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Genomic Variation in Parthenogenetic Lizard *Darevskia armeniaca*: Evidence from DNA Fingerprinting Data

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Microsatellites, or short tandem repeats, are abundant across genomes of most organisms. It is evident that the most straightforward and conclusive way of studying mutations in microsatellite-containing loci is to use clonally transmitted genomes or DNA sequences inherited in multigeneration pedigrees. At present, little is known about the origin of genetic variation in species that lack effective genetic recombination. DNA fingerprinting in 43 families of the parthenogenetic lizard species *Darevskia armeniaca* (131 siblings), using $(GACA)_4$, $(GGCA)_4$, $(GATA)_4$, and $(CAC)_5$ probes, revealed mutant fingerprints in siblings that differed from their mothers in several restriction DNA fragments. In some cases, the mutant fingerprints detected in siblings were also found in population samples. The mutation rate for new restriction fragment length estimated by using multilocus probes varied from 0.8×10^{-2} to 4.9×10^{-2} per band/per sibling. Probably, the most variations detected as restriction fragment length polymorphism have germ-line origin, but somatic changes of $(CAC)_n$ fingerprints in adult lizards were also observed. These results provide new evidence of existing unstable regions in genomes of parthenogenetic vertebrate animals, which provide genetic variation in unisexual populations.

Multilocus DNA fingerprinting has proved useful for assaying genetic variation in a number of clonal fish (Turner et al. 1990; Schartl et al. 1991; Elder and Schlosser 1995; Umino et al. 1997) and lizard (Kan et al. 1998; Tokarskaya et al. 2001; Martirosyan et al. 2002) species. Genomic variability in parthenogenetic reptiles is particularly interesting because these species provide an example of breeding system in which there is no male contribution to the genome of the progeny (Darevsky 1992). Caucasian rock lizards *Darevskia armeniaca* (Lacertidae) are widely distributed in Armenia and southern Georgia. They are truly parthenogenetic, all-female, meiotic, diploid species of hybrid origin. For example, *D. armeniaca* originated from interspecific hybridization between *Darevskia valentini* and *Darevskia mixta* (Darevsky 1992). Existing as unisexual lineages, this species is characterized by some level of clonal diversity detected by allozymes (Fu et al. 2000) and low level of mitochondrial DNA variation (Moritz et al. 1992). Clonal diversity may arise as a result of mutations, multiple hybridization events, or some level of recombination, which may occur during continued clonal reproduction and the evolution of species (Murphy et al. 2000).

Recently, we have shown that clonally reproducing lizards of the genus *Darevskia* (*Darevskia unisexualis*, *D. armeniaca*, *Darevskia dahli*, and *Darevskia rostombekovi*) possess species-specific DNA fingerprints, which are practically identical for all individuals in each population studied when the M13 minisatellite probe was used; however, they display some level of intrapopulation variation with microsatellite probes of different types (Kan et al. 1998; Tokarskaya et al. 2001; Ryskov et al. 2003; Martirosyan et al. 2002). Observations of polymorphic fingerprint patterns in the population samples of *D. armeniaca* (Tokarskaya et al. 2001) revealed the importance of studying inheritance of microsatellite-containing DNAs in families of *D. armeniaca*. In this study, we fingerprinted 43 parthenogenetic families (131 siblings) of *D. armeniaca* using various microsatellite probes and compared our findings with those relating to the congeneric species *D. unisexualis* (Tokarskaya et al. 2004). Taking into consideration all our investigations, we suggest that germ-line and somatic mutations should make significant contribution in overall genetic variation of parthenogenetic populations.

Materials and Methods

Specimens and Populations

Reproductively mature females of *D. armeniaca* were collected during late June of 2000 to June 2001 from natural habitats of

