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≪**Research Note**≫

# Chicken and Quail Microsatellite Markers Reveal Polymorphisms in Guinea Fowl

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Microsatellite markers are tandem repeats of short nucleotides which are abundant, evenly distributed and highly polymorphic in eukaryotic genomes. As such, they are a useful tool in poultry breeding. Microsattelite markers have been identified in genomes of poultry such as chickens and quail. Exchange of marker information between chickens, quail and other avian species such as guinea fowl would be an important step towards the construction of a high-resolution comparative genetic map of these poultry species of agricultural importance. Using the polymerase chain reaction, chicken and quail microsatellite markers were tested to see if they would be suitable in amplifying guinea fowl loci. Eighty three percent (30/36) and 73 percent (19/26) of chicken and quail primers, respectively, amplified individual loci in guinea fowl. Fifty percent (15/30) and 47 percent (9/19) of the amplified loci using chicken and quail primers, respectively, were found to be polymorphic. These very few chicken and quail microsatellite markers that seem to reveal guinea fowl loci could be used as anchor points for comparative mapping. However, more effort should be committed to developing guinea fowl-specific markers since those of chickens and quail may not be sufficient for studies in guinea fowl.

**Key words**: guinea fowl, microsatellite markers, polymorphisms

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## Introduction

The construction of genomic maps for farm animals has become an indispensable tool in the study of genetics underlying the control of economically important traits (Kayang *et al.,* 2000). The construction of these maps has been greatly enhanced by the development of highly polymorphic DNA markers, particularly microsatellite markers (Tautz and Renz, 1984; Stallings *et al.,* 1991; Georges and Andersson, 1996). Microsatellites have been designated the markers of choice in genome mapping and linkage analysis owing to their dispersion throughout genomes, high level of polymorphism due to high variation in the number of repeat units (Tautz, 1989), codominance mode of inheritance and the ease with which they can be typed by the polymerase chain reaction (PCR). They have been effectively used in analyses of genetic diversity and evolution of animal genomes (Gupta *et al.,* 1996; MacHugh *et al.,* 1997; Takahashi *et al.,* 1998; Zhang *et al.,* 2002).

Linkage maps of polymorphic markers are being developed in agriculturally important species such as poultry. The chicken (*Gallus gallus*) is the most researched of the avian species with more than 800 microsatellite markers isolated (Groenen *et al.,* 2000). The Japanese quail (*Coturnix japonica*) has also been recognized as agriculturally important, thus attempts have been made to isolate microsatellite markers for genome mapping of this avian species. However, much remains to be done in other economically important avian species, such as the guinea fowl (*Numida meleagris*). Guinea fowl production is an established and profitable enterprise in European markets such as France, Italy and Belgium. Guinea fowl is also raised commercially on farms in Canada (Nova Scotia Department of Agriculture and marketing, 1997). However, poor growth rate and feed efficiency is a draw back to profitability in guinea fowl production enterprises.

At present genetic information of guinea fowl which may facilitate genetic improvement programs is very scanty. When compared to other avian species such as chickens, quail and turkey, for instance, no genetic maps are available yet. In view of this, mapping efforts in this species would not only be essential in the genetic improvement of guinea fowl per se but could also be of great economic benefit to other closely related species.

Studies have shown that markers developed for a species may be useful in others. For instance, microsatellite marker exchange has been reported within closely related avian species (Primmer *et al.,* 1996; Richardson *et al.,* 2000).

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Smith *et al.* (2000) used primers designed for turkey EST-targeted regions to cross-amplify guinea fowl, quail and pigeon DNA. The guinea fowl and pigeon sequences showed the lowest similarity with the turkey sequences. In recent studies, chicken (Kayang *et al.,* 2003) and quail (Kayang *et al.* 2002) microsattelite markers revealed polymorphic loci in helmeted guinea fowl. Since the chicken is the most widely studied of the phasianidae family, attempts have been made to use the large number of chicken-specific microsatellites available to develop DNA markers for the turkey (Reed *et al.,* 1999, 2000) and Japanese quail (Pang *et al.,* 1999; Inoue-Murayama, 2001).

In the present study chicken and Japanese quail microsatellites were tested for polymorphism and their suitability in amplifying guinea fowl loci. This would be a step forward towards the construction of a comparative genetic map in phasianidae and creating avenues for improving traits of economic importance in these avian species.

## Materials and Methods

### *Samples and DNA Extraction*

Guinea fowl blood samples were obtained from 80 each of unrelated males and females in random bred population. Whole blood samples (about 0.5 m*l*) were collected from the brachial vein into 1.5 m*l* eppendorf tubes containing 0.5M EDTA as anticoagulant. DNA was isolated using Dr. Gentle® DNA Isolation kit (Takara Bio Inc., Shiga, Japan) and its concentration and relative purity were determined using a fluorometer(Bio-Rad Laboratories Inc., Hercules, CA, USA). In the initial screening, equal quantities of DNA samples from ten birds of either sex were pooled and eight pools from each population were used for PCR amplifications. Thereafter, individual birds were typed for polymorphism. DNA was diluted to approximately 25 ng/$\mu l$ with deionized distilled water prior to PCR amplifications.

