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Genetic Diversity of Green Peafowl (*Pavo muticus*) in Northern Thailand Based on Mitochondrial DNA Control Region Sequences

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ABSTRACT.– The population size of green peafowl (*Pavo muticus* L.) has declined dramatically in Thailand. It is currently found in two geographically isolated regions in the northern and western areas. Here, we assessed the level of genetic variation of this endangered bird species to examine the influence of habitat fragmentation. We collected moulted feather and egg specimens of wild individuals from three populations in the North: Wiang Lor Wildlife Sanctuary (WLO-1 and WLO-2) and Tappaya Lor Non-Hunting Area (TPL). Mitochondrial control region sequences were obtained and analyzed. The results revealed that *P. muticus* in all populations showed a high level of haplotype diversity (0.784, n = 72), implying high genetic diversity. The haplotype diversity in WLO-1 population was higher than in other two populations. On phylogenetic trees and a haplotype network, the haplotypes of *P. muticus* individuals did not form a separate cluster, regardless of their distribution ranges, and three haplotypes were found in the three populations. By combining the present results with the previous data, our genetic investigations revealed that *P. muticus* populations in Thailand exhibited a low genetic difference (4.31%) between the two regional populations with no statistical significance. This suggests that there is no subdivision of wild populations.

KEY WORDS: D-loop, genetic variation, mitochondrial DNA, peafowl, phylogeny

INTRODUCTION

Green peafowl (*Pavo muticus* Linnaeus, 1766) is a well-known galliform bird in the family Phasianidae which is highly threatened in many countries in East and South-east Asia (IUCN, 2014). It is commonly found in tropical forests, particularly in mixed deciduous and dry dipterocarp forests, as well as in the agricultural areas adjacent to forests for foraging (Johnsgard, 1999; BirdLife International, 2015). In Thailand, this species was historically distributed across almost all of Thailand except in the central valley of the Chao Phraya River and the northeastern and eastern provinces (Delacour, 1977). In the last few decades, however, the green peafowl population has undergone a serious decline owing to habitat loss and fragmentation, environmental pollution and human persecution (McGowan et al., 1999; Meckvichai et al., 2007; Pinthong, 2009), resulting in local extinction in some areas; such as in the southern part of Thailand. Therefore, the endangered green peafowl is currently considered as one of the nationally protected species by the Thailand's Office of Natural Resources and Environmental Policy and Planning (ONEP) and is listed under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Nowadays, within Thailand, green peafowl is found only in two geographically isolated regions in the northern and western areas of the country. Within each region, they are distributed in patchy areas particularly close to shallow streams or

rivers with exposed sand bars, for example, along the Ping, Ing, Yom and Nan river basins in the North and in the Huai Kha Khaeng, Huai Tab Salao, Huai Song Tang and Mae Klong basins in the West (Ponsena, 1988; Meckvichai et al., 2007). The population sizes of the western populations are larger than the northern populations, with the largest population being found in Huai Kha Khaeng Wildlife Sanctuary (HKK), with an estimate of 300 birds (McGowan et al., 1999).

Most studies on green peafowl in Thailand have principally focused on their ecology, particularly distribution range and habitat use, and some behaviors (e.g., Ponsena, 1988; Arrathrakorn, 2001; Saridnirun et al., 2016; Sukumal et al., 2017). However, research on genetic variability and structure is relatively rare, particularly in the wild populations, probably because it is not easy to find and catch them in nature. Since habitat fragmentation can increase the chance of genetic drift and inbreeding, and so potentially result in a reduced genetic diversity that could directly influence the survivability and adaptability of the animals (Frankham, 1995). Therefore, knowledge of the current genetic diversity and structure of wild populations is necessary for long-term conservation strategies and management of the species to maintain and increase the population size.

