Allelic Diversity Changes in 96 Canadian Oat Cultivars Released from 1886 to 2001

Yong-Bi Fu,* Gregory W. Peterson, Graham Scoles, Brian Rossnagel, Daniel J. Schoen, and Ken W. Richards

ABSTRACT

There is longstanding concern that modern plant breeding reduces crop genetic diversity. Such reduction may have consequences both for the vulnerability of crops to changes in their pests and diseases and for their ability to respond to changes in climate and agricultural practices. This concern, however, has not been well validated in recent molecular studies of genetic diversity of several crop species. The objective of this study was to assess allelic diversity changes in 96 Canadian oat (*Avena sativa* L.) cultivars released from 1886 to 2001 by means of 30 simple sequence repeats (SSRs). A total of 62 alleles were found from 11 informative SSR loci. Thirty-nine alleles were detected infrequently (*frequency* ≤ 0.15) among the cultivars and only two alleles were observed frequently (*frequency* ≥ 0.95). Analyses of the dynamics of SSR alleles over time in these oat cultivars revealed random patterns of allelic change at three loci, shifting patterns of change at one locus, increasing patterns of change at two loci, and decreasing patterns of change at five loci. Significant decrease of alleles was detected in cultivars released after 1970 and also in some specific breeding programs. Three different band-sharing analyses of the genetic diversity of the grouped cultivars, however, failed to detect significant diversity changes among cultivars released from different breeding periods or programs. These findings indicate that allelic diversity at particular loci, rather than average genetic diversity, is sensitive to oat breeding practices. They also indicate the need for attention to be paid to oat germplasm conservation.

Concern has often been expressed that modern plant breeding techniques reduce crop genetic diversity (Vellve, 1993; Clunier-Ross, 1995; Tripp, 1996). Such reduction may have consequences both for the vulnerability of crops to their pests and diseases and for their ability to respond to changes in climate or agricultural practice (Clunier-Ross, 1995; FAO, 1998). To address these concerns, data are needed that allow objective quantification of the changes that have occurred in genetic diversity of the major agricultural crop species (Duvick, 1984; Swanson, 1996; Tripp, 1996; Donini et al., 2000). With the advent of molecular genetic techniques, assessments of crop genetic diversity have increased in number (Karp et al., 1997). Many assessments, in fact, suggest that the reduction of the genetic diversity accompanying plant improvement has been negligible (Donini et al., 2000; Russell et al., 2000; Lu and Bernardo, 2001; Christiansen et al., 2002; Fu et al., 2002, 2003). For example, Donini et al. (2000) showed that plant breeding resulted in a qualitative, rather than quantitative, change in genetic diversity of 55 dominant UK winter wheat (*Triticum aestivum* L.) varieties released from 1934 to 1994. As well, despite a significant (35%) reduction of microsatellite alleles per locus in eight current maize (*Zea mays* L.) inbreds, Lu and Bernardo (2001) still failed to detect a significant difference in genetic diversity between current and historical maize inbreds. While these results appear to refute the concern that modern breeding reduces diversity, they are based on relatively small amounts of data. It would be useful, therefore, to extend studies of allelic dynamics accompanying intensive breeding over longer periods of time and to many varieties.

Oat breeding in Canada began in the late 1800s to meet the demand of the growing Canadian livestock industry (Welsh et al., 1953; McKenzie and Harder, 1995). Selection and hybridization from the 1900s to the 1930s at Agriculture and Agri-Food Canada (AAFC) experimental farms and several Canadian agricultural colleges generated several highly productive cultivars such as Liberty and Legacy. Backcrossing of rust resistant genes into 'Rodney' and 'Pendek' in the 1960s produced several highly successful cultivars such as Harmon, Dumont, and Robert. Introduction of wild oat (*A. sterilis* L.) germplasm in the 1970s further enhanced the development of many cultivars with genes for resistance to both stem rust (cause by *Puccinia graminis* f. sp. *avenae* Eriks. & E. Henn) and crown rust (caused by *P. coronata* Corda var. *avenae* Eriks.). So far, the breeding programs have developed and released as many as 130 registered cultivars, most of which have made significant impacts on the economy of western Canada (McKinnon, 1998). In spite of impressive achievements in yield and disease resistance, concern about narrowing of the oat gene pool is warranted, as cultivar development in Canada since 1930 has been largely based on a genetic foundation of fewer than 10 parental lines. This situation is also likely true for the oat breeding programs in the USA, as most USA oat germplasm utilized for cultivar development before 1970 traced back to only seven landrace varieties introduced from Europe (Coffman, 1977).