### *Amplification of Genomic DNA*

Thirty six and twenty two chicken and quail microsatellite primers were synthesized (Operon Technologies, Alameda, CA, USA) and used to initiate PCR amplifications in a PTC 100 thermal cycler (MJ Research Inc., Waltham, MA, USA). These primers were chosen based on likelihood to yield amplification in chickens (Cheng *et al.,* 1995) and quail (Kayang *et al.,* 2000). The nucleotide sequence and annealing temperatures of the chicken and quail primers are given in Table 1 and Table 2, respectively. PCR reactions were carried out in a final volume of 25 $\mu l$ consisting of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris HCl, pH 8.0, 1% Triton X-100, 200 $\mu$M of each deoxyribonucleotide, 100 pM of primer, 100 ng of genomic DNA and 2.5 units of *Taq* DNA polymerase (Sigma-Aldrich, St. Louis, MO, USA). The PCR cycling parameters were as follows: Initial denaturation at 95℃ for 9 min, and 30 cycles at 95℃ for 30 sec, 44–60℃ for 1 min and 72℃ for 1 min for denaturation, primer annealing and extension, respectively. A final extension at 72℃ was carried out for 5 min. Negative control reaction was prepared for each primer employed in PCR amplification to identify contamination from reactions with non-target template DNA. All amplifications were repeated two times to ensure reproducibility. Twenty microliters of the PCR amplified products were analyzed on a 1.5% agarose gel (Fisher Scientific, Fair Lawn, NJ, USA) stained with 0.1 $\mu$g/m*l* ethidium bromide. The PCR products were separated in 1 X TBE buffer in a Fisher Biotech midigel electrophoresis system (Fisher Scientific, Fair Lawn, NJ, USA), at 80 volts for 120 min.

### *Fragment Visualization and Data Analysis*

Electrophoresis was performed at 100 volts for 240 min in 0.5 X TBE buffer. The gels were photographed under ultraviolet (UV) light. The size of RAPD bands was estimated using either 100 bp ladder or pGEM® DNA molecular weight markers (Promega, Madison, WI, USA) which were included in each gel. The microsatellite profiles resulting from PCR amplification of guinea fowl genomic DNA using chickens and quail microsatellite primers were analyzed manually. The amplified DNA bands (fragments) were scored as binary matrix 1 (if band present) and 0 (if band absent) for individual DNA pools within chickens and guinea fowl populations.

### *Sequencing of DNA Fragments*

To estimate the size of amplicons for individual loci, genomic DNA from individual/single guinea fowls was amplified using the chicken and quail specific primers. Ten loci each revealed from chicken and quail primers were sequenced; however, only two loci each from the primers G01598 and AB035859 were presented in Table 3. The DNA fragments were excised from agarose gel and purified using the Qiagen MiniElute Gel Extraction Kit (Qiagen, Inc., Turnberry Lane, CA, USA). Blunt ended PCR products of approximately 200 bp and 400 bp both derived from PCR amplification of guinea fowl genomic DNA using either primer G01598 or AB035859 were cloned into PCR-TRAP Cloning Vector (GeneHunter Corporation, Nashville, Tennessee, USA) and sequenced using the ABI 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). These sequences can be accessed through GenBank with accession numbers AY 862543, AY862544, AY862545 and AY862546. A homology search with sequences registered in GenBank was carried out using the NCBI databases using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

## Results and Discussion

Reproducible amplification profiles of guinea fowl genomic DNA using chicken and quail specific primers are presented in Table 1 and Table 2, respectively. A total of 36 and 26 microsatellite primers which amplified specific loci in chicken and quail, respectively, were used to amplify guinea fowl genomic DNA. Eighty three percent (30/36) and 73 percent (19/26) of the chicken and quail primers, respectively, amplified individual loci in guinea fowl. A total of 194 and 199 alleles were amplified in guinea fowl using chicken and quail specific microsatellite

Table 1. **Chicken microsatellite Primers used in amplifying guinea fowl genomic DNA using the polymerase chain reaction (PCR)**

