalized populations can play a role in selection by grazing animals (Cahn and Harper, 1976b). Harberd (1963) used distribution of rare leaf marks to show that clones can occupy an area of up to 3 m^2, indicating that clonal spread is important to white clover maintenance.

To understand how white clover populations persist over decades, it is necessary to characterize their population dynamics. An important first step is to characterize genetic variation in this obligately outcrossing species. In studies to measure rates of seedling recruitment, researchers (Champness and Morris, 1948; Charlton, 1977; Chippindale and Milton, 1934) found greater than 200 seed m^{-2} to a depth of 10.5 cm, which produced seedlings. These seeds probably came from just a few clones in a field that prolifically produced flowers, as the remaining clonal patches (based on leaf marks) generate

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Abbreviations: AMOVA, analysis of molecular variance; NTSYS, numerical taxonomy and multivariate analysis system; NY, New York; PA, Pennsylvania; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; UPGMA, unweighted paired group method of arithmetic averages; VT, Vermont.

few flowers (Burdon, 1980; Turkington et al., 1979). In another study, Lee et al. (1993) found sufficient genetic variation in two Australian cultivars for exploitation in development of new cultivars. The variability was observed in newly established clonal plants taken from 42 farms that had been sown to cultivar Irrigation 25 to 42 yr previously. Genetic coefficients for variation of 11 plant characters ranged from 12 to 38% for 150 genotypic clones of this cultivar. Burdon (1980) reported that nearly every clone of 50 collected and established from a 60-yr-old permanent grassland in North Wales differed significantly from every other clone in at least one genetically inherited character.

It seems contradictory that white clover populations can exhibit diverse genetic variability when their primary mechanism for growth is vegetative propagation, or clonal spread. This may be due to survival of genetically different white clover plants that are best adapted to the microenvironment at a particular point in time. Burdon (1980) argued that variable microenvironment selects for growth of individuals best adapted genetically to a given set of environmental conditions. Thus, the variable environment may be a major factor in generating the high genetic diversity of white clover individuals in a population. An additional mechanism for diversity may be that of seedling recruitment throughout the growing season. However, this mechanism has only been shown to operate in southern pastures where heat and drought kill off white clover plants each summer.

RAPD (randomly amplified polymorphic DNA) markers can be utilized to examine population structure and population dynamics of white clover. Molecular genetic markers are unaffected by environmentally induced variations in plant growth and morphology; they can be accurately used to discriminate among many varieties of plant species (Bellamy et al., 1996; Wallner et al., 1996; Wang et al., 1994), and they have been used to assess genetic variation in plants (Newbury and Ford-Lloyd, 1993; Huff et al., 1993; Waugh and Powell, 1992). To understand how white clover populations persist under continuous grazing management for many decades, we determined RAPD profiles for white clover individuals sampled from pastures in PA, NY, and VT. Genetic distance variance of individuals from the selected locations was assessed to examine genetic variability of white clover populations across wide cultivar and indigenous backgrounds.

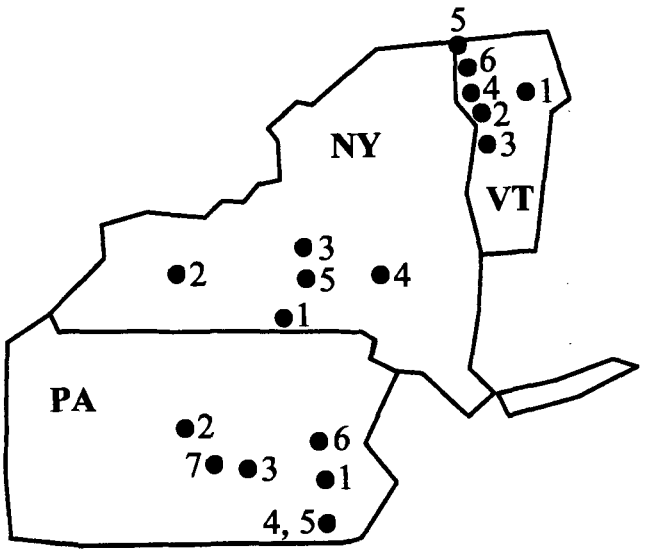

Fig. 1. Approximate locations of 18 white clover populations randomly sampled in the northeastern U.S. Each location (1–4 ha) was sampled at intervals of about 10 m across two transects, with 25 individuals sampled from each transect.