The non-coding control region or displacement-loop (D-loop) region is considered to be the most variable portion of the mitochondrial DNA (mtDNA) genome (Desjardins and Morais, 1990; Randi and Lucchini, 1998). Therefore, it is suitable as a molecular marker for investigating genetic variation and phylogenetic relationships within species. The genetic variability in two natural populations of green peafowl in Huai Hong Khrai Royal Development Study Center (HHK; northern Thailand) and HKK (western Thailand) was previously reported in a preliminary study based on mtDNA control region sequences. The results showed a high level of genetic diversity in the two populations (Sawangtham et al., 2015). However, the sample sizes and number of locations analyzed were both small, and so those results need confirmation and expanding upon by an analysis of a more extensive sample collection to understand the local and overall population genetic diversity within and between wild populations of this endangered bird species in Thailand. Therefore, the present study examined the genetic diversity and structure of green peafowl in the two localities in the North: Wiang Lor Wildlife Sanctuary (WLO) in Phayao Province and Tappaya Lor Non-Hunting Area (TPL) in Chiang Rai Province. These are recently-discovered populations near Ing River in which the information on population genetics of each location is unknown. The results gained from this study are discussed with the previous study in order to better understand the green peafowl's genetic diversity in Thailand.

MATERIALS AND METHODS

Study areas and sampling

Geographically, WLO can be considered as a fragmented area comprised of two geographically isolated fragments, namely WLO-1 and WLO-2, which are at least 15 km apart and separated from each other by roads and human settlements (Fig. 1). However, WLO-1 is adjacent to TPL, only 1.5 km apart and separated from each other by the road and river, whereas WLO-2 is about 40 km from TPL. According to this geography, we therefore considered WLO-1,

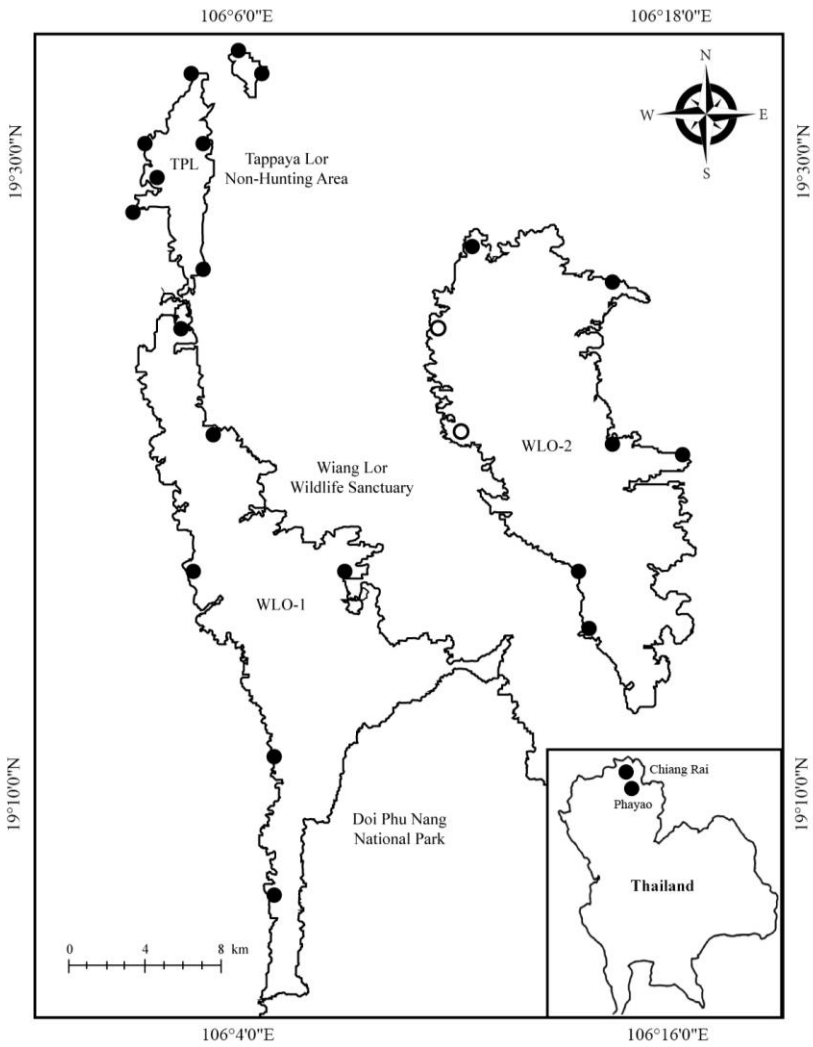

FIGURE 1. Map of Thailand showing the approximate locations of WLO in Phayao Province and TPL in Chiang Rai Province. The enlarged map of WLO-1, WLO-2 and TPL showing the locations of collection sites. Sites with and without feather collection are represented by black and white circles, respectively.