We conducted a molecular assessment of the genetic diversity in 96 oat cultivars released in Canada from 1886 to 2001. These cultivars represent the majority of the oat cultivars registered in Canada since 1886 and typify the core germplasm used in the major Canadian oat breeding programs. Analyses of these 96 oat cultivars using 442 amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995) confirmed the narrowness of the Canadian oat gene pool and detected a nonsignificant trend of reduction in variable AFLP loci since 1886 (Fu et al., unpublished results). These findings were encouraging, but how applicable they are with respect to the whole oat genome remains unclear. The objective of this study was to assess allelic diversity changes in 96 Canadian oat cultivars released from 1886 to 2001 by means of 30 SSRs.

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Abbreviations: bp, base pair; AFLP, amplified fragment length polymorphism; PCR, polymerase chain reaction; PGRC, Plant Gene Resources of Canada; SSR, simple sequence repeat.

MATERIALS AND METHODS

Plant Materials

Ninety-six Canadian oat cultivars (Table 1) were selected from a panel of 129 cultivars maintained at Plant Gene Resources of Canada (PGRC). The selection was based on pedigree analyses, agronomic and economic importance, and representation of different eras of oat breeding in Canada. Consultation was made with several oat breeders and researchers in the selection of the cultivars. The reliability of the information collected for each cultivar was verified by comparison with data from the literature (Welsh et al., 1953; Baum, 1969; McKenzie and Harder, 1995) and from the related online information resource on oat pedigree (N. Tinker, 2002, personal communication).

DNA Extraction

Approximately 10 to 15 seeds of each cultivar were randomly selected from the accession in the PGRC oat collection and grown in the greenhouse at the AAFC Saskatoon Research Centre. Young leaves were collected from 10 5-d-old seedlings, bulked for each cultivar, freeze-dried with a Labconco Freeze Dry System (Kansas City, MO, USA) for 3 to 5 d, and stored at –80°C. From each bulked sample, dry leaves were finely chopped and ground to a fine power in a 2-mL Eppendorf tube with two 3-mm glass beads on a horizontal shaker. Genomic DNA was extracted by means of DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, Ontario, Canada) according to the manufacturer's directions. Extracted DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical Co., St. Louis, MO, USA), followed by dilution to 10 ng μL^{-1} for SSR analysis.

SSR Analysis

On the basis of reported polymorphism, 30 SSR primer pairs were selected and assayed in this study: AM1, AM2, AM3, AM4, AM5, AM6, AM15, AM19, AM21, AM23, AM25,

Table 1. Ninety-six Canadian oat cultivars chosen for study with their release year, origin, and accession number.

Cultivar	Year†	Program‡	CN§	Cultivar	Year	Program	CN
Banner	1886	I.US	53004	Random	1971	LRC	1790
Joanette	1889	I.SW	53273	Scott	1972	ECORC	32988
Alaska	1900	I.US	53097	Gemini	1973	ECORC	32986
Old Island Black	1900	I.UN	52996	Alma	1974	SCRDC	33005
Swedish Select	1902	I.SW	53052	Elgin	1974	OAC	33003
Sixty Day	1905	I.RU	53083	Hudson	1974	CRC	33004
Victory	1911	I.SW	53419	Laurent	1977	MDC	33979
Legacy	1920	ECORC	34096	Foothill	1978	ECORC	33964
Gopher	1923	I.US	53661	Sentinel	1978	ECORC	33965
Gold Rain	1926	I.SW	53714	Cascade	1979	LRC	36153
Hajira	1926	I.AL	1954	Lamar	1979	SCRDC	39341
Early Triumph	1927	MDC	53877	Manic	1979	SCRDC	2216
Vanguard	1930	CRC	54306	Fidler	1980	CRC	37174
Bell	1932	I.SC	34079	Donald	1982	ECORC	51839
Cartier	1932	MDC	53891	Dumont	1982	CRC	42932
Eagle	1937	I.SW	54059	Kamouraska	1982	SCRDC	42933
Erban	1937	OAC	54307	OAC Woodstock	1982	OAC	43400
Lanark	1939	MDC	54258	Calibre	1983	CDC	42931
Mabel	1939	MDC	54259	Baldwin	1985	MDC	17843
Valor	1940	CDC	54542	Jasper	1985	LRC	17733
Ajax	1941	CRC	54558	Marion	1985	SCRDC	17834
Brighton hulless	1941	ECORC	54561	Riel	1985	CRC	17835
Exeter	1942	CRC	54559	Tibor hulless	1985	ECORC	17824
Roxton	1943	MDC	54539	Nova	1986	SCRDC	99036
Beaver	1945	ECORC	54764	Capital	1987	SCRDC	45129
Larain	1945	LRC	56416	Robert	1987	CRC	99039
Abegweit	1947	ECORC	55041	Cluan	1988	SCRDC	99037
Beacon	1947	ECORC	54850	Derby	1988	CDC	46754
Garry	1947	CRC	54964	Newman	1988	ECORC	45979
Fortune	1948	CDC	2939	Quamby	1988	SCRDC	99038
Lanark	1948	ECORC	55042	Appalaches	1989	SCRDC	99040
Torch hulless	1951	CDC	57115	Sylva	1989	SCRDC	99041
Rodney	1953	CRC	56534	Ultima	1989	SCRDC	99049
Scotian	1953	ECORC	57057	Waldern	1990	LRC	18133
Shefford	1953	MDC	56809	AC Lotta hulless	1991	ECORC	18135
Simcoe	1953	OAC	56639	AC Stewart	1991	ECORC	18134
Shield	1956	ECORC	57063	AC Belmont hulless	1992	CRC	52130
Vicar hulless	1956	CRC	57141	AC Hunter	1992	ECORC	46738
Fredericton	1957	ECORC	4655	AC Preakness	1993	CRC	99045
Fundy	1957	ECORC	57138	AC Baton hulless	1994	ECORC	99047
Glen	1957	MDC	57450	AC Percy hulless	1994	ECORC	18136
Pendek	1959	LRC	28892	AC Fregeau hulless	1996	ECORC	99048
Russell	1960	ECORC	57374	AC Rebel	1996	CRC	99043
Harmon	1965	CRC	4686	AC Ernie hulless	1997	ECORC	99046
Kelsey	1966	IHRS	4748	CDC Bell	1998	CDC	99044
Sioux	1966	CRC	5106	AC Mustang	1999	LRC	99042
Fraser	1967	ARC	34093	AC Pinnacle	1999	CRC	99034
Grizzly	1967	UOA	1807	AC Ronald	2001	CRC	99035