MATERIALS AND METHODS

Population Sampling

Trifoliate leaf samples of white clover were collected from 18 locations in fall of 1996 between 72° and 79° W longitude and between 39° and 45° N latitude (Table 1). Seven locations were in central and eastern Pennsylvania, five were in western New York, and six were in the northwestern quadrant of Vermont (Fig. 1). No two populations were closer than 27 km

Table 1. Properties of 18 locations in the Northeast where white clover populations were randomly sampled.

Location	Latitude	Longitude	Elevation	Mean summer temp†	Annual precip.	Years since seeding	Years in pasture
	deg min		m	°C	mm		
PA1	40 21.4′	76 05.1′	109.7	21.2	1080	4th, in alfalfa	2nd
PA2	40 52.2′	77 50.1′	353.6	19.4	1240	3rd, in tall fescue	3rd
PA3	40 34.8′	77 16.9′	189.0	20.7	1264	Never seeded in wc‡	16th
PA4	39 49.7′	76 03.6′	128.0	21.3	1192	Never seeded in wc 7th	40th
PA5	39 49.7′	76 03.6′	128.0	21.3	1192		7th
PA6	41 04.1′	76 04.3′	298.7	19.6	990	Never seeded in wc	35th
PA7	40 38.0′	77 38.0′	231.6	nd	nd	Never seeded in wc	5th
NY1	42 02.1′	76 42.8′	274.3	19.7	879	Never seeded in wc	13th
NY2	42 44.8′	78 03.8′	481.6	17.6	1181	2nd, in Ladino	2nd
NY3	42 57.1′	75 58.1′	365.8	19.9	857	4th, in Ladino	25th
NY4	42 38.8′	75 21.8′	487.7	18.8	1114	3rd, in wc	8th
NY5	42.30.0′	76.18.9′	341.4	18.7	951	10th, in Ladino & orchardgrass	10th
VT1	44.19.2′	72 32.9′	365.8	17.3	1046	5th, in wc	5th
VT2	44 11.7′	73 20.9′	45.7	19.1	895	Never seeded in wc	22nd
VT3	43 51.5′	73 07.9′	128.0	19.0	970	Never seeded in wc	42nd
VT4	44 27.7′	73 11.3′	91.4	19.1	895	9th, in wc	9th
VT5	45 00.6′	73 13.1′	45.7	19.2	899	Never seeded in wc	9th
VT6	44 44.9′	73 08.1′	73.2	19.2	899	3rd, in wc	3rd

† June, July, August, and September, 1996.
‡ wc, white clover.

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from each other. Four PA locations (PA1, PA2, PA6, and PA7) were also randomly sampled in the summer; no effort was made to sample the same individuals on both dates. Each population was in a managed, permanent dairy pasture varying from 2 to 42 yr of continuous intensive rotational grazing (Table 1). One location had been seeded 1 yr previously, three locations had been seeded 2 yr previously, and six locations were seeded more than 3 yr previously with commercial white clover cultivars. The remaining eight locations had never been seeded. The locations were selected to give a range of years in pasture and a range of years since commercial white clover seeding to provide a range of population histories. Each sample was from a distinct white clover genet.