| Locus Name | GenBank Number[1] | Repeat Array | Forward Primer (5′–3′) | Reverse Primer (5′–3′) | Ta[2] | At[3] | Ap[4] | Typical Size (bp) | Amplicon Size (bp) |
|---|---|---|---|---|---|---|---|---|---|
| *ADL127* | G01736 | $(AT)_{12}(AC)_8$ | GAACCAGCAATTATATTAAATA | TTAACACAAAAGAACCAGGCAG | N/A[5] | 0 | 0 | 148 | — |
| *ADL132* | G01740 | $(CA)_9$ | GCTGTTTCTAAGCCATCTTC | CCACTTGGAAGTAATCTCCT | 52 | 2 | 0 | 190 | 300– 550 |
| *ADL134* | G01754 | $(CA)_{17}$ | TTCCATAAGCCATCAATCAG | TTTTCCTCTCCCTCCATTTT | N/A | 0 | 0 | 150 | — |
| *ADL110* | G01723 | $(CA)_9$ | TGCTTGGGAGTTCAGATACA | TAGAATGAGAAACAAAAGGC | 46 | 5 | 1 | 124 | — |
| *ADL186* | G01745 | $(CA)_{14}$ | GAGTGCTGCCTTCAAGTATC | ACCCAAGAATTGCTCTGATG | N/A | 0 | 0 | 109 | 150– 800 |
| *ADL122* | G01733 | $(A)_{19}$ | TGAAATACCAAGGCATCTGT | TGGCTAAGAAAGTGGAACTG | N/A | 0 | 0 | 231 | — |
| *ADL172* | G01594 | $(AC)_{18}$ | CCCTACAACAAAGAGCAGTG | CTATGGAATAAAATGGAAAT | 49 | 5 | 0 | 160 | 300– 800 |
| *ADL180* | G01602 | $(TG)_{15}$ | ACCAGAGCATCTACTACTGAAGA | AAACCTGGAAATGAAAGCAT | 47 | 6 | 0 | 145 | 150– 900 |
| *ADL166* | G01588 | $(TG)_{15}$ | TGCCAGCCCGTAATCATAGG | AAGCACCACGACCCAATCTA | 53 | 7 | 2 | 136 | 150– 900 |
| *ADL193* | G01614 | $(GT)_{18}$ | TTGAGACCAGAACAGGAAAT | CAAACGCAGGCAGGAAGCAG | 51 | 3 | 1 | 218 | 550– 700 |
| *ADL181* | G01603 | $(CA)_{12}$ | CCAGTGAAATTCATCCTTTT | CAATCTTTTGTGGGGTATGG | 48 | 6 | 0 | 181 | 250– 800 |
| *ADL187* | G01608 | $(TG)_{22}$ | AATTGTTTGTTTACGCTTCT | GCTGGTGGCACAGATGAGAG | 48 | 3 | 1 | 104 | 150– 700 |
| *ADL176* | G01598 | $(GT)_{12}$ | TTGTGGATTCTGGTGGTAGC | TTCTCCCGTAACACTCGTCA | 52 | 5 | 0 | 195 | 150– 900 |
| *ADL185* | G01607 | $(CA)_{16}$ | CATGGCAGCTGACTCCAGAT | AGCGTTACCTGTTCGTTTGC | 52 | 9 | 1 | 137 | 150–1100 |
| *ADL167* | G01589 | $(GT)_9$ | AACTTCCTCTTGGTTGATAA | CTGTTTAGCCTCCCTCATAA | 46 | 6 | 0 | 175 | 200–1000 |
| *ADL171* | G01593 | $(TG)_{18}$ | ACAGGATTCTTGAGATTTTT | GGTCTTAGCAGTGTTTGTTT | 46 | 10 | 1 | 106 | 250–1250 |
| *ADL161* | G01585 | $(GT)_{12}$ | TGGAACTTTTCCCTATGTTA | AAGGAGTCAATTGTAGCACA | N/A | 0 | 0 | 140 | — |
| *ADL163* | G01587 | $(GT)_{13}$ | TGTGTAGCCTACAGGATTGC | AGCCAAAATGGAGGTTCTGG | 51 | 6 | 0 | 173 | 150–1100 |
| *ADL154* | G01579 | $(GT)_{26}$ | GCTGCCACCTTCAAAACCTG | CTCACCATCTCATTCTTCAT | 50 | 8 | 0 | 165 | 200–1000 |
| *ADL159* | G01583 | $(GT)_{15}$ | GCCATTATTTTTCCCTGTGT | CTCCCCANAGTCATTAGCAG | 49 | 8 | 0 | 114 | 150–1100 |
| *ADL157* | G01581 | $(TG)_{25}$ | CTCTGTCAGGAAGGGGTGTA | GTGCCTGTCCTCTGTTTCAT | 52 | 4 | 2 | 159 | 100–1100 |
| *ADL158* | G01582 | $(CA)_{12}$ | TGGCATGGTTGAGGAATACA | TAGGTGCTGCACTGGAAATC | 52 | 6 | 0 | 217 | 100–1000 |
| *ADL145* | G01570 | $(TG)_9$ | CGTGGTGTTGTGTATCATTT | CTCTTTTGCAGTCCTCCTAC | 48 | 10 | 1 | 119 | 150–1000 |
| *ADL149* | G01574 | $(CA)_{11}$ | ATAGCATACACCCAGCCACC | GAATAAGAATGTTNCCCTGC | 55 | 8 | 2 | 227 | 100–1250 |
| *ADL138* | G01563 | $(AC)_{10}$ | GCTTCAGGAACTATTTACAT | CTTTAGAGATTATGCCAGTA | 44 | 5 | 1 | 124 | 150– 750 |
| *ADL144* | G01569 | $(GT)_{13}$ | TCAGAAAAGGAAAACAAAA | TTATCACCAAGTCAGCCATC | 50 | 3 | 0 | 200 | 100– 650 |
| *ADL129* | G01559 | $(GT)_9$ | CAGTTTTTGCCCCTCTCTTG | TGTGNTTTTTTTTTCTTCCA | N/A | 0 | 0 | 155 | — |
| *ADL136* | G01561 | $(TG)_{10}TC(TG)_{10}$ | TGTCAAGCCCATCGTATCAC | CCACCTCCTTCTCCTGTTCA | 52 | 10 | 0 | 147 | 200–1500 |
| *ADL125* | G01558 | $(TG)_9$ | TAAACGGGGAATGTTAGGCA | TGGAAAATAAATAGAAGGCA | 45 | 11 | 0 | 144 | 150– 900 |
| *ADL126* | G01735 | $(TTG)_6T_{15}$ | ATCATCCAGCATAGCTTTGT | CTTGCTCTTCTCTTTTTCCA | 45 | 7 | 1 | 99 | 100– 850 |
| *ADL118* | G01729 | $(CA)_{10}A_{10}$ | GATCACTCTTAGATGCCACA | AGAGAGGGGTTACAAGGCTG | 48 | 5 | 0 | 162 | 100– 550 |
| *ADL123* | G01557 | $(TG)_{13}$ | GCTGTGTCAAGATTAGAATCAC | AACAATGAAAAACACTACCTGA | 46 | 4 | 1 | 137 | 150– 800 |
| *ADL109* | G01554 | $(AC)_{13}$ | ATCTCCATAACTTCTGCTGC | AAAAATAAAATATCTCCCAG | 46 | 7 | 0 | 215 | 350–1150 |
| *ADL111* | G01724 | $(TG)_{15}(T_4G)_5T_7$ | CCTTCCTGACCTTCCACTTC | CCACAAAAATACCCACCATC | 49 | 8 | 2 | 133 | 200–1000 |
| *ADL102* | G01547 | $(GT)_{18}$ | TTCCACCTTTCTTTTTTATT | GCTCCACTCCCTTCTAACCC | 47 | 8 | 1 | 96 | 300–1100 |
| *ADL106* | G01550 | $(TG)_9$ | CATTCTCTGATTCTGCCTTT | AACTCCTGGTGTGCTACAA | 48 | 7 | 3 | 151 | 100–1000 |