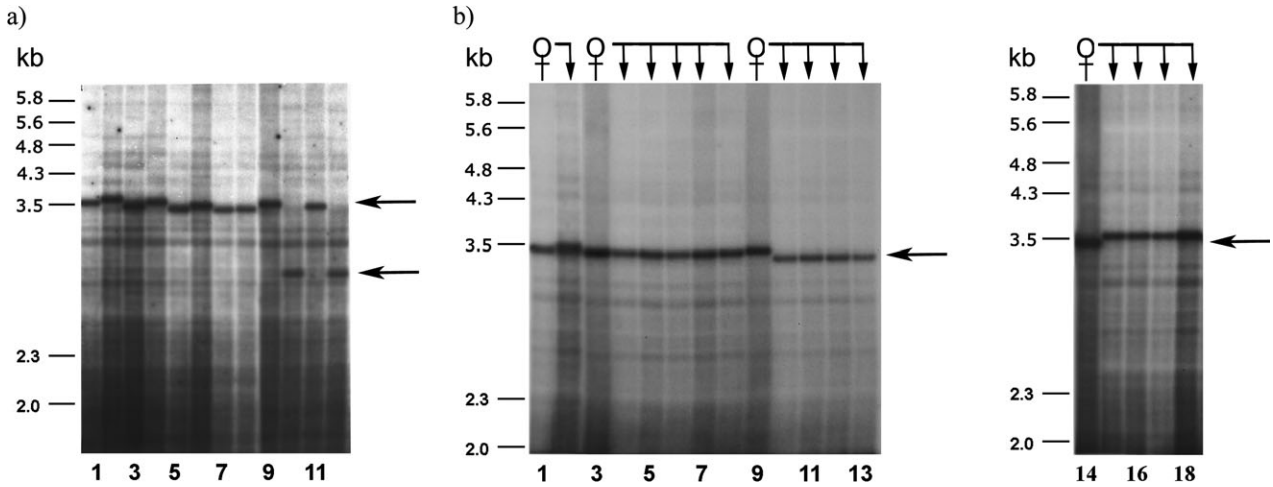

Figure 1. $(GACA)_n$ fingerprinting in populations (**a**) and families (**b**) of *Darevskia armeniaca*. Selected samples were taken from northern (a, lanes 1–4, 9–12), central (a, lanes 5–8), and western (b, lanes 1–18) populations of Armenia. Variable bands are indicated by the arrows. Lanes 3–8 (b) demonstrate an example of family where mutations were not found. DNA fragment sizes are given in kilobases.

western, northern, southern, and central Armenia. Each mature female was maintained in separate enclosure in the laboratory until they began to produce eggs. The eggs were incubated under laboratory conditions for 30 days, opened, and embryos were immediately frozen in the liquid nitrogen. Liver, lung, heart, muscles, and other organs were taken from freshly killed adult animals and frozen in liquid nitrogen. Fresh blood was stored in 0.05 M EDTA, pH 8.0, at 4 °C.

DNA Isolating and Fingerprinting

DNA samples from lizard blood, tissues, and whole embryos were isolated by standard phenol–chloroform extraction with the use of Proteinase K. DNA was digested with *Alu*I, *Bsu*RI, *Hin*fI, and *Mva*I restriction enzymes (Fermentas) and consequently blot hybridized with ^{32}P-labeled probes: $(CT)_9$, $(GT)_9$, $(CAC)_5$, $(CTG)_5$, $(CAG)_5$, $(GATA)_4$, $(GACA)_4$, $(GGCA)_4$ as was previously described (Ryskov et al. 1988; Tokarskaya et al. 2001).

Results

In the first set of experiments, genomic DNA of *D. armeniaca* was cut with the use of 4 restriction enzymes and hybridized with each of the 8 oligonucleotide probes (see Materials and

