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Genetic Diversity of Myanmar and Indonesia Native Chickens Together with Two Jungle Fowl Species by Using 102 Indels Polymorphisms

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ABSTRACT: The efficiency of insertion and/or deletion (indels) polymorphisms as genetic markers was evaluated by genotyping 102 indels loci in native chicken populations from Myanmar and Indonesia as well as Red jungle fowls and Green jungle fowls from Java Island. Out of the 102 indel markers, 97 were polymorphic. The average observed and expected heterozygosities were 0.206 to 0.268 and 0.229 to 0.284 in native chicken populations and 0.003 to 0.101 and 0.012 to 0.078 in jungle fowl populations. The coefficients of genetic differentiation (*Gst*) of the native chicken populations from Myanmar and Indonesia were 0.041 and 0.098 respectively. The genetic variability is higher among native chicken populations than jungle fowl populations. The high *Gst* value was found between native chicken populations and jungle fowl populations. Neighbor-joining tree using genetic distance revealed that the native chickens from two countries were genetically close to each other and remote from Red and Green jungle fowls of Java Island. (**Key Words:** Genetic Markers, Indels Polymorphism, Jungle Fowl, Native Chicken)

INTRODUCTION

Nowadays, it is increasingly recognized that insertion and deletion (indels) polymorphisms are an important source of genetic as well as phenotypic diversity (Brandström and Ellegren, 2007). Indels can be genotyped with simple procedures based on size separation as compared to complex and heterogenous mutation patterns of microsatellites and without the necessity of applying special equipment for high-throughput genotyping that is relatively costly for small or medium scale studies using microsatellite and SNP markers (Väli et al., 2008). Moreover, indels markers have many genetic advantages for analytical use: they are widely spread throughout the genome, all of the polymorphisms derive from a single mutation event and they have reduced mutation rates (Natalle et al., 2010). It was reported that the draft sequence of the chicken genome was composed of approximately 1.2 Gb which included 447,388 indels polymorphisms (2011, November) in the National Center for Biotechnology Information (NCBI) Entrez SNP database (www.ncbi.nih.gov/sites/entrez?db=snp). This clearly indicated that indels could form a very common class of polymorphism over the chicken genome and might be important genetic markers. The present study focused on indels polymorphisms as genetic markers for studying genetic diversity in chicken population.

Chickens are good models for studying the genetic basis of phenotypic traits because of the extensive diversity among domestic chickens (Wong et al., 2004). There are more than 18 million stocks of chickens being raised in the world (FAO Statistics, 2009). Native chickens, as the most adaptable and geographically widespread livestock species, form an integral part of the Myanmar and Indonesian ecosystem. Native chickens possess unique adaptive traits that permit them to survive and reproduce under harsh climatic, nutritional and management conditions typically associated with low input-output production systems (Mwacharo et al., 2006).

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Myanmar is the largest country on main land South East Asia with a total land area of 676,577 sq Km. Myanmar possesses tropical and sub tropical climates and rich with diverse species of animal and wild relatives of livestock. Latest estimates indicate that there are 105 million domesticated birds raised in Myanmar, out of which 94 million are chicken, mostly 78.7 million birds (84%) kept under backyard production systems (Burgos et al., 2009).

The land area of Indonesia covers 1,919,445 sq Km, spreading from Aceh Province in the north west of Sumatra to the Western part of Papua. It is a large tropical island in South East Asia, which shelters a large number and variety of wild as well as domesticated animals. According to livestock statistic from 2007, Indonesia has an estimated standing population of 620 million chickens which include 317 million native/village chicken (51%) kept by rural farmers (Ministry of Agriculture -2007 cited by Bambang et al., 2009).

Although these native chickens are not available for commercial use, they are raised as dual- purpose for meat and eggs, providing cash income in times of need. It is interesting to examine the genetic variability among native chicken from two south East Asia countries using molecular markers. Therefore, the present study was conducted to evaluate the indels polymorphisms as genetic markers to characterize the genetic variability of the native chicken populations from Myanmar and Indonesia and to determine the genetic relationship among Myanmar and Indonesia native chicken populations and two jungle fowl species.