[1] GenBank Accession number of sequences from which PCR primers were designed. [2] Annealing temperature (℃) in PCR amplification of guinea fowl genomic DNA. [3] Alleles (total) per bird per primer pair. [4] Average number polymorphic alleles per primer pair. [5] Optimum temperature for primer annealing was not applicable; no amplification at all temperatures tested during optimization.

primers, respectively. The total number of alleles per primer pair (locus) ranged from 2 to 11 and 3 to 15 in chicken and quail specific primers with an average of 6.5 and 10.4 alleles per locus, respectively (Table 1). In previous studies, Crooijmans *et al.* (1996) and Vanhala *et al.* (1998) estimated the number of alleles per primer to be 5.2 and 5.7, respectively, for a commercial broiler line. The range and size of amplicons of the microsatellite markers observed in this study is explained by Smith *et al.* (1996) as follows: First, the primers may be annealing to similar regions of the genomes of chickens and guinea fowl, but the length of the target region for amplification may be variable even though a high sequence homology may exist. Second, the primers may be annealing at different regions of the genomes of these two avian species. Thus, amplifications from these regions would result in unique products in chickens and guinea fowl. A third explanation is that these primers may be binding to regions of the genome of chickens and guinea fowl with lower sequence homology. The expected amplicon size from the chicken (Cheng *et al.,* 1995) and quail (Kayang *et al.,* 2000) microsatellite primers ranged from 96–231 and 125–251 base pairs. However, these primers also revealed new loci beyond the estimated range and while these loci did not contain microsatellites as expected, some were polymorphic. These loci could be a valuable resource for mapping the avian genome.

Fifty percent (15/30) and 47 percent (9/19) of the

Table 2. **Quail microsatellite Primers used in amplifying guinea fowl genomic DNA using the polymerase chain reaction (PCR)**

