

CHAPTER III

RESULTS

3.1 Sample collections

In this study can be collected bloodstain and feather papillae from various parts of Thailand at least three different locations and each were collected location least 3 individuals for DNA extraction. Bloodstain and feather can be easily managed in the laboratory, convenience for working in the field and can be preserved at the room temperature. In natural habitat, blood samples are difficult to be collected from the wild but feathers are easier to be collected along their trails. Only two species which collected from feather papillae; Green Peafowl feathers were collected from Ping Watershed (Amphoe Li, Lumpun province and Amphoe Chomthong, Chiang Mai province) and Great Argus feathers were collected from Hala Bala wildlife sanctuary, Narathivas province. The advantage of collection both bloodstain and feather are non invasive method and need not to kill the animals.

3.2 DNA extraction

The QIAamp® DNA Mini Kit extraction method is easy, quickly and convenience although it was more expensive but it worth for a lot of sample. Extracted genomic DNA of 63 individuals on the filter paper and they were dissolved in 150 µl of AE buffer. An amount of each extracted DNA was estimated by 0.8% agarose gel electrophoresis. Quality of genomic DNA of four gallopheasants; 11 Great Argus (*Argusianus argus argus*), 13 Red Junglefowl (*Gallus gallus spadiceus*), 12 Siamese Fireback (*Lophura diardi*), 18 Green Peafowl (*Pavo muticus imperator*) and a partridge; 9 Crested Wood Partridge (*Rollulus roulroul*) were extracted from bloodstains and feathers. An amount of each extracted DNA was estimated by 1.8% agarose gel electrophoresis compared with the PhiX174/*Hinf* I and M23 1.5 kb + 100 bp DNA ladder marker and the most DNA

products were pure enough for amplifying and yielded genomic DNA are shown in Figure 3.1-3.5.

All samples of extracted DNA of Great Argus (*Argusianus argus argus*) show good quality and high molecular weight DNA. Only one extracted DNA samples was contained RNA contamination which was appeared as smeared bands at the bottom line of agarose gel (Lanes 9). However, RNA contaminant did not interfere the success of amplification reactions. Some extracted DNA samples which show good quality and high molecular weight DNA were even through, repeat extraction and amplification but it is still not be amplified RAPD fragment.

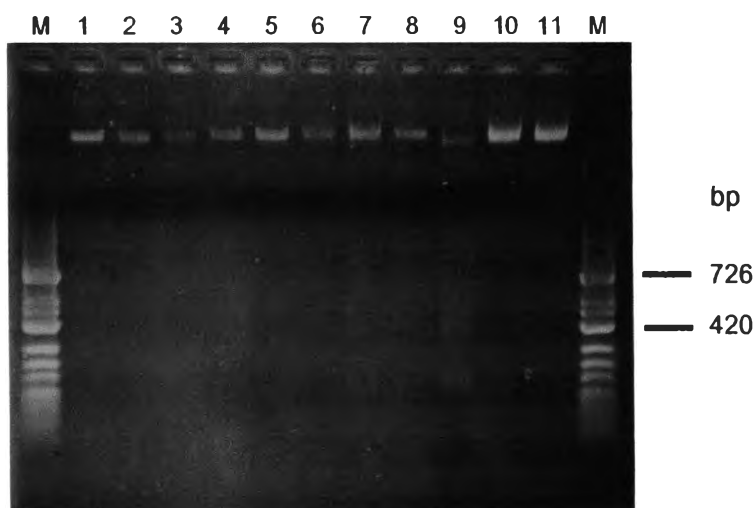


Figure 3.1 Extracted genomic DNAs from bloodstains of Great Argus (*Argusianus argus argus*) using QIAamp® kit method were electrophoresed with 0.8% agarose gel and stained with ethidium bromide.

Lane M	=	PhiX174/ <i>Hinf</i> I standard marker
Lane 1-3	=	Hala Bala wildlife sanctuary (HLBL)
Lane 4-6	=	Khao Pratabcharng wildlife research and breeding station (PTC)
Lane 7-9	=	Khao Soi Dao wildlife research and breeding station (SD)
Lane 10-11	=	Individual farm in Amphoe Sattaheep, Chonburi Province (STH)

All samples of extracted DNA of Red Junglefowl (*Gallus gallus spadiceus*) show good quality and high molecular weight DNA.

