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短　報
Note

Genetic Structure of the Domestic Emu Population in Abashiri on the Basis of Mitochondrial and Microsatellite DNA Polymorphism

By

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Summary：The emu (*Dromaius novaehollandiae*) is a ratite native to Australia. Various products, including oils, meat, and eggs, can be obtained from the emu, making it a useful industrial animal. The genetic improvement of the emu is essential for the development of emu farming. To estimate the genetic diversity of the domestic emu population in Abashiri, we investigated mitochondrial DNA (mtDNA) and microsatellite DNA polymorphisms. The D-loop region of mtDNA was sequenced, and two haplotypes were detected: 15792C/16114G (a-haplotype) and 15792T/16114A (b-haplotype), with respective frequencies of 0.96 and 0.04. Therefore, the a-haplotype was overwhelmingly prevalent in the Abashiri population. Additionally, four microsatellite loci were genotyped, and polymorphism was detected at all markers. The average number of alleles at these markers was 7.25, and the average observed heterozygosity (H_O) was 0.52, compared to an average expected heterozygosity (H_E) of 0.59. Therefore, we speculated that high genetic diversity was maintained in the Abashiri emu population.

Key words：emu, mitochondrial DNA, microsatellite DNA

Introduction

The emu (*Dromaius novaehollandiae*) is a ratite native to Australia that provides oils, meat, and eggs[1]. Emu oil, which contains massive amounts of unsaturated fatty acids, has been especially used in therapeutics[2-9] and cosmetics[10]. In addition to these useful traits, emus possess a mild character and adaptation ability, encouraging their agriculture in various regions[11]. In the city of Abashiri, which is located in eastern Hokkaido, Japan, a pair of emus were first introduced from a farm in the USA in 1999, and were supplemented by 20 individuals from farms of Australia and Japan. Currently, the population of farmed emus in Abashiri is approximately 540 individuals, which are largely bred as livestock for the production of oils, meat, and eggs to stimulate the local economy. Hatching, breeding, and feed conditions have previously been investigated to determine the optimal conditions for emus in cold regions[11-14].

However, unlike in other livestock species, scarcely any genetic improvement has been conducted in the emu. To improve the productivity of the emu population in Abashiri, its genetic structure must be understood. Mitochondrial DNA (mtDNA) and microsatellite DNA are useful genetic markers for individual identification and the determination of parentage in various organisms. In this study, we investigated genetic diversity in the Abashiri emu population using polymorphisms of the mtDNA D-loop region and four known microsatellite DNA markers.

Materials and Methods

Sample collection

We collected feather samples from 83 chicks hatched in 2013 at the emu farm, Okhotsk Emu Land in the city of Abashiri. These 83 individuals may contain sibling and consanguinity, because they were produced by random mating in the large-scale rearing.

DNA extraction and sexing

Genomic DNA was extracted from the feathers using DNAzol reagent (Life Technologies, Grand Island, NY). Sexing via PCR-RFLP analysis using *Bgl*II (New England Bio Labs, Ipswich, MA) on the *ESEX* gene was per-

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formed according to previously described methods[15].

mtDNA analysis

We amplified a large mtDNA fragment using KOD FX (TOYOBO, Osaka, Japan) with the primers emu_H14335_LA (5'-ATT TAC ACT CAT ATT TAT CCC TCT CCT AAT C-3') and emu_L4034_LA (5'-GTA ATA GTT GAA CCC GTA ATA AGA CTA AGT G-3'), which were designed from a reference sequence (NC_002784)[16]. Amplification was conducted at 94℃ for 2 min, followed by 40 cycles of 98℃ for 10 s and 68℃ for 8 min. The PCR products were separated on 1% agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). DNA sequencing of the D-loop region was performed for 23 individuals using an Applied Biosystems 3730xl DNA Analyzer (Life Technologies) with the following primers: emu_L15709 (5'-TAT CAG GCA TGG ACT ACA TTC AAT ATA C-3') and emu_H16416 (5'-GAG GAG GGT GGA AAT ACC ATA AC-3').

Microsatellite DNA analysis

DNA samples from 83 individuals were amplified using AmpliTaq Gold DNA polymerase (Life Technologies) with fluorescence-labeled primers (*Dn28*, *Dn35*, *Dn06*, and *emu18*)[17,18]. PCR was conducted at 95℃ for 10 min, followed by 35 cycles of 94℃ for 30 s, 64℃ for 30 s, and 72℃ for 1 min. The PCR products were separated on a Beckman CEQ8000 instrument (Beckman Coulter, Fullerton, CA) using a size standard, and the fragment sizes were determined using fragment analysis software. The heterozygosities of the four loci, based on the genotype data sets obtained from fragment analysis, were estimated using GenePop 4.2[19].

Results and Discussion

Sexing based on PCR-RFLP analysis identified the sexes of all chicks. The numbers of males and females were 48 and 35, respectively, and this ratio was consistent with a theoretical value of 1:1 ($P > 0.1$).

We sequenced a 444-bp segment of the D-loop region, compared it among 23 individuals, and detected two nucleotide substitution sites, at 15,792-nt and 16,114-nt of the reference sequence (NC_002784)[16]. Although the 15792C/16114G haplotype (a-haplotype) was found in nearly all individuals, the 15792T/16114A haplotype (b-haplotype) was detected in one individual, yielding a ratio of 0.96 to 0.04 (Table 1).