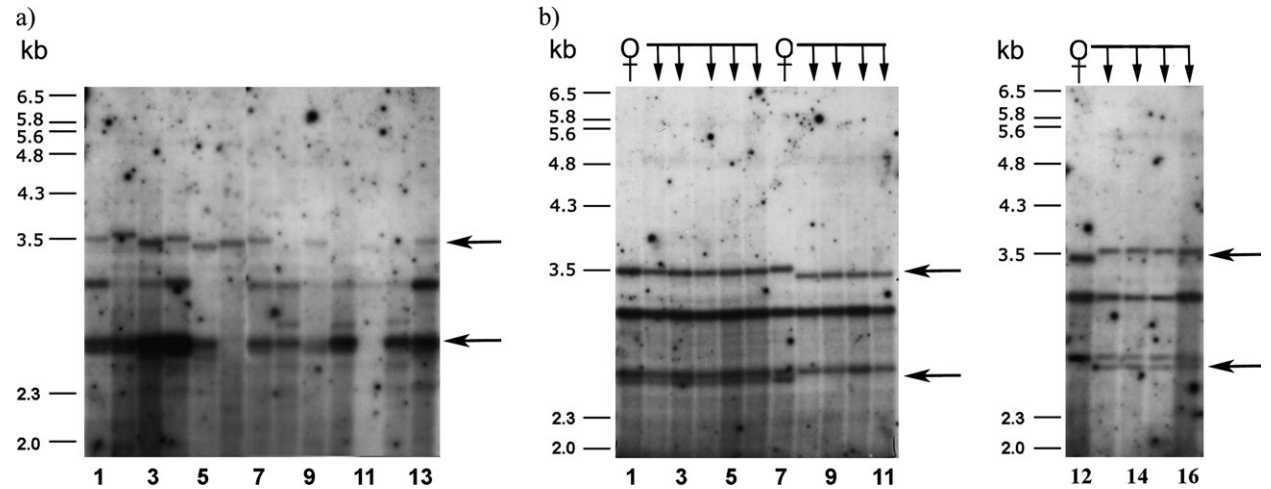

Figure 2. $(GGCA)_n$ fingerprinting in populations (**a**) and families (**b**) of *Darevskia armeniaca*. Selected samples were taken from northern (a, lanes 1–4, 7–13), central (a, lanes 5 and 6), and western (b, lanes 1–16) populations of Armenia. Lanes 1–6 (b) demonstrate an example of family where mutations were not found. Variable bands are indicated by the arrows. DNA fragment sizes are given in kilobases.

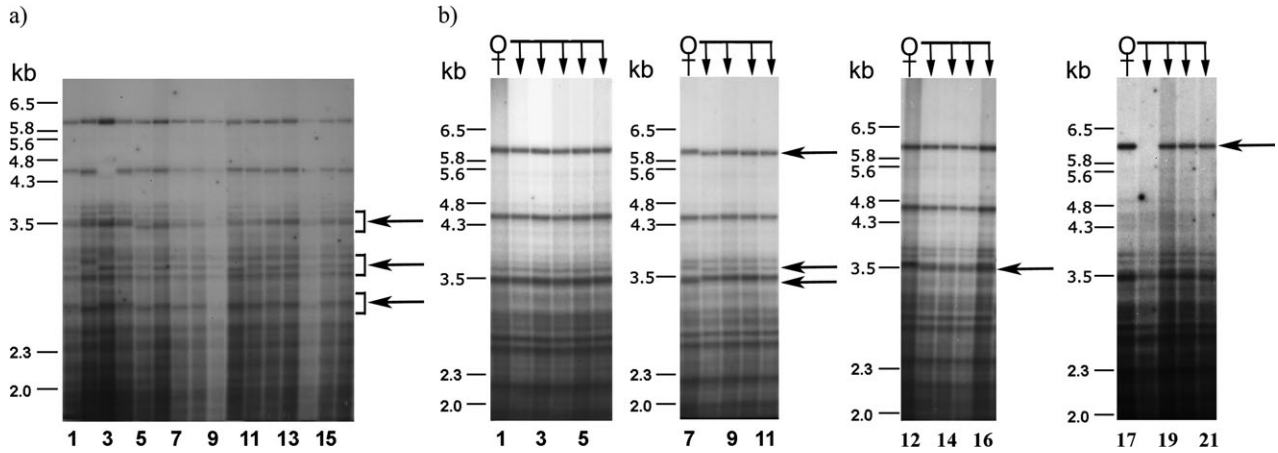

Figure 3. $(GATA)_n$ fingerprinting in populations (**a**) and families (**b**) of *Darevskia armeniaca*. Selected samples were taken from northern (a, lanes 1–4, 10–16), central (a, lanes 5–9; b, lanes 17–21), and western (b, lanes 1–15) populations of Armenia. Lanes 1–5 (b) demonstrate an example of the family where mutations were not found. Variable bands are indicated by the arrows and square brackets. DNA fragment sizes are given in kilobases.