MATERIALS AND METHODS

In the present study, we used 11 native chicken populations and two jungle fowl species. In Myanmar native chickens, we analyzed three populations from Yangon (Yan) and Pegu (Peg) of lower Myanmar and from Mandalay (Man) of central Myanmar. In Indonesian native chicken, we analyzed four populations of local chicken and four populations of local varieties: The local chicken populations were collected from Semarang (Sem), Kendal (Ken), Yogyakarta region (Yog) of the central Java, and Karawang region (Kar) of west Java. The populations of local varieties were Ayam Kedu (AK) from Temanggun (AK-Tem) and Solo (AK-Sol) of the central Java, Black Kedu (BK) from Semarang (BK-Sem) and Ayam Alab (AA) from Temanggun (AA-Tem). Two jungle fowls species from Java, Red jungle fowl (*Gallus gallus bankiva*, RJF) and Green jungle fowl (*Gallus varius*, GJF) were also examined. Blood samples were obtained from Yan (n = 27), Peg (n = 13), Man (n = 40), Sem (n = 24), Ken (n = 40), Yog (n = 57), Kar (n = 15), AK-Tem (n = 10), AK-Sol (n = 19), BK-Sem (n = 23), AA-Tem (n = 20), RJF (n = 3), GJF (n = 6) respectively.

Genomic DNA was extracted from blood samples using standard phenol-chloroform extraction protocols (Sambrook, 1989). Suitable indels markers were selected by using the NCBI Entrez SNP database. The primer sequences used in this study were listed in Table 1.

In primer design, indels sequences of 20 to 30 bp along the every 10 Mb of chromosome length were selected. The PCR amplifications were performed in a 10 μl reaction volume, which included 6.15 μl of distilled water, 1 μl of 10× reaction buffer, 0.8 μl of dNTP (2.5 mM), 0.25 units of Ex Taq™ (Takara Bio Inc, Otsu, Japan), 10 ng of template DNA, 25 μM each of forward and reverse primers. The PCR reaction was performed with an initial denaturation for 2 min at 94°C followed by 28 to 32 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 48 to 65°C and extension for 30 s at 72°C and final extension for 7 min at 72°C in Takara PCR thermal cycler - TP650 (Takara Bio Inc). The PCR products and a 100 bp DNA ladder (Sib Enzyme Ltd, Russia) were electrophoresed on 1 to 2% agarose gel electrophoresis in 1×TBE. The indel polymorphisms were identified through a Toyobo FAS III UV-Transilluminator (Toyobo, Osaka, Japan) after ethidium bromide staining for 15 to 30 min. Genotypes of indel polymorphisms were determined by size difference between the PCR fragments.

Statistical analysis

We calculated allele frequencies from genotyping data by direct counting. The proportion of polymorphic loci (*Ppoly*) was calculated as the ratio of polymorphic loci to the total number of loci analyzed. Chi square (χ^2) approximation was used to test Hardy-Weinberg Equilibrium (HWE) at each locus (Weir, 1996). To estimate the genetic variability, we calculated the average observed heterozygosity ($\overline{H}_O$), the average expected heterozygosity ($\overline{H}_E$) of each population and degree of genetic differentiation (*Gst*) (Nei, 1973). $\overline{H}_E$ was estimated by Nei's formula (Nei, 1978). To assess the genetic relationships between 13 populations, pair wise standard genetic distance (Ds) (Nei, 1972) was computed using PHYLIP ver. 3.69 (Felsenstein, 2009). From the data of the genetic distance matrix, we constructed phylogenetic tree by using neighbor- joining (NJ) method (Saitou and Nei, 1987) implemented by MEGA software ver.4.1 (Tamura et al., 2007).

RESULTS

Indels polymorphisms

The mean (±SE) *Ppoly* value of each population is listed in Table 2. Out of 102 indels markers, 97 markers (*Ppoly* =

95%) were polymorphic in native chicken populations, all of which were in HWE (Table 1). The *Ppoly* was higher in Indonesian native chicken populations (*Ppoly* = 94%) than Myanmar native chicken populations (*Ppoly* = 92%) (Table 2). One marker (m 66) was monomorphic only in Indonesian native chicken populations whereas three markers (m 11, m 33 and m 92) were monomorphic only in Myanmar native chicken populations.

