The Thai Journal of Veterinary Medicine

Volume 40
Issue 4 *December, 2010*

Article 7

12-1-2010

Tetranucleotide Microsatellite Markers for Molecular Testing in Thai Domestic Elephants (Elephas maximus indicus)

Duangsmorn Suwattana

Jutarat Jirasupphachok

Sumolya Kanchanapangka

Weerapong Koykul

Original Article

Tetranucleotide Microsatellite Markers for Molecular Testing in Thai Domestic Elephants (*Elephas maximus indicus*)

Duangsmorn Suwattana[1*] Jutarat Jirasupphachok[1] Sumolya Kanchanapangka[2] Weerapong Koykul[2]

Abstract

Eleven microsatellite loci were evaluated for genetic profiles of Thai domestic elephants (*Elephas maximus indicus*) and their suitability as genetic markers for molecular testing. A total of 66 animals were tested. 10 out of 11 loci could be amplified and they demonstrated polymorphism with allelic numbers per locus ranging from 7 (LaT06) to 39 (LaT18). Values of expected heterozygosity (He) and Polymorphic Information Content (PIC) were between 0.6449 (LaT17) – 0.9593 (LaT05) and 0.5934 (LaT17) – 0.9578 (LaT05), respectively. Analysis of the ten microsatellite markers revealed Combined Exclusion Probability (CEP) of 99.99998783% or 1.2167×10^{-7} and 99.91% confident for individual verification, suggesting that using all these loci together as a set of genetic markers is an extremely powerful tool for the unique identification. In addition, this set of microsatellite markers provides a qualified system for fingerprinting purposes and parentage testing in Thai domestic elephants.

Keywords: Microsatellite, polymorphism, Thai domestic elephant

[1]*Department of Animal Husbandry, Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok 10330.*
[2]*Department of Anatomy, Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok 10330.*
Corresponding author E-mail: *Duangsmorn.S@chula.ac.th*

บทคัดย่อ

เครื่องหมายไมโครแซทเทลไลท์ชนิดเตตร้านิวคลีโอไทป์เพื่องานด้านอณูพันธุศาสตร์ในช้างไทย

ดวงสมร สุวัฒฒน[1*] *จุฑารัตน์ จิระศุภโชค*[1] *สุมลยา กาญจนะพังคะ*[2] *วีระพงศ์ โกยกุล*[2]

การศึกษาไมโครแซทเทลไลท์ดีเอ็นเอชนิดเตตร้านิวคลีโอไทด์จำนวน 11 ตำแหน่ง สำหรับประยุกต์ใช้งานด้านอณูพันธุศาสตร์ในช้างไทย โดยเก็บตัวอย่างเลือดช้างเลี้ยงในแถบภาคกลางและตะวันออกเฉียงเหนือ จำนวนทั้งสิ้น 66 เชือก นำมาสกัดดีเอ็นเอและตรวจหาข้อมูลพันธุกรรมโดยวิธีปฏิกิริยาลูกโซ่ พบว่า สามารถเพิ่มจำนวนไมโครแซทเทลไลท์ดีเอ็นเอได้ 10 ตำแหน่ง ยกเว้น LaT07 โดยเครื่องหมายทุกตำแหน่งแสดงรูปแบบความหลากหลายอยู่ระหว่าง 7-39 อัลลีล ค่าเฮเทอโรไซโกซิตี้ และค่าโพลีมอร์ฟิกอินฟอร์เมชันคอนเทนท์ (PIC) อยู่ระหว่าง 0.6449-0.9593 และ 0.5934-0.9578 ตามลำดับ ผลการวิเคราะห์ข้อมูล พบว่า เมื่อนำเครื่องหมายพันธุกรรมชนิดไมโครแซทเทลไลท์ทั้ง 10 ตำแหน่งมาใช้งานพร้อมๆ กัน จะทำให้มีประสิทธิภาพสูงในการตรวจวิเคราะห์ข้อมูลพันธุกรรมของช้างไทย เมื่อนำไปใช้เป็นข้อมูลลายพิมพ์ดีเอ็นเอเฉพาะตัวของช้างไทยให้ผลที่ถูกต้องแม่นยำในการตรวจสอบความสัมพันธ์ทางสายเลือดสูงถึง 99.99998783% หรือ 1.2167×10^{-7} และมีความแม่นยำสูงถึง 99.91% เมื่อใช้ตรวจสอบระบุตัวตนของสัตว์ แสดงให้เห็นว่า เครื่องหมายทางพันธุกรรมชุดนี้ สามารถนำไปประยุกต์ใช้งานด้านอณูพันธุศาสตร์ในช้างไทยได้เป็นอย่างดี