Methods) in order to identify hypervariable and the most informative restriction enzyme–hybridization probe combinations. Whereas probes $(GACA)_4$, $(GGCA)_4$, $(GATA)_4$, and $(CAC)_5$, in combination with restriction enzyme *Mva*I, provided the DNA fingerprints with clear-cut polymorphic bands that were optimal for monitoring genomic alterations, the other enzyme–probe combinations resulted in complex and poor resolved band patterns superimposed in some cases on strong hybridization smear (data not shown). Using hybridization probes $(GACA)_4$, $(GGAT)_4$, and $(GATA)_4$, variable DNA fragments were detectable among population samples and were also found as mutant fragments in some family samples (Figures 1–3). The mean mutation rate of new restriction fragment length estimated by using multilocus probes varied from 0.8×10^{-2} to 1.3×10^{-2} per band/per sibling (Table 1). In some cases (e.g., see Figure 3b, lanes 7–11), more than one mutant DNA fragment was revealed. It is interesting that in one case (Figure 3b, lane 18) in the size range between 6.5 and 5.8 kb, the disappearance of the whole restriction fragment, which was present in mother and in other 3 siblings fingerprinting patterns, was observed. Probably it is connected with the appearance of the new restriction sites in the range of this fragment. The newly formed short restriction fragments can be masked in low electrophoretic region of the fingerprint pattern. It should be noted that mutant fragments were detected by both $(GACA)_4$ and $(GGCA)_4$ probes in 8 siblings from 2 families. Moreover, in population and family samples, the $(GGCA)_4$ and $(GACA)_4$ probes at hybridization revealed some common ~3.5-kb fragments, but with different intensity. Clustering of different simple repetitive motifs or intermingling could be an explanation of this phenomenon, but not of the high level of similarity among probes (Lioi and Galasso 2002). The observation of mutant $(GACA)_4$, $(GATA)_4$, and $(GGCA)_4$ fingerprints in families of *D. armeniaca* that resulted from changes of restriction fragments for all siblings of a single brood suggests a germ line rather than somatic origin of mutations.

Table 1. DNA fingerprinting in parthenogenetic families of *Darevskia armeniaca*

Probes	No. of siblings examined	No. of mutant siblings	Total no. of mutant bands	Average no. of bands scored per sibling (SE)	Frequency of mutations per sibling (%)	Mutation rate value per sibling per band[a]
$(GACA)_4$[b]	131	19	19	18.4 (0.4)	14.5	0.008
$(GGCA)_4$[c]	131	19	27	16.2 (0.12)	17.56	0.013
$(GATA)_4$[d]	131	17	30	24.7 (0.8)	12.98	0.009
$(CAC)_5$[e]	131	129	129	20.5 (0.1)	98.47	0.049

[a] Mutation rate value per sibling/per band was calculated according to Bois et al. (1998) as number of mutant sibling bands divided by average number of bands per individual and divided by total number of siblings tested. For example, 19/18.4/131 = 0.008 (where 19 is the number of mutant siblings, 18.4 is the average number of $(GACA)_4$ fingerprint bands, and 131 is the total number of siblings tested).

[b] Bands were scored between 4.8 and 2.3 kb.

[c] Bands were scored between 3.5 and 2.0 kb.

[d] Bands were scored between 6.5 and 2.3 kb.

[e] Bands were scored between 9.4 and 2.0 kb.