WLO-2 and TPL as different populations isolated by geographic barriers.

Because green peafowl birds are relatively large and very wary birds, it is hard to catch them in nature to take blood or tissue samples. Thus, noninvasive samples such as feathers are excellent specimens for a study of mtDNA diversity in wild populations. Therefore, this study used feathers as a source for DNA extraction. We extensively collected moulted feather specimens of green peafowl from 22 collection sites, of which six, eight and eight sites were from WLO-1 (47 feathers), WLO-2 (80 feathers) and TPL (104 feathers), respectively (Fig. 1). We collected these specimens in May 2015 and discriminated individuals in each location based on two criteria: distance and sex. In the former case, since it has been reported

that the home range size of green peafowl in breeding season was about 148 hectares or 2 km in distance (Ponsena, 1988), we collected feather specimens from within collection sites that were at least 2 km away from each other, which means that we were initially restricted to one individual from each of six sites from WLO-1, eight from WLO-2 and eight from TPL (Fig. 1). For the latter criterion, we identified additional individuals within each site based on morphological differences in the feathers between males and females as previously reported (Meckvichai and Arsirapoj, 2009). In addition to these two criteria, we also performed DNA sequencing of some remaining specimens within a site for screening of different mtDNA haplotypes to increase the sample sizes for each site. Additionally, egg samples (broken or un-hatched eggs) of green peafowl were also obtained. Eight and two eggs were collected from WLO-1 and TPL, respectively, preserved in 95% (v/v) ethanol and kept in the laboratory at room temperature for subsequent DNA extraction.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from egg shell membranes or from feather roots with a pulp, by cutting into small pieces, using the FavorPrep™ tissue genomic DNA extraction mini kit (Favorgen Biotech Corp.) following the manufacturer's protocol. The extracted DNA was supplemented with 60 µl of elution buffer (pH 8.0). The control region fragment was amplified by PCR using the species-specific PMDFU-F and PMDFU-R primers as described by Sawangtham et al. (2015). The PCR reaction was performed in a total volume of 50 µl. The reaction mixture contained approximately 30–50 ng of DNA template, 0.5 µM of each primer and 1Xpremix of EmeraldAmp®GT PCR master mix (Takara, Japan). Negative controls were included in all reactions. The PCR proceeded on a BioRad thermal cycler with a PCR reaction cycle of 95°C for 4 min followed by 40 cycles of 95°C for 40 sec, 60.6°C for 1.15 min and 72°C for 3 min, and then a final 72°C for 10 min. The reaction was terminated at 4°C. The size of the amplification product was checked by electrophoresis through 0.8% (w/v) agarose gel, stained with SYBR®Safe DNA gel stain (Invitrogen™) and visualized via blue light. The desired PCR products were subsequently sent to Bioneer Inc., Daejeon, South Korea for commercial purification and sequencing services. Each PCR product was sequenced in both directions using the forward and reverse primers.

Sequences and the phylogenetic and haplotype network analyses

Seventy-two sequences were obtained and subsequently aligned and edited manually using Clustal W (Thompson et al., 1994) via the program included in version 6.06 of MEGA (Tamura et al., 2013). The number of haplotypes (H), number of variable sites (v), mean number of pairwise nucleotide differences (k), haplotype diversity (h) and nucleotide diversity (π) were estimated for each population and the overall population (WLO-1 + WLO-2 + TPL) using DnaSP version 5.10.01 (Librado and Rozas, 2009). Mean transition/ transversion (ts/tv) ratio and genetic distance within and between populations were estimated using Kimura's two-parameter method via MEGA. We additionally used Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010) to calculate Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) values for the test of selective neutrality.