คำสำคัญ: ไมโครแซทเทลไลท์ ความหลากหลาย ช้างไทย

[1]*ภาควิชาสัตวบาล คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ 10330*

[2]*ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ 10330*

***ผู้รับผิดชอบบทความ E-mail:** Duangsmorn.S@chula.ac.th*

Introduction

There are two populations of elephants (*Elephas maximus indicus*) in Thailand: those in the wild and those that have been domesticated and reproduced. The number of wild elephants has plummeted due to illegal poaching and loss of habitat. Furthermore, wild elephants have been held captive and spuriously identified as domestic elephants. Therefore, conservation plans and effort are urgently needed along with development of techniques required for correct identifications of elephants based on their genetics. Determination of the genetic polymorphism has been extensively used in animal breeding and selection since it provides accuracy and efficiency in individual identification and parentage testing (Bruford and Wayne, 1993). Microsatellite loci which belong to Asian elephants (*Elephas maximus*) and African elephants (*Loxodonta africana*) have been investigated (Nyakaana and Arctander, 1998; Eggert et al., 2000; Fernando et al., 2001; Comstock et al., 2002; Archie et al., 2003; Kongrit et al., 2008; Thitaram et al., 2008; Gobush et al., 2009). However, those loci were tested in elephants of different regions of the world and they might not represent patterns of microsatellite loci in Thai domestic elephants, as first reported with a small number of tetranucleotide markers by Suwattana et al. (2007). Thus, an application of microsatellite loci in the determination of unique fingerprinting or genetic profile and parentage testing will further support the conservation plans for elephants in Thailand.

Materials and Methods

Blood samples were collected from 66 captive elephants from two regions of Thailand, the central and northeastern parts, including 17 related animals from 6 families and other 49 genetically unrelated individuals. DNA was isolated from whole blood samples using QIAamp® DNA Blood Minikit according to the manufacturer's instructions. PCR amplifications were performed using the primers for 11 tetranucleotide microsatellite loci including LaT05, LaT06, LaT07, LaT08, LaT13, LaT16, LaT17, LaT18, LaT24, LaT25 and LaT26, with the expected size between 166-392 bp. Two to three times PCR-amplifications per DNA sample per microsatellite marker were performed at the annealing temperature of 52-56°C (Archie et al., 2003; Suwattana et al. 2007). Each microsatellite locus was identified by DNA sequencing before all the samples were preceded. The PCR products were preliminarily tested for size by 2.0% agarose gel electrophoresis and analyzed by using Applied Biosystems 3130 Series System with the GeneMapper® program. Number of alleles were calculated to obtain frequency of alleles, expected heterozygosity (He) (Nei, 1978), Polymorphic Information Content (PIC) (Botstein et al., 1980), Exclusion Probability (EP) and Combined Exclusion Probability (CEP) (Jamieson and Taylor, 1997).

Results

Microsatellite loci amplifications by PCR were successful in ten out of eleven markers tested. Nevertheless, the locus LaT07 was unable to yield an amplification product although PCR conditions were carefully adjusted. The observed marker sizes of all loci were 238 to 573 (Table1). The same result of an individual sample with all the loci by the repeated PCR amplifications was exhibited. The sequencing analysis of all markers proved to be microsatellite DNA with three kinds of sequences as a motif of $(GGAT)_n$ at loci LaT26, LaT24, LaT16 and LaT17; that of $(CCAT)_n$ at loci LaT25, LaT18, LaT13 and LaT06, and a core sequence of $(AGAT)_n$ at locus LaT08.

Table 1 Statistical analysis showing number of alleles, observed size (bp), expected heterozygosity (He), polymorphic information content (PIC), exclusion probability (EP) and combined exclusion probability (CEP) of the ten microsatellite markers.