| Locus Name | GenBank Number[1] | Repeat Array | Forward Primer (5′–3′) | Reverse Primer (5′–3′) | Ta[2] | At[3] | Ap[4] | Typical Size (bp) | Amplicon Size (bp) |
|---|---|---|---|---|---|---|---|---|---|
| *GUJ003* | AB035814 | $(CA)_9$ | AGGGAAGAAGCAACTGTTC | ATTCCAGAATCTGGACTGG | 48 | 15 | 0 | 146 | 150–1600 |
| *GUJ006* | AB035816 | $(CA)_{14}$ | TGGGATGATAATGAGGTACGG | AGGATAGCATTTCAGTCACGG | 55 | 12 | 1 | 125 | 100–1500 |
| *GUJ008* | AB035818 | $(CA)_{10}$ | CATGGTTATCAACCTGCAGA | ACATGCCAGTCCTTCACAAT | N/A[5] | 0 | 0 | 174 | — |
| *GUJ010* | AB035820 | $(CA)_{15}$ | TTCCTTCTGGGTGCTGCTCA | CATAGACACATCCCTCCCTC | N/A | 0 | 0 | 156 | — |
| *GUJ011* | AB035821 | $(CA)_{11}$ | GAGCATTTCTAGTCTGTCTC | GATCAATACACAGGCTAAGG | N/A | 0 | 0 | 164 | — |
| *GUJ013* | ABO35823 | $(CA)_{10}$ | ACCAAACCCGAGATCCGACA | AGCGTTCGCGTTCCTCTTTC | 60 | 11 | 1 | 139 | 200–1400 |
| *GUJ017* | AB035827 | $(CA)_{14}$ | AGAGAGATTAGAGGAGCTGC | GGCACTAAAACCATCGAGAG | 60 | 13 | 3 | 165 | 100–1850 |
| *GUJ019* | AB035829 | $(CA)_{21}$ | GGGGGCTGTAGGTCTGGATC | ATCGGGCACGCGAGGACCAT | 50 | 7 | 0 | 191 | 150– 800 |
| *GUJ021* | AB035831 | $(CA)_{11}$ | GAGCATTTCTAGTCTGTCTC | GATCAATACACAGGCTAAGG | 55 | 9 | 0 | 209 | 200–1100 |
| *GUJ023* | AB035833 | $(CA)_7TA(CA)_{11}$ | GAGAGGTACAGCAACACTTT | CGTTTCTTTCTGGAGTGTCT | 55 | 13 | 2 | 237 | 100–1800 |
| *GUJ025* | AB035835 | $(CA)_9$ | CCTGAGCGAATACACAACTG | AGTGTTAGGTGAGGACTGCT | 60 | 9 | 1 | 245 | 150–1150 |
| *GUJ027* | AB035837 | $(CA)_{15}$ | TTCACAGATGACAATCTAGC | CTGCAAGTAACAGAAGGTAA | 55 | 8 | 0 | 176 | 100– 900 |
| *GUJ028* | AB035838 | $(CA)_9$ | TGAACAAAGCAGAAAGGAGC | CCTTACCTACATGAAACGTC | 55 | 12 | 1 | 150 | 200–1500 |
| *GUJ029* | AB035839 | $(CA)_{11}CT(CA)_2$ | GAGCATTTCTAGTCTGTCTC | ATACACAGGCTAAGGAAACC | 55 | 8 | 0 | 152 | 250–1100 |
| *GUJ031* | AB035841 | $(CA)_9$ | AAGGGCAGGGGCTGGGAACA | CGCCTCTGCGGTGTGCAACT | N/A | 0 | 0 | 164 | — |
| *GUJ034* | AB035844 | $CG(CA)_2(CA)_9$ | CGTAACGGTCCAATATGGAT | TCCAGATGCAGAGGTATTT | 55 | 10 | 0 | 247 | 300–1750 |
| *GUJ035* | AB035845 | $(CA)_{14}$ | AATACTGGTTTTGTGATGGC | GGGCAATAAAAGAAAGACTG | 55 | 14 | 1 | 152 | 250–1500 |
| *GUJ036* | AB035846 | $(CA)_9TA(CA)_4$ | CTTTCATTGCTTTTGCCT | CACTAAAGATTGGCTAACAG | N/A | 0 | 0 | 151 | — |
| *GUJ037* | AB035847 | $(CA)_{10}C(CA)_2$ | CCATTCCTCCATCGTTCTGA | GGGAAGGAGTGTAGGAAAGA | N/A | 0 | 0 | 182 | — |
| *GUJ039* | AB035849 | $(CA)_{19}$ | CAAAGAGCAGAGGGAATGGA | CCGAGAGATGGGTTTTTTCC | 60 | 9 | 0 | 188 | 150–1400 |
| *GUJ042* | AB035852 | $(CA)_8$ | TCAGTGCCTTTGTGTTGTCC | ACAGCCTTCCCCAAATTCCT | 55 | 12 | 3 | 191 | 150–1850 |
| *GUJ044* | AB035854 | $(CA)_{16}$ | GCCTTGAAACCTGAGTGATC | TGCATTTCAGCAGCTCTCAG | 55 | 10 | 0 | 206 | 200–1500 |
| *GUJ045* | AB035855 | $(CA)_{18}$ | ACATGCACCACCATTCTTGC | CATGCACAAATGAGCGTGCA | 60 | 8 | 0 | 251 | 100–1400 |
| *GUJ046* | AB035856 | $(CA)_9$ | GCCATGTTTGTCACCTTGCA | ACTGGTTGGGACTGAAGGAT | N/A | 0 | 0 | 236 | — |
| *GUJ048* | AB035858 | $(CA)_{14}$ | AACGCATACAACTGACTGGG | GGATAGCATTTCAGTCACGG | 55 | 8 | 0 | 140 | 250–1100 |
| *GUJ049* | AB035859 | $(CA)_{11}$ | GAAGCAGTGACAGCAGAATG | CGGTAGCATTTCTGACTCCA | 55 | 3 | 2 | 243 | 500–1500 |