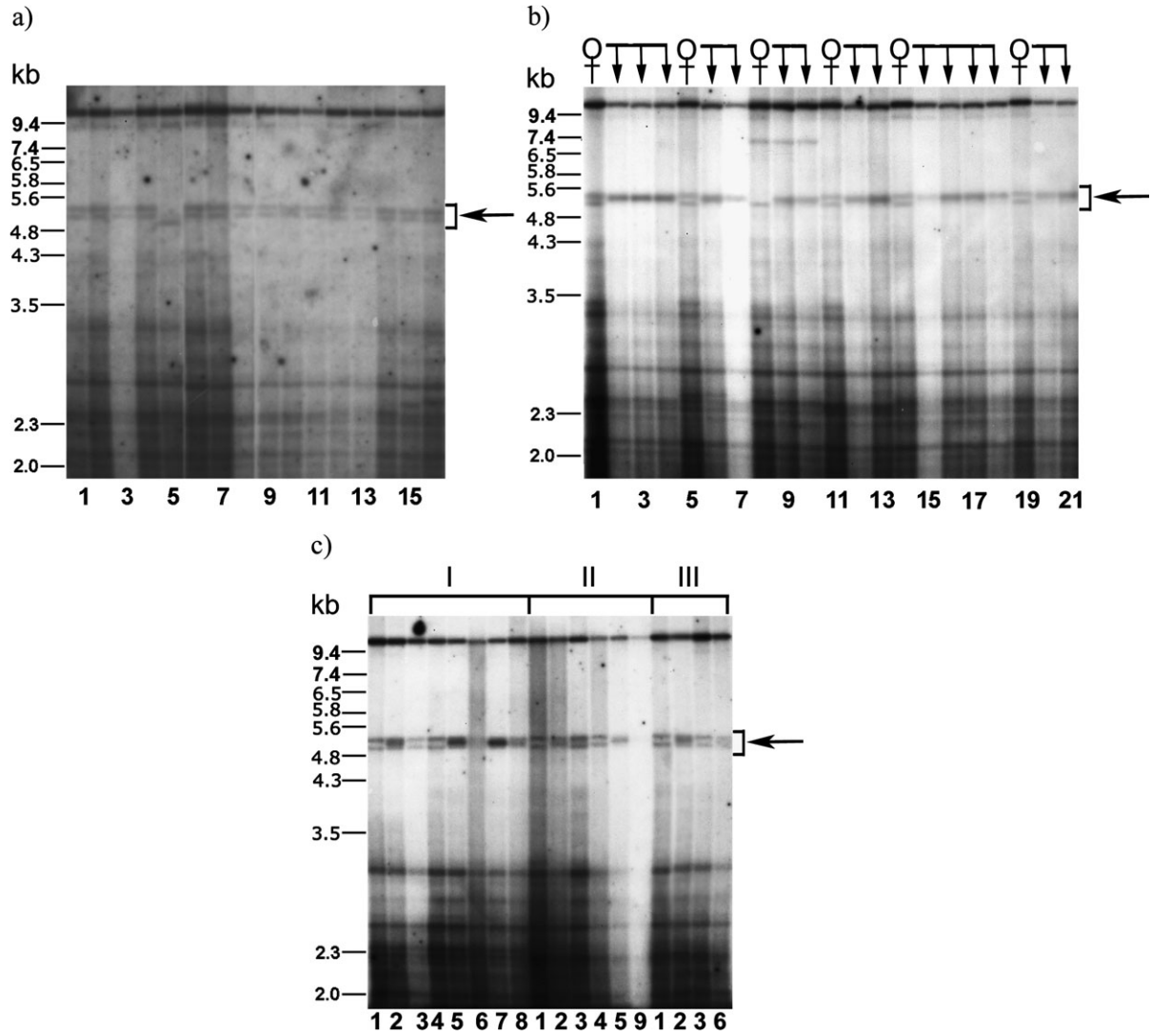

Figure 4. $(CAC)_n$ fingerprinting in populations (**a**), families (**b**), and tissues (**c**) of *Darevskia armeniaca*. Selected samples were taken from central (a, lanes 1–13), northern (a, lanes 14–16; b, lanes 11–13), southern (b, lanes 1–7; b, lanes 8–10), and western (b, lanes 14–21) populations of Armenia. (c) Tissues—1: blood; 2: liver; 3: lung; 4: ovary; 5: heart; 6: intestine; 7: brain; 8: bilious bubble; and 9: muscles of 3 adult lizards (I, II, and III). Variable bands are indicated by the arrows and square brackets. DNA fragment sizes are given in kilobases.

All microsatellite probes used revealed mutant fingerprints in *D. armeniaca* siblings and show different levels of intrafamily variability in the parthenogenetic families, but the highest level of variability was observed using $(CAC)_5$ probe (Table 1). Typical cases are shown in Figure 4. One can see that practically identical $(CAC)_5$ fingerprints in population samples of *D. armeniaca* were obtained (Figure 4a). On the other hand, almost all the sibling $(CAC)_5$ fingerprint patterns were different from those of their mother's (Figure 4b). Mutant DNA fragments were revealed in the electrophoretic region between 5.6 and 4.8 kb. We suppose that this should be a result of somatic changes leading to tissue mosaicism in these animals. For example, somatic mutations are frequent in mice and occur mainly during the first few cell divisions after fertilization (Gibbs et al. 1993). To identify somatic mosaicism, DNA from various tissues of 3 parthenogenetic lizards was further analyzed, and fingerprint patterns differed between organs and tissues of these animals (Figure 4c). Thus, various restriction fragments length polymorphisms are detectable in a population, families, and tissues DNA samples of *D. armeniaca* using microsatellite probes.