†Year of cultivar release or registration.
‡The code for the origin or breeding program from which a cultivar was developed. ARC = Agassiz Research Centre; CDC = Crop Development Center, Univ. of Saskatchewan; CRC = Cereal Research Centre, Winnipeg; ECORC = Eastern Cereal and Oilseed Research Centre, Ottawa; I = Introduced from Algeria (I.AL), Russia (I.RU), Scotland (I.SC), Sweden (I.SW), Unknown (probably Canada, I.UN), and USA (I.US); IHRS = Indian Head Research Station; LRC = Lacombe Research Centre; OAC = Ontario Agriculture College, Univ. of Guelph; MDC = Macdonald College, McGill Univ.; SCRDC = Soil and Crops Research and Development Centre, Sainte-Foy; and UOA = Univ. of Alberta.
§CN = the cultivar accession number in the PGRC oat collection.

AM26, AM27, AM28, AM30, AM31, AM38, AM40, AM41, AM42, AM83, AM87, AM91, AM102, AM112, AM115, HVM3, HVM4, HVM34, and HVM44. The AM-SSRs were isolated from *A. sativa* (Li et al., 2000; Pal et al., 2002) and the HV-SSRs from barley (*Hordeum vulgare* L.) (Liu et al., 1996). The polymerase chain reaction (PCR) contained 50 ng template DNA, 1× buffer (Promega, Madison, WI, USA), 1.5 m*M* $MgCl_2$, 200 μ*M* each of dNTP, 10 pmol of each primer, and 1 unit of *Taq* polymerase (Invitrogen, Carlsbad, CA, USA). Different "Touchdown" PCR programs were used for different primers depending on their melting temperatures (Liu et al., 1996; Li et al., 2000; Pal et al., 2002). PCR products were separated on a sequencing gel containing 6% (w/v) polyacrylamide, 7 *M* urea and 1× TBE at 85-W constant power for 3 h (BioRad sequencing system, Hercules, CA, USA). The gel was fixed, stained, and dried with a DNA silver staining kit (Promega, Madison, WI, USA).

Data Analysis

To generate a dataset of SSR allele counts for each cultivar, DNA fragments amplified by SSR primer pairs were identified on the basis of their size in base pairs measured with a 10-bp DNA ladder (Invitrogen, Carlsbad, CA, USA), and compared with the size reported in the literature (Liu et al., 1996; Li et al., 2000; Pal et al., 2002). Frequencies of the scored alleles were calculated with respect to primer, breeding period, and breeding program. To identify the patterns of allelic changes for each locus, the numbers of alleles detected in the cultivars were assessed in chronological order from 1886 to 2001. Such assessment was also performed for groups of cultivars on the basis of their release periods and originating programs.

