GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic diversity of the Arctic fox using SRAP markers

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Genet. Mol. Res. 12 (4): 6176-6183 (2013)
Received October 29, 2012
Accepted April 10, 2013
Published December 4, 2013
DOI http://dx.doi.org/10.4238/2013.December.4.4

ABSTRACT. Sequence-related amplified polymorphism (SRAP) is a recently developed molecular marker technique that is stable, simple, reliable, and achieves moderate to high numbers of codominant markers. This study is the first to apply SRAP markers in a mammal, namely the Arctic fox. In order to investigate the genetic diversity of the Arctic fox and to provide a reference for use of its germplasm, we analyzed 7 populations of Arctic fox by SRAP. The genetic similarity coefficient, genetic distance, proportion of polymorphic loci, total genetic diversity (Ht), genetic diversity within populations (Hs), and genetic differentiation (Gst) were calculated using the Popgene software package. The results indicated abundant genetic diversity among the different populations of Arctic fox studied in China. The genetic similarity coefficient ranged from 0.1694 to 0.0417, genetic distance ranged from 0.8442 to 0.9592, and the proportion of polymorphic loci was smallest in the TS group. Genetic diversity ranged from 0.2535 to 0.3791, Ht was 0.3770, Hs was 0.3158, Gst was 0.1624, and gene flow (Nm) was estimated at 2.5790. Thus, a high level of genetic diversity and many genetic relationships were found in the populations of Arctic fox evaluated in this study.

Key words: Arctic fox; SRAP marker; Genetic diversity; Germplasm

INTRODUCTION

The Arctic fox, also known as the Blue fox (*Alopex lagopus*), has a long history of artificial breeding, particularly in China (Guo, 2003). A small, inbred breeding population, along with other issues, has led to degradation of the species, poor-quality hides, and performance degradation, resulting in a decrease in the Arctic fox population (Zhang, 2001). Therefore, as breeding of the Arctic fox is of great economic importance, this study was conducted to analyze the genetic diversity of Arctic fox populations in China (Tan et al., 2007). With the use of molecular markers, the genetic diversity of populations can be evaluated and used to improve populations over time, and adjust the breeding structure of populations to improve their productivity (Zhang et al., 2009). Sequence-related amplified polymorphism (SRAP) technology is a novel and efficient genetic marker system, which reveals genetic variation in open reading frames (ORFs) among related organisms (Li and Quiros, 2001). Because of its simplicity and efficiency, SRAP has been used in the construction of genetic maps (Li and Quiros, 2001), comparative genetic studies (Li et al., 2003), and examinations of genetic diversity (Riaz et al., 2001; Ferriol et al., 2003) in many plant species. It has also been used to study genetic variation in the giant river prawn, *Macrobrachium rosenbergii* (Zhou et al., 2006). However, to our knowledge, the present study is the first use of SRAP in a study of a mammalian species.

The objective of this study was to investigate genetic variability among different populations of *A. lagopus* using the novel SRAP marker technique. Polymerase chain reaction (PCR)-based SRAP markers were adopted to study the genetic variability, total genetic diversity, genetic diversity within populations, and genetic differentiation among 7 *A. lagopus* populations. This novel mammalian study will be of great importance for genetic studies of other mammals and will lay the foundation for systematics studies of other mammals at the molecular level.

MATERIAL AND METHODS

Animal and data sources

Samples were collected from the Great Khingan Tuqiang Forestry Bureau, Heilongjiang, China, in November 2007. Body weight, body length, and abdominal circumference were measured from 297 Arctic foxes (91 females and 206 males). Skin length and guard hair length data of the animal hide were obtained from 421 foxes (209 females and 212 males). Therefore, data were collected for 421 Arctic foxes (Table 1). The experimental sample population was divided into 7 populations based on different breeding groups. The groups used to investigate genetic diversity in this study are listed in Table 2.

Table 1. Production performance sample data for *Alopex lagopus*.

Arctic fox test samples	No. with weight records	No. with body length records	No. with abdominal circumference records	No. with animal hide and skin length records	No. with guard hair length records
Female	91	91	91	209	209
Male	206	206	206	212	212
Total		297		421	

Table 2. *Alopex lagopus* population groups.

Population group No.	Name of population	Group ID
1	Guo feeding groups	GDL
2	Liu feeding groups	LGH
3	Lv feeding groups	LYX
4	Taishang feeding groups	TS
5	Qingdao feeding groups	QD
6	An feeding groups	AN
7	Li feeding groups	LI

Genomic DNA extraction

Total genomic DNA was extracted from 0.2 g muscle tissue from a total of 718 samples, following the procedure described in a previous study (Baixiu, 2000). DNA samples were stored at -20°C until use.