Markers	LaT05	LaT06	LaT08	LaT13	LaT16	LaT17	LaT18	LaT24	LaT25	LaT26
Number of Alleles	39	7	38	12	15	8	19	24	21	28
Observed Size (bp)	363-567	238-418	274-482	246-308	273-418	297-355	434-573	294-435	326-529	334-488
He	0.9593	0.8162	0.9567	0.9239	0.8405	0.6449	0.9005	0.8426	0.9151	0.9325
PIC	0.9578	0.7912	0.9550	0.9189	0.8265	0.5934	0.8924	0.8240	0.9088	0.9285
EP	0.9181	0.6253	0.9130	0.6812	0.6937	0.3593	0.7989	0.8464	0.8273	0.8636
CEP	0.9999998783 or 1.2167 x10^{-7}									

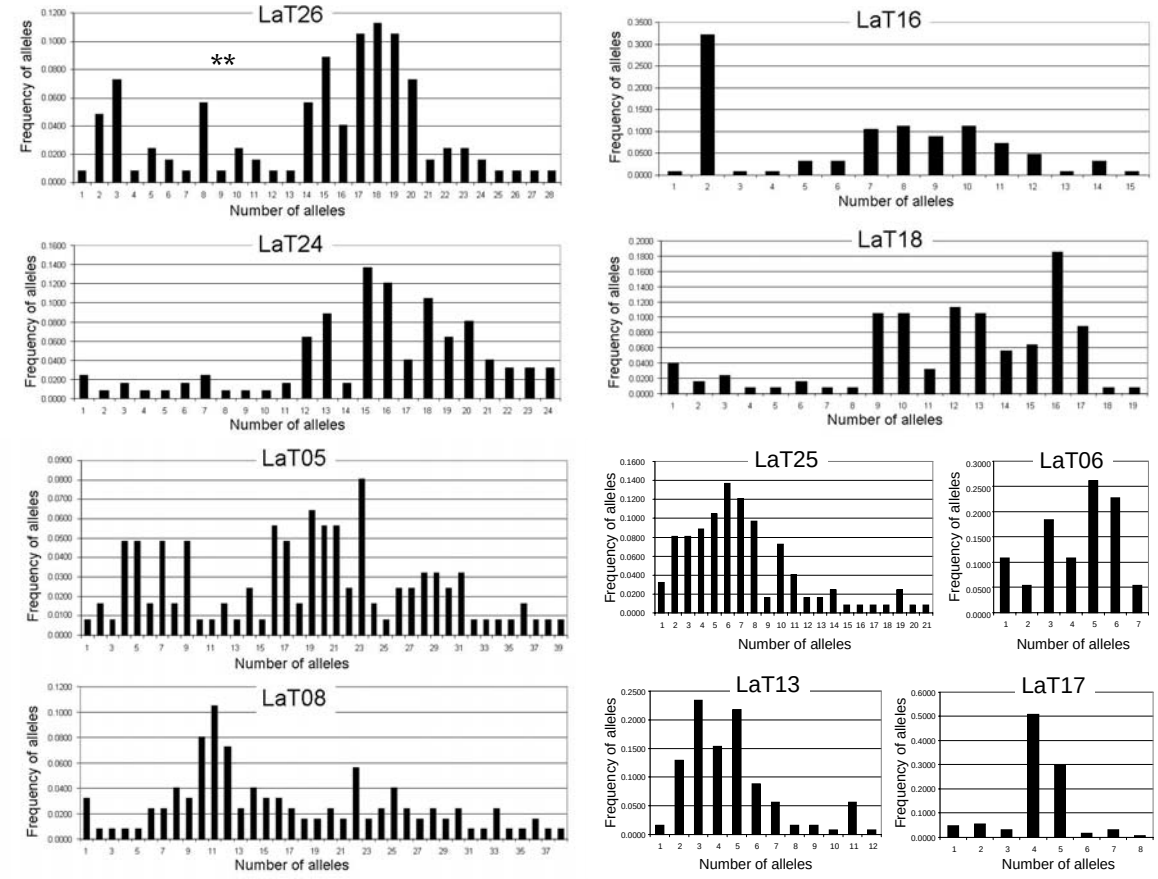

Figure 1. Diagrams representing DNA fragments of each microsatellite locus, arranged for sizes from the shortest to the longest.