Table 1. Summary of primer sequences for 102 indels loci

Loci	Chr: Reference NCBI	Chromosomal position	Forward primer (5'-3')	Reverse primer (3'-5')	Size range (bp)	TA (°C)	HWE (x^2 value)
m 1	1: rs15186483	2380713	agctattcagggaggggatg	ggatgcctgtttctggaaga	287-311	60	0.012
m 2	1: rs14796695	11865023	caggtagcttggggaatcag	ttcttgtctgggccaaatgt	222-252	60	0.002
m 3	1: rs14803637	29610732	gggaaagataaaatggcttga	cagatccacacttcagggaaa	263-292	60	0.028
m 4	1: rs15224689	32491050	aacccatgggggtcttgtat	tagggaacagatggggaaaa	300-328	60	0.004
m 5	1: rs1524406	41678486	agcatcagcaaggtgctctt	agacaaagcttccctggaca	272-299	60	0.023
m 6	1: rs15272331	56388055	gccccttcaagcatgatatt	ctcctttcagctgcagatttc	297-321	60	0.017
m 7	1: rs13879446	67455476	agaatgccctttggattcct	tcagcgggttatacagagca	265-295	60	0.113
m 8	1: rs14844703	73142545	aggcttacggttggagacaa	aactgcaactccctccagtg	241-271	60	0.005
m 9	1: rs13893049	86184937	gagagctgggttctccagtg	ttctttttgctggctgcttt	223-252	60	1×10^{-5}
m 10	1: rs15340628	92114321	tgttttgccagcatgacatt	gctgtgggagaaacaacaca	252-289	60	3×10^{-4}
m 11	1: rs15354680	100316232	tgctcacagcaaacctaacg	acgcaggctagaggacttca	265-288	60	0.353
m 12	1: rs15381205	112800871	tacagctcaggccacatgaa	gggtccaaaagaaagacatca	270-297	60	0.001
m 13	1: rs15402998	123160898	ataacgcaaagggagggatg	gtttgcctgtggtgttgtga	221-255	60	0.013
m 14	1: rs14888652	135638781	atgggctggtgaactgtagc	ccatctggccttagcaaatc	349-374	60	0.001
m 15	1: rs15443072	143204968	gggaaactctgcattgctgt	ataatgctgccactcctgct	298-329	60	1×10^{-5}
m 16	1: rs15477016	159215305	gggatgagcttcaaagtgga	acccaacaaggacgtgtca	268-296	60	0.051
m 17	1: rs15467491	162349692	cctccttgagctctgacacc	tcctgtgggtcttttccaac	174-203	60	2.6×10^{-4}
m 18	1: rs13968127	170697123	aacagaagcctcctgccttt	aggatggtttggtttgcttg	276-304	60	0.183
m 19	1: rs13986305	186019671	aaaaagaagtgctgtctgaagaaaa	gaaaacgcgaaatgaatggt	257-287	60	0.266
m 20	1: rs15550068	196892451	cagaccctacaagccacaca	ttctcagaataattttgccagttg	301-324	60	0.001
m 21	1: rs15554369	200599560	tgacttccccattcattggt	gcactttcacagcctcccta	300-326	60	0.010
m 22	2: rs15879191	7098042	attttgcttgacgtgcagtg	ctgcagcctttgggagtatc	258-291	65	1.3×10^{-4}
m 23	2: rs15891921	11463166	tccctttttcaggctgtgatt	ctctaggccaagcatctggt	251-276	60	5.7×10^{-5}
m 24	2: rs14151659	22996352	agggatatttgggacgaagg	gccttttcacacatccaggt	298-329	60	0.055
m 25	2: rs15953427	37759827	tctatcaggccttgcacctt	ttacttgagggtgcccaatc	254-282	60	0.011
m 26	2: rs15971881	44143530	tctctgcactctccaaagca	tgtgagcaccgagcaataag	254-293	65	0.162
m 27	2: rs15102430	57732225	gtagaaggccaaccaaccaa	caaccaaagagggaacatgc	253-279	65	n/a
m 28	2: rs16011387	61748980	tgcaagctcttgttcattgc	ccgctttgctacatcctctc	261-291	65	0.019
m 29	2: rs16040761	78554543	tcagaaggatcgcatgaaga	tcattttgagaaaagacaggaactt	235-262	55	0.106
m 30	2: rs16054197	87816451	aaccacattctggggatgaa	aaccacctcccgtaacagtg	272-296	60	0.005
m 31	2: rs15132229	99174129	accagcccttaaaccgttg	aatctcaaagcccacccagt	276-299	60	0.069
m 32	2: rs16088091	106532884	gcacagctatgcccaaaataa	ctctgcctctggtggagact	276-299	60	0.005
m 33	2: rs14235853	116697385	catgcctgttgcttttacca	ccttgttgaggcatgtcagt	257-277	65	0.001
m 34	2: rs15147812	123445602	cccctccaacctaagtcattc	gtgcattccccatcatttct	285-314	60	0.002
m 35	2: rs14250138	131679775	ccagccctgtaggagagagtt	agctgcaggacatgaggtct	229-255	60	0.001
m 36	2: rs15165839	145558702	aggcttgaggatacgcttca	cagcccaacaggttaccataa	265-295	65	0.003
m 37	2: rs16144735	152495037	ctgccacagttcaagaagca	ttccaagtaggcgaataccg	247-272	55	0.031
m 38	3: rs15267007	9421750	caaaatgtgcgacttttcct	gaaactggccgtggtacaat	272-300	60	0.116
m 39	3: rs16229905	18459587	tgacagaatcaggggaaaatg	gccttctatcaaagccagca	274-293	55	0.064
m 40	3: rs15299757	26141512	aagaaggagctaccgcactg	actgcctggcaagtgaagat	236-261	55	n/a
m 41	3: rs16248030	32579565	ggtttccagcaacaggagaa	ggatgagccaaaattggaga	261-286	55	0.008
m 42	3: rs15336669	44685823	ccaggttgtgtacgcagaga	tttgccttccacgtttgtct	236-265	60	0.001
m 43	3: rs15364542	58211486	gccatttctgccactgtctt	ggaaatggatcctgcaaaaa	337-360	60	n/a

