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GENETIC AND HISTORICAL RELATIONSHIPS AMONG GEOTHERMALLY ADAPTED *AGROSTIS* (BENTGRASS) OF NORTH AMERICA AND KAMCHATKA: EVIDENCE FOR A PREVIOUSLY UNRECOGNIZED, THERMALLY ADAPTED TAXON[1]

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Agrostis species have been known to evolve ecotypes rapidly in response to unusual edaphic conditions. The geographic distribution of *Agrostis* taxa in Lassen Volcanic National Park (California) and Yellowstone National Park (Wyoming) in the United States and the Valley of the Geysers (Kamchatka Peninsula) in Russia suggests that *Agrostis scabra* might have independently evolved morphologically similar ecotypes several times. We used RAPDs to show that, contrary to expectation, the thermal populations are not independently evolved, but instead constitute a single taxon that currently has four names. A UPGMA including the four thermal and nine nonthermal *Agrostis* taxa showed that the thermal cluster divides into geographically distinct subclusters, but that two morphologically distinct thermal taxa do not cluster independently. Even though currently confused with the thermal populations, nonthermal *A. scabra* is not closely related. An analysis of molecular variance (AMOVA) showed significant differentiation between the thermal populations and the nonthermal species sampled in this study. Splitting a hypothesized thermal operational taxonomic unit (OTU) into its components (geographically separated populations) does not greatly affect the partitioning of variation among OTUs. All thermal populations therefore should be assigned to the same taxon, but its taxonomic rank cannot be determined at this time.

Key words: *Agrostis*; bentgrass; geothermal; Gramineae; heat tolerance; Kamchatka, Russia; Lassen Volcanic National Park, California; Poaceae; thermal; Yellowstone Naitonal Park, Wyoming.

The genus *Agrostis* can be confusing taxonomically, and morphology is not a reliable indicator of genetic affinity. Populations that look alike may be genetically distinct, and phenotype can be greatly affected by growth conditions (Davies, 1953). Species in this genus are particularly known for their ability to form morphologically similar ecotypes in response to heavy metals and other unusual edaphic conditions (Jain and Bradshaw, 1966; McNeilly, 1968; Wu et al., 1975; Hogan and Courtin, 1977; Karataglis, 1980a, b; Archambault and Winterhalder, 1995). Such *Agrostis* ecotypes are often interfertile, and cross-pollinating, but the selection pressure exerted by differing soil characteristics is great enough to maintain genetic differences between populations only meters apart. Based on experimental manipulations and field observations, Karataglis et al. (1980b) and Archambault and Winterhalder (1995) estimated that the formation of *Agrostis* ecotypes is rapid and probably frequent, with the same ecotype evolving independently in different locations.

Two *Agrostis* taxa in geothermally influenced habitats of Yellowstone National Park, Wyoming, USA, have geographic distributions that suggest ecotype formation in response to soil temperature extremes or unique soil chemistry (M. T. Tercek, personal observations). *Agrostis rossiae* Vasey is endemic to Yellowstone thermal areas (Swallen, 1948), while *Agrostis scabra* Willdenow occurs both in thermal areas and in a variety of nonthermal habitats throughout the northern latitudes (Hitchcock, 1950; M. T. Tercek, personal observations). The thermal form of *A. scabra*, despite keying out as *A. scabra* in the local floras, differs from the nonthermal form in its shorter stature (8–20 cm vs. 15–30 cm), more rapid growth, and annual habit, all characteristics shared with *A. rossiae* (Table 1). *Agrostis rossiae* can be distinguished from *A. scabra* by its compressed, rather than spreading panicle (Hitchcock, 1950). In 1999, one of the present authors (M. T. Tercek) discovered that every Yellowstone thermal *Agrostis* population (*A. rossiae* as well as thermal *A. scabra*) is surrounded by a nonthermal *A. scabra* population that appears to be reproductively isolated from the adjacent thermal population by its later flowering time. Seeds of thermal *Agrostis* populations germinate from December to January, when all nonthermal areas of the park are covered with snow. The thermal plants are killed by rising soil temperatures in mid-June. Nonthermal *A. scabra* populations do not initiate new growth from their perennial roots until late June, when the snow has completely melted, and they flower in mid-July to mid-August. A number of populations were found to be growing around a relatively localized heat source (e.g., a single steam vent), and in these cases, the nonthermal population was distributed concentrically around the thermal population.

Thermal *Agrostis* populations are rare and quite small, ranging in size from a single plant to a population covering approximately 100 m in diameter. For this reason, *A. rossiae* has

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TABLE 1. Morphological differences among *Agrostis rossiae*, thermal *A. scabra*, and nonthermal *A. scabra*, based on an examination of herbarium and field specimens.