The numbers of alleles from the 10 loci surveyed were detected from seven for LaT06 to thirty-nine for LaT05 (Table1), yielding a mean value of 21.1 alleles per locus. The frequency of alleles at each microsatellite locus is shown in figure 1. The expected heterozygosity (He), Polymorphic Information Content (PIC), Exclusion Probability (EP) and Combined Exclusion Probability (CEP) of the tested loci are listed in Table1. The mean PIC value was 0.8596 while the mean expected heterozygosity value was 0.8732. Not all markers were equally informative in this study. The LaT05 locus showed the

highest allele polymorphism and displayed the highest exclusion probability as well. The LaT16 locus had one allele with a much higher frequency than the others while the LaT07 had two out of eight alleles with very high frequencies and did not show heterozygote deficiency.

A comparison of microsatellite loci was established for the efficacy in molecular testing. Based on Polymorphic Information Content (PIC) values, all markers appeared to be highly to very highly efficient and desirable markers in parentage testing and individual identification, in a decreasing degree as LaT05, LaT08, LaT26, LaT24, LaT25, LaT18, LaT16, LaT13, LaT17 and LaT06, respectively. Results from the analysis of alleles of all markers by matching data from 2 elephants at a time were compared with pedigree and family records. It was found that when the 10 markers were analyzed simultaneously as an individual DNA fingerprinting or genetic profile, parentage testing results were in accordance with the family history. The combined probability of parentage exclusion (CEP) value for the 10 microsatellite loci was 0.9999998783 or 1.2167×10^{-7} efficacy while the test of individual identification was shown to be 99.91% accuracy.

Discussion

The purpose of this investigation was to evaluate the efficiency and accuracy of the use of tetranucleotide microsatellite polymorphism for parentage testing and individual identification in Thai domestic elephants. Microsatellite loci used in this study, were already found to exist in African elephants (*Loxodonta africana africana*) (Archie et al., 2003). We demonstrated that ten tested loci could be PCR-amplified in Thai elephants, indicating that African and Asian elephants (*Elephas maximus*) share the conserved regions especially in tandem repeated DNA (Eggert et al., 2000). Corresponding to the previous study by Suwattana et al. (2007), microsatellite locus LaT07 was confirmed not to be amplified, suggesting that the primer for this locus may not be appropriate for Thai elephants. The same figures of PCR products from many repeated PCR reactions in the samples proved the qualification of all other tested markers.

It is noteworthy that the allele size and number of each locus were different when tested on different groups of animals as shown for loci LaT06, LaT08, LaT13, LaT16, LaT24, LaT25 and LaT26, reported by Archie et al. (2003), Thitaram et al. (2008) and our results in this study. It was found that tetranucleotide loci were highly polymorphic with the greater allele number than those of dinucleotide and trinucleotide microsatellite loci although the dinucleotides appeared to have mutation rates 1.5-2 times higher than the tetranucleotides (Chakraborty et al., 1997; Fernando et al., 2001). In addition, the very high numbers of alleles of all microsatellite markers in this study indicated that there was still high genetic diversity among the captive elephants in Thailand. The heterozygosity and PIC values were also considered to be high to very high since they were more than 0.50 and most of them were relatively closed to 1.00.

Analysis of CEP values for the accuracy of interpretation is useful when pedigree and family records of the elephants are unclear and ambiguous. High variability of allelic microsatellite markers implies their high effectiveness for fingerprinting purposes, individual identification and parentage testing. The CEP value is a measure of the ability of a certain panel of markers in order to identify genetic paternity, excluding all other candidates. The probability of exclusions, the cumulative value in this study, was 0.9999998783 or 1.2167×10^{-7} when all 10 markers were considered, indicating that the chance of testing inaccuracy is approximately one in a million. Such a high value would be useful especially for situations in which there are many parental candidates and no known parent is available. The probability of two random animals having identical genotypes was estimated at 99.91% confidence, which indicated that this microsatellite panel is theoretically sufficient for individual identification of any elephant in Thailand. However, additional effective microsatellite markers should be considered in order to achieve higher efficiency of testing. Typically, the greater the allelic diversity and the size of the microsatellite locus, the more informative it is, because these properties provide unique alleles to individual animals (O'Reilly et al., 1998; Norris et al., 2000; Boudry et al., 2002). Hence, certain microsatellite markers can be considered as an extremely powerful system for fingerprinting purposes, individual identification and parentage testing in Thai elephants.