Table 1. Summary of primer sequences for 102 indels loci (Continued)

Loci	Chr: Reference NCBI	Chromosomal position	Forward primer (5'-3')	Reverse primer (3'-5')	Size range (bp)	TA (°C)	HWE (x^2 value)
m 44	3: rs15372369	65825652	gctttaaaagaagccgagca	ccagaattcccaatttttcaa	297-326	60	0.069
m 45	3: rs15380325	70288536	aggatccttggtcaatgtgg	cagttcaggcagatccatca	273-297	60	0.005
m 46	3: rs15409517	84493460	tccttctaagatgcggcaat	cagtttggggttgggtaaaa	250-281	60	0.004
m 47	3: rs15427052	94441494	tgacaacgcatcacagcata	gcttccgtattacccagcag	260-289	60	1.5×10^{-5}
m 48	3: rs16338959	108511888	atattgggaactcgctgtgg	atgtcacacaaagcctgctg	250-276	60	0.009
m 49	3: rs14413503	111873254	acatggcacttgatgaagca	aattggcttttggacacctg	263-284	60	0.023
m 50	4: rs16359295	9542599	tataaatggggtgggtgtgg	caccaaaagcagaaatgcaa	281-305	60	0.007
m 51	4: rs14432370	14488517	atttttgatctgggcacgaa	gaggcaggaggtggaagag	227-252	60	0.001
m 52	4: rs14438548	24411304	aggcagacagatgtggaaga	caggaatacaaagccgcagt	253-282	60	0.009
m 53	4: rs16384349	32708827	gcaaccctgaagaaaaccaa	gcaaagcagagtttggaacc	258-287	60	0.004
m 54	4: rs16399900	46455122	ttgcagcaaaagggaagatt	tggaggaatgcagctgacta	280-306	65	0.001
m 55	4: rs16414997	56511101	tctgtgcagattcggtatgg	gtcactgcccttcagcaaat	243-272	60	0.009
m 56	4: rs16429149	68586191	catcagcttccctttgtga	tctcattgctcattgtacagctc	222-250	60	0.116
m 57	4: rs16431940	70693757	ttagcttcccccaaacactg	aacagcggctcattcattct	289-314	60	0.001
m 58	4: rs16449188	89179812	agcatctcagccttccttca	ctgggctcatacccatgtct	260-289	60	4.1×10^{-4}
m 59	4: rs15642550	90357511	gctcatgcatggaatttgtg	tcctgtgctctccatctatgc	264-290	60	0.017
m 60	5: rs14508864	1531872	tgtcacaaatgcaggaggtg	cagacctcaaagcatcacaca	281-311	60	0.001
m 61	5: rs16463699	10884770	aggctccaagtgctgtgatt	ccaaaataaagtcccgacaa	242-276	60	0.001
m 62	5: rs16478463	26549515	ccttgcatctcctccttcag	ggagggaaagggtcaatgat	238-266	60	0.012
m 63	5: rs14529374	31922437	tggtcatgatggtttggaga	gtgcaggacattttgcttga	268-297	60	0.001
m 64	5: rs14542642	48345965	tcctaatgtcggtcatgctg	caagtctgtggccaggaagt	270-299	60	0.006
m 65	5: rs16508335	51981730	ggcagaggagagcagaaatg	tgctgtttgtccgaagtttg	261-291	60	0.017
m 66	5: rs15745605	60173479	ccacccgagtcctaagtctg	tcttcatggggaaggaagtg	242-271	60	2.5×10^{-7}
m 67	6: rs14570404	9359709	gctgttcacttggtcttgc	cgaggactgaaggaaatgaca	326-349	60	0.023
m 68	6: rs14580218	19952173	gctctgcttccctcctttct	ttgtgatccacacctgcatt	279-305	60	0.001
m 69	6: rs15811157	29943028	ttttgttaaccaggggcaat	gtagcatctgcagcccaaat	334-363	60	0.004
m 70	6: rs15823004	36962704	tgcctcagtctggtctgttg	tgcatgagggttcagaagtg	279-308	60	0.003
m 71	7: rs14604441	7009917	agcatcacaccaactgcaag	cattcctccagagcttcctc	294-314	60	0.006
m 72	7: rs16591682	18682408	gctgctataagctgcccatc	ggcaagcaggaaatgaagag	284-306	60	1.5×10^{-4}
m 73	7: rs14622212	29604888	ttaaagccagcacacaatgc	catccagcagtcagcctttt	331-360	60	3.8×10^{-4}
m 74	7: rs16615778	37651290	gaggatatgggcaagtctgg	tccccttgtcctgctgttat	324-350	60	0.026
m 75	8: rs15908922	9253123	ttttcatgggtagttcattagaga	atgctgccttccataactgc	307-327	60	0.004
m 76	8: rs16636129	19782818	gcgtcagagtgtgaaatgct	agcacgctgttcctctgaat	302-324	60	0.128
m 77	8: rs16649376	29327443	catttggggcagcagtattc	cacctcccaaacttgcatct	339-363	60	0.0159
m 78	9: rs16664917	4736922	tcctcctggaacttcctcct	ttcagtttgccttggtcctc	320-345	60	0.0123
m 79	9: rs14683671	24883034	gtcgcagcttcagaaaggac	ctgtacacaaacggcgatgt	286-311	60	0.148
m 80	9: rs14672349	13452188	attgaaagcgaccattccag	gccttctgaaacctaccaagtt	277-302	60	0.001
m 81	10: rs15572293	8045518	tgcacaaaactaatcttgctgtct	ggtgttgtcaatcctgtttgc	309-330	60	0.021
m 82	10: rs14012832	20119574	ggccaggatctcaaaacaga	tccccttgtatgtcctctgc	319-346	60	0.003
m 83	11: rs15611781	9281820	ctgcctccaggcctttctat	gcacaagaatcaccagcaag	319-348	60	0.001
m 84	11: rs14018578	1883678	ccagggctatggaatgctta	cactggctgactgcagatgt	319-347	60	1.3×10^{-5}
m 85	11: rs14693330	20791178	tttgagcaccaccgtgagta	cccagctcaagagtcgaaac	290-315	60	0.045
m 86	12: rs15648972	9133627	cggtcctcatgttgtcaaag	gacaatgcacagctgcataaa	305-327	62	0.001
m 87	12: rs15672210	19136567	aagggcagagaactgttcca	tgggttggagggtatcttca	334-364	60	0.024
m 88	13: rs15695194	9834883	ggtgggtaatccagtctctcc	cttcaggctcaacaggaacc	293-326	60	0.002
m 89	13: rs15706498	15621848	agcgcacacatttgcattag	aggctgaggaaggttgtcct	299-325	60	0.031
m 90	14: rs14077825	9856807	tgtttggcatacctgtgcat	taggaagaaagggctctgct	315-336	60	0.213

