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Genetic Variability and Its Ecological Implications in the Clonal Plant *Carex scopulorum* Holm. in Colorado Tundra

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Abstract

We assayed the amount and distribution of genetic variability in the clonal polyploid plant *Carex scopulorum* var. *scopulorum*. We used 5 polymorphic protein loci to sample 7 populations on the tundra in Rocky Mountain National Park, Colorado, U.S.A. *Carex* has levels of variability comparable to those found in nonclonal species. In the loci studied, transmission is disomic, as in diploid species. Of 148 tillers tested, 59.5% had different 5-locus genotypes. The distributions of genotypes fit Hardy-Weinberg expectations at 4 loci in most populations. These results suggest that there is significant cross-pollination and establishment by seed in this tundra plant. There were significant differences in allele frequencies between adjacent populations occupying wet versus moist environments at alcohol dehydrogenase, an enzyme known to be relevant to metabolism under oxygen-limiting conditions. There was no relationship between geographic distance and genetic distance at the scale we studied.

Introduction

Arctic and alpine tundra communities contain a large number of plant species that can reproduce clonally (Billings and Mooney, 1968; Bliss, 1971). Such clonal species are often perceived to have important advantages in the rigorous tundra environments. The clonal habit provides: (1) the opportunity to maintain specific individuals, presumably adapted to specific sites, for long periods of time; (2) the ability to reproduce copies of an adapted genotype without depending on the uncertainties of sexual reproduction; and (3) the competitive advantage of expanding in space without giving up previous footholds. These advantages of clonal reproduction are discussed in some detail in Jackson et al. (1985).

Two important consequences of extended dependence upon clonal reproduction for a species are possible:

1. *The development of single clones with very extensive distributions.* The existence of such large clones, often spanning several hectares, has been documented or at least suggested in a number of situations (Harberd, 1967; Silander, 1985; Grant et al., 1992).
2. *The presence of somewhat lower levels of genetic variability in comparison to species that reproduce primarily or solely via sexual reproduction.* This perspective has been held for a long time (e. g., Stebbins 1950; Gustafsson, 1947; Silander, 1985). However, these characteristics have seldom been compared explicitly between clonal and nonclonal species. Results to date suggest that, in fact, clonal species can be as variable as nonclonal ones (Silander, 1985; Ellstrand and Roose, 1987; Hamrick and Godt, 1989).

Carex scopulorum Holm. is a common species inhabiting subalpine forests and tundra in the western U.S.A. It is able to grow clonally via rhizomes. Three varieties are recognized: *scopulorum*, *bracteola*, and *pruinophylla*. *Carex*, like other Cyperaceae, have diffuse rather than localized centromeres. Perhaps as a result, polyploidy, aneuploidy, and chromosome fragmentation are common (Davies, 1956; White, 1978). Chromosome numbers are variable, with $2N = 36$, 37, 38, 39, and 40 reported for *C. scopulorum*. Other numbers are also reported for related species; occasional trivalents have been noted, suggesting that at least some chromosomes are in trisomic complements (Standley, 1985). We worked with var. *scopulorum*, for which chromosome numbers have not been previously reported. The goal of our study was to analyze the genetic constitution of individual shoots in order to document (1) levels of genetic variability; (2) potential for sexual reproduction as indicated by estimates of fit to Hardy-Weinberg expectations; (3) extent of clonal growth; (4) the relationship between genetic variability and geographic distance; and (5) possible patterns of microevolution, i. e., the association between environmental heterogeneity and genetic variability.

Materials and Methods

STUDY SITE AND COLLECTIONS

The site is located on Trail Ridge (3800–3950 m a.s.l.) within Rocky Mountain National Park, in the Rocky Mountains, Colorado, U.S.A. (40°25′N, 105°50′W).

We collected leaf samples from plants from a variety of locations representing diverse microenvironments (Table 1). Each location will be referred to as a population. There are no boundaries around these locations, which are usually separated from one another by 200 to 4000 m. The exceptions are populations 1 and 2, where our objective was to compare the genetic constitution of individuals inhabiting two very different micro-environments, one with and the other without standing water. Five populations (C-3, 6, 7, 8, 9) were analyzed for 5 electrophoretic loci. An additional 2 populations (C-1, 2) were analyzed for 2 loci only. At C-1, 11 pairs of plants were also analyzed at 3 loci to determine whether individuals within 10 cm or less of one another were likely to be the same clone. At C-3, sampling was also done on a fine scale, collecting plants systematically every 1–2 m along three transects. The transects were at 5-m intervals, with A-B and B-C separated by 5 m and A-C by 10 m. In other populations, individual shoots sometimes were collected at distances usually 1–2 m, but up to 10 m, of one another over an area spanning 200–1500 m^2, depending on the population.