[1] GenBank Accession Number of sequences from which primers were designed. [2] Annealing temperature (℃) in PCR amplification of guinea fowl genomic DNA. [3] Alleles (total) per bird per primer pair. [4] Average number polymorphic alleles per primer pair.
[5] Optimum temperature for primer annealing was not applicable; no amplification at all temperatures tested during optimization.

chicken and quail-specific primers (loci), respectively, were found to be polymorphic in guinea fowl. These polymorphic alleles accounted for approximately 11% and 6.6% of the total number of alleles in all chicken and quail-specific primers tested, respectively. The number of polymorphic loci in guinea fowl per chicken or quail specific primer pairs ranged from 1–3. While repetitive DNA sequences constitute substantial portions of most eukaryotic genomes and they represent an enormous, largely untapped reservoir of genetic variation, these results suggest that primers designed to amplify chicken and quail repetitive elements may not be efficient in revealing these loci in the guinea fowl. In most part this may be attributed to the low genetic similarity among chicken, quail and guinea fowl (Smith *et al.,* 2000).

As shown in Table 1 and Table 2, 26% and 21% of the chicken and quail specific primers successfully amplified loci in guinea fowl at the annealing temperature optimized for chickens and quail, respectively. Chicken and quail-specific primers have been successfully tested in other phasianidae on the assumption that since they are related species, their simple sequence repeat loci could be similar. However, success in DNA amplification seems to vary based on stringency of amplification reactions (Inoue-Murayama *et al.,* 1998). In previous studies, about 92 and 69% of chicken specific primers amplified products in turkeys at Low (4 mM $MgCl_2$) and high (1.5 mM $MgCl_2$) stringency reactions, respectively (Levin *et al.,* 1995; Liu *et al.,* 1996). The low success rate in amplifying guinea fowl loci with chicken-specific (26%) and quail-specific (21%) primers may be attributed to the relatively high stringency that was applied in our amplification reactions (1.5 mM $MgCl_2$ coupled with high primer annealing temperatures).

By sequencing specific PCR products, it was confirmed that chicken and guinea fowl specific primers amplified specific loci in guinea fowl. We observed that these guinea fowl sequences derived from specific PCR amplification shared very low sequence homology with chickens (about 20–30 percent), not with quail. This may be attributed to the fact that these specific loci in chickens and quail have not been sequenced and therefore not available in GenBank databases. It may also be just the mere lack of sequence similarity and greater genetic distance among these three avian species. Pang *et al.* (1999) showed that although eight out of eleven chicken markers with specific

Table 3. **Simple sequence motifs derived from amplification of guinea fowl genomic DNA using chicken and quail specific primers**

| Organism | Locus Name | Primer Accession Number[1] | GF[2] Seq.[3] Accession Number[4] | Length of Repeat[5] | Repeat | Annotation |
|---|---|---|---|---|---|---|
| Chicken | *ADL176* | G01598 | AY862543 | 1 | 5′-CCCCCCC-3′ | 5′-$(C)_7$-3′ |
| | | | | 1 | 5′-TTTTTT-3′ | 5′-$(T)_6$-3′ |
| | | | | 2 | 5′-ATATAT-3′ | 5′-$(AT)_3$-3′ |
| | | | | 3 | 5′-CCACCA-3′ | 5′-$(CCA)_2$-3′ |
| | | | | 4 | 5′-CTGTCTGT-3′ | 5′-$(CTGT)_2$-3′ |
| | | | AY862546 | 1 | 5′-TTTTTTTTT-3′ | 5′-$(T)_9$-3′ |
| | | | | 1 | 5′-GGGGGGGGG-3′ | 5′-$(G)_9$-3′ |
| | | | | 2 | 5′-GTGT-3′ | 5′-$(GT)_2$-3′ |
| | | | | 2 | 5′-CTCT-3′ | 5′-$(CT)_2$-3′ |
| | | | | 3 | 5′-CAACAA-3′ | 5′-$(CAA)_2$-3′ |
| | | | | 3 | 5′-TAATAA-3′ | 5′-$(TAA)_2$-3′ |
| | | | | 3 | 5′-GAAGAA-3′ | 5-$(GAA)_2$-3′ |
| Quail | *GUJ049* | AB035859 | AY862544 | 1 | 5′-AAAAAAA-3′ | 5′-$(A)_7$-3′ |
| | | | | 1 | 5′-GGGGGG-3′ | 5′-$(G)_6$-3′ |
| | | | | 1 | 5′-TTTTTT-3′ | 5′-$(T)_6$-3′ |
| | | | | 1 | 5′-CCCC-3′ | 5′-$(C)_4$-3′ |
| | | | AY862545 | 1 | 5′-TTTTT-3′ | 5′-$(T)_5$-3′ |
| | | | | 1 | 5′-CCCC-3′ | 5′-$(C)_4$-3′ |
| | | | | 2 | 5′-TGTGTG-3′ | 5′-$(TG)_3$-3′ |
| | | | | 2 | 5′-CTCTCT-3′ | 5′-$(CT)_3$-3′ |
| | | | | 2 | 5′-AGAGAG-3′ | 5′-$(AG)_3$-3′ |
| | | | | 3 | 5′-CTGCTG-3′ | 5′-$(CTG)_2$-3′ |