Table 1. Summary of primer sequences for 102 indels loci (Continued)

Loci	Chr: Reference NCBI	Chromosomal Position	Forward Primer (5'-3')	Reverse Primer (3'-5')	Size range (bp)	TA (°C)	HWE (x^2 value)
m 91	14: rs15740439	11675614	aggcatgccagaacattcat	ggtcttttccagcctgagtg	278-301	60	0.215
m 92	15: rs14094135	9649390	tagttcccagtggtgtgtgg	agggtgtctcttcagcctca	310-334	60	0.001
m 93	15: rs15783434	10844586	taattgattcagcgcagagc	ccagccagcttcattgagat	291-315	60	0.005
m 94	16: rs15026709	133330	actcattgggaatggactcg	cacgtccctctccatgtttt	331-346	60	0.005
m 95	17: rs15790503	9512181	ctcagcccttgctttctttg	ttggattctccctcatttgc	282-311	48	n/a
m 96	17: rs15027282	10043385	ccacaacgactcggttaagaa	gtcattgctgggaacctcat	287-315	60	0.203
m 97	18: rs15818344	3551368	ttcagttttggtgtcgctca	ttctctgagcctgccagaat	300-323	60	0.029
m 98	19: rs14121581	6252496	cgccacacataaatcagtcg	ccttgttgctacctggctgt	349-376	60	n/a
m 99	20: rs14277689	9478707	cgaggatgacctgtggtgta	tcctgaaagcttttgtgtgc	326-355	65	0.004
m 100	20: rs16174629	11941342	accatgggctgttctttgaa	ggcaggttgtgaaggatagc	323-347	65	0.004
m 101	21: rs16179814	3159840	acaaccgctcgacagaaagt	agttgacctcccctggaaat	284-310	65	5.8×10^{-5}
m 102	22: rs16183765	3842053	tcagggacatcccagaagac	gcaccagaaatgctctctcc	339-368	65	0.046

HWE-Hardy-Weinberg Equilibrium Test (n/a = Not applicable; the rest of the markers are non significant at $p<0.05$).