To assess the significance of the observed difference in allelic counts between the cultivars released in different breeding periods or programs, a permutation method was applied. Specifically, an allele was chosen, and on the basis of its observed frequency of occurrence among all 96 cultivars, it was randomly allocated to the 96 cultivars without replacement regardless of cultivar origin or release year. This step was repeated for the other alleles identified in this study, followed by counting the number of alleles for the "artificial" cultivars from a known period or program. The difference in allelic counts between two groups of "artificial" cultivars was calculated and compared with the actual observed difference. This permutation of alleles was repeated 10 000 times. The numbers of alleles in these "artificial" cultivars was averaged over 10 000 runs to generate the expected and standard deviation of number of alleles for the cultivars in each group of interest. The proportion of the 10 000 runs, in which the difference in allelic counts was larger than the observed allelic difference, gave the probability of detecting the allelic difference between two cultivar groups. The simulation was done by a SAS program written in SAS IML (SAS Institute, 1996) for the different breeding periods and programs, and it is available from the senior author.

To assess the change in diversity in the cultivars released from different breeding periods or programs, average diversity was measured by three commonly employed similarity (or band-sharing) methods. The simple matching method, first described by Sokal and Michener (1958) and later applied by Apostol et al. (1993), defines the similarity as $S_{ij} = (a + d)/(a + b + c + d)$, where S_{ij} is the similarity between two individuals i and j, a is the number of bands present in both i and j, b is the number of bands present in i and absent in j, c is the number of bands present in j and absent in i, and d is the number of bands absent from both i and j. The second method, proposed by Dice (1945) and later applied by Nei and Li (1979), calculates the similarity as $S_{ij} = (2a)/(2a + b + c)$. The third method, described by Jaccard (1908), estimates the similarity as $S_{ij} = a/(a + b + c)$. Note that a, b, and c used in the last two methods are the same as in the first method. In this study, dissimilarity (i.e., 1− similarity) was calculated by a SAS program written in SAS IML.

RESULTS

Ten out of 30 SSR primer pairs (AM2, AM4, AM6, AM23, AM27, AM30, AM41, AM83, HVM34, and HVM-44) displayed unscorable banding patterns, whereas nine (AM15, AM19, AM21, AM26, AM28, AM40, AM91, HVM3, and HVM4) revealed monomorphic bands only. The remaining 11 primer pairs (Table 2) detected a total

Table 2. Numbers of simple sequence repeat alleles and variation pattern over time for each primer pair in Canadian oat cultivars and comparisons of observed (O) and expected (E) alleles relative to those in cultivars released before 1930.

Primer pair	Breeding period and number of cultivars								Variation pattern
	Pre-1930 13	1930s 7	1940s 11	1950s 12	1960s 5	1970s 13	1980s 21	1990s 14	
AM1	8	7	8	6	4	5	8	4	Decreasing
AM3	6	3	5	4	4	5	7	6	Random
AM5	8	5	7	6	3	4	6	6	Decreasing
AM25	2	2	2	2	2	2	2	2	Random
AM31	6	6	5	3	4	2	2	2	Decreasing
AM38	5	3	5	4	1	3	3	2	Decreasing
AM42	2	3	3	3	2	3	3	3	Shifting
AM87	1	2	2	3	2	3	3	2	Increasing
AM102	2	1	2	2	2	2	3	4	Increasing
AM112	2	2	3	3	2	1	3	2	Decreasing
AM115	1	2	2	1	1	2	2	1	Random
O-Total†	43	36	44	37	27	32	42	34	
O-Lost‡		16	13	17	19	18	14	18	
O-New§		9	14	11	3	7	13	9	
E-Total¶	41.9	33.4	39.7	40.9	28.9	41.9	48.2	43.0	
E-SD#	2.8	3.0	2.9	2.9	3.0	2.8	2.6	2.8	
Prob(E > O)††		0.681	0.814	0.146	0.277	0.007	0.048	0.011	

† O-Total = the total number of alleles detected in the cultivars of a specific breeding period.
‡ O-Lost = the total number of alleles undetected in the cultivars of a specific breeding period relative to those present in the 13 cultivars released before 1930.
§ O-New = the total number of new alleles detected in the cultivars of a specific breeding period relative to those present in the 13 cultivars released before 1930.
¶ E-Total = the total number of alleles expected to be detected in the cultivars of a specific breeding period.
# E-SD = the standard deviation of the number of alleles expected to be detected in the cultivars of a specific period.
†† Prob(E > O) = the proportion of the 10 000 random permutations showing that the simulated difference in the number of alleles between cultivars before 1930 and in the breeding period of interest was larger than the observed difference.

of 62 alleles, with an average of 5.6 alleles per primer pair. Primer pair AM5 detected 12 alleles (the largest number) and AM25 and AM115 only two alleles each. The frequency distribution of alleles in the 96 cultivars is shown in Fig. 1A. There were two alleles having occurrence frequencies of ≥0.95 in the cultivars and 39 alleles with frequencies of ≤0.15. Among the 39 infrequent alleles, there were four and 16 alleles with frequencies of ≤0.02 and ≤0.05, respectively. An examination of the oat linkage maps with SSR markers (Pal et al., 2002; Wight et al., 2003) revealed only five of the 11 SSR loci were mapped on five linkage groups (AM3 on the linkage group 36; AM42 on 11; AM87 on 24; AM102 on 22; and AM112 on 2). This implies that the 11 SSR loci assayed here may be widely distributed over oat chromosomes.