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TABLE 1

Locations, habitats, sample sizes, and interlocation distances of collections

Location	Microenvironment	Population designation	Sample sizes	Interpopulation distances[a]
Iceberg Pass				
Standing water	Very wet	C-1	39	1 ⇔ 2
Sloping meadow	Moist	C-2	41	10–30 m
Moist depression	Moist	C-3	42	1 ⇔ 3
				1000 m
Forest Canyon Overlook				
Willow bog	Wet	C-6	48	6 ⇔ 9
				300 m
Meadow	Moist	C-7	37	7 ⇔ 9
				200 m
Culverts	Variable	C-8	23	3 ⇔ 6–9
				3,000+ m
Standing water	Very wet	C-9	12	1 ⇔ 6–9
				4,000+ m

[a] Interpopulation distances are shown between pairs of numbered populations joined by double-ended arrows; for example, the distance between individuals of 1 and 2 is 10 to 30 m.

ELECTROPHORESIS

Samples of leaf material were collected in the field and kept cold until processing, which involved grinding in chilled mortars with a grinding buffer described in Mitton et al. (1979). General electrophoretic methods are also described in Mitton et al. (1979). Five loci were reliably scorable and polymorphic. These include anodal peroxidase (PER-AN) with 3 alleles, cathodal peroxidase (PER-CATH) with 3 alleles, phospho-gluco-isomerase (PGI) with 3 alleles, an esterase (EST) with 2 alleles, and alcohol dehydrogenase (ADH) with 4 alleles. In order for ADH to express itself, it appears useful to keep samples in closed plastic bags (i. e., anoxic conditions) and refrigerated for several days. This method was not tested rigorously, but samples treated in this fashion produced higher levels of activity than samples that were processed within 1–2 days of being collected.

Interpopulational differences in genetic constitution were tested with a G test. For each population, there were two categories, one consisting of the number of most common alleles, the other representing the total of all other alleles. Only populations with sample size >20 were used in the test. Within each population, fit to Hardy-Weinberg equilibrium was tested with a χ^2 test using expected and observed numbers of genotypes in several categories.

Results

PATTERNS OF CLONAL GROWTH

The number of 5-locus genotypes that is theoretically possible with the numbers of alleles observed is $3 \times 3 \times 3 \times 2 \times 4 = 216$. Many of these would necessarily be rare because of the low frequencies of certain alleles and the small numbers of plants that were typed for all loci. Of the 4 ADH alleles, 1 is very rare ($<2\%$); if it is eliminated from the calculations, the number of genotypes theoretically possible is 162, although only 148 could potentially be observed because we analyzed 148 plants. Of these, 88 were found in the 148 individuals that were typed at 5 loci. That is, 59.5% of the individuals were identifiably different from one another. The distribution of genotypes is shown in Table 2. In addition, we compared two habitat types that differ in moisture availability and associated soil hypoxia to determine whether this feature influenced clone number. Habitats 6 and 9 are characterized by wet conditions and had 45 separate clones represented within a total sample of 57. Habitats 3, 7, and 8 were moist rather than wet and had 60 clones represented in 91 samples tested. The difference is not statistically significant ($\chi^2 = 2.34$, $P > 0.10$).

TABLE 2

Number of genotypes observed in Carex *($N = 148$)*

	Distribution						
No. genotypes	62	14	8	1	2	2	1
No. individuals representing that genotype	1	2	3	4	5	6	8

A limited attempt was made to estimate the probability that ramets (or tillers) adjacent to one another are of the same or different clones. The sampling scheme is described in "Materials and Methods." Of 11 such pairs of tillers at C-1, with members of a pair growing within 10 cm of each other, 7 had the same genotype at the 3 loci tested, while the other 4 were different from one another at 1 or more loci. All other materials collected within one population consisted of tillers, often growing as at C-3 within 1–2 m of one another; these nearby samples often represented separate clones. For example, 3 transects were run within 5 m of one another at population 3. Transect 3A had 11 genotypes in 13 samples, transect 3B had 6 genotypes in 12 samples, and transect 3C had 10 genotypes in 17 samples. Furthermore, adjacent transects 3A and 3B had only 3 genotypes in common, while transects 3B and 3C had only 1 genotype in common, and only 1 genotype (of a total of 27) was found in all 3 transects. Another way to summarize clonality is to divide the number of clones, N_c, into the number of shoots sample, N_s. Overall, the N_s/N_c is $148/88 = 1.68$. In the wet areas it is $57/45 = 1.27$, while in the moist area it is $91/60 = 1.51$.