SRAP procedure

The SRAP technique was followed as described by Li and Quiros (2001) with slight modifications. Nineteen different primer combinations were employed, including 8 forward primers and 11 reverse primers (Table 3). Each 25-µL PCR mixture consisted of 1.0 µL genomic DNA, 2.5 µL PCR buffer, 200 µM dNTPs, 4 mM $MgCl_2$, 0.5 µM primer, 1.25 U Taq polymerase (TaKaRa), and sterile double-distilled water. Samples were subjected to the following thermal amplification profile: 5 min of initial denaturation at 94°C, then five cycles of 1 min denaturation at 94°C, 1 min annealing at 35°C and 1 min elongation at 72°C, followed by 35 cycles with an annealing temperature of 50°C, and a final elongation step of 5 min at 72°C. Separation of amplified fragments was accomplished on 6% denaturing acrylamide gels [acrylamide:bisacrylamide (19:1), 1X TBE] at 90 V for 2.5 h. Gels were stained with 0.1% $AgNO_3$ solution and then digitally photographed. Primer banding patterns that were difficult to score and primers that failed to amplify consistently were excluded. Consequently, 88 primer combinations were selected and utilized in the *A. lagopus* study population.

Table 3. Primer sequences used for SRAP analysis.

Forward primer	Reverse primer
me1: 5'-TGAGTCCAAACCGGATA-3'	em1: 5'-GACTGCGTACGAATTAAT-3'
me2: 5'-TGAGTCCAAACCGGAGC-3'	em2: 5'-GACTGCGTACGAATTTGC-3'
me3: 5'-TGAGTCCAAACCGGAAT-3'	em3: 5'-GACTGCGTACGAATTGAC-3'
me4: 5'-TGAGTCCAAACCGGACC-3'	em4: 5'-GACTGCGTACGAATTTCA-3'
me5: 5'-TGAGTCCAAACCGGAAG-3'	em5: 5'-GACTGCGTACGAATTAAC-3'
me6: 5'-TGAGTCCAAACCGGTAA-3'	em6: 5'-GACTGCGTACGAATTGCA-3'
me7: 5'-TGAGTCCAAACCGGTCC-3'	em7: 5'-GACTGCGTACGAATTCAA-3'
me8: 5'-TGAGTCCAAACCGGTGC-3'	em8: 5'-GACTGCGTACGAATTCTG-3'
	em9: 5'-GACTGCGTACGAATTCGA-3'
	em10: 5'-GACTGCGTACGAATTCAG-3'
	em11: 5'-GACTGCGTACGAATTCCA-3'

Data analysis

Photographs of denaturing acrylamide gels showing different banding patterns were

digitized and analyzed. SRAP fragments were scored for the presence or absence of the specific allele, classified as "1" or "0", in each sample, respectively. The distance matrix and similarity coefficients were constructed using the Popgene (version 3.2) software package. Total genetic diversity (H_T), within-population diversity (H_S), genetic differentiation (G_{ST}), and gene flow (N_M) were calculated using the Popgene Genetic Analysis (version 3.2) software package.

RESULTS

Using the SRAP primer combinations, 88 primer pairs were analyzed in 718 samples. Primer pairs em6-me11 showed the clearest and best amplification (Figure 1). Representative results are shown in Figure 2. The optimum annealing temperature for the selected primers, evidenced by stronger amplification, was determined to be 49°C (lane 4). Therefore, this temperature was used for subsequent amplifications. PCR products were detectable on agarose gel, although fewer polymorphisms were visible than on non-denaturing polyacrylamide gel. Thus, PCR products were separated on 6% non-denaturing polyacrylamide gel (acrylamide:bisacrylamide, 19:1).

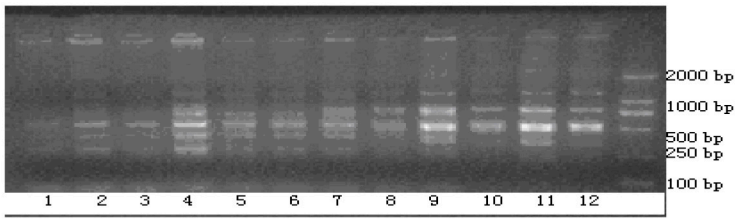

Figure 1. Determination of optimum annealing temperature of selected primers. *Lane 1* = 45°C; *lane 2* = 45.5°C; *lane 3* = 46.9°C; *lane 4* = 49°C; *lane 5* = 51.4°C; *lane 6* = 53.8°C; *lane 7* = 56.2°C; *lane 8* = 58.6°C; *lane 9* = 60.9°C; *lane 10* = 63°C; *lane 11* = 64.5°C; *lane 12* = 65°C. Amplification was strongest at 49°C.