Taxon	Panicle	Leaf sheaths	Leaf blades (cm)	Height (cm)	Approximate time to flowering in field
Agrostis rossiae	Compressed	Grip stem loosely	1–2 × 0.3–0.5	8–20	4 wk
Thermal *A. scabra*	Spreading	Grip stem loosely	1–2 × 0.3–0.5	8–20	4 wk
Nonthermal *A. scabra*	Diffuse, spreading	Grip stem tightly	3–5.5 × 0.1–0.2	15–30	6 mo

become a management priority for the National Park Service (Boyce, 1991; J. Whipple, National Park Service, personal communication). Dorn (1989) reported that *A. rossiae* is restricted to the Upper Geyser Basin, where Old Faithful geyser is located. However, many more populations have since been found (M. T. Tercek, personal observation; J. Whipple, National Park Service, personal communication). Although there are many thermal areas in Yellowstone, *A. rossiae* and thermal *A. scabra* populations occur only in the western part of the park. In most cases, a particular thermal population consists of only one of those taxa, not both. The thermal habitats in which *Agrostis* taxa occur tend to be located on glacial deposits at a slightly higher elevation than nearby hot springs and consequently contain numerous fumaroles (steam vents) and relatively little geothermal water and are permeated by steam and other trace gasses, including hydrogen sulfide, which acidifies the soil (pH 3.0–4.5). These habitats are usually dominated by the moss *Racomitrium canescens*, and contain a heat-tolerant grass, *Dichanthelium lanuginosum*, and a small number of annual forbs.

Because of the consistent geographic association of the thermal and nonthermal populations, we initially hypothesized that the thermal populations may have arisen independently in each thermal area as an ecotype of nonthermal *Agrostis scabra* (Jain and Bradshaw, 1966; McNeilly, 1968; Wu et al., 1975; Hogan and Courtin, 1977; Karataglis, 1980a, b; Archambault and Winterhalder, 1995). Preliminary greenhouse experiments showed that *Agrostis rossiae*, thermal *A. scabra*, and nonthermal *A. scabra* maintain their morphological differences (Table 1) when grown from seed in a variety of soils and under both hot and cold temperature extremes, suggesting that they are genetically distinct (M. T. Tercek, unpublished data). Furthermore, these taxa experience no reduction in seed set when their inflorescences are isolated from external pollen sources, suggesting that they are capable of self-pollination or are apomictic. The present study was part of a larger effort to determine the ecological and genetic factors contributing to the endemism of *A. rossiae*, the historical relationships between the thermal and nonthermal *Agrostis* populations, and whether or not ecotype formation is taking place in response to selection pressures imposed by the geothermal habitats. These questions have practical as well as theoretical interest: if *A. rossiae* is merely an ecotype of nonthermal *A. scabra*, it may not be worthy of conservation effort.

MATERIALS AND METHODS

Plant populations were located with the help of National Park Service botanist J. Whipple and herbarium specimen data (Montana State University [MONT, Bozeman, Montana, USA] and Yellowstone National Park [YELLO] herbaria). Three hundred seventy-four individuals from 27 populations were included in the RAPDs analysis (Table 2). Plants were collected from nine *Agrostis rossiae* populations and four thermal *A. scabra* populations. To put the thermal populations in a larger geographic and phylogenetic context, plants were also collected from populations of seven of the nine other *Agrostis* species known to occur in Wyoming (Dorn, 2001), and *A. scabra* var. *geminata* was collected from a thermal plant community in the Boiling Springs Lake thermal area of Lassen Volcanic National Park, California, USA (Gillett et al., 1995). Samples were collected over the entire geographic range of each population, with an effort to collect plants at least 1 m from each other. Because some populations were quite small, this was not always possible. Approximately 5–8 cm of leaf was collected from each plant and stored in silica gel until DNA was extracted. In addition to the field-collected plants, a single specimen was obtained from a thermal community located in the Valley of the Geysers, Kamchatka Peninsula, Russian Federation. Eight seeds from the latter specimen were propagated, and the progeny were identified as *Agrostis pauzhetica* Probatova, which is apparently endemic to that region (Probatova, 1984). *Agrostis scabra* var. *geminata* and *A. pauzhetica* resemble thermal *A. scabra*, but they sometimes have awned lemmas. Voucher specimens for all taxa are deposited at the Tulane University herbarium (NO).