One to three trifoliate leaves were taken from the same stolon to insure that a sample represented a single genet. Each location (1–4 ha) was sampled at 10-m intervals across two transects, with 24 individuals sampled along each transect. Leaf tissue from each individual was placed in a moist paper towel and stored on ice until they were processed in the laboratory. Trifoliate leaves of an individual were blotted dry and 100 mg fresh weight placed in a 2-mL, cone-shaped-bottom plastic microcentrifuge tube. Samples were lyophilized and stored at −70°C. A total of 1056 individuals were sampled.

Template DNA Preparation

Genomic DNA preparation was based on the procedure of Steiner et al. (1995). Five 4-mm diam. glass beads were added to each lyophilized sample, the tubes placed in a Vortex Genie[1] platform, and the samples ground by vortexing them for 20 min. One-hundred mg of each sample was extracted with 400 μL of ROSE buffer and diluted as described (Steiner et al., 1995). This nonpurified, diluted genomic DNA preparation was used for PCR amplifications of DNA sequences. DNA concentrations were determined by comparison of intensity of ethidium bromide stained bands with DNA standard on agarose electrophoretic gels (Sambrook et al., 1989).

DNA Amplification

PCR amplifications were conducted in 0.65 mL PCR reaction tubes in a final volume of 15 μL. Reaction components consisted of 3 μL of 170-fold diluted genomic DNA (10 ng), 10 m*M* Tris-HCl (pH 8.3), 10 m*M* KCl, 2.5 m*M* $MgCl_2$, 0.2 m*M* each of dATP, dTTP, dGTP, and dCTP, 0.1 μ*M* primer, and 3.3 units of Stoffel fragment TAQ polymerase (Perkin Elmer Cetus, Norwalk, CT). Reactions were controlled in a Perkin Elmer Cetus 480 thermocycler as follows: 1 cycle of 4 min at 96°C, 50 s at 36°C, and 1 min 45 s at 72°C; 42 cycles of 50 s at 94°C, 50 s at 36°C, and 1 min 45 s at 72°C; 1 cycle of 50 s at 94°C, 50 s at 36°C, and 2 min 30 s at 72°C; and held at 4°C. Amplified DNA sequences were separated in agarose gels (14 g agarose kg^{-1} water) in 0.5 × TBE buffer, stained in ethidium bromide, and photographed under UV illumination (Sambrook et al., 1989).

On the basis of results of Bullitta (1995) and Steiner et al. (1998), six primers were selected and tested for their ability to generate polymorphic markers for white clover. On the basis of the quality and quantity of their amplified fragments, three of those primers were chosen for this study (OPA08, OPB14, and OPH12; Operon Technologies, Inc., Alameda, CA). They produced 54 polymorphic RAPD markers from white clover genomic DNA. All distinct bands scored that were reproducibly scorable by three individuals were used for the RAPD profiles. The markers ranged in size from 250 bp to 1.5 kb in length; they were scored as present (1) or absent (0). Fragment size was determined by comparison with the 100-bp ladder from Life Technologies (Gaithersburg, MD).

Statistical Analysis of RAPD Profiles

Genetic distance among individuals was measured as an Euclidean metric distance E, that was calculated between all possible pairwise combinations of molecular genetic markers (RAPD bands) for individual plants, on the basis of Huff et al. (1993),