Table 2. The genetic variability from 13 populations of native chickens and two jungle fowl species

Population	No. of samples	P_{Poly}	±SE	$\overline{H}_O$	±SE	$\overline{H}_E$	±SE
Yan	27	0.901	0.034	0.216	0.004	0.266	0.004
Peg	13	0.713	0.045	0.218	0.005	0.239	0.004
Man	40	0.871	0.034	0.229	0.005	0.263	0.005
Sem	24	0.861	0.035	0.258	0.004	0.269	0.004
Ken	40	0.901	0.029	0.242	0.004	0.276	0.004
Yog	57	0.901	0.029	0.268	0.005	0.284	0.004
Kar	15	0.792	0.041	0.231	0.004	0.263	0.004
AK-Tem	10	0.772	0.041	0.239	0.005	0.255	0.004
AK-Sol	19	0.881	0.032	0.250	0.004	0.278	0.004
BK-Sem	23	0.743	0.044	0.207	0.004	0.242	0.004
AA-Tem	20	0.812	0.039	0.206	0.004	0.229	0.004
RJF	3	0.218	0.041	0.101	0.005	0.078	0.004
GJF	6	0.039	0.019	0.003	0.002	0.012	0.003

Yan = Yangon; Peg = Pegu; Man = Mandalay; Sem = Semarang; Ken = Kendal; Yog = Yogyakarta; Kar = Karawang; AK-Tem = Ayam Kedu from Temmanggun; AK-Sol = Ayam Kedu from Solo; BK-Sem = Black Kedu from Semarang; AA-Tem = Ayam Alab from Temmanggun; RJF = Red jungle fowl; GJF = Green jungle fowl.

In two jungle fowl species, RJF showed more polymorphic loci (22 markers, *Ppoly* = 21%) than GJF (4 markers, *Ppoly* = 3.9%) (Table 2).

Genetic variability

The $\overline{H}_O$ and $\overline{H}_E$ values ranged from 0.206 to 0.268 and from 0.229 to 0.284 in 11 native chicken populations (Table 2). In the jungle fowls, RJF showed higher $\overline{H}_O$ and $\overline{H}_E$ values (0.101 and 0.078) than GJF (0.003 and 0.012). Among the Myanmar and Indonesian native chickens, the *Gst* value was 0.041 for Myanmar and 0.098 for Indonesia (Table 3). The two jungle fowl species showed the highest genetic differentiation between them with *Gst* value of 0.436. The *Gst* observed between two jungle fowl species and Myanmar native chicken (0.213 to RJF and 0.264 to GJF) was higher than the *Gst* between two jungle fowl species and Indonesian native chicken (0.162 to RJF and 0.186 to GJF). The *Gst* among 13 populations was

Table 3. Coefficient of genetic differentiation (*Gst*) in various subsets of 13 populations of native chickens and two jungle fowl species estimated from 102 indels loci

Subset	*Gst*
Among Myanmar native chickens	0.041
Among Indonesia native chickens	0.098
Among Myanmar and Indonesia native chickens	0.119
Between 2 jungle fowl species	0.436
Between Myanmar native chicken and RJF	0.213
Between Myanmar native chicken and GJF	0.264
Between Indonesia native chicken and RJF	0.162
Between Indonesia native chicken and GJF	0.186
Among 13 subpopulations	0.227

RJF = Red jungle fowl; GJF = Green jungle fowl.

Table 4. Pair wise genetic distance matrix between 13 populations of native chickens and two jungle fowl species

	Yan	Peg	Man	Sem	Ken	Yog	Kar	AK-Tem	AK-Sol	BK-Sem	AA-Tem	RJF	GJF
Yan		0.078	0.034	0.082	0.063	0.063	0.099	0.087	0.072	0.139	0.102	0.405	0.367
Peg			0.062	0.156	0.133	0.136	0.146	0.165	0.143	0.200	0.150	0.342	0.333
Man				0.093	0.065	0.067	0.110	0.088	0.075	0.174	0.118	0.337	0.347
Sem					0.040	0.049	0.088	0.048	0.054	0.129	0.057	0.326	0.344
Ken						0.012	0.046	0.017	0.009	0.092	0.086	0.288	0.289
Yog							0.055	0.026	0.019	0.103	0.089	0.287	0.294
Kar								0.076	0.049	0.093	0.124	0.282	0.247
AK-Tem									0.023	0.114	0.093	0.308	0.337
AK-Sol										0.100	0.099	0.318	0.335
BK-Sem											0.147	0.304	0.263
AA-Tem												0.342	0.334
RJF													0.251
GJF													

Yan = Yangon; Peg = Pegu; Man = Mandalay; Sem = Semarang; Ken = Kendal; Yog = Yogyakarta; Kar = Karawang; AK-Tem = Ayam Kedu from Temmanggun; AK-Sol = Ayam Kedu from Solo; BK-Semarang = Black Kedu from Semarang; AA-Tem = Ayam Alab from Temmanggun; RJF = Red jungle Fowl; GJF = Green jungle Fowl.

calculated as 0.227.