DNA was extracted following the methods of Doyle and Doyle (1990). DNA samples were quantified with a spectrophotometer and diluted with TE (Tris-EDTA) buffer. Each 25-μL polymerase chain reaction (PCR) contained 10 mmol/L Tris-HCl, 50 mmol/L KCl, 0.8 mmol/L dNTPs; 1 unit *Taq* polymerase, 0.2 μmol/L primer, 3 mmol/L $MgCl_2$, and approximately 2 ng of template. The PCR reactions were treated for 3 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 40°C, and 1 min at 72°C, followed by a final extension step of 15 min at 72°C (Golembiewski et al., 1997). Amplifications were resolved on a 1.5% agarose gel (150 V for 5 min, followed by 100 V for 2 h) and stained with ethidium bromide. Forty random decamer primers (RAPDs primers 701–740, University of British Columbia, Vancouver, British Columbia, Canada) were screened and four were chosen for their repeatability and quality of staining (primers 702, 713, 719, 721).

The scoring of each individual was based on at least two separate PCR reactions. The NTSYSpc 2.1 genetics package (Rohlf, 2000) was used to produce UPGMA phenograms of all individuals, using the Jaccard, Simple Matching, and Dice similarity coefficients. Population gene frequencies were calculated using the methods of Lynch and Milligan (1994), and NTSYSpc was used to produce population-level UPGMA phenograms based on Nei's (1978) unbiased genetic distance, which is suitable for small population sizes. The UPGMA phenograms based on other similarity measures had the same topology (not shown). The population-level data were also visualized with the principal components analysis module of NTSYSpc. The FREETREE program (Pavlicek et al., 1999) was used to produce 500 bootstrap pseudoreplicates of the population-level data and to calculate the level of confidence that could be assigned to each cluster in the original UPGMA phenogram.

The Arlequin genetics package (Schneider et al., 2000) was used to perform an analysis of molecular variance (AMOVA), which uses bootstrapped permutations of the data to test an hypothesized population genetic structure. Arlequin's AMOVA module was configured to test five different hypothesized population structures. The first hypothesis treated *A. rossiae*, thermal *A. scabra*, Kamchatka thermal *Agrostis* (*A. pauzhetica*), and Lassen thermal *Agrostis* (*A. scabra* var. *geminata*) as separate operational taxonomic units (OTUs). The other three hypotheses combined the thermal populations into successively larger OTUs, and the final hypothesis grouped the thermal populations with nonthermal *A. scabra*, which was determined by the UPGMA to be most closely related to the thermal populations. Because the AMOVA tests for levels of differentiation among groups of taxa, *A. idahoensis*, *A. exarata*, and *A. variabilis* were also included in the analysis as outgroups because the principal components analysis indicated that they were more closely related

TABLE 2. Location information for populations from which *Agrostis* specimens were collected for RAPD genetic analysis. All populations with an abbreviation ending in AR are *Agrostis rossiae*; all those ending in TAS are thermal *Agrostis scabra*; all ending in NAS are nonthermal *A. scabra*. The first part of each abbreviation indicates the population's location. Thus, MAR and MNAS are, respectively, *A. rossiae* and nonthermal *A. scabra* that grow adjacent to each other, around the same thermal heat source. YNP = Yellowstone National Park.