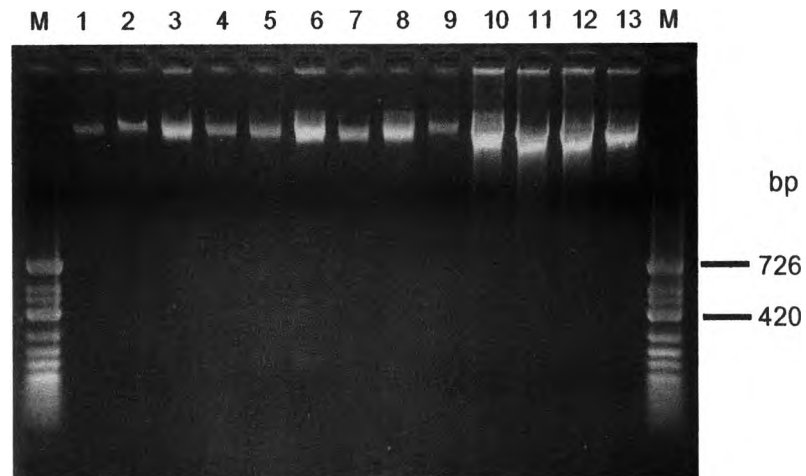


Figure 3.2 Extracted genomic DNAs from bloodstains of Red Junglefowl (*Gallus gallus spadiceus*) using QIAamp® kit method were electrophoresed with 0.8% agarose gel and stained with ethidium bromide.

Lane M	=	PhiX174/ <i>Hinf</i> I standard marker
Lane 1-3	=	Huay Kha Kaeng Wildlife Sanctuary (HKK)
Lane 4	=	Huay Yang Parn wildlife research and breeding station (HYP)
Lane 5-7	=	Huay Kha Kaeng Wildlife Sanctuary (HKK)
Lane 8-10	=	Phatthalung wildlife research and breeding station (PTL)
Lane 11-13	=	Satun (ST)

All samples of extracted DNA of Siamese Fireback (*Lophura diardi*) show good quality and high molecular weight DNA.

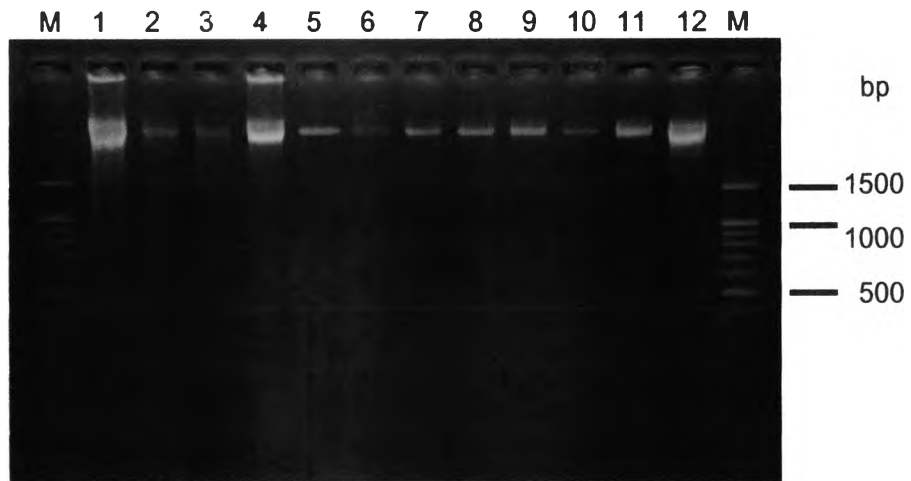


Figure 3.3 Extracted genomic DNAs from bloodstains of Siamese Fireback (*Lophura diardi*) using QIAamp® kit method were electrophoresed with 0.8 % agarose gel and stained with ethidium bromide.

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Phu Khieu wildlife research and breeding station (PK)
Lane 4-6	=	Individual farm in Amphoe Sattaheep, Chonburi Province (STH)
Lane 7-9	=	Khao Pratabcharng wildlife research and breeding station (PTC)
Lane 10-12	=	Khao Soi Dao wildlife research and breeding station (SD)

All samples of extracted DNA of Green Peafowl (*Pavo muticus imperator*) show good quality and high molecular weight DNA. Some extracted DNA samples contained RNA contamination as visualized by smeared bands at the bottom of agarose gel.