Assessments of the number of alleles per locus over the release years revealed four pattern types that we refer to as Random, Shifting, Increasing, and Decreasing (Fig. 2 and Table 2). Alleles detected with primer pairs AM3, AM25, and AM115 displayed a random pattern of change over time, meaning that alleles detected in the early years of oat breeding were randomly present in cultivars that were developed later on. These three primer pairs consist of different sequences and numbers of repeats in their products. Primer pair AM42 showed a shifting pattern of predominance in two of the three

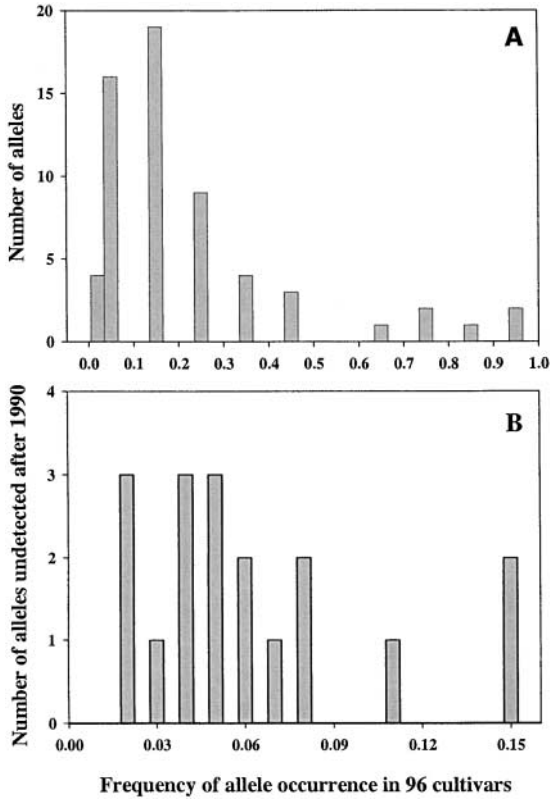

Fig. 1. The distributions of 62 SSR alleles (A) and 18 alleles undetected in the oat cultivars released since 1990 (B) with respect to their occurrence frequencies in all 96 oat cultivars. Fig. 1A also separately illustrates four and 16 SSR alleles of occurrence frequency less than or equal to 0.02 and 0.05, respectively. Note that different scales are presented in the axes of Fig. 1A-B.

alleles from the early to the later years. Two primer pairs, AM87 and AM102, showed an increasing number of alleles in cultivars released from the 1930s to the 1970s. These two primer pairs detected alleles that share the same repeat sequence (AC) yet have a different numbers of repeats. Five other primer pairs (AM1, AM5, AM31, AM38, and AM112) displayed a pattern of decreasing number of alleles in cultivars released after 1950. For example, primer pair AM31 detected seven alleles present in cultivars released before 1950, but only two alleles were detected in cultivars released after 1980. Two primer pairs, AM31 and AM38, displayed alleles that share the same repeat sequence (GAA) with different numbers of repeats (23 and 8, respectively; Table 2), while the other three primer pairs (AM1, AM5, and AM112) detected alleles that only partially share the same AG sequence.

The observed numbers of alleles detected with each primer pair in cultivars of each specified period are in agreement with the patterns of allelic change per primer as described above (Table 2). The total numbers of alleles detected in the cultivars released in each period appear to show little change over the periods under study, but this could be biased by the unequal numbers of cultivars assessed in each period (Table 2). The 10 000 permutations of the allele frequency data give the expected number of alleles in the cultivars for each given period (and its standard deviation), as well as the probability that the difference (i.e., between observed and expected numbers of alleles) is significant. Thus, taking into account the difference in number of cultivars assessed in each period, revealed that the cultivars released after 1970 had significantly ($P < 0.007$) fewer alleles than those released before 1930.