GENETIC VARIABILITY AND INTERPOPULATION DIFFERENCES

Most populations sampled had genotypic frequencies that fit closely to Hardy-Weinberg expectations at all but the esterase locus, which showed heterozygote deficiencies in 3 of 5 populations. There was significant interpopulation heterogeneity in allele frequencies at all loci tested (Table 3). At 4 of 5 loci, these differences were not associated with identifiable environmental conditions. However at ADH there was significant interpopulation differentiation associated with moisture conditions (Table 4). This differentiation was especially pronounced when comparing nearby populations occupying adjacent habitats whose primary difference was in moisture conditions. In population C-6, a wet, willow-dominated bog, the frequency of allele 3 was 0.7, whereas in the nearby ($\leq$100 m) drier meadow (C-7) it was only 0.3. Several kilometers away, population C-1 occupies a pond with standing water all summer, and population C-2 is an adjacent meadow. At (very wet) C-1, the frequency of allele 3 was almost fixed (0.97), whereas at (drier) C-2 it was 0.72, still a high frequency but significantly lower than at C-1.

The calculation of genetic distances between 4 populations analyzed for all 5 loci indicates that there is no relationship, on this scale, between genetic distance and physical distance (Table 5). For example, populations 6 and 7, or 6 and 8, are separated by about 300–500 m, but the genetic distance separating them is comparable to that between populations separated by 3000 m, e. g., population 3 and populations 6, 7, and 8.

Discussion

There is a high level of genetic heterogeneity within these populations of *Carex scopulorum*. This result contradicts the notion that on the tundra large clones dominate the landscape because clonal

TABLE 3

Genotypic and allelic frequencies in population samples. For every locus, interpopulation differences tested with G-test; significance indicated in parentheses after locus. Only populations with N > 20 used in test. Within populations, fit to Hardy-Weinberg expectations tested with χ^2 test. Significance levels are 5.99 for P < 0.05, indicated as (*); 9.21 for P < 0.01 (**); and 10.60 for P < 0.005 (***) for 2 d. f.

Locus population	N	Commonest allele	Expected (E) and observed (O) numbers of genotypes: 11 + 33 + 13 E	11 + 33 + 13 O	12 + 23 E	12 + 23 O	22 E	22 O	χ^2
PGI ($P < 0.005$)									
3	42	0.690	4.0	8	17.9	10	20.0	24	8.2**
6	38	0.635	6.4	4	22.2	17	19.4	17	2.4
7	37	0.851	0.8	2	9.4	7	26.8	28	2.4
8	23	0.870	0.4	1	5.2	4	17.4	18	1.2
9	12	0.750	0.7	1	4.5	4	6.8	7	0.1
ADH ($P < 0.001$)									
3	37	0.905	0.3	1	6.3	5	30.3	31	1.6
6	47	0.777	2.3	2	16.3	17	28.3	28	0.1
7	36	0.306	17.4	17	15.3	16	3.4	3	0.1
8	23	0.522	5.3	3	11.6	16	6.3	4	3.6
9	12	0.542	2.5	2	6.0	7	3.5	3	0.4
PER-AN ($P < 0.005$)									
3	41	0.866	0.7	2	9.5	7	30.7	32	2.8
6	47	0.745	3.1	1	17.9	22	26.1	24	2.5
7	37	0.932	0.2	0	4.7	5	32.2	32	0.2
8	23	0.674	2.4	1	10.1	13	10.5	9	1.9
9	12	0.917	0.0	0	1.8	2	10.1	10	0.1
PER-CATH ($P < 0.001$)									
3	29	0.828	0.9	2	8.3	6	19.9	21	2.2
6	47	0.543	9.8	13	23.3	17	13.8	17	3.5
7	32	0.672	3.5	4	14.1	13	14.5	15	0.2
8	19	0.821	0.1	1	2.8	1	16.1	17	7.7**
9	11	0.591	1.8	2	5.3	5	3.8	4	0.04
EST ($P < 0.001$)									
3	36	0.889	.04	2	7.1	4	28.4	30	6.9*
6	37	0.521	10.8	15	23.5	15	12.3	17	6.1*
7	35	0.586	6.0	13	17.0	3	12.0	19	23.7***
8	23	0.522	5.3	5	11.5	12	6.3	6	0.05
9	12	0.800	1.0	1	5.0	5	6.0	6	0.0008