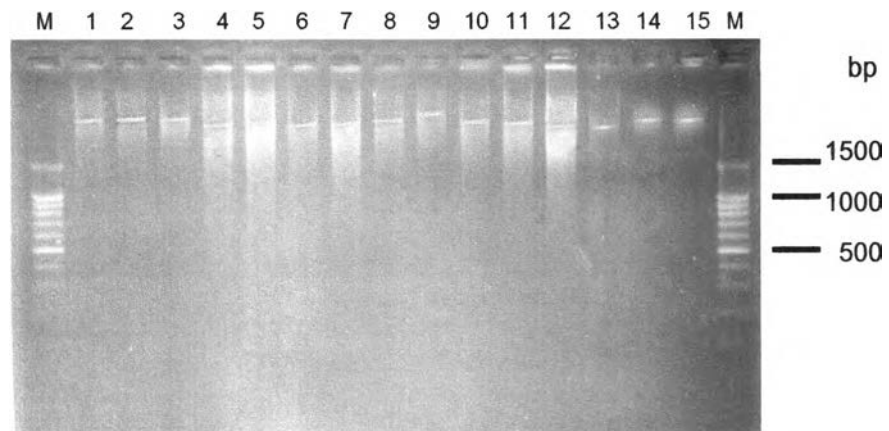


Figure 3.4 Extracted genomic DNAs from bloodstains of Green Peafowl (*Pavo muticus imperator*) using QIAamp® kit method were electrophoresed with 0.8 % agarose gel and stained with ethidium bromide.

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Khao Pratabcharng wildlife research and breeding station (PTC)
Lane 4	=	Huay Yang Parn wildlife research and breeding station (HYP)
Lane 5	=	Pong Wild, Payao province (P)
Lane 6	=	Lumpang Wild, Lumpang province (LP)
Lane 7	=	Chiang Khong Wild, Chiang Rai province (CK)
Lane 8-10	=	Surin Wild (SR)
Lane 11-12	=	Phu Khieu wildlife research and breeding station (PK)
Lane 13-15	=	Khao Soi Dao wildlife research and breeding station (SD)

All samples of extracted DNA of Crested Wood Partridge (*Rollulus roulroul*) show good quality and high molecular weight DNA.

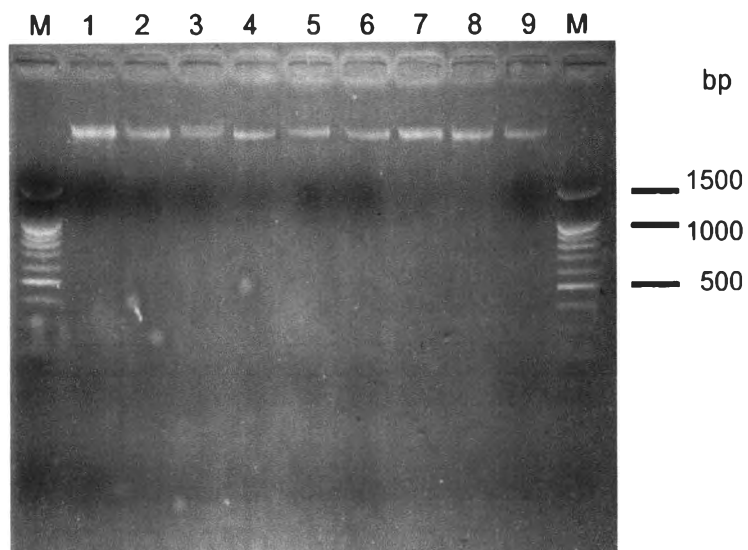


Figure 3.5 Extracted genomic DNAs from bloodstains of Crested Wood Partridge (*Rolluluss roulroul*) using QIAamp® kit method were electrophoresed with 0.8 % agarose gel and stained with ethidium bromide.