The average number of new alleles per cultivar was 1.29 for the 1930s, 1.27 for the 1940s, 0.92 for the 1950s, 0.60 for the 1960s, 0.53 for the 1970s, 0.62 for the 1980s, and 0.64 for the 1990s (Table 2). Clearly, the cultivars released before 1960 had one fold more new alleles than those released after 1960. The cultivars with the most number of new alleles are Erban, Brighton hulless, and Shield, each having five new alleles. They were released in 1937 from the University of Guelph, in 1941 from ECORC at Ottawa and in 1956 from ECORC, respectively. Eighteen alleles were undetected in cultivars released after 1990 and their allelic frequencies ranged from 0.0104 to 0.1458 with an average of 0.0544 (Table 2, Fig. 1B). These undetected alleles came from six different SSR loci (i.e., 4 in AM1, 1 AM3, 3 AM5, 5 AM31, 4 AM38, and 1 AM112), indicating the allelic reduction is not restricted to a single chromosomal segment.

Permutation assessments of the detected numbers of alleles in cultivars released from different breeding programs (Table 3) showed that the cultivars generated from the breeding program at Sainte-Foy, QC, had significantly ($P = 0.024$) fewer alleles than a group of cultivars introduced to Canada from other countries. The permutations also revealed marginally fewer alleles in cultivars released from the breeding programs at Guelph and Ottawa than in the introduced cultivars. These differences are consistent with the relatively small

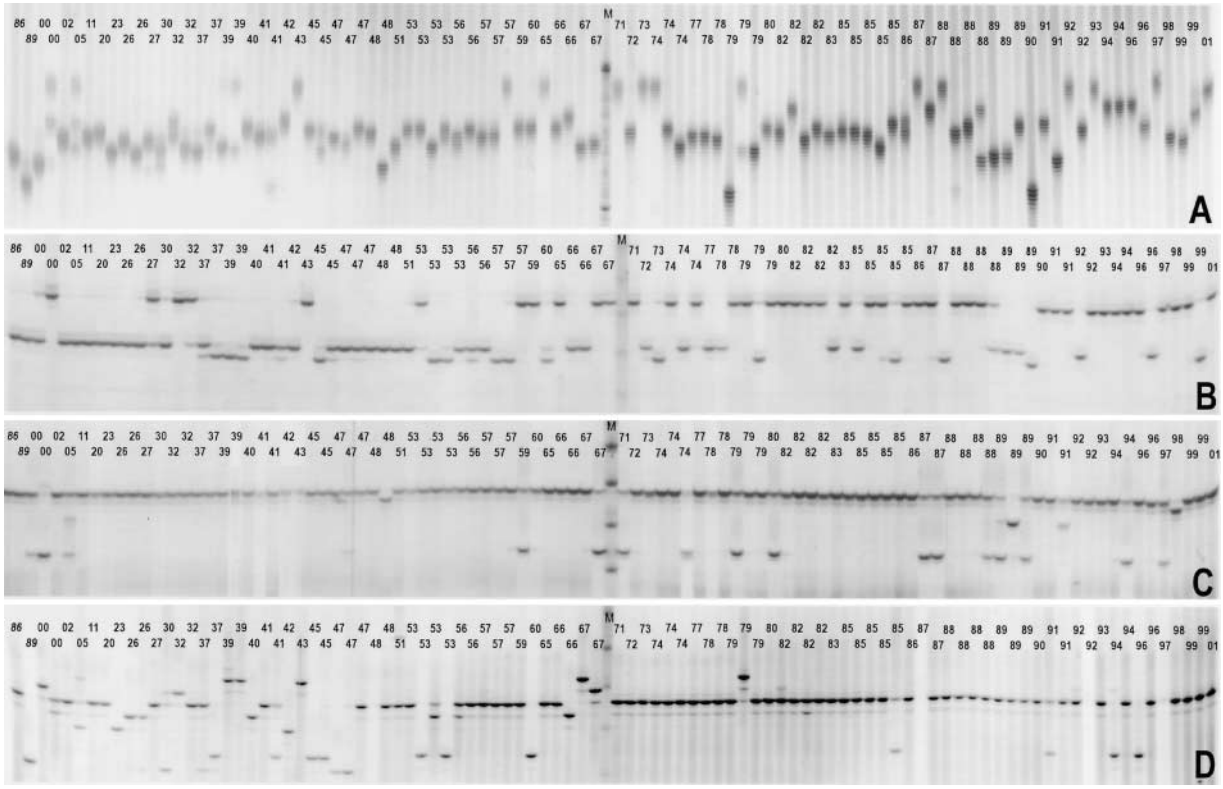

Fig. 2. Four silver staining gels that illustrate the four allelic change patterns over the 115 years of Canadian oat breading. A, Random (from AM3); B, Shifting (from AM42); C, Increasing (from AM102); and D, Decreasing (from AM31). In each gel, samples from 96 Canadian oat cultivars are arrayed from left to right in a chronological order, from 1886 to 2001. The order parallels that in Table 1. Only the last two digits of the release year are given for each cultivar. *M* is the DNA ladder.

numbers of new alleles detected for the cultivars from each breeding program. For example, the numbers of new alleles versus the total number of alleles detected in cultivars from different breeding programs were 7:33 for Sainte-Foy, 5:20 for Guelph, and 14:45 for Ottawa.