growth is common and sexual reproduction is difficult due to unpredictable and inclement weather. Our results suggest that while adjacent tillers are often, though not always, of the same genotype, tillers separated by 1–2 m usually belong to different individuals. About 59% of individuals sampled represent different genotypes, a similar value to the 66% of plants having different genotypes in a grass species inhabiting Kansas prairie (Karman and Briske, 1985). This value of 59% is higher than all but 4 of the 29 studies of clonal species reviewed by Ellstrand and Roose (1987) and indicates that *Carex scopulorum* populations in this area are more diverse than those of other species occupying lower elevations or latitudes. Our estimate represents a lower boundary because if more polymorphic loci were used, more individuals could be differentiated. For example 19 of the 29 studies reported by Ellstrand and Roose (1987) used more than five loci.

The relationship between genetic variability and clone numbers can also be compared to results from other studies by dividing the total number of samples, N_s, into the number of genetically different clones, N_c. In this study, using only clones identified using all 5 loci, $N_s/N_c = 148/88 = 1.68$. This represents a comparable or greater level of genetic heterogeneity than in most other species of *Carex* studied so far, all of which were sampled at comparable inter-ramet distances of 1–4 m. These other studies had a greater likelihood of differentiating among clones, as they all used larger numbers of polymorphic loci than our study. For example, *Carex lasiocarpa* Ehrh. and *C. pellita* Muhl. ex Willd. were sampled in boreal and forested regions of North America. The mean N_s/N_c for *C. lasiocarpa* was 4.7, with a range of 1.0–40.0. In *C. pellita*, the mean N_s/N_c was 6.1 (range 1.4–22.0). The number of polymorphic alleles was 12 for both species (McClintock and Waterway, 1993). In arctic *Carex* from Eurasia with 8 polymorphic loci, means and ranges are as follows: *C. bigelovii* (4.6, 2.2–7.0), *C. lugens* (1.3, 1.1–1.6), *C . ensifolia* (2.9, 1.4–2.7), and *C. stans* (7.1, 1.2–19.0). The first two are primarily outcrossing, while the latter species have mixed-mating systems (Stenstrom et al., 2001). *Carex*

TABLE 4

Frequencies of ADH alleles in wet vs. moist environments

Location	Population	N	Alleles 1 + 2	Alleles 3 + 4	Significance
Pond margins	C-1	39	0.03	0.97	
					$P < 0.05$
Adjacent meadow	C-2	41	0.28	0.72	
Willow bog	C-6	48	0.22	0.78	
					$P < 0.001$
Adjacent meadow	C-7	37	0.69	0.31	

N indicates sample sizes. Differences in numbers of alleles tested with χ^2.

TABLE 5

Geographic (m) and genetic (Nei, 1972) distances among populations sampled. Only populations with N ≥ 20 and with all 5 loci recorded are shown

		Genetic Distance			
		3	6	7	8
Geographic distance	3	—	0.092	0.112	0.085
	6	3,000	—	0.110	0.083
	7	4,000	300	—	0.039
	8	3,000	500	200	—

bigelovii was also studied in Iceland, using 7 loci, and had a mean N_s/N_c of 1.9, with a range of 1.6–2.1 (Jonsson et al., 1996). *Carex uvula* in the Alps has a value of 7.7, and apparently shows little recruitment via sexual progeny (Steinger et al., 1996).

There is significant genetic variability in this and other species of alpine and arctic *Carex* as well as other species that are either primarily (Steinger et al., 1996; Escaravage et al., 1998) or entirely (Diggle et al., 1998) dependent upon establishment by asexual propagules. This variability may be maintained either by random events such as recurrent mutation and drift or by selection generated by the heterogeneous environments encountered in these settings. The association between specific environments and specific genotypes and allele frequencies in *Carex scopulorum* and frequencies of specific clones in the asexual *Polygonum viviparum* (Diggle et al., 1998) are strongly suggestive in this regard.