- | | | |
|----------|---|--|
| Lane M | = | 1.5 kb+100 bp standard marker |
| Lane 1 | = | Individual Farm in Bangkok (BKK) |
| Lane 2-3 | = | Khok Mai Rua wildlife research and breeding station (KMR) |
| Lane 4-6 | = | Amphoe Sattaheep, Chonburi Province (STH) |
| Lane 7-9 | = | Khao Pratabcharng wildlife research and breeding station (PTC) |

3.3 Determination of genetic variation among intra- and inter-specific taxa or species of five gallopheasants and partridge

RAPD analysis had been increasingly used for determination of intraspecific population differentiation and identification of genetic markers at different taxonomic level in avian. This is possible due mainly to its flexibility and less time consuming. Based on the fact that arbitrary sequence of oligonucleotides (usually 10-12 mers) are required for this technique, the most suitable RAPD primers for a particular application can be selected from unlimited number of arbitrary oligonucleotides (Weising et al., 1995).

RAPD method was used to determine genetic variations in all five species by modification from Peyachoknagul (2002). Previously, the PCR profile of 40 cycles (denaturation at 94 °C for 1 minute, annealing at 37 °C for 1 minute and extension at 72 °C for 2 minutes) was carried out the RAPD products with a DNA Thermal Cyclic.

In the preliminary studied primers were screen and only fifteen out of hundred primers (OPA-18, OPC-02, OPC-10, OPM-10, OPP-03, OPP-09, OPP-10, UBC-02, UBC-98, UBC-101, UBC-104, UBC-132, UBC-133, UBC-135 and UBC-137) can be produced DNA band. Finally, only 5 out of fifteen primers (OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135) were used as the markers for investigating the genetic variation of RAPD-PCR products.

Genetic diversity within interspecies were produced totally 98 scorable bands ranging from 200 bp to 1500 bp alleles number of 16, 23, 22 and 21 scorable bands were generated by primer OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135, respectively (Table 3.1-3.2).

Variation of each species produced totally 233 scorable bands, alleles. Total number of 69, 37, 34, 49 and 53 loci were found in Great Argus, Red Junglefowl, Siamese Fireback, Green Peafowl and Crested Wood Partridge, respectively. Each

marker; OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135 was generated 6-17, 6-17, 4-14, 4-12 and 7-11 scorable bands, respectively (Table 3.3). Three species have intra specific band; Great Argus has 3 specific bands, Crested Wood Partridge has 2 specific bands and Green Peafowl has only one specific band. Two species; Red Junglefowl and Siamese Fireback have no intraspecific band (Table 3.4). The scoring data of all RAPD patterns generated from each primer among inter- and intraspecies are shown in Appendix B.

Table 3.1 Shown RAPD products of interspecies; Number of scorable bands, size range, total no. of monomorphic/polymorphic bands and Percentage of polymorphic bands of five primers.

Primer	No. of scorable bands	Size range (bp)	Total no. of monomorphic/polymorphic bands	Percentage of polymorphic bands per site
OPA-18	16	320-1500	0/16	100.00
OPC-02	16	250-1300	0/16	100.00
OPP-03	23	250-1200	0/23	100.00
UBC-133	22	350-1500	0/22	100.00
UBC-135	21	380-1400	0/21	100.00

Table 3.2 Shown candidate species specific bands of interspecies; primer, species, size of loci, total no. of monomorphic/polymorphic bands and Percentage of polymorphic bands.

Primer	Species	Size of loci that found candidate species specific bands (bp)	M/P bands	%P
OPA-18	Gallus	650 (HYP) and 540 (ST)	2/5	71.43
	Argusianus	-	0/6	100.00
	Rollulus	700 (STH)	0/5	100.00
	Lophura	850 (PTC), 800 (PTC) and 500 (STH)	3/4	57.14
	Pavo	-	2/0	0.00
OPC-02	Gallus	-	2/2	50.00
	Argusianus	750 (STH)	3/1	25.00
	Rollulus	320 (PTC)	2/5	71.43
	Lophura	1000 (PK) and 620 (PTC)	2/2	50.00
	Pavo	-	2/1	33.33
OPP-03	Gallus	680 (ST)	4/2	33.33
	Argusianus	520 (HLBL), 300 (HLBL) and 280 (PTC)	0/6	100.00
	Rollulus	700 (PTC)	2/1	33.33
	Lophura	-	0/7	100.00
	Pavo	780 (LP)	5/1	16.67
UBC-133	Gallus	-	3/4	57.14
	Argusianus	500 (HLBL) and 380 (HLBL)	3/6	66.67
	Rollulus	1100 (KMR) and 1000 (KMR)	4/5	55.56
	Lophura	1200 (PK), 1050 (STH) and 900(STH)	3/5	62.50
	Pavo	-	4/0	0.00
UBC-135	Gallus	600 (ST)	6/4	40.00
	Argusianus	-	0/7	100.00
	Rollulus	700 (STH), 650 (PTC) and 400 (KMR)	2/7	77.78
	Lophura	520 (PTC)	7/1	12.50
	Pavo	-	8/2	20.00