$$E = \{\varepsilon_{xy}^2\} = n\left[1 - \frac{n_{xy}}{n}\right]$$

where n is the total number of polymorphic bands and n_{xy} is the number of bands shared by the individuals x and y. The Euclidean metric distance between any two individuals is equivalent to the number of observed band differences out of the total number of markers surveyed. Euclidean distance matrices were produced for all populations using the RAPDdistance V 1.04 freeware package developed by John Armstrong, Adrian Gibbs, Rod Peakall, and George Weiller (Australian National University, Canberra; gopher://life.anu.edu.au:/). The Euclidean distance matrix was used as data input for all statistical analyses. NTSYS-pc (version 1.8, Exeter Software, Setauket, NY; Rohlf, 1996) was used to perform the following analyses: dendrogram (based on the unweighted paired group method of arithmetic averages, UPGMA), cophenetic correlation coefficients, and matrix correlations using the Mantel test (Smouse et al., 1986; Rohlf, 1996). The analysis of molecular variance (AMOVA) procedure (Excoffier et al., 1992) was used to estimate variance components for the RAPD phenotypes, partitioning the total variation among individuals within populations, between populations within a state (PA, NY, VT), and between states. AMOVA (version 1.55) was provided by Laurent Excoffier, Department of Anthropology and Ecology, University of Geneva, 12 rue. G. Reviliode, 1227 Carouge, Switzerland (http://anthropologie.unige.ch/LGB/software/win/amova/). AMOVA estimates these nested variance components and calculates values of *F*-statistic analogues termed Φ-statistics. Significance levels for variance component estimates and Φ_{st} are computed by non-parametric permutation procedures. The genetic distance between any two particular populations was represented by its Φ_{st} value, which is equivalent to the proportion of the total variance that is partitioned between two populations, and is herein referred to as interpopulation distance.

The validity of RAPD profiles and population analyses was tested by dividing the four PA summer and fall populations into two equivalent subpopulations of 24 individuals. Thus, two subpopulations from each location and date comprised a random sample of individuals in the field collected on the same date. The eight subpopulations were converted to distance matrix files and compared by AMOVA.

To facilitate AMOVA calculations, population size was reduced to 42. A single interpopulation distance matrix of 22 populations was created from 28 overlapping matrices, because of the limitation of the AMOVA program to analyze only 255 individuals at one time. This procedure is valid because those cells that overlapped had identical Φ_{st} values. This matrix was used to develop possible genetic relationships of the populations within and among states.

The four locations sampled in summer were removed from the data set to leave only populations that were sampled in

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the fall. The resulting 18 by 18 interpopulation distance matrix was used to estimate population relatedness by algorithms of the NTSYS-pc. Dendrograms were produced by SAHN (sequential, agglomerative, hierarchical, and nested) cluster analysis (UPGMA). To test for goodness of fit of the clustering to the hierarchical data matrix, the SAHN output was converted to a cophenetic similarity values matrix. The distance matrix for the 18 populations was plotted against the cophenetic matrix.

RESULTS

RAPD Profiles of Genomic DNA

Each of the three primers produced 14 to 22 polymorphic markers (bands) in replicate amplifications (Table 2). As the number of polymorphic markers increased, the number of unique genotypes increased linearly (r^2 = 0.95). DNA profiles were assigned from 54 molecular markers to each of 48 individuals sampled from each of 22 white clover populations in PA, NY, and VT; 1038 individuals out of 1056 were identified as possessing a unique RAPD profile (Table 2). Of the 22 populations, 14 of them contained 48 unique DNA profiles (genotypes), and the remaining eight populations contained 43 to 47 unique genotypes, indicating some individuals had matching genotypes. When using fewer RAPD markers, for example 22 from primer OPH12, we found only 208 unique genotypes. Use of two or three primers increased the number of unique individuals, but use of a fourth primer did not change the number of unique individuals, thus indicating that a saturation point was reached.

Genotypes that matched and were sampled consecutively in the field, may be members of clonal patches. In seven instances, a twin genotype was found within transect lengths of less than 20 m in fields in PA and NY. In one PA location, a triplet genotype was found over a distance of 30 m and a quadruplet genotype was found within the location. Genotypes that matched in the field, but were separated by other genotypes, may be members of a fragmented clonal patch. Eight of the locations had matching genotypes, but with individuals of different genotypes interspersed. One individual from NY was identical to two from PA and another from NY was identical to an individual from VT. Because these identical individuals were separated by more than 50 km, it seems unlikely that they originated from the same clone. Our data is not capable of distinguishing whether individuals with matching RAPD profiles are identical because they are clones or because they have a common ancestor.