Genetic distance and phylogenic analysis

The *Ds* distances are shown in Table 4. The *Ds* distances between the native chicken populations ranged from 0.034 to 0.078 within Myanmar, from 0.009 to 0.147 within Indonesia and from 0.063 to 0.200 between the two countries.

The smallest (0.063) was observed between the Ken and Yan population and between the Yan and Yog populations. The largest (0.200) was obtained from between BK-Sem and Peg populations. The genetic distances between native chicken populations and the two jungle fowl species ranged from 0.247 to 0.405.

The NJ tree constructed from the *Ds* distances between the 13 populations gave two major clades as shown in Figure 1. The first clade was composed of Myanmar and Indonesian native chicken populations. The second clade was composed of RJF and GJF, which was located outside the first clade.

DISCUSSION

Indels polymorphisms

The results revealed both of the native chicken populations showed polymorphisms in most of the indels

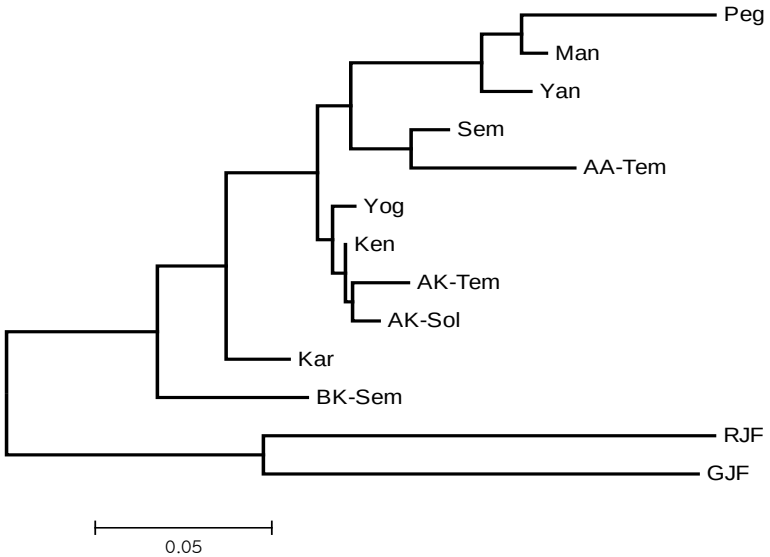

Figure 1. Neighbor-joining (NJ) tree constructed by genetic distance matrix from 13 populations of native chickens and two jungle fowl species. Yan = Yangon; Peg = Pegu; Man = Mandalay; Sem = Semarang; Ken = Kendal; Yog = Yogyakarta; Kar = Karawang; AK-Tem = Ayam Kedu from Temmanggun; AK-Sol = Ayam Kedu from Solo; BK-Sem = Black Kedu from Semarang; AA-Tem = Ayam Alab from Temmanggun; RJF = Red jungle fowl; GJF = Green jungle fowl.

loci (greater than 90%). It is larger than the previous study of Väli et al. (2008) and their study stated that 81 and 76 out of 94 indels markers (86.2% and 80.9%) could be validated as polymorphic loci in dogs (n = 7) and wolves (n = 18). Jungle fowl populations especially in GJF showed much fewer polymorphic indels loci (4%) than native chicken populations. These results suggest that the indels polymorphisms examined here have been acquired in the chickens after the separation of ancestral species of the GJF and chickens.

Genetic variability

In this study, the $\overline{H}_O$ and $\overline{H}_E$ values were 0.206 to 0.268 and 0.229 to 0.284 in native chicken populations and 0.003 to 0.101 and 0.012 to 0.078 in two jungle fowl species respectively. The $\overline{H}_E$ values of the present study were close to those of the gene constitution of blood groups in Myanmar and Indonesian native chickens (0.299 and 0.279) (Yamamoto et al., 2010). The $\overline{H}_E$ values in the present study were higher when compared to the range of egg white protein polymorphisms estimated from native fowl populations in Asia ($\overline{H}_E$ = 0.089 to 0.170, Kinoshita et al., 2004) and blood protein variation in Myanmar native chicken ($\overline{H}_E$ = 0.198, Okamoto et al., 2004). However the $\overline{H}_E$ values of the present study were lower than the heterozygosity of four calpain gene polymorphisms in Myanmar and Indonesian native chickens revealed 0.388 and 0.389 respectively (Okumura et al., 2006). These differences may have arisen from difference in the sample size, sample population and source of genetic markers in each study.