Phylogenetic relationship constructions were performed using MEGA for the neighbor-joining (NJ) analysis (Saitou and Nei, 1987) and PhyML version 3.0 for the maximum likelihood (ML) analysis (Guindon et al., 2010). The NJ tree was constructed using Kimura's two-parameter model (Kimura, 1980) according to the ts/tv ratio. For ML tree construction, the Akaike information criterion (AIC) algorithm was conducted to determine the best-fitting model for nucleotide substitution. For the present data set of control region sequences, the GTR + I model with a proportion of invariant sites of 0.926 was chosen. A total of 1,000 bootstrap replications were performed for both NJ and ML trees to assess the confidence in the tree topology (Felsenstein and Kishino, 1993). The nucleotide sequence of the control region of the Indian peafowl (*P. cristatus*; KF444060) was used as the outgroup. In addition, in order to examine and visualize the relationships among mtDNA haplotypes, a median-joining network analysis of the haplotypes was conducted using NETWORK version 4.6.1.3 (Bandelt et al., 1999). We constructed a haplotype network with the default parameters based on the variable nucleotides of the alignment sequences.

Population structure analyses

In order to make a clearer picture on how much genetic differentiation there is between northern and western Thai green peafowl populations, we included our previous data from HHK (northern Thailand; n = 27) and HKK (western Thailand; n = 22) (Sawangtham et al., 2015) for population genetic structure analyses. A hierarchical analysis of molecular variance (AMOVA) included in Arlequin was used to examine the degree of genetic differentiation among the groups (a group of pooled northern WLO-1, WLO-2, TPL and HHK populations versus a western group of the HKK population). We also estimated the population genetic differentiation (F_{ST}) and gene flow (N_m) between pairwise populations with significant level at $p < 0.001$. Estimation of gene flow was calculated with the equation $N_m = [(1/F_{ST}) - 1]/2$ (Weir and Cockerham, 1984; Bei et al., 2014).

RESULTS

Genetic diversity of green peafowl in WLO-1, WLO-2 and TPL

We were able to obtain feather specimens from 20 of 22 collection sites, missing two sites in WLO-2 (Fig. 1). According to individual identification criteria and egg collection, a total of 72 green peafowl individuals were identified, of these 28, 17 and 27 were from WLO-1, WLO-2 and TPL, respectively. The control region segment was successfully amplified and sequenced for those individuals, and the nucleotide sequence was 1,090 bp in length. A total of 16 haplotypes were identified based on 19 (1.74%) variable sites of the entire alignment sequences (Table 1). All of the haplotype sequences were deposited in the GenBank under accession numbers MG838888-MG838903 for Hap1 to Hap16 haplotypes, respectively. The most variations were detected in the 5' domain I of control region (approximately base position 1-360 bps) and several numbers of tandem repeats were found in the 3' domain III. Sixteen transitions and three transversions with no insertion or deletion (indels) were observed. The mean ts/tv ratio was 3.34 and the average nucleotide composition was A = 27.7%, T = 31.3%, C = 28.1%, G = 12.9%, with a higher A + T (59.0%) than G + C (41.0%), showing AT bias.

TABLE 1. Nineteen variable sites and 16 haplotypes based on 1,090 bp of the mtDNA control region were detected from 72 green peafowl individuals. The number of individuals and frequency of each haplotype are shown in the two columns on the right.

Haplotype	Variable sites in sequences																				n	% freq.
	29	43	99	100	123	147	148	151	155	171	173	186	235	260	312	493	790	836	1008		
Hap1	C	T	T	T	C	A	C	A	T	C	C	C	C	T	G	T	C	T	A		31	43.1
Hap2	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		2	2.8
Hap3	T	.	C	.	.	.	.	.	.	T	.	.	T	.	.	C	.	.	.		9	12.5
Hap4	T	A	C	.	.	.	.	.	.	T	.	.	T	.	.	C	.	.	.		1	1.4
Hap5	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.		1	1.4
Hap6	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T		3	4.2
Hap7	.		.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.		6	8.3
Hap8	.		.	.	.	G	.	.	C	.	T	T	.	C	.	C	.	C	.		8	11.1
Hap9	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.		2	2.8
Hap10	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T		1	1.4
Hap11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T		1	1.4
Hap12	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.		2	2.8
Hap13	.	.	.	C	.	.	.	T	C	.	.	.	.	.	A	.	T	.	.		2	2.8
Hap14	.	.	.	C	.	.	.	.	C	.	.	.	.	.	A	.	T	.	.		1	1.4
Hap15	.	A	.	C	.	.	.	.	C	.	.	.	.	.	A	.	T	.	.		1	1.4
Hap16	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.		1	1.4