Table 2. Primer attributes and summary of RAPD markers found in 22 white clover populations from 18 locations in Pennsylvania, New York, and Vermont.

Primer	Primer sequence	Number of scorable bands	Number of unique genotypes
OPA-08	5′-GTGACGTAGG	14	95
OPB-14	5′-TCCGCTCTGG	18	240
OPH-12	5′-ACGCGCATGT	22	423
Combined		54	1038

Population Analyses

We made comparisons of populations within and between geographic groups of populations on the basis of 48 individuals per population. Locations PA3 and PA7, NY3 and NY5, and VT3 and VT6 were examined as three groups, each with two populations, by AMOVA. The variance among the three groups was 2.3% (P = 0.34, ns) of the total, indicating that populations grouped by state were indistinguishable from each other. The variance among populations within states was 24.3% ($P < 0.001$), and the variance within populations was 73.4% ($P < 0.0001$) of the total. Results obtained for seven remaining population combinations from each of the three states gave essentially the same results (data not shown). Together these results establish that among states, white clover populations are not different from each other, and that white clover populations within a pasture population are genetically highly diverse.

AMOVA analysis of the RAPD profiles from four PA locations (counties PA1, PA2, PA6, and PA7) showed that at each location the genetic makeup of white clover populations had significantly changed from summer to fall. The variance among sampling dates at each of the four locations (PA1-1 and PA1-2, PA2-1 and PA2-2, PA6-1 and PA6-2, PA7-1 and PA7-2; 24 individuals per subpopulation) ranged from 23 to 30% ($P < 0.001$) of the total. The temporal change in genetic makeup of the populations could be due to seasonal succession of genotypes, seedling recruitment, collecting a different set of individuals during the second sampling, or failure to reproducibly generate RAPD markers. To test these possibilities, each of the eight populations was divided into two equivalent subpopulations of 24 individuals. Each subpopulation then comprised a random sample of individuals in the field collected at the same date. To assess the genetic makeup of the subpopulations, we compared them within each location and for each date. Variance among subpopulations from the same sample date and same locations was 0% (Table 3), while variance within each subpopulation was essentially 100%. For example, AMOVA variance among subpopulations for two groups of 24 from PA1-1 was 0% and within subpopulations was essentially 100%; a similar result was found for PA1-2 (Table 3). On the

Table 3. Analysis of molecular variance (AMOVA) for white clover RAPD profiles of four locations in which both summer and fall sampling was conducted. Pairs of subpopulations consisted of two random samples of 24 individuals from the same sampling date.

Field	Variance (%)		*P*-Value among subpopulations
	Among subpopulations	Within subpopulations	
PA1-1†	0	101.4	0.83
PA1-2‡	0	101.8	0.69
PA2-1†	0	101.8	0.86
PA2-2‡	1.1	101.1	0.81
PA6-1†	0	100.8	0.70
PA6-2‡	0	101.6	0.94
PA7-1†	0.2	99.8	0.40
PA7-2‡	0	100.5	0.57

† Summer.
‡ Fall.

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other hand, when 48 individuals of PA1-1 and of PA1-2 were analyzed by AMOVA, among population variance was 23.5% and within population variance was 76.7%. Essentially the same results were found for the three other locations that were sampled on two dates.

Because the genetic makeup of populations changed significantly from summer to fall, the four locations sampled in the summer were removed from the data set. The interpopulation distance matrix in Table 4 was assembled from overlapping matrices produced by AMOVA. The SAHN cluster analysis (NTSYS-pc) using the UPGMA algorithm produced the dendrogram in Fig. 2. The results revealed groups of six (PA2-2, PA3, NY2, PA5, NY4, PA6-2) and 10 populations (PA4, NY1, VT4, VT5, VT6, NY3, PA7-2, VT1, VT3, VT2), with two populations as single branches (PA1-2, NY5). However, the goodness of fit for the clustering to the hierarchical data matrix, as determined by the cophenetic correlation coefficient, was poor $r = 0.68$).