With respect to the different breeding periods, average diversity increased in cultivars released before 1950 but decreased thereafter (Table 4). Similar trends were observed for the three dissimilarity measures. Genetic diversity, however, did not significantly differ among cultivars released in different breeding periods.

A nonsignificant difference of the average diversity was also observed for cultivars released from different breeding programs, although the average diversities of some grouped cultivars appeared to vary. For example, the difference in the average diversity calculated by Dice's method between the introduction cultivars and those released from the breeding program at Saskatoon was relatively large (0.530 − 0.456 = 0.076), but the large standard deviations (i.e., ≥0.12) of these diversity estimates made the significance test of the diversity difference less sensitive. In addition, comparisons of the

Table 3. Numbers of simple sequence repeat alleles observed in Canadian oat cultivars released from specific breeding programs and comparisons of observed (O) and expected (E) alleles relative to those in cultivars introduced from other countries.

Program†	Cultivar count	Alleles					
		O-total‡	O-lost§	O-new¶	E-total#	E-SD††	Prob (E > O)‡‡
Introductions	12	42			40.8	2.9	
ECORC (Ottawa)	25	45	11	14	50.4	2.5	0.064
CRC (Winnipeg)	18	43	9	10	46.3	2.6	0.173
SCRDC (Sainte-Foy)	12	33	16	7	40.8	2.9	0.024
MDC (Montreal)	9	35	16	9	36.9	3.0	0.279
LRC (Lacombe)	7	30	19	7	33.4	3.0	0.170
CDC (Saskatoon)	6	30	18	6	31.3	3.0	0.329
OAC (Guelph)	4	20	27	5	26.1	3.0	0.058
ARC, IHRS and UOA	3	19	23	0	22.6	2.9	0.156

† The coding of each program (See Table 1).
‡ O-Total = the total number of alleles detected in the cultivars of a breeding program.
§ O-Lost = the total number of alleles undetected in the cultivars of a breeding program relative to those present in the 12 introduction cultivars.
¶ O-New = the total number of new alleles detected in the cultivars of a breeding program relative to those present in the 12 introduction cultivars.
# E-Total = the total number of alleles expected to be detected in the cultivars of a breeding program.
†† E-SD = the standard deviation of the number of alleles expected to be detected in the cultivars of a breeding program.
‡‡ Prob(E > O) = the proportion of the 10 000 random permutations showing that the simulated difference in the number of alleles between the introduction cultivars and those from the specific breeding program was larger than the observed difference.

Table 4. Average genetic diversity of Canadian oat cultivars released in different breeding periods and programs, calculated using Dice's method, simple match coefficient (SMC), and Jaccard's method.

Program/period†	Cultivar count	Average dissimilarity (and standard deviation)‡		
		Dice	SMC	Jaccard
Period				
Pre-1930	13	0.426 (0.115)	0.165 (0.054)	0.588 (0.118)
1930s	7	0.508 (0.126)	0.202 (0.048)	0.663 (0.124)
1940s	11	0.538 (0.120)	0.206 (0.047)	0.688 (0.103)
1950s	12	0.479 (0.121)	0.195 (0.051)	0.634 (0.128)
1960s	5	0.476 (0.104)	0.177 (0.037)	0.638 (0.093)
1970s	13	0.426 (0.125)	0.167 (0.048)	0.586 (0.132)
1980s	21	0.434 (0.118)	0.172 (0.049)	0.593 (0.120)
1990s	14	0.418 (0.120)	0.155 (0.047)	0.567 (0.130)
Program				
Introductions	12	0.456 (0.120)	0.177 (0.053)	0.617 (0.119)
ECORC (Ottawa)	25	0.506 (0.138)	0.196 (0.056)	0.653 (0.138)
CRC (Winnipeg)	18	0.505 (0.144)	0.190 (0.056)	0.657 (0.136)
SCRDC (Sainte-Foy)	12	0.438 (0.123)	0.173 (0.050)	0.595 (0.120)
MDC (Montreal)	9	0.440 (0.123)	0.179 (0.049)	0.601 (0.124)
LRC (Lacombe)	7	0.389 (0.154)	0.158 (0.065)	0.542 (0.162)
CDC (Saskatoon)	6	0.530 (0.162)	0.203 (0.063)	0.670 (0.168)
OAC (Guelph)	4	0.308 (0.096)	0.124 (0.037)	0.463 (0.119)
ARC, IHRS and UOA	3	0.392 (0.045)	0.151 (0.008)	0.561 (0.045)

† The coding of each program (see Table 1).
‡ Three band-sharing methods used in this study were Dice's method (Dice, 1945), simple match coefficient (Sokal and Michener, 1958), and Jaccard's method (Jaccard, 1908).

estimates from the three band-sharing methods indicate similar patterns of variation for cultivars released from different breeding programs or periods, although the average diversities obtained with Dice's method and Jaccard's coefficient were larger than those obtained with the simple match coefficient.