Kimura's two-parameter genetic distance between populations ranged from 0 to 0.009, indicating low genetic differentiation among the three populations, and was the same as the genetic distances detected within each population. The overall diversity indices were 0.784, 0.0033 and 3.63 for h, π and k respectively (Table 2), indicating high level of genetic diversity in WLO-1, WLO-2 and TPL populations. In addition, these three parameter values in the WLO-1 population were higher than in other two populations. Moreover, no statistical significance for Tajima's D or Fu's F_S was observed for each and overall populations (Table 2) suggesting the absence of selective selection detected in this study.

The phylogenetic relationships inferred from the NJ tree produced similar topologies with the ML tree, and so we show the ML tree with the bootstrap support values of both analyses (Fig. 2). On the ML tree, the green peafowl did not cluster by geographic area, since individuals from WLO-1, WLO-2 and TPL were nested within the same clade. The network analysis of the 16 haplotypes showed a similar

TABLE 2. Genetic diversity parameters estimated for each and overall populations of wild green peafowl in northern Thailand.

Population	n	v	*k*	H	*h* ± S.D.	π ± S.D.	Tajima's *D* (*p*-value)	Fu's F_S (*p*-value)
TPL	27	13	3.07	9	0.724 ± 0.088	0.0028 ± 0.00062	–0.302 (0.412)	–0.848 (0.364)
WLO-1	28	18	4.86	11	0.873 ± 0.040	0.0044 ± 0.00053	0.177 (0.625)	–0.582 (0.424)
WLO-2	17	10	2.31	6	0.706 ± 0.106	0.0021 ± 0.00051	–0.804 (0.232)	–0.179 (0.481)
Overall pop.	72	19	3.63	16	0.784 ± 0.043	0.0033 ± 0.00038	–0.219 (0.476)	–2.471 (0.207)

Notes: v, number of variable sites; *k*, the average number of pairwise nucleotide differences; H, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity.

outcome with the phylogenetic analyses. From the mtDNA haplotype network (Fig. 3), three haplotypes (Hap1, 3 and 7) were observed in all three populations and four were found in only two areas; of these, one (Hap12), two (Hap8 and 9) and one (Hap6) haplotypes were found in WLO-1 and WLO-2, WLO-1 and TPL and WLO-2 and TPL, respectively. Moreover, the Hap1 haplotype was positioned at the center and showed the highest frequency (43.1%) of occurrence, being shared by 31 individuals and so is the most common haplotype. While Hap8, the most divergent haplotype, might have derived from Hap1 it would have required at least seven mutational changes.