DISCUSSION

We have compared RAPD molecular markers in 22 populations of white clover at 18 locations in three northeastern states (PA, NY, VT). Our data show that these populations are genetically indistinguishable between the three states, but are genetically different between pastures within states, and between summer and fall sampling dates. That a white clover population can undergo a genetic shift in two months (summer to fall) at first seems surprising, since white clover is thought to spread in pastures primarily through development and establishment of clonal plants from stolon nodes. Temporal change in the genetic makeup of populations could be due to seasonal succession of ecotypes, seedling recruitment, sampling of a different set of individuals, or failure to generate reproducibly RAPD markers. Because of our sampling technique, we probably did not sample the same clones at the four locations on the two dates. When each of the four populations was split into two subpopulations, AMOVA calculations showed that subpopulations of all pairs were alike. Thus, the combination of daily changes in plant growth, animal grazing, and microenvironment over a two-month time period may be the driving force in changing population structure. These results establish that although each subpopulation was genetically diverse, they were not different from each other when sampled on the same date. Thus, change in genetic makeup of the four populations from summer to fall was due to a population shift of white clover individuals and not because of improper sampling or failure of the method to generate reproducibly RAPD markers. That AMOVA variance among each pair of subpopulations was 0% clearly shows that the two subpopulations from the same population taken on the same day are indistinguishable from each other. That AMOVA variance among populations was 23.5% clearly shows that the two populations from the same location taken on different dates had different genetic makeups.

It may also appear surprising that white clover populations were different among the 18 locations, but not different among the three states (PA, NY, VT). We found considerable genetic distance variance within white clover populations (60–75%) and among populations within the three states (25–35%). In contrast, we found low genetic distance variance among the three states (1–10%). These findings indicate that the populations of the three states likely represent the northeastern region without any further subdivision in structure.

Newly established plants (ramets), whether growing from an internode or from a seedling, likely were able to establish because that genotype was particularly well adapted to the microenvironment and to the seasonal conditions at that time. During that same period, other clones may have stopped growing because those conditions did not favor that genotype. Thus, such a shift in ecology from summer to fall could partly explain the observed shift in white clover population genetics. Furthermore, the high density of clones in pastures is also consistent with these arguments. Cahn and Harper (1976a) reported 1.5 to 6 clones in a 10-cm^2 area and Trathan (1983; cited in Turkington, 1996) found 48 to 50 clones per m^2 area based on 11 and 12 morphologically

Table 4. Interpopulation distance matrix Φ_{st}† for the 18 locations of fall-sampled white clover populations in the northeastern USA.