Abbreviation	Taxon	Sample size	Location description	Universal Transverse Mercator (UTM) coordinates
AcrossLSAR	*Agrostis rossiae*	15	YNP: Across the Firehole River from Lone Star Geyser.	12T 515220 4918097
AHQAR	*Agrostis rossiae*	20	YNP: Agrostis Headquarters, thermal basin near Lone Star Geyser trail.	12T 514821 4919760
BHBAR	*Agrostis rossiae*	16	YNP: Off-trail thermal basin near Lone Star Geyser trail.	12T 514714 4919718
BHTAR	*Agrostis rossiae*	15	YNP: Off-trail thermal basin near Lone Star Geyser trail.	12T 514738 4919711
BLSanAR	*Agrostis rossiae*	16	YNP: Upper Geyser Basin, thermal depression near Black Sand Pool.	12T 511777 4923781
IHAR	*Agrostis rossiae*	17	YNP: Off-trail thermal basin near the the Howard Eaton trail.	12T 515352 4919281
MAR	*Agrostis rossiae*	17	YNP: Base of the Mallard Lake Trail, behind Old Faithful Lodge.	12T 514555 4922868
PSAR.1	*Agrostis rossiae*	17	YNP: Upper Geyser Basin, Pine Spring Thermal Area.	12T 511997 4923638
PSAR.2	*Agrostis rossiae*	19	YNP: Upper Geyser Basin, Pine Spring Thermal Area.	12T 512151 4923746
FTAS	thermal *Agrostis scabra*	18	YNP: Lower Geyser Basin, near Narcissus Geyser.	12T 515944 4932443
NORTAS	thermal *Agrostis scabra*	15	YNP: Norris Geyser Basin, near back basin overlook trail.	12T 523246 4952877
NTPTAS	thermal *Agrostis scabra*	15	YNP: Norris Geyser Basin, southwest edge of One Hundred Spring Plain.	12T 523002 4953104
TBTAS	thermal *Agrostis scabra*	14	YNP: Twin Buttes thermal area, near the top of the southwest butte.	12T 509460 4931328
Kamchatka	*Agrostis pauzhetica*	8	Valley of the Geysers, Kamchatka Pennisula, Russian Federation.	N/A
Lassentherm	*Agrostis scabra* var. *geminata*	6	Lassen Volcanic National Park, California, USA, Boiling Springs Lake.	N/A
AHQNAS	nonthermal *Agrostis scabra*	19	YNP: Agrostis Headquarters, off-trail near Lone Star Geyser trail.	12T 514821 4919760
FNAS	nonthermal *Agrostis scabra*	17	YNP: Lower Geyser Basin, Firehole Lake Drive, Narcissus Geyser.	12T 515944 4932443
MNAS	nonthermal *Agrostis scabra*	14	YNP: Base of Mallard Lake Trail, behind Old Faithful Lodge.	12T 514555 4922868
PSNAS	nonthermal *Agrostis scabra*	10	YNP: Upper Geyser Basin, Pine Spring Thermal Area.	12T 512151 4923746
Capillaris	*Agrostis capillaris*	11	YNP: Near the Mallard Lake trail, Old Faithful Area.	N/A
Exarata	*Agrostis exarata*	17	YNP: Thermal drainage on road near Madison Junction.	N/A
Humilis	*Agrostis humilis*	18	Swampy area near shore of Beartooth Lake, Shoshone National Forest.	N/A
Idahoensis1	*Agrostis idahoensis*	7	YNP: Dog's-Head trail to Shoshone Lake.	N/A
Idahoensis2	*Agrostis idahoensis*	3	YNP: Near the outlet to Otter Creek, Hayden Valley.	N/A
Mertensii	*Agrostis mertensii*	8	East slope of Pat O'Hara Peak, Shoshone National Forest, Wyoming.	N/A
Thurberiana	*Agrostis thurberiana*	19	Shore of Grassy Lake Reservoir, Targhee National Forest, Wyoming.	N/A
Variabilis	*Agrostis variabilis*	3	Near Dorf Lake, Gallatin National Forest, Montana.	N/A

to the thermal cluster than other sampled taxa (Table 4). The AMOVA procedure also provides estimates of the population differentiation statistic, F_{ST}, which is calculated as the percentage of the bootstrapped variation that is *not* partitioned within the populations (Excoffier et al., 1992). These AMOVA F_{ST} estimates were compared to G_{ST} values calculated following the methods of Nei (1973), which is equivalent to F_{ST} when there are only two alleles possible (Nei, 1973). Culley et al. (2002) have shown that the G_{ST} of Nei (1973) is not always comparable to the G_{ST} used in the widely cited review by Hamrick and Godt (1989). We chose Nei's formula because it is used more frequently in the literature (Culley et al., 2002) and can be directly compared to the recent review of RAPDs studies by Nybom and Bartish (2000). In the Nei (1973) method, the values of total expected heterozygosity (H_t) for a taxon and within-population heterozygosity (H_s) are averaged over all polymorphic loci and then entered into the formula $G_{ST} = (H_t - H_s)/H_t$, where $H_s = 1/L \Sigma\ 1 - p_i^2 - q_i^2$, p_i is the frequency of the amplified allele at the ith locus in a population, q_i is the frequency of the null allele for the ith locus, and L is the number of loci.

RESULTS

The four primers produced 60 scorable bands. All of the bands were polymorphic among the populations, but there was little polymorphism within populations. The mean within-population heterozygosity (H_s) was less in the endemic thermal