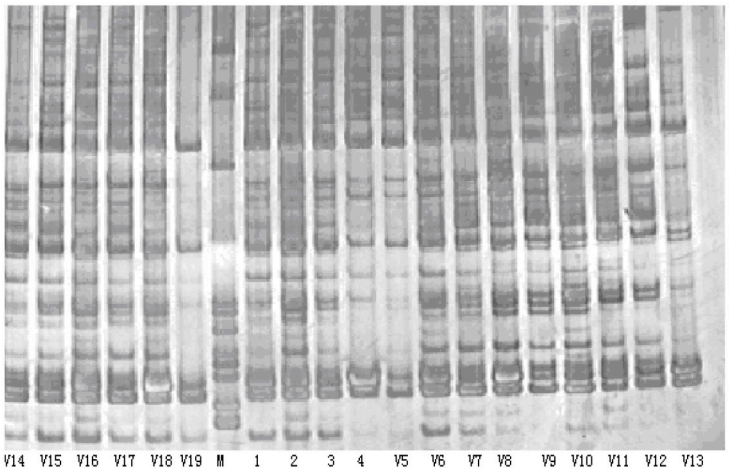

Figure 2. Non-denaturing polyacrylamide gel separation. *Lane M = pBR322*/*Msp*I.

Genetic variation among the 7 populations of Arctic fox

The similarity coefficients and genetic distances among the 7 study groups are summarized in Table 4. The similarity coefficient was lowest (0.0417) between groups TS and LI, and was highest (0.1694) between groups LYX and QD. The genetic distance was lowest (0.8442) within groups QD and LYX, and was highest (0.9592) within groups LI and TS. The small similarity coefficients between or within groups indicated that there was a marked difference in genotypes, demonstrating that the animals studied were from different regions. Genetic variation in these populations may be the result of genetic drift caused by different evolutionary paths followed over the foxes' long history. The proportions of polymorphic loci observed in the study groups are summarized in Table 5. The proportion of polymorphic loci was found to be the smallest (71.43%) in group TS, indicating that genetic information of this group was low. Nei's genetic diversity index and Shannon's information index are shown in Table 6.

Table 4. Analysis of genetic similarity and genetic distance.

Pop. ID	LYX	TS	LI	QD	GDL	AN	LGH
LYX	0	0.8889	0.8909	0.8442	0.8904	0.8846	0.9139
TS	0.1177	0	0.9592	0.9183	0.9339	0.8791	0.8898
LI	0.1155	0.0417	0	0.8929	0.9262	0.8806	0.9354
QD	0.1694	0.0852	0.1133	0	0.9520	0.9360	0.8968
GDL	0.1161	0.0684	0.0766	0.0492	0	0.9518	0.9565
AN	0.1226	0.1289	0.1271	0.0661	0.0494	0	0.9408
LGH	0.0900	0.1168	0.0668	0.1089	0.0444	0.0610	0

GDL = Guo feeding groups; LGH = Liu feeding groups; LYX = Lv feeding groups; TS = Taishang feeding groups; QD = Qingdao feeding groups; AN = An feeding groups; LI = Li feeding groups.

Table 5. em11-me6 primers amplified groups at many loci.

	GDL		LGH		LYX		TS		QD		AN		LI	
	TNL	NPL	TNL	NPL	TNL	NPL	TNL	NPL	TNL	NPL	TNL	NPL	TNL	NPL
em11-me6	21	19	21	16	21	19	21	15	21	18	21	17	21	20
Proportion of polymorphic loci	90.48%		76.16%		90.48%		71.43%		85.71%		80.95%		95.24%	

TNL = total number of loci; NPL = number of polymorphic loci; GDL = Guo feeding groups; LGH = Liu feeding groups; LYX = Lv feeding groups; TS = Taishang feeding groups; D = Qingdao feeding groups; AN = An feeding groups; LI = Li feeding groups.

Table 6. Analysis of genetic diversity index.

Number	Group name	Gene diversity (Nei, 1973)	Shannon's Information index (Lewontin, 1972)
1	LYX	0.3525	0.5151
2	TS	0.2735	0.4056
3	LI	0.3791	0.5477
4	QD	0.2535	0.3773
5	GDL	0.3183	0.4686
6	AN	0.2755	0.4126
7	LGH	0.3581	0.5310

In accordance with the theory of Nei (1973), stating that genetic variation in populations can be reflected by the genetic diversity index, the results of this study show great variation within group LI (0.3791).