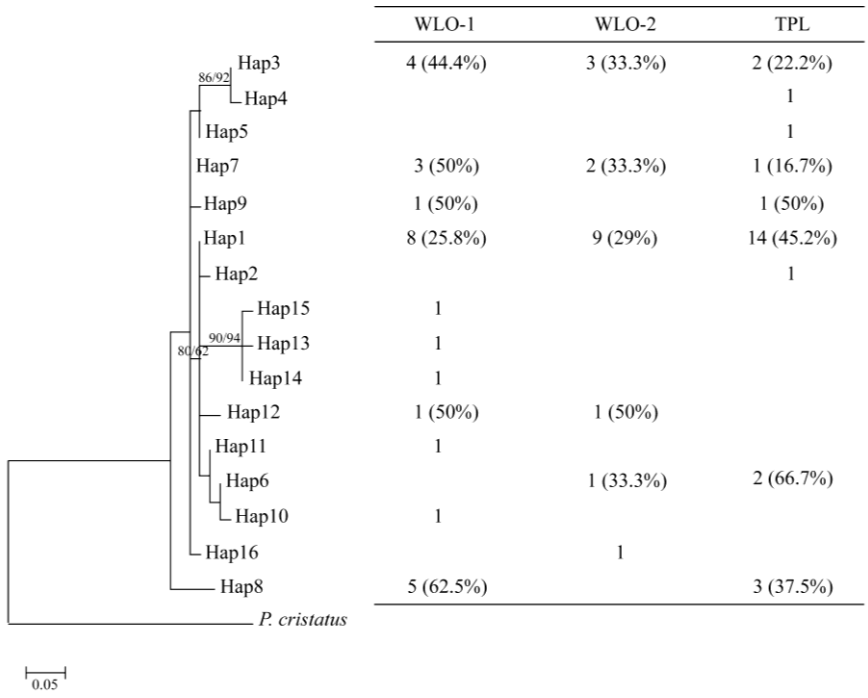

FIGURE 2. Phylogenetic ML tree of 16 haplotypes derived from 72 sequences constructed based on the 1,090 mtDNA control region sequences. The table besides the tree represents the distribution and frequency of haplotypes in the WLO-1, WLO-2 and TPL populations. Numbers next to each node represent the ML/NJ bootstrap support for that node when ≥ 50%.