In contrast to our a priori expectations for a polyploid and aneuploid species, allelic products are inherited in disomic fashion. For example, if a tetraploid species were involved and each allele behaved independently, tetrasomic inheritance could be expected. That is, each individual could have 1, 2, 3, or 4 alleles. In the case of a monomeric enzyme, a homozygote would have only 1 band, but there could be many different heterozygotes. Certain genotypes, including those with alleles 1-2-2-2 or 1-1-2-2, or 1-1-1-2, would give 2 bands of varying intensities, while other genotypes, such as 1-2-2-3, would give 3 bands and others, such as 1-2-3-4, would give 4 bands. Aneuploidy and the associated extra, or missing, single chromosomes would add to the complexity. We saw no such patterns in our gels. It should also be remembered that in *Carex*, the complexity of chromosome variation is due primarily to the presence of diffuse centromeres, which causes chromosomes either wholly or in pieces to be transmitted in rather less organized fashion than in species with chromosomes possessing single, well-defined centromeres (Stebbins, 1950). This mode of transmission makes the observed disomic patterns even more surprising. The banding patterns we saw in the tetraploid *Carex* were no different from those seen in diploid *Pinus* species., *Arceuthobium* species., and other species with which we are familiar. In addition, the fit of the populations to Hardy-Weinberg expectations implies the existence of a straightforward Mendelian, diploid pattern of recombination. The reason for such disomic inheritance is not clear. It may be due to diploidization of loci that were formerly polysomic, as is the case in a number of ancient polyploids (see review in Haufler, 1987). Alternatively, it may reflect a mechanism of strict autosyndetic (i. e., "diploid") chromosome association between members of chromosome pairs from different genomes. However such a scenario presupposes that this *Carex* is an allopolyploid. Given the lack of information about the origins of polyploidy in *Carex* and other Cyperaceae and the aneuploid series routinely observed in these taxa (Stebbins, 1950; Davies, 1956; Grant, 1971) no clear mechanism can be invoked at this time.

The genetic architecture of these *Carex* populations is complex. There is no clear relationship between genetic distance and geographic distance. This pattern suggests that at the scale we sampled, random events, including bottlenecks, highly localized physical and biotic conditions, or a combination of these factors, are more important in shaping these populations than isolation by distance. We observed a similar pattern in *Deschampsia caespitosa* populations studied in the same area (Gehring and Linhart, 1992).

The patterns of variability shown by ADH suggest that specific loci can be sensitive to specific environmental features, in this case water logging and the associated hypoxic soil conditions. These differences are not associated with extent of clonality, which is similar in both habitat types. This enzyme is important under hypoxic conditions to reduce the damage to plant tissues associated with the buildup of ethanol generated by alcoholic fermentation (Freeling and Bennett, 1985). Variation in ADH has been documented for other plant species occupying habitats with variable levels of soil hypoxia (Brown et al., 1974, 1980; Marshall et al., 1973). Small-scale differentiation for many traits has also been noted in natural populations of other species (Linhart and Grant, 1996), and these traits include specific allozyme loci (Mitton et. al., 1977; Grant and Mitton, 1977; Rainey et. al., 1987).

The fact that most populations show genotypic distributions that fit Hardy-Weinberg expectations implies strongly that seeds are produced via sexual reproduction and that the pollination associated with this reproduction involves a good deal of outcrossing. If self-pollination were the sole or primary mode of fertilization, such inbreeding would be very likely to produce deviations from Hardy-Weinberg expectations. Comparisons with other *Carex* species indicate that *C. scopulorum* shows a fit to Hardy-Weinberg expectations similar to those of other outcrossing *Carex* and is therefore likely to also have an outcrossing mating system (McClintock and Waterway, 1993; Jonsson et al., 1996; Stenstrom et al., 2001; Huh, 2001).

The combination of small clone size and fit to Hardy-Weinberg expectations provides indirect evidence that regeneration via seeds is likely to be common in this species and other *Carex* listed above. This conclusion, along with results of studies of other genera in both arctic and alpine tundras, indicates that sexual reproduction is a common component of the ecology of plants in these habitats (e. g., Gugerli, 1997; Tolvanen and Henry, 2000; Forbis, 2003).

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