[1] GenBank Accession Number for nucleotide sequences from which primers were designed. [2] Guinea Fowl, [3] Sequences, [4] GenBank Accession numbers of guinea fowl nucleotide sequences derived from PCR amplification of genomic DNA using chicken and quail specific primers. [5] Length of repeats in base pairs.

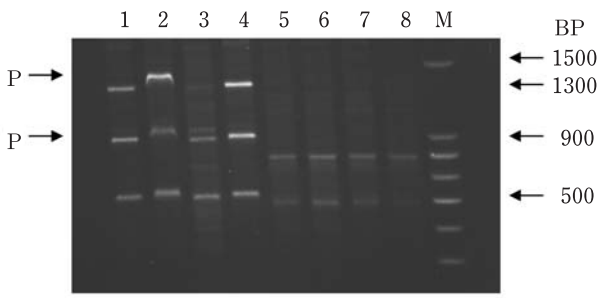


Fig. 1. **Comparison of guinea fowl and chicken DNA profiles derived from the amplification of genomic DNA using a quail specific primer *GUJ049*.** PCR products were separated on a 1.2% agarose gel. The DNA amplification profiles of guinea fowls and chickens are presented in lanes 1–4 and lanes 5–8, respectively. Lanes 2 and 3 revealed polymorphic loci denoted as P. The size of these polymorphic loci was approximately 1,300 and 900 base pairs. Lane M contains 1 kb DNA ladder molecular size markers (Invitrogen, Carlsbad, CA, USA). BP depicts size of DNA fragments in base pairs.

PCR amplification in quails amplified microsatellite loci sequence, homology of these markers with chicken sequences was detected in only six of them.

Table 3 illustrates simple sequence motifs derived from amplification of guinea fowl genomic DNA using the chicken and quail specific primers. These simple sequence motifs derived from amplification of guinea fowl genomic DNA using chicken specific primers consisted of repeat units ranging from 1 (homopolymeric tract) to 4 nucleotides in length. On the other hand, amplification of guinea fowl genomic DNA with quail specific primers yielded repeat units ranging from 1 to 3 nucleotides in length. The chicken and quail specific primers G01598 and AB035859 revealed simple sequence motifs consisting of $(GT)_{12}$ and $(CA)_{11}$ in chicken and quail, respectively. However, the respective loci in guinea fowl that were revealed using the chicken specific primer G01598 consisted shorter dinucleotide repeats such as $(GT)_2$ and much longer homopolymeric tracts such as $(T)_9$ and $(G)_9$ (GenBank Accession Number AY862546). It was also observed in this study that of the guinea fowl sequences reported in this study also exhibited homology with chicken sequences.

In summary, several chicken and quail-specific primers successfully amplified polymorphic loci in guinea fowl. However, the homology between these guinea fowl sequences derived from the amplification of genomic DNA and existing sequences of chicken and quail in the GenBank databases was low. These findings suggest that although chicken and quail microsatellite markers amplified guinea fowl loci, they may not sufficiently serve

as markers for the guinea fowl. However, these markers revealed additional loci in guinea fowl, some of which were polymorphic. These new loci could be a valuable resource for mapping the avian genome.

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## References

Cheng HH, Levin I, Vallejo RL, Khatib H, Dodgson JB, Crittenden LB and Hillel J. Development of a genetic map of the chicken with markers of high utility. Poultry Science, 74: 1855–1874. 1995.

Crooijmans RPMA, Groen AF, van Kampen AJ, van der Beek AS, van der Poel JJ and Groenen MAM. Microsatellite polymorphisms in commercial broiler and layer lines estimated using pooled blood samples. Poultry Science, 75: 904–909. 1996.

Georges M and Anderson L. Livestock genomics comes of age. Genome Research, 6: 907–921. 1996.

Groenen MA, Cheng HH, Bumstead N, Benkel BF, Briles WE, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, de Leon AP, Soller M, Takahashi H and Vignal A. A consensus linkage map of the chicken genome. Genome Research, 10: 137–147. 2000.

Gupta PK, Balyan HS, Sharma PC and Ramesh B. Microsatellites in plants: a new class of molecular markers. Current Science, 70: 45–54. 1996.