Location	PA1-2	PA2-2	PA3	PA4	PA5	PA6-2	PA7-2	NY1	NY2	NY3	NY4	NY5	VT1	VT2	VT3	VT4	VT5	VT6
PA1-2	0.0000																	
PA2-2	0.2842	0.0000																
PA3	0.3355	0.0957	0.0000															
PA4	0.3984	0.1363	0.2257	0.0000														
PA5	0.3967	0.2059	0.1788	0.3092	0.0000													
PA6-2	0.4493	0.2014	0.2438	0.2355	0.3247	0.0000												
PA6-2	0.3094	0.2963	0.3417	0.2976	0.3442	0.4296	0.0000											
NY1	0.4114	0.2015	0.2603	0.0941	0.3601	0.2549	0.3500	0.0000										
NY2	0.3025	0.1241	0.1737	0.2021	0.1507	0.3229	0.2672	0.2791	0.0000									
NY3	0.4024	0.1926	0.3270	0.2532	0.3782	0.3347	0.3371	0.2901	0.2100	0.0000								
NY4	0.3612	0.1176	0.1943	0.2446	0.3225	0.2709	0.3732	0.3166	0.2475	0.3079	0.0000							
NY5	0.3650	0.2221	0.2320	0.2519	0.3594	0.3441	0.3291	0.2347	0.2503	0.3416	0.3205	0.0000						
VT1	0.3671	0.2303	0.2917	0.2350	0.3292	0.3601	0.2195	0.3329	0.2322	0.3208	0.3375	0.3367	0.0000					
VT2	0.3495	0.1637	0.2794	0.1801	0.3154	0.3039	0.2144	0.3002	0.1920	0.1870	0.2616	0.3558	0.1655	0.0000				
VT3	0.2793	0.1830	0.2245	0.1695	0.2996	0.2246	0.2170	0.1799	0.2258	0.2604	0.2870	0.2283	0.1653	0.1725	0.0000			
VT4	0.4575	0.2213	0.2823	0.1960	0.3493	0.2614	0.3414	0.2631	0.2874	0.3037	0.3212	0.3894	0.3100	0.1819	0.2200	0.0000		
VT5	0.4765	0.2439	0.2987	0.2106	0.3352	0.3305	0.3838	0.2774	0.2430	0.2725	0.3446	0.4025	0.3218	0.2280	0.2856	0.1731	0.0000	
VT6	0.3945	0.2452	0.3089	0.1931	0.3591	0.2616	0.3175	0.2055	0.2700	0.2114	0.3342	0.3427	0.3001	0.2095	0.1974	0.2344	0.1637	0.0000

† Φ_{st} value, which is equivalent to the proportion of the total variance that is partitioned between two populations. All blank cells in the upper matrix diagonal have a value of zero, indicating all corresponding values shown iu the lower matrix diagonal are highly significant.

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distinct leaf mark patterns, respectively. Because we sampled at 10-m intervals, we could have only detected clonal patches of greater than 10 m in length, when we should expect several hundred clones to be present within that distance interval. Thus, the variable geography, climate, ecology, and soils within the three-state (PA, NY, VT) area sampled may reflect the highly adaptable nature of white clover.

Previous studies have only looked at variance within populations. Turkington (1996) found the extent of genetic diversity in white clover populations similar to that found in populations taken from clearly different environments (see also Gliddon and Trathan, 1985). Genetic variance within the populations studied by us was based on 54 RAPD molecular markers, which are specific DNA sequences unaffected by environment. Previous studies on genetic variability were based on morphological markers, or phenotypic characters, which are subject to variation caused by differential expression under changing environmental conditions.

The significance of our findings to understanding pasture ecology and pasture management interactions can be interpreted in a number of ways. The fact that some locations were seeded before 2 or 3 yr sampling means that some populations were less naturalized than other, more established populations. Nevertheless, we were looking at population structures determined by changing ecotypes against a background of more slowly recruited new genotypes (cultivar versus indigenous genetic background). We assumed that the white clover populations sampled by us existed under conditions in which seedling recruitment, vegetative establishment, immigration, emigration, and death were balanced within the context of their environment and their genetic background. However, these processes must be in a constant state of flux because of the relentless herbivore predation on the pastures. Because of the demonstrated plasticity of white clover with respect to ecological adaptation (Burdon, 1980; Turkington, 1996; Turkington and Harper, 1979a, b), we view the observed genetic variability to be a result of differential growth of white clover genets. This variation was therefore a result of establishment of different genotypes primarily through mechanisms of exploratory vegetative and less so through seedling recruitment. In both cases, establishment of a different clonal plant or a new seedling has the same net effect: establishment of a genet that complements the ecological characteristics of the growth spot and fills a competition void. This could account for the extensive genetic variability and for the rapid seasonal change in the genetic makeup of white clover populations demonstrated by our data. Thus, because no two populations were alike, our results suggest that microenvironment is important for shaping population structure in white clover. However, our data do not differentiate between mechanisms of (i) introduction of new heterozygous individuals through seedling recruitment, or (ii) differential growth of genets from the existing genetically diverse population of bare stolons.