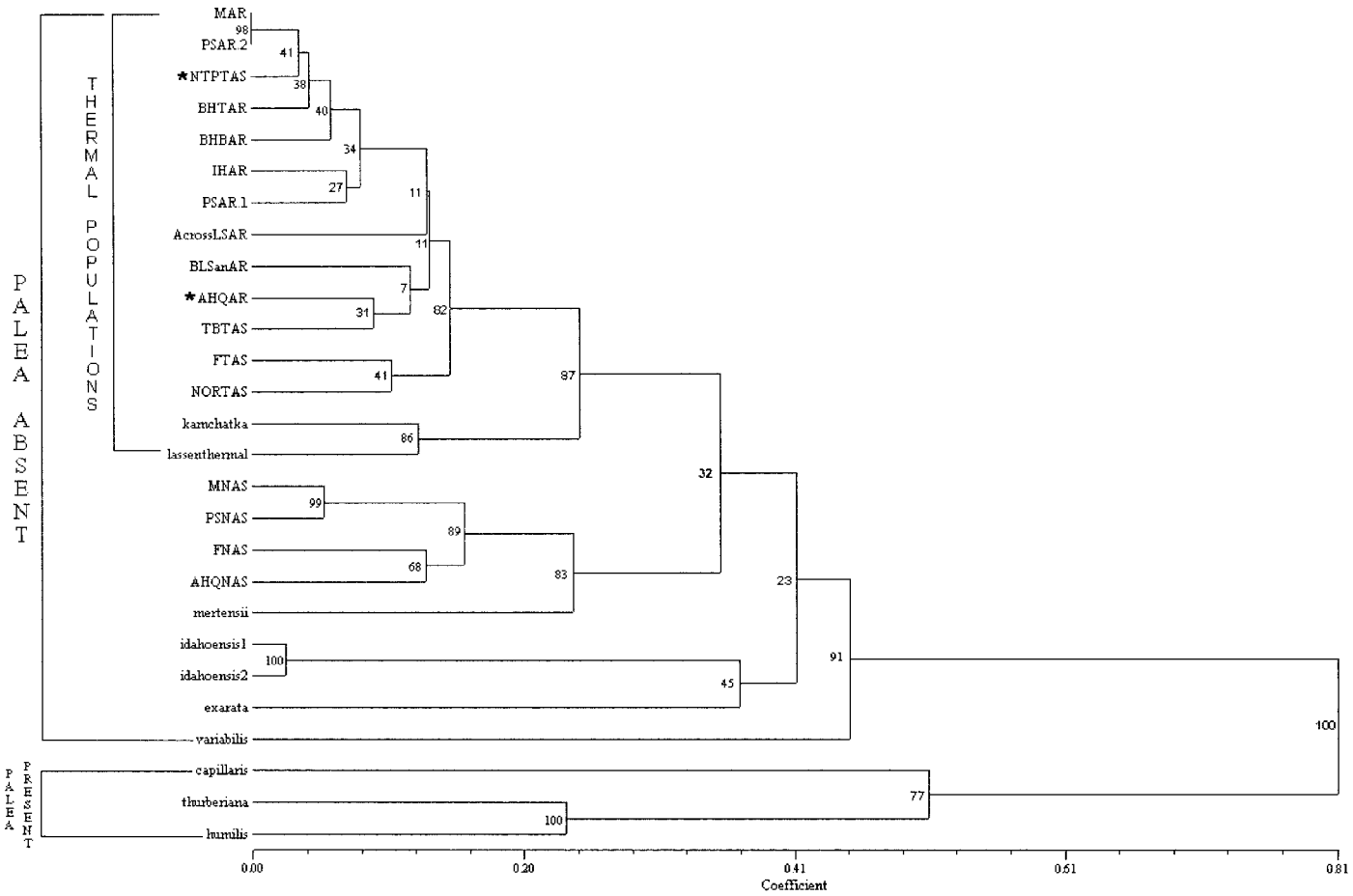

Fig. 1. UPGMA of *Agrostis* taxa collected from populations in Wyoming, California (USA), and the Kamchatka Peninsula (Russian Federation), based on 60 putative RAPDs loci and Nei's (1978) unbiased similarity. See Table 2 for an explanation of population name abbreviations. The numbers at each node indicate the bootstrap support for that cluster. Asterisks indicate *A. rossiae* or thermal *A. scabra* that cluster within the other taxon.

populations (*A. rossiae* = 0.0114, thermal *A. scabra* = 0.0097) than in the nonthermal populations (0.0236), but the range of values in the latter was greater. G_{ST} was uniformly high (0.8011–0.9400) (Table 3). A sample photograph of an RAPD gel is available as Supplemental Data accompanying the online version of this article.

In the UPGMA, all Yellowstone thermal populations form a single cluster, with the Kamchatka (*A. pauzhetica*) and Lassen (*A. scabra* var. *geminata*) thermal populations grouping as the next most similar. *Agrostis rossiae* and thermal *A. scabra* were intermingled on the phenogram and did not cluster independently (Fig. 1). The apparent interchangeability of *A. rossiae* and thermal *A. scabra* on the phenogram was confirmed by the FREETREE bootstrap analysis, which showed poor support (7–41%) for the terminal clusters of the thermal portion of the phenogram, even though there was good support for the clustering of all thermal populations as a single unit (87%) and for the clustering of the Kamchatka and Lassen thermal populations (86%). Nonthermal *A. scabra* was the taxon most similar to the thermal populations; however, this grouping had low bootstrap support (32%), which indicates that many of the alternative trees generated by this procedure had other taxa in that position. Phenogram topology was not affected by the similarity coefficient used (Jaccard, Dice, Simple Matching, or Nei's [1978] unbiased similarity), and the UPGMA of all 374 individuals had the same topology as the UPGMA of the population-level data, with individuals from each population grouping together (not shown). Furthermore, a population-level UPGMA using raw RAPDs band frequencies (percentage of occurrence of each band in each population) was identical to one in which gene frequencies were calculated using the methods of Lynch and Milligan (1994), which correct for the dominance of RAPDs bands.