Inoue-Murayama M, Kayang BB, Kimura K, Ide H, Nomura A, Takahashi H, Nagamine Y, Takeda T, Hanada H, Tatsuda K, Tsudzuki M, Matsuda Y, Mizutani M, Murayama Y and Ito S. Chicken microsatellite primers are not efficient markers for Japanese quail. Animal Genetics, 32: 7–11. 2001.

Inoue-Murayama M, Nomura A, Ide H, Kimura K, Tatsuda K, Takahashi H, Nagamine Y, Hanada H, Takeda T, Tsudzuki M and Ito S. Application of chicken microsatellite markers to Japanese quail and Chinese painted quail. Animal Genetics, 29 (Suppl. 1): 46. 1998.

Kayang BB, Inoue-Murayama M, Nomura A, Kimura K, Takahashi H, Mizutani M and Ito S. Fifty microsatellite markers for Japanese quail. The Journal of Heredity, 91: 502–505. 2000.

Kayang BB, Inoue-Murayama M, Hoshi T, Matsuo K, Takahashi H, Minezawa M, Mizutani M and Ito S. Microsatellite loci in Japanese quail and cross-species amplification in chicken and guinea fowl. Genetics Selection Evolution, 34: 233–253. 2002.

Kayang BB, Inoue-Murayama M, Takahashi H, Minezawa M, Tsudzuki M, Mizutani M and Ito S. Twenty-eight new microsatellite loci in chicken and their cross-species amplification in Japanese quail and helmeted guinea fowl. Animal Science Journal, 74: 255–259. 2003.

Levin I, Cheng HH, Baxter-Jones C and Hillel J. Turkey microsatellite DNA loci amplified by chicken-specific primers. Animal Genetics, 26: 107–110. 1995.

Liu Z, Crooijmans RPMA, van der Poel JJ and Groenen MAM. Use of chicken microsatellite markers in turkey: a pessimistic view. Animal Genetics, 27: 191–193. 1996.

MacHugh DE, Shriver MD, Loftus RT, Cunningham P and Bradley DG. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and Zebu cattle (*Bos taurus* and *Bos indicus*). Genetics, 146: 1071–1086. 1997.

Nova Scotia Department of Agriculture and marketing. Guinea Fowl. http: //www.gov.ns.ca/nsaf/elibrary/archive/lives/feather/guineas1.htm. 1997.

Pang SWY, Ritland C, Carlson JE and Cheng KM. Japanese quail microsatellite loci amplified with chicken-specific primers. Animal Genetics, 30: 195–199. 1999.

Primmer CR, Moller AP and Ellegren H. A wide-range survey of cross-species microsatellite amplification in birds. Molecular Ecology, 5: 365–378. 1996.

Reed KM, Mendoza KM and Beattie CW. Utility of chicken-specific microsatellite primers for mapping the turkey genome. Animal Biotechnology, 10: 137–141. 1999.

Reed KM, Mendoza KM and Beattie CW. Comparative analysis of microsatellite loci in chicken and turkey. Genome, 43: 796–802. 2000.

Richardson DS, Jury FL, Dawson DA, Salgueiro P, Komdeur J and Burke T. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in sylviidae species and their cross-species amplification in other passerine birds. Molecular Ecology, 9: 2226–2231. 2000.

Smith E, Shi L, Drummond P, Rodriguez L, Hamilton R, Powell E, Nahashon S, Ramlal S, Smith G and Foster J. Development and characterization of expressed sequence tags for the turkey (*Meleagris gallopavo*) genome and comparative sequence analysis with other birds. Animal Genetics, 31: 62–67. 2000.

Smith EJ, Jones CP, Bartlett J and Nestor KE. Use of randomly amplified polymorphic DNA markers for the genetic analysis of relatedness and diversity in chickens and turkeys. Poultry Science, 75: 579–584. 1996.

Stallings RL, Ford AF, Nelson D, Torney DC, Hilderbrand CE and Moyzis RK. Evolution and distribution of (GT)n repetitive sequence in mammalian genomes. Genomics, 10: 807–815. 1991.

Tautz D and Renz M. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research, 12: 4127–4138. 1984.

Tautz D. Hypervariability of simple sequences as a general source for polymorphic markers. Nucleic Acids Research, 17: 6463–6471. 1989.

Takahashi H, Nirasawa K, Nagamine Y, Tsudzuki M and Yamamoto Y. Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphisms. The Journal of Heredity, 89: 543–546. 1998.

Vanhala T, Tuiskula-Haavisto M, Elo K, Vilkki J and Makitanila A. Evaluation of genetic variability and genetic distance between eight chicken lines using microsatellite markers. Poultry Science, 77: 783–790. 1998.

Zhang X, Leung FC, Chan DKO, Chen Y and Wu C. Comparative analysis of allozyme, random amplified polymorphic DNA, and microsatellite polymorphism on Chinese native chickens. Poultry Science, 81: 1093–1098. 2002.