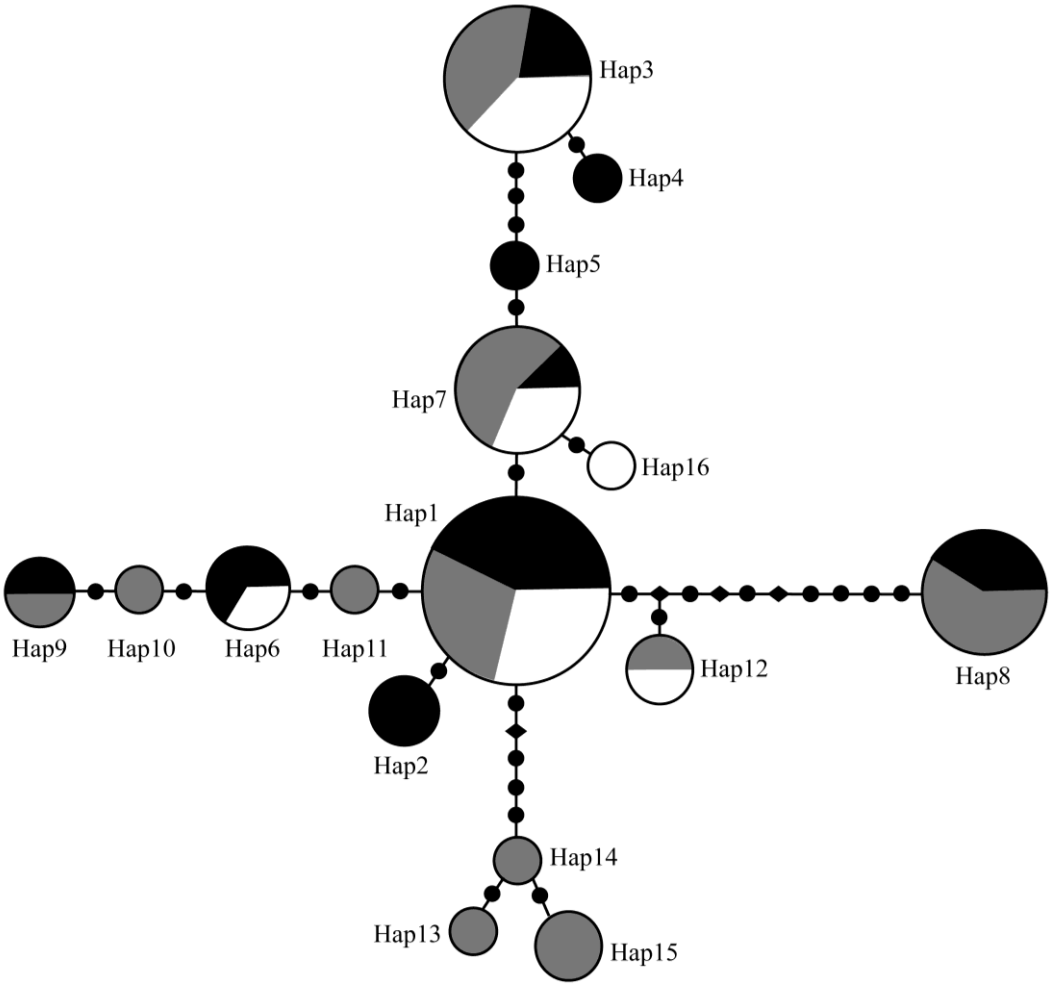

FIGURE 3. Median-joining network for the 16 haplotypes of green peafowl based on the polymorphic sites of the control region sequences. Circles represent the haplotypes that are connected by maximum parsimonious. The sizes of circles correspond to the frequency of the haplotypes. The black small circles between the links indicate numbers of mutational changes. The black small squares represent the missing haplotypes. Individuals collected from TPL, WLO-1 and WLO-2 are shown in black, light grey and white, respectively.

Population structure of green peafowl in Thailand

A hierarchical molecular variation analysis among the groups (a group of pooled northern WLO-1, WLO-2, TPL and HHK populations versus a western group of the HKK population) and within populations revealed a low genetic difference (4.31%) with no significant genetic subdivision between the two regional populations (p = 0.025). Most of genetic variations were detected from different individuals within populations (94.12%), whereas variations among populations (1.57%) were very small

TABLE 3. Analysis of molecular variance (AMOVA) of green peafowl samples from two geographic regions of Thailand (northern and western)[a] based on the mtDNA control region sequences.

Source of variation	d.f.	Sum of square	Percentage of variation	*p*-value
Among groups	1	5.14	4.31	0.025
Among populations within groups	3	7.18	1.57	0.176
Within populations	116	197.27	94.12	0.199
Total	**120**	**209.60**		

[a]Northern = TPL + WLO-1 + WLO-2 + HHK; Western = HKK
Significant level at $p < 0.001$

(Table 3). This was also consistent with the low pairwise F_{ST} values (ranging from –0.008 to 0.090) that were not significantly different from zero for all population-pairwise comparisons (Table 4), indicating a lack of significant genetic differentiation among populations. The estimated gene flow (N_m) values among these five populations ranged from 5.06 (WLO-1 vs HKK) to ∞ (WLO-1 vs HHK and WLO-2 vs TPL) (Table 4), indicating sufficient gene flow among the five populations. Gene flow was higher among populations within a region rather than between regions, where WLO-1 and HKK population comparison had a lowest value of N_m (Table 4).

DISCUSSION

Genetic diversity of green peafowl in WLO-1, WLO-2 and TPL

The vertebrate mtDNA control region is typically composed of the three domains: the central conserved domain II and the flanking hypervariable 5' domain I and 3' domain III (Desjardins and Morais, 1990; Randi and Lucchini, 1998); our results also support this. In the present study, most of variable nucleotides were observed in domain I rather than in the other two domains. However, the number of variable sites was small compared with those found

TABLE 4. Population pairwise F_{ST} value (below the diagonal) and gene flow (above the diagonal) for comparisons between populations.

Population	TPL	WLO-1	WLO-2	HHK	HKK
TPL	–	49.50	∞	35.21	12.66
WLO-1	0.010	–	11.70	∞	5.06
WLO-2	– 0.008	0.041	–	14.21	16.74
HHK	0.014	0.000	0.034	–	6.26
HKK	0.038	0.090	0.029	0.074	–

Significant level at $p < 0.001$

in other related species, such as Hume's pheasant *Syrmaticus humiae* (Bei et al., 2014) and Elliot's pheasant *S. ellioti* (Jiang et al., 2007), suggesting low genetic divergence in control region sequence in *P. muticus*. In addition, the observation of A+T base rich and the presence of tandem repeats in the 3' flanking domain III, which are unique characteristics of avian mtDNA control region (Jiang et al., 2007), suggests that the haplotype sequences in this study were unlikely to have been amplified from a nuclear pseudogene.

Among three populations analyzed, green peafowl in WLO-1 population exhibited the highest level of diversity both in terms of *h* and π. Although WLO-2 is a relatively larger area than TPL, a lower value of *h* in WLO-2 population (0.706) than in TPL population (0.724) may result from the smaller sample size examined from WLO-2. Rather a higher diversity may be found if the numbers of samples were increased. Thus, the genetic diversity of the WLO-2 population awaits further investigation. On average, the observed level of *h* detected in this study was 0.784, indicating high haplotype diversity in green peafowl in these three populations. However, this value was relatively low when compared with a previous study in HHK and HKK populations (h = 0.851, n = 49) (Sawangtham et al., 2015), and compared with other Galliformes species, such as *S. humiae* (h = 0.853, n = 73) (Bei et al., 2014), *Tragopan caboti* (h = 0.97, n = 53) (Dong et al., 2010) and *S. ellioti* (h = 0.992, n = 33) (Jiang et al., 2007).