TABLE 3. Genetic diversity of *Agrostis* taxa collected in Wyoming, California (USA), and the Kamchatka Peninsula (Russian Federation), based on 60 putative RAPDs loci: mean within-population expected heterozygosities (H_{pop}) and population differentiation (G_{ST}) indices. Values were not calculated for *A. pauzhetica* (Kamchatka thermal *Agrostis*) because all the individuals analyzed were the progeny of a single mother plant. *Agrostis mertensii*, *A. variabilis*, and *A. scabra* var. *geminata* (Lassen thermal *Agrostis*) were not included because of their small sample sizes. Means for each category were weighted by the sample size of each population.

Population	Mean H_{pop}	Range of H_{pop}	G_{ST}
Agrostis rossiae	0.0110	0.0000–0.0379	0.8670
Thermal *Agrostis scabra*	0.0097	0.0059–0.0163	0.8833
Nonthermal *Agrostis scabra*	0.0239	0.0083–0.0597	0.8011
All nonthermal *Agrostis*	0.0236	0.0060–0.0710	0.9400

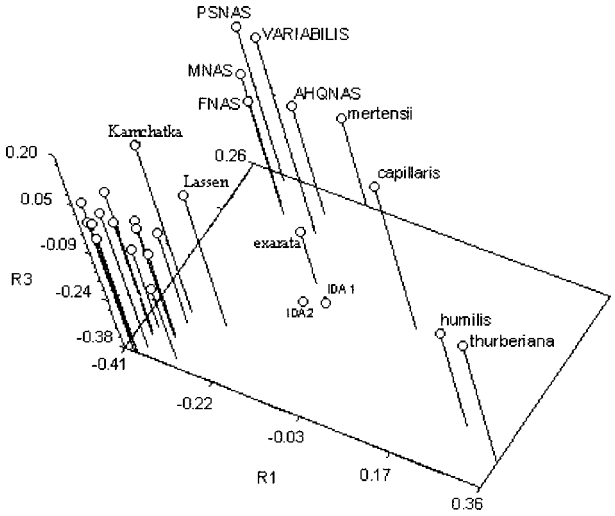

Fig. 2. Principal components analysis of 60 putative RAPDs loci for *Agrostis* taxa collected from populations in Wyoming, California (USA), and the Kamchatka Peninsula (Russian Federation). All thermal *Agrostis* populations cluster in the left corner of the plot. Unlabeled points are *Agrostis rossiae* and thermal *A. scabra* populations. See Table 2 for meanings of population abbreviations

The principal components analysis showed the same relationships among the OTUs as the UPGMA, with the first three axes explaining 55% of the variance (Fig. 2). The thermal populations clustered together, with the Kamchatka and Lassen thermal populations plotting slightly outside the Yellowstone group. Interestingly, nonthermal *A. scabra*, *A. exarata*, *A. variabilis*, and *A. idahoensis* were approximately equidistant from the thermal cluster, which agrees with the low bootstrap support shown for the grouping of nonthermal *A. scabra* as the sister taxon of the thermal populations.

The AMOVA tests of the five hypothesized population genetic structures indicate that the portion of the variation distributed within the hypothesized OTUs (34.76–36.69%) is not greatly affected by the conflation of all the thermal populations into one group (hypotheses 1–4), but this increases with the addition of nonthermal *A. scabra* (Table 4). In every case, the AMOVA F_{ST} estimates calculated among OTUs were significant (i.e., nonzero, $P < 0.0001$), indicating a significant difference between hypothesized OTUs, and these values (mean F_{ST} for all AMOVA analyses = 0.9526) were similar to the value of 0.9391 calculated for this subset of populations with Nei's (1973) formula (Table 3). With one exception (populations MAR and PSAR.2, where $F_{ST} = 0$), fixation indices calculated by Arlequin between all possible pairs of populations ranged between 0.69 and 0.99, and they were all significantly nonzero (data not shown).