Acknowledgement

This study was financially supported by Chulalongkorn University Centenary Academic Development Project and Faculty of Veterinary Science Innovation Center for Veterinary Biotechnology (2008-2009), Chulalongkorn University. We thank the elephant owners in the central and northeastern parts of Thailand, who allowed us to collect blood samples.

References

Archie, E.A., Moss, C.J. and Alberts, S.C. 2003. Characterization of tetranucleotide microsatellite loci in the African Savannah elephant (*Loxodonta africana africana*). Mol. Ecol. Notes. 3: 244-246.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 182-190.

Bruford, M.W. and Wayne, R.K. 1993. Microsatellites and their application to population genetic studies. Curr. Op. Genet. Devel. 3: 939-943.

Boudry, P., Collet, B., Cornette F., Hervouet, V. and Bonhomme, F. 2002. High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by

microsatellite-based parentage analysis of multifactorial crosses. Aquaculture 204: 283-296.

Chakraborty, R., Kimmel, M., Stivers, D.N., Davison, L.J. and Deka, R. 1997. Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. Proc. Natl. Acad. Sci. USA. 94: 1041–1046.

Comstock, K.E., Georgiadis, N., Pecon-Slattery, J., Roca, A.L., Ostrander, E.A., O'Brian, S.J. and Wasser, S.K. 2002. Patterns of molecular genetic variation among African elephant populations. Mol. Ecol. 11: 2489-2498.

Eggert, L.S., Ramakrishnan, U., Mundy, N.I. and Woodruff, D.S. 2000. Polymorphic microsatellite DNA markers in the African elephant (*Loxodonta africana*) and their use in the Asian elephant (*Elephas maximus*). Mol. Ecol. Notes. 9: 2223-2225.

Fernando, P., Vidya, T.N.C. and Melnick, D.J. 2001. Isolation and characterization of tri- and tetranucleotide microsatellite loci in the Asian elephant (*Elephas maximus*). Mol. Ecol. Notes. 1: 232-233.

Gobush, K., Kerr, B. and Wasser, S. 2009. Genetic relatedness and disrupted social structure in a poached population of African elephants. Mol. Ecol. 18: 722-734.

Jamieson, A. and Taylor, S.S. 1997. Comparisons of three probability formulae for parentage exclusion. Anim. Genet. 28: 397-400.

Kongrit, C., Siripunkaw, C., Brockelman, W.Y., Akkarapatumwong, V., Wright, T.F. and Eggert, L.S. 2008. Isolation and characterization of dinucletide microsatellite loci in the Asian elephant (*Elephas maximus*). Mol. Ecol. Res. 8: 175-177.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89: 583-590.

Norris, A.T., Bradley, D.G. and Cunningham, E.P. 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. Aquaculture: 182: 73-83.

Nyakaana, S. and Arctander, P. 1998. Isolation and characterization of microsatellite loci in the African elephant, *Loxodonta africana*. Mol. Ecol. 7: 1436-1437.

O'Reilly, P.T., Herbinger, C. and Wright, J.M. 1998. Analysis of parentage determination in Atlantic salmon (*Salmo salar*) using microsatellites. Anim.Genet. 29: 363-370.

Suwattana, D., Koykul, W., Jirasupphachok, J. and Kanchanapangka, S. 2007. Microsatellite polymorphism and parentage control in Thai domestic elephants (*Elephas maximus*). Thai J. Vet. Med. 37(4): 33-38.

Thitaram, C., Thongtip, N., Somgird, C., Colenbrander, B., van Boxtel, D.C.J., van Steenbeek, F. and Lenstra, J.A. 2008. Evaluation and selection of microsatellite markers for an identification and parentage test of Asian elephants (*Elephas maximus)*. Conserv. Genet. 9: 921-925.