Table 3.3 Shown primer, species, number of scorable bands, size range, number of monomorphic/polymorphic bands, percentage of polymorphic bands and candidate species specific bands. Resulting from RAPD analysis in intraspecies

Primer	Species	Size range (bp)	No. of scorable bands	No. of monomorphic / polymorphic bands	Percentage of polymorphic bands	Candidate species specific bands
OPA-18	Great Argus	280-1200	17	0/17	100.00	1
	Red Junglefowl	400-1000	6	5/1	16.67	0
	Siamese Fireback	280-850	10	3/7	70.00	0
	Green Peafowl	350-1400	11	0/11	100.00	0
	Crested Wood Partridge	380-800	9	0/9	100.00	0
OPC-02	Great Argus	250-1300	17	1/16	94.12	3
	Red Junglefowl	500-1200	6	2/4	66.67	0
	Siamese Fireback	300-800	7	2/5	71.43	0
	Green Peafowl	320-1000	10	0/10	100.00	0
	Crested Wood Partridge	250-650	9	2/7	77.78	0
OPP-03	Great Argus	280-1500	14	0/14	100.00	1
	Red Junglefowl	400-1500	8	2/6	75.00	0
	Siamese Fireback	650-1500	6	0/6	100.00	0
	Green Peafowl	400-1100	10	0/10	100.00	0
	Crested Wood Partridge	550-1200	4	0/4	100.00	1
UBC-133	Great Argus	450-1200	10	1/9	90.00	0
	Red Junglefowl	400-1400	8	4/4	50.00	0
	Siamese Fireback	400-900	4	1/3	75.00	0
	Green Peafowl	400-1300	9	0/9	100.00	1
	Crested Wood Partridge	300-1100	12	1/11	91.67	0
UBC-135	Great Argus	250-1200	11	1/10	90.91	0
	Red Junglefowl	300-1300	9	2/7	77.78	0
	Siamese Fireback	400-1100	7	2/5	71.43	0
	Green Peafowl	350-1200	9	0/9	100.00	0
	Crested Wood Partridge	300-800	10	1/9	90.00	1

Table 3.4 Shown species, primer, population and size of loci that found candidate population specific bands. Resulting from RAPD analysis in intraspecies

Species	Primer	Population	Size of loci that found candidate population specific bands (bp)
Great Argus	OPA-18	HLBL	800
	OPC-02	HLBL	650
		PTC	700, 500
	OPP-03	PTC	1400
Green Peafowl	UBC-133	Ping	700
Crested Wood Partridge	OPP-03	PTC	1200
	UBC-135	KMR	500