DISCUSSION

The UPGMA phenogram based on 60 RAPDs loci suggests that all thermal *Agrostis* populations, regardless of geographical location, comprise a previously unrecognized taxon that is currently referred to under four different names. In Yellowstone National Park, the thermal *A. scabra* populations are conflated with nonthermal *A. scabra*, and *A. rossiae* is recognized as a separate species (Dorn, 2001). In Lassen Volcanic National Park, California, this thermal taxon is called *A. scabra* var. *geminata* (Gillett et al., 1995), and in Kamchatka, it is called *A. pauzhetica* (Probatova, 1984). The same grouping of all thermal plants was obtained when the data were analyzed at the population and individual levels; the individuals from each population all clustered together as discrete groups. Contrary to our original hypothesis, the thermal populations do not appear to be independently evolved ecotypes of parapatric, nonthermal *A. scabra*. There is no relationship between geographic proximity and the genetic distance between thermal and nonthermal populations. Instead, the UPGMA shows that all nonthermal *A. scabra* populations, as a group, are distantly related to the thermal taxa. Indeed, the fact that nonthermal *A. scabra* did not plot any nearer to the thermal taxon than did *A. exarata*, *A. idahoensis*, or *A. variabilis* on the principal components analysis (Fig. 2), combined with the low bootstrap support for its UPGMA clustering with the thermal populations, suggests that nonthermal *A. scabra* may not be the sister taxon of the thermal populations. It is possible that the nonthermal sister taxon of the thermal populations was not included in the present study but may be found near other sites of thermal activity in the Pacific ring, where *Agrostis* species have been reported to occur, e.g., the Aleutian Islands or Big Windy Preserve, Alaska, USA (Juday, 1998).

TABLE 4. Partitioning of genetic variation and population differentiation indices (F_{ST}) of *Agrostis* taxa collected in Wyoming, California (USA), and the Kamchatka Peninsula (Russian Federation), based on 60 putative RAPDs loci, as determined by analysis of molecular variance (AMOVA) testing of eight hypothesized operational taxonomic units (OTUs). Parentheses indicate groups of populations. Kamchatka Thermal = *A. pauzhetica*, Lassen Thermal = *A. scabra* var. *geminata*. Mean AMOVA F_{ST} estimate = 0.9525. Nei's (1973) G_{ST} = 0.9391.

Population structure tested	Percentage of variation among OTUs	Percentage of variation among populations within OTUs	Percentage of variation within populations	AMOVA-derived F_{ST} estimate (sum of first two columns)
(*A. rossiae*) + (thermal *A. scabra*) + (Kamchatka thermal) + (Lassen Thermal) + (nonthermal *A. scabra*) + (*A. variabilis*) + (*A. exarata*) + (*A. idahoensis*)	58.14	36.42	5.44	0.9456
(*A. rossiae* + thermal *A. scabra*) + (Kamchatka thermal) + (Lassen Thermal) + (nonthermal *A. scabra*) + (*A. variabilis*) + (*A. exarata*) + (*A. idahoensis*)	60.61	34.76	4.63	0.9537
(*A. rossiae* + thermal *A. scabra*) + (Kamchatka thermal + Lassen Thermal) + (nonthermal *A. scabra*) + (*A. variabilis*) + (*A. exarata*) + (*A. idahoensis*)	60.54	34.86	4.63	0.9530
(All thermal populations) + (nonthermal *A. scabra*) + (*A. variabilis*) + (*A. exarata*) + (*A. idahoensis*)	58.86	36.69	4.46	0.9555
(All thermal populations + nonthermal *A. scabra*) + (*A. exarata*) + (*A. variabilis*) + (*A. idahoensis*)	50.68	45.54	3.79	0.9622