The H_T and H_S values of the study groups are summarized in Table 7. H_T was found to be 0.3770 and H_S was 0.3158, indicating that total genetic diversity is greater than genetic diversity within populations, and thus, substantial genetic variation exists between the experimental groups. G_{ST} was found to be 0.1624. The amount of genetic differentiation between populations reflects the action of evolutionary processes, since these differences reflect divergence of populations from a common ancestral population. Genetic differentiation that is occurring in the process of Arctic fox speciation is of particular interest based on the relatively high value of N_M (2.5790), indicating some level of gene flow between the groups.

Table 7. Analysis of the total population genetic structure using primers em11-me6.

H_T	H_S	G_{ST}	N_M*
0.3770	0.3158	0.1624	2.5790

H_T = total genetic diversity; H_S = genetic diversity within populations; G_{ST} = coefficient of genetic differentiation; N_M* = gene flow among populations; [N_M* = 0.5 (1 - G_{ST}) / G_{ST} (McDermott and McDonald, 1993)].

DISCUSSION

The *A. lagopus* populations studied were found to be highly polymorphic, possibly due to their complex growing environments and genetic backgrounds. Another possibility is that the polymorphic nature of the Arctic fox populations was caused by genetic drift. In this study, a novel PCR-based SRAP marker technique was successfully used to assess genetic differentiation among 7 Arctic fox populations. The results of this study provide molecular tools for further studies of the fox population in China. PCR-based SRAP markers have the potential for a variety of applications for genetic research and for practical breeding programs in mammals. Indeed, SRAP has already been successfully applied in polymorphism studies of plants, such as cotton (Lin et al., 2004), sugarbeet (Wang et al., 2007), peach and nectarine (Ahmad et al., 2004), watermelon (Li et al., 2007; Wang et al., 2009), and wheat, among others.

SRAP markers were previously found to be more efficient than inter simple sequence repeat (ISSR) markers for evaluating population diversity (Huang et al., 2012). Furthermore, SRAP analysis has been used for accurate molecular identification of traditional Chinese medicinal materials in the genus *Paris*, and provided molecular evidence for taxonomy and identification of different *Paris* species (Xin et al., 2011).

SRAP markers reflect the actual genetic diversity of endophytic fungi strains of the genus *Taxus*, and could therefore be used for identification of endophytic fungi from this genus (Ren et al., 2012). In addition to identification, SRAP markers may also offer information for protecting and/or exploiting economically important species, such as *Curcuma wenyuji* (Leng et al., 2009). Song et al. (Song et al., 2010) demonstrated that ISSR and SRAP markers were both effective and reliable for assessing the degree of genetic variation in *Salvia miltiorrhiza*, while Cheng et al. (Cheng et al., 2007) showed the potential for SRAP fingerprinting in the identification of the Chinese herb, Radix Polygoni Multiflori. Finally, SRAP markers have recently been used to study genetic variation of parasites with significance to human and animal health (Alasaad et al., 2008).

Using SRAP markers, we were able to obtain good amplification and subsequently identify genetic polymorphism in our study population. This study provides the first data of

genetic variation using SRAP markers in mammals, thus demonstrating that this technology is suitable for further molecular characterization in *A. lagopus*. Furthermore, the results of this study demonstrate that the use of SRAP technology provides a more efficient method for examining genetic diversity in mammals, thus enabling identification of phylogenetic relationships among populations of *A. lagopus*. Currently, studies based on fragment sequencing are in progress in order to shed light on the relationship between genotype and phenotype in this group. In addition, it is necessary to increase the sample size of the Arctic fox to gain further insight into genetic diversity within *A. lagopus*, and to detect molecular markers that allow discrimination between genotypes. In conclusion, the SRAP marker system is a simple and efficient marker system that provides several advantages over other systems. The technology is simple, with a reasonable throughput rate, provides the ability to disclose numerous co-dominant markers, allows easy isolation of bands for sequencing, and most importantly, targets ORFs directly (Youssef et al., 2011), a feature that could be adapted for a variety of purposes in mammals, as the dominant SRAP markers could provide more accurate information on population genetic diversity compared to traditional methods. SRAP molecular genetic distances based on haploid derived from different dikaryotic parents should also be useful for predicting hybrid performance in outbreeding populations of *A. lagopus*.

CONCLUSIONS

SRAP genetic markers can be used in mammals with good results. In this study, 7 Arctic fox populations showed higher genetic diversity between, than within, populations. Genetic similarity coefficients ranged from 0.1694 to 0.0417, and genetic distances ranged from 0.8442 to 09592. The proportion of polymorphic loci was smallest in the group TS, and Nei's genetic diversity index ranged from 0.2535 to 0.3791. The values of Ht, Hs, Gst, and Nm, were 0.3770, 0.3158, 0.1624, and 2.5790, respectively. Therefore, high-level genetic diversity and genetic relationships were found among different populations of the Arctic fox using SRAPs.

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