DISCUSSION

The SSR analyses conducted in this study reveal the first, clear-cut molecular evidence for the negative impacts of oat breeding on the genetic diversity. Four different patterns of allelic change were identified in the Canadian oat germplasm: random at three loci, shifting at one locus, increasing at two loci, and decreasing at five loci. Most importantly, a significant decrease in SSR allele number was detected in materials from more recent breeding periods and from specific breeding programs. The reduction in SSR allele number is of special concern in terms of genetic resource conservation and plant breeding. If this pattern of allelic reduction is reflective of the genome as a whole, future efforts to improve this species through selective breeding may be hindered by lack of diversity.

The increase in the average genetic diversity of cultivars released from 1930 to 1950 may well reflect the consequences of extensive hybridization performed from 1920 to 1940 (Table 4). This hybridization not only generated impressive increases in oat yields, but also simultaneously broadened the genetic background of the released cultivars by employing genetically diverse lines. The decrease in the average genetic diversity of cultivars released since 1950 (Table 4) may in part be explained by breeding efforts to utilize almost all of the known crown and stem resistance genes, which was accomplished by backcrossing to resistant parental lines (after 1950) and introgression of resistance from three to four wild oat lines to several specific rust races (in the 1970s). This effort may have reduced diversity in some chromosomal segments. This reasoning is supported by the observation of allelic reductions at five SSR loci in cultivars released since 1950 (e.g., Fig. 2D) as well as significant allelic reduction after 1970 (Table 2). Thus, identifying these specific chromosomal regions marked by these SSR loci and determining if these regions are associated with rust-resistant genes would help verify the diversity changes observed in the assessed gene pool. However, only one such locus (AM112) was mapped (Pal et al., 2002) and whether the mapped region harbors any rust-resistant genes remains unknown.

The findings presented here, along with those in barley (Russell et al., 2000) and maize (Lu and Bernardo, 2001), appear to indicate that allelic diversity at particular loci, rather than average genetic diversity, is sensitive to plant breeding practices. This seems to be true as selective improvement largely focuses on certain chromosomal regions with the aim of introducing desirable novel alleles. This implies that diversity assessment of the gene pool would be most informative when allelic diversity itself is the target of study. Thus, evaluation of allelic diversity requires proper selection of effective molecular tools such as multiallelic SSR markers and DNA sequencing of individual genes of interest, rather than random amplified polymorphic DNA (RAPD) (Williams et al., 1990) and AFLP markers with limited polymorphism per locus. This reasoning is supported by the comparisons of AFLP and SSR findings on these 96 oat cultivars. For example, analyses of 442 polymorphic AFLP bands did not reveal clear patterns of diversity change (Fu et al., unpublished results) concordant with the breeding efforts over time as did the SSR analyses presented here. Also, AFLP analyses detected only a nonsignificant trend of fixing 1% of variable AFLP loci over the 115 yr of oat breeding, while significant decrease of some SSR alleles was detected in cultivars

released after 1970. This reasoning also suggests that analyses of specific chromosomal regions associated with genes for breeding targets would yield more information on the impacts of plant breeding. The linkage and QTL maps of many crop species established in recent years would enhance such analyses.

With the genetic narrowing of the oat germplasm, there is a need for continuous diversification of oat breeding materials for sustainable breeding programs in the future. To facilitate the diversification of germplasm, conservation of genetically diverse germplasm is a prerequisite and is critical for long-term breeding efforts. Eventually, the introgression of new genes or incorporation of new gene complexes will be needed in some breeding programs to overcome a possible "genetic ceiling" in oat improvement, to avoid genetic vulnerability to biotic stresses, and to widen crop adaptation to new environments. Thus, attention needs to be paid to integrated efforts in the conservation of oat germplasm and exploration for new sources of desirable alleles. While it is not clear how general these findings are with respect to other crop species, further studies of other important crop species with effective molecular tools would not only allow us to understand the impacts of plant breeding on plant genomes, but also facilitate the efforts of conserving and diversifying breeding materials for sustainable crop improvements.

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