In the present study, the heterozygosities of native chickens were higher than the heterozygosities of ancestral species. This finding was similar to the earlier report of Väli et al. (2008). They reported that the values of $\overline{H}_O$ and $\overline{H}_E$ were 0.268 and 0.355 in dogs and 0.194 and 0.261 in wolf by using indels markers.

The *Gst* values of Myanmar and Indonesian native chicken populations were 0.041 and 0.098, indicating that the degree of genetic differentiation is higher in Indonesian native chicken than Myanmar native chicken. This might be related to the sample population of Indonesian native chicken, which contained local chicken varieties like BK, AA, and AK, and there may be some degree of genetic differentiation between them.

The *Gst* of present study was higher than those of Myanmar and Indonesian native chicken (0.024 and 0.020) estimated in the genetic constitution of four calpain gene polymorphisms (Okumura et al., 2006). However *Gst* of Myanmar native chicken was close to the range of 0.001 to 0.039 (Kinoshita et al., 2004) estimated from egg white protein polymorphism of local populations in Asian countries (Myanmar, Indonesia, China, Nepal, Vietnam and Laos). The *Gst* of Indonesian native chicken was close to blood protein polymorphisms found in four chicken breeds from Yunnan Province of China (*Gst* = 0.075, Okamoto et al., 2003) and Nepal (*Gst* = 0.093, Maeda et al., 1992).

The *Gst* between Myanmar and Indonesian native chickens was 0.119, indicating that the genetic differentiation between them was not large. Therefore, Myanmar and Indonesia native chickens can be regarded as genetically close populations. The *Gs*t values between Myanmar native chicken populations and two jungle fowl species (0.213 to RJF and 0.264 to GJF) are greater than between Indonesian native chicken populations and two jungle fowl species (0.162 to RJF and 0.186 to GJF). It may be due to the fact that the small *Gst* was obtained between populations in a close geographic area. However, highest *Gst* (0.436) was observed between RJF and GJF from Java Island.

The *Gst* value in this study may be higher than the value of other studies. According to the review of Theresa et al. (2002), a wide range of *Gst* values usually results from uneven allele frequency distributions across populations at some loci. In the present study, the major allele was the same in all populations at 87 indels loci. Whereas uneven allele frequency distributions were observed in the remaining 15 loci: the major allele in most populations was minor in some populations, which may contribute to high *Gst* value.

Genetic distance and phylogenic analysis

The low genetic distance observed among native chicken populations (0.009 to 0.200), may be reflecting the fact that genetically these populations are not greatly isolated from each other. In addition, the average genetic distances among native chicken populations (0.088) observed in present study is close to the findings of Yamashita et al. (1994) by DNA fingerprinting analysis among the stock of domestic fowls (0.104). The larger genetic distances were found between native chicken populations and two jungle fowl species from Java Island.

The topology of the NJ tree showed that Myanma**r** native chickens and Indonesian native chickens form a respective cluster in one clade whereas RJF and GJF from Java Island formed another clade, suggesting that native chickens are genetically closely related to each other and remote from jungle fowls of Java Island.

In the previous studies of Yamashita et al. (1994) and Okumura et al. (2006), the GJF was located far away from native chicken populations and it is consistent with the hypothesis that domestication of the chicken may have started from RJF. However, RJF from Java Island comprises a different clade that was far away from native chicken populations, which agreed with the un-rooted neighbor

joining (NJ) population tree of Niu et al. (2002). In their NJ tree, domestic fowls belonged to the same cluster as *G. g. gallus* and *G. g. spadiceus* in Thailand and its adjacent areas, whereas *G. g. bankiva* from Indonesian Islands formed a separate cluster. Furthermore Akishinonomiya et al. (1994) stated that the domestic fowl from Indonesian Island had large genetic differences compared with *G.g.bankiva* from the same region.

The present study examined the genetic characteristics of the Myanmar and Indonesia native chickens and two jungle fowl species from Java Island by using indels polymorphisms as genetic marker. The genetic variability is higher among native chicken populations and lower in the two jungle fowl species. The high genetic differentiation occurred between native chicken populations and two jungle fowl species from Java Island. The native chickens from two countries were genetically close to each other and remote from jungle fowls of Java Island. Although the indels markers showed low heterozygosity compared to microsatellite markers, they can demonstrate close genetic variability and phylogenic topology to other studies cited above. Therefore, indels polymorphisms are efficient for studying genetic diversity of populations.

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