Genetic distance among populations being about 1% indicates that green peafowl exhibited low genetic differentiation between populations, leading to the random distribution of haplotypes across the three geographical areas in phylogenetic trees; the low F_{ST} values also support this. Such low genetic differentiation and the presence of shared haplotypes among the three populations may result from gene flow as N_m values among the three-pairwise population comparisons (ranged from 11.7 to ∞) are larger than 4, indicating sufficient gene flow which could homogenize gene pools among populations. However, the green peafowl can fly weakly and spends most of its time on the ground for foraging, nesting and rearing its chicks. It is commonly found in the areas with more open understorey vegetation mostly within 1–2 km of stream or water sources (Ponsena, 1988; Brickle, 2002; Saridnirun et al., 2016). Although, the bird had a larger home range during the non-breeding season (177.23 ha) than during the breeding season (25.72 ha) (Sukumal et al., 2017), and the genetic exchange between populations might rarely occur, the presence of shared haplotypes could be explained by the fact that the birds may have formerly lived in a continuous area before habitat fragmentation and so retain their ancestral haplotypes. In other word, habitat fragmentation may have occurred too recently (probably less than 100 years) and have no detectable effect at the genetic level. Thus, further study on green peafowl distribution and movement among these areas are required for detecting the migration ability and frequency.

Hap1 is the most frequent haplotype on the mtDNA haplotype network and is also present in all three populations (wide geographic distribution), meaning that it is also an ancestral haplotype. Hap7 and Hap3 are also present in all three populations but they are not as frequent. Several haplotypes (9/16) are present only in one area and very few individuals. These are highly unlikely to be ancestral and are probably newly derived. Hap13, Hap14 and Hap15 group together in

the network, and also in the tree, where they form a clade. Remarkably, these haplotypes come from the same collection site in WLO-1 and are closely related with each other, suggesting that Hap13 and Hap15 might have derived from Hap14.

Population structure of green peafowl in Thailand and implications for conservation

Despite habitat fragmentation, our findings revealed that wild *P. muticus* in Thailand still showed high level of genetic diversity in terms of haplotype diversity with lack of significant genetic differentiation between the northern and western populations. A high level of individual variations detected within populations leads to a low genetic differentiation between regional populations. Moderate to high levels of gene flow detected between the two geographical regions and among the five populations also support this conclusion. Similar results were found in genetic structure of other related species, such as Hume's pheasant (*S. humiae*) (Bei et al., 2014) and Cantabrian capercaillie (*Tetrao urogallus cantabricus*) (Liukkonen-Anttila et al., 2004), in which habitat fragmentation has no influence on genetic divergence. Our results suggest that there is no subdivision of wild populations of green peafowl in all populations analyzed. The species can, therefore, be considered as a single management unit. Subsequent introduction of this bird across populations to reduce the chance of inbreeding is possible and maybe helpful, especially in small populations. However, further assessment species heterozygosity and inbreeding levels within populations using microsatellite markers is needed for long-term and effective conservation plans. Additionally, since microsatellite loci have a high level of polymorphism (Ashley and Dow, 1994; Grueber et al., 2008), they can be applied for more accurate individual identification from noninvasive samples (Lobo et al., 2015).

Habitat destruction and high hunting pressure are two major threats for green peafowl populations. Negative population trends and habitat fragmentation are projected to continue (McGowan et al., 1999; BirdLife International, 2015). This situation could subsequently lead to dramatic decreases or extinction of wild populations in some local areas in the future. Therefore, sustainable maintenance of the green peafowl population size requires conservation genetics studies and conservation management actions, including habitat protection, reforestation, hunting prohibition and education to raise awareness of the local people living in agroforest ecosystem that are shared with this bird.

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