Discussion

Previously, high level of genomic variability was detected by DNA fingerprinting using of the probes $(CAC)_5$ and $(GACA)_4$ in natural populations and laboratory lines of clonal fish species, *Poecilia formosa* and *Rivulus marmoratus*

(Turner et al. 1990, 1992). The authors suggested that mutations, subsequent to the founding of clonal lineages, should be an important source of observed variation, although all fingerprints tested were clonally stable for at least 3 generations. DNA fingerprinting was employed to analyze *Poecilia formosa* siblings of single brood (Schartl et al. 1991). It was found that all the individuals display almost identical fingerprint patterns, which could be expected due to apomictic breeding. However, with the $(GATA)_4$ and $(GAA)_6$ probes, truly hypervariable loci were detected that give rise to variable restriction fragment length.

Recently, remarkable intrafamily variation of $(GATA)_n$-, $(TCT)_n$-, and $(TCC)_n$-containing DNA fragments (Ryskov et al. 2003; Tokarskaya et al. 2004) was shown in congeneric parthenogenetic species *D. unisexualis* using the oligonucleotide fingerprinting. It was observed that mutation rate of the new restriction fragment length with $(GATA)_4$ probe was as high as 0.9×10^{-2} per microsatellite band/per sibling. The case of somatic variation of $(GATA)_n$ detectable loci was also described in adult *D. unisexualis* lizards (Tokarskaya et al. 2004). Mutation rate of the new restriction fragment length estimated by using multilocus probes in *D. armeniaca* (this study) and *D. unisexualis* (Tokarskaya et al. 2004) was of the same order as the one that had been previously reported for humans (Jeffreys et al. 1991) and mice (Bois et al. 1998). These values are also within the range of values reported for individual microsatellite loci in bisexual species (from 10^{-2} to 10^{-4} per locus per gamete per generation) (Weber and Wong 1993; Ellegren 2000; Gardner et al. 2000; Brohede et al. 2002).

Despite the continued accumulation of data on mutation rate and sequence organization of microsatellite-containing loci in various species (Orti et al. 1997; Colson and Goldstein 1999; Neff and Gross 2001; Cruz et al. 2005), the general picture of their instability remains largely unclear. Recent studies have shown that different processes are actually involved in the creation of microsatellite-containing loci variability. Among such processes, there are sister chromatid exchange, asymmetry and polarity in the distribution of mutations, single-nucleotide substitutions, deletions and insertions in flanking regions, and genesis of mobile elements (Goldstein and Polloch 1997; Ellegren 2000; Wilder and Hollocher 2001; Li et al. 2002). It should be noted that the differences in fragment size detected by DNA fingerprinting appear to be too large to reflect microsatellite repeat number variation. Rather, this reflects mutations and/or epigenetic modifications in restriction sites or other kinds of genomic alterations, including mutations, which may occur during somatic development (Gibbs et al. 1993; Brookfield 2003).

Theoretically, instability of the hybrid karyotype characteristic of parthenogenetic species of the genus *Darevskia* (Darevsky 1992) may also lead to chromosomal rearrangements resulting in the observed diversity of DNA fingerprints influenced by such factors as selection or perhaps the age of species. Nevertheless, the results of this study provide new evidence of existence unstable regions in parthenogenetic genome, detectable with microsatellite probes, which provide sources of genetic variation observed in unisexual populations. It is evident that gene cloning and sequencing of allelic variants of microsatellite-containing loci may give more detailed information on the nature of their variability. Such experiments were commenced by us using genomic library of *D. unisexualis* (Korchagin et al. 2004) and characterization of recombinant clones corresponding to variable microsatellite loci and now they are in progress.

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