Agrostis rossiae and thermal *A. scabra* are intermingled on the UPGMA, despite the fact that they maintain separate morphologies (Table 1) when grown under uniform conditions (M. T. Tercek, unpublished data). The low bootstrap values assigned to the terminal branches of the thermal portion of the UPGMA indicate that our data set do not support any definite conclusions regarding the separation of these two taxa. This result could be explained by a possibly recent divergence of *A. rossiae* and thermal *A. scabra* or by continued hybridization between the two. With regard to the first possibility, it is well known that gene trees do not always agree with species trees (Avise, 1989; Maddison, 1997). The markers used in the present study may be too conservative to resolve such recently diverged populations. With regard to the second possibility, no morphological hybrids of *A. rossiae* and thermal *A. scabra* have been observed. However, even if infrequent hybridization was occurring, it is possible that morphological differences may be maintained by natural selection despite the detection of gene flow by neutral markers (Nesbitt et al., 1995; Harrison et al., 1997; Olfelt et al., 2001). *Agrostis rossiae* and thermal *A. scabra* do have mostly separate geographical distributions, which could reflect a difference in habitat requirements. *Agrostis rossiae* is restricted to the vicinity of the Firehole River drainage, while thermal *A. scabra* occurs primarily north and south of this area. Laboratory experiments have shown that they differ in tolerance to soil acidity (M. T. Tercek, unpublished data). However, transplantation experiments have shown that *A. rossiae* and thermal *A. scabra* both survive to maturity in each other's habitat, and the population differentiation indices (F_{ST}, G_{ST}) suggest that there is low gene flow between *Agrostis* populations. Our Nei (1973) G_{ST} values of 0.8011–0.9400 (Table 3) were higher than those reported for self-pollinating taxa (0.7) by Nybom and Bartish (2000), and the AMOVA-derived F_{ST} values calculated between all possible pairs of populations (F_{st} = 0.69–0.99, data not shown) indicate that gene flow seldom occurs between thermal *Agrostis* populations that are in some cases only meters apart. These findings contradict Despain's (1990) speculation that seed dispersal among *A. rossiae* populations is frequently achieved by ungulates that migrate between Yellowstone's thermal areas during the winter, but these findings do agree with experiments that suggest *A. rossiae* and thermal *A. scabra* are self-pollinating or apomictic, having no reduction in seed set when isolated from external pollen sources (M. T. Tercek, unpublished data). Preliminary attempts to cross-pollinate *A. rossiae* and thermal *A. scabra* were unsuccessful, but more rigorous experimentation is needed before a definite conclusion can be reached.

Self-pollination is also consistent with the low within-population heterozygosities found for the thermal *Agrostis* taxa (*A. rossiae* = 0.0114, thermal *A. scabra* = 0.0097). These values are even lower than the mean cited for self-pollinating taxa (0.091) by Nybom and Bartish (2000). However, the nonthermal taxa in our study had similarly low heterozygosities (Table 3), consistent with results reviewed by Gitzendanner and Soltis (2000), who found a correlation between diversity estimates in rare species and their widespread congeners. The low genetic diversity found in this study is therefore not likely due to the small population sizes of the thermal taxa, but instead to something that is shared by other members of the genus, e.g., self-pollination. James and Brown (2000) reported similar within-population heterozygosity (0.0053–0.0224) for the Australian endemic *A. adamsonii*.

The AMOVA showed that grouping all the thermal populations together in a single OTU produces a situation in which there is more variation partitioned among the OTUs than within them (Table 4). Splitting this hypothesized thermal OTU into its component parts does not greatly change the partitioning of variation, but grouping it with its closest relative, nonthermal *A. scabra*, increases the variation within OTUs from 35% to 46% (Table 4). It therefore seems reasonable to consider all the thermal populations as a single taxonomic unit that is separate from nonthermal *A. scabra*. Olfelt et al. (2001) used an AMOVA to reach a similar conclusion in their delineation of *Sedum* taxa.

The phenogram presented here (Fig. 1) may serve as a working hypothesis of the historical relationships among the study taxa until data from more explicitly phylogenetic methods become available. Cladistic methods are often considered preferable to phenetic methods for hypothesizing historical relationships (Li, 1999), and some authors have pointed out that RAPDs bands may not always be homologous among taxa (e.g., Rieseberg, 1996). However, the results of the present study corroborate the morphological taxonomic work of Bjorkmann (1960) and Carlbom (1968) in their separation of taxa that possess a palea (*A. capillaries*, *A. humilis*, and *A. thurberiana*) from those that do not, and they confirm the grouping of *A. mertensii* with nonthermal *A. scabra*, which could be predicted from their morphological similarity (M. T. Tercek, personal observation). More generally, several authors have found RAPD-based phenograms to be similar to trees produced by other methods, including morphological cladistics (e.g., James and Brown, 2000; Fjellheim et al., 2001; Olfelt et al., 2001), inter-simple sequence repeats (ISSR) (Ayres and Strong, 2001), restriction fragment length polymorphisms (RFLP) (Nocelli et al., 1999), and isozymes (e.g., Sun and Wong, 2001).

In conclusion, RAPD data suggest that thermal *Agrostis* populations of North American and Kamchatka, regardless of their geographic location, are historically related and may comprise a monophyletic taxon, although explicitly cladistic methods, based on other characters, should be used to confirm this conclusion. Field botanists and systematists should be aware that nonthermal *A. scabra* can be easily confused with thermal *A. scabra* (Hitchcock and Cronquist, 1973; Dorn, 2001), but they can be distinguished morphologically (Table 1). Additional research is needed before the thermal populations can be given taxonomic rank. In particular, it is important to verify the apparent consanguinity of *A. rossiae* and thermal *A. scabra*. Because they are morphologically distinct, appear to be autogamous, and probably do not hybridize in the field, they are best treated as separate species until more information is available.

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