Consequently, only two haplotypes were detected in the Abashiri population, indicating two maternal lineages with major (a) and minor (b) haplotypes. In a previous report, four substitution sites were detected in the D-loop for emus of this region[20]. Although the majority of Australian individuals show the a-haplotype, the minor b-haplotype has been observed in New Zealand individuals and in the ancient species[20]. Therefore, we speculated that mtDNA diversity of the emu is generally poor, and that the Abashiri population contains two largely divergent maternal lineages derived from both farmed and ancient origins.

Table 1 mtDNA haplotypes observed in the Abashiri emu population

Haplotype	Nucleotide position (nt)		Frequency ($n = 23$)
	15792	16114	
a	C	G	0.96
b	T	A	0.04

Nucleotide position according to the reference sequence (NC_002784).

We genotyped four microsatellite loci in 83 individuals and detected polymorphisms in all of the microsatellite markers. The numbers of alleles in *Dn28*, *Dn35*, *Dn06*, and *emu18* were six, five, nine, and nine, respectively, and the average number of alleles were 7.25. The fragment sizes of *Dn28*, *Dn35*, *Dn06*, and *emu18* were 98–122, 127–143, 227–381 and 153–175 bp, respectively (Table 2). The allele frequencies showed deflection in all markers except *Dn28*, and some minor alleles were detected in all loci. The major allele sizes and frequencies of *Dn35*, *Dn06*, and *emu18* were 141 (0.84), 277 (0.51), and 165 bp (0.51), respectively. The observed heterozygosities (H_O) ranged from 0.02 to 0.78, with an average of 0.52, and the expected heterozygosities (H_E) ranged from 0.28 to 0.73, with an average of 0.59 (Table 2). Among these markers, a remarkable deficiency of heterozygosity was observed for *Dn35*. We speculated that this result may have been caused by the presence of null alleles or by random genetic drift due to taking data from only one generation. However, it has previously been reported that the number of alleles and heterozygosity of *Dn28* were poor in a Chilean emu population[18]. These results suggest that the emu population of each country may have a different genetic composition. Although slightly lower heterozygosity was observed in the Abashiri population compared to that of previous studies[18,19], heterozygosity was over 0.5 at all loci except *Dn35*. Therefore, we speculated that high genetic diversity is maintained in the Abashiri emu population. Additionally, the power of discrimination (P_D) values of *Dn28*, *Dn35*, *Dn06*, and *emu18* were 0.87, 0.54, 0.79, and 0.88, respectively (Table 2). The high P_D values of *Dn28* and *emu18* indicate their usefulness as genetic markers for parentage testing of emus.

This study represents the first report to investigate genetic structure in a Japanese emu population. This genetic information will be useful for the development of

Table 2 Number of alleles, allele frequency, and heterozygosity of four microsatellite markers observed in the Abashiri emu population

Markers	N_A	Allele size (bp)	Alleles (upper) and Allele frequency (lower)									H_E/H_O	P_D
Dn28	6	98 - 122	*98*	*110*	*113*	*116*	*119*	*122*				0.73/0.69	0.87
			0.01	0.02	0.37	0.25	0.27	0.08					
Dn35	5	127 - 143	*127*	*129*	*139*	*141*	*143*					0.28/0.02	0.54
			0.01	0.01	0.12	0.84	0.01						
Dn06	9	227 - 381	*227*	*275*	*277*	*279*	*281*	*283*	*305*	*377*	*381*	0.66/0.57	0.79
			0.01	0.06	0.51	0.27	0.08	0.05	0.01	0.01	0.01		
emu18	9	153 - 175	*153*	*157*	*159*	*161*	*165*	*167*	*171*	*173*	*175*	0.70/0.78	0.88
			0.02	0.02	0.02	0.08	0.51	0.07	0.01	0.11	0.14		

N_A, H_E, H_O, and P_D indicate number of alleles, expected heterozygosity, observed heterozygosity, and power of discrimination respectively.

genetic markers to select emu pedigrees with desirable productive traits.

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ミトコンドリアおよびマイクロサテライト DNA 多型に基づく網走エミュー集団の遺伝的構造

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要約：エミュー（*Dromaius novaehollandiae*）はオーストラリア原産の走鳥類であり，エミューオイル，食肉および卵などを生産することから，オホーツク地域の新規産業動物として期待されている。しかし，エミューの生産形質における遺伝的改良はほとんど実施されておらず，それは今後のオホーツクにおけるエミュー産業の発展に必須となることが予測される。我々はミトコンドリア DNA（mtDNA）およびマイクロサテライト DNA 多型を指標として，網走におけるエミュー集団の遺伝的多様性を調査した。D-loop 領域の塩基配列を決定し，23 個体間において比較した結果，2 ヶ所の塩基置換サイトが検出され，2 種類のハプロタイプの存在が認められた（a- ハプロタイプ：15792C/16114G および b- ハプロタイプ：15792T/16114A）。それらの頻度は，それぞれ 0.96 および 0.04 であり，網走集団の多くは a- ハプロタイプに占められていた。一方，4 座位のマイクロサテライト DNA について 83 個体のジェノタイピングを行った結果，そのすべてにおいて多型が認められ，平均アレル数は 7.25，平均ヘテロ接合率は 0.52（H_O）および 0.59（H_E）であった。したがって，網走におけるエミュー集団は高い遺伝的多様性を保持することが示唆された。

キーワード：エミュー，ミトコンドリア DNA，マイクロサテライト DNA

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