Whether or not a field had been seeded in a commer-

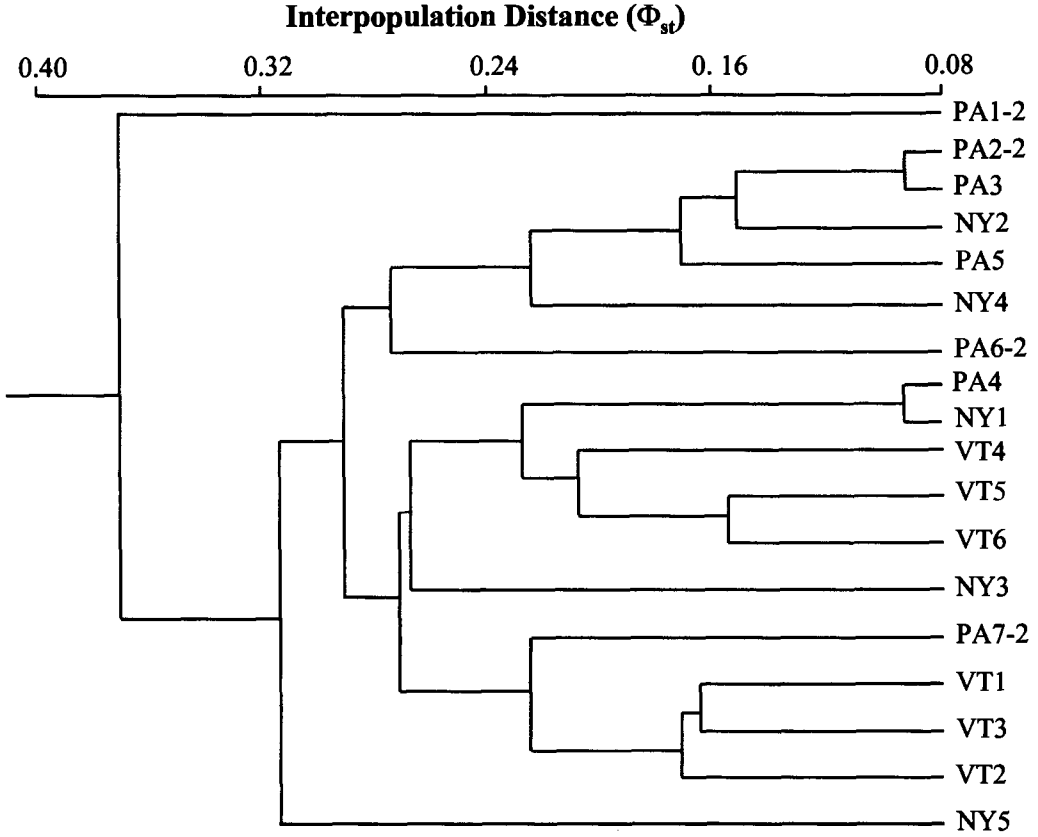

Fig. 2. Dendrogram (nested clusters) of 18 white clover populations showing their possible relationships. NTSYS-pc was used to produce the phenogram.

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cial white clover cultivar by the grazier apparently did not have an effect on the rapidly changing genetic diversity over a two-month grazing period. The PA1 location was seeded with a local pasture mix and was in its second year as pasture; the PA2 location was in its fourth year after seeding with tall fescue; the PA6 location had never been seeded during 35 yr in pasture; and the PA7 location had never been seeded during 5 yr in pasture. The PA1 location was grazed by sheep and the others were grazed by dairy cattle. In spite of these different histories (Table 1), population genetic diversity at each location changed over two months through natural selection pressure or through differential growth among individuals within a highly variable population genetic background. This suggests that white clover, whether as commercial cultivars or indigenous ecotypes, readily adapts to changing environments. None of the factors listed in Table 1 correlated with the clustering shown in Fig. 2.

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