3.4 RAPD analysis

3.4.1 Analysis of genetic polymorphism in interspecies

Primer OPA-18

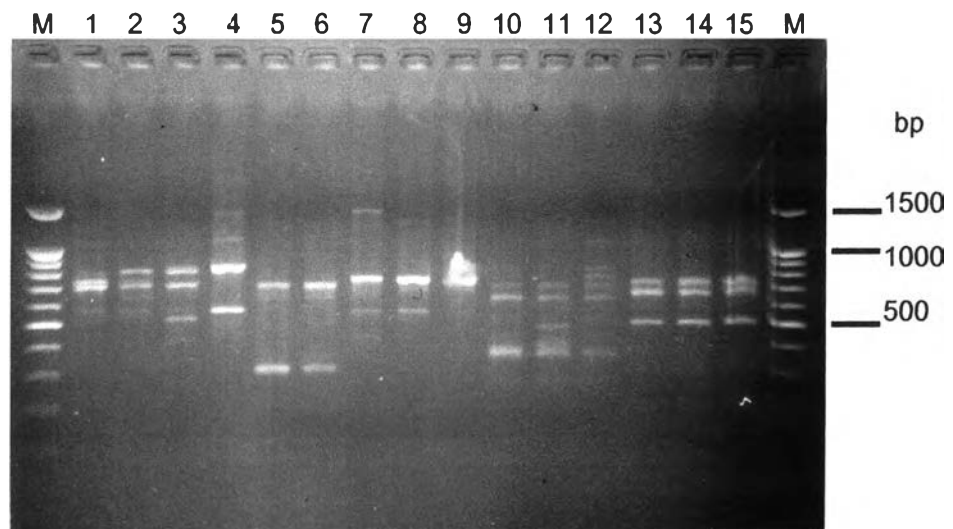


Figure 3.6 RAPD patterns of interspecies generated from primer OPA-18

Primer OPC-02

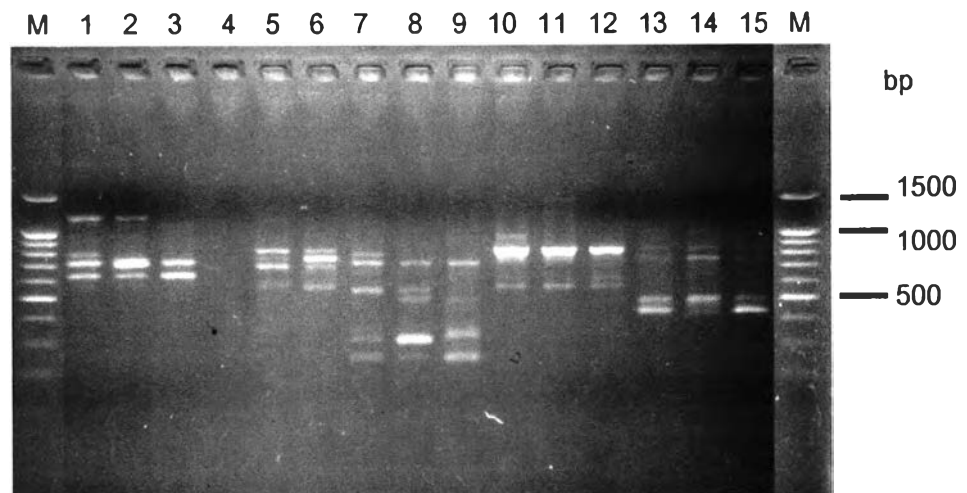


Figure 3.7 RAPD patterns of interspecies generated from primer OPC-02

Primer OPP-03

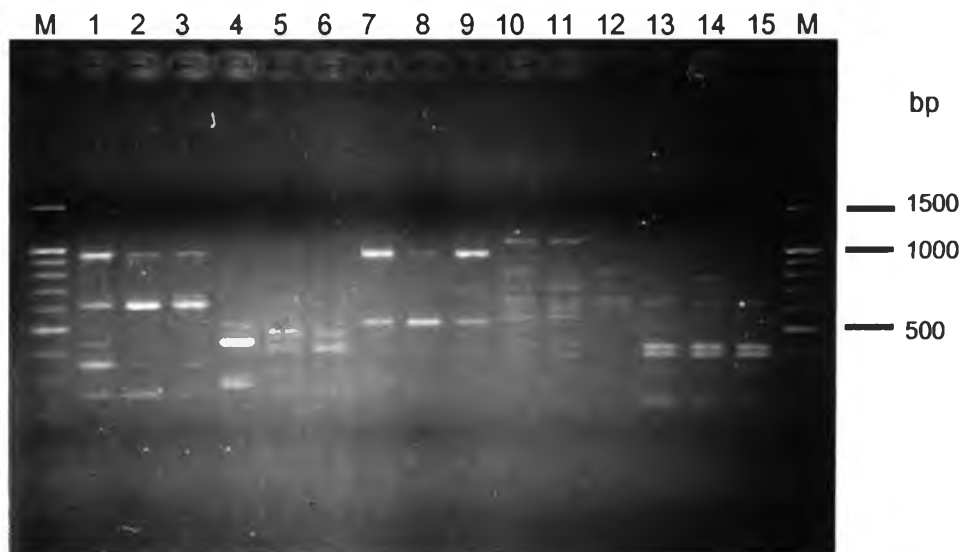


Figure 3.8 RAPD patterns of interspecies generated from primer OPP-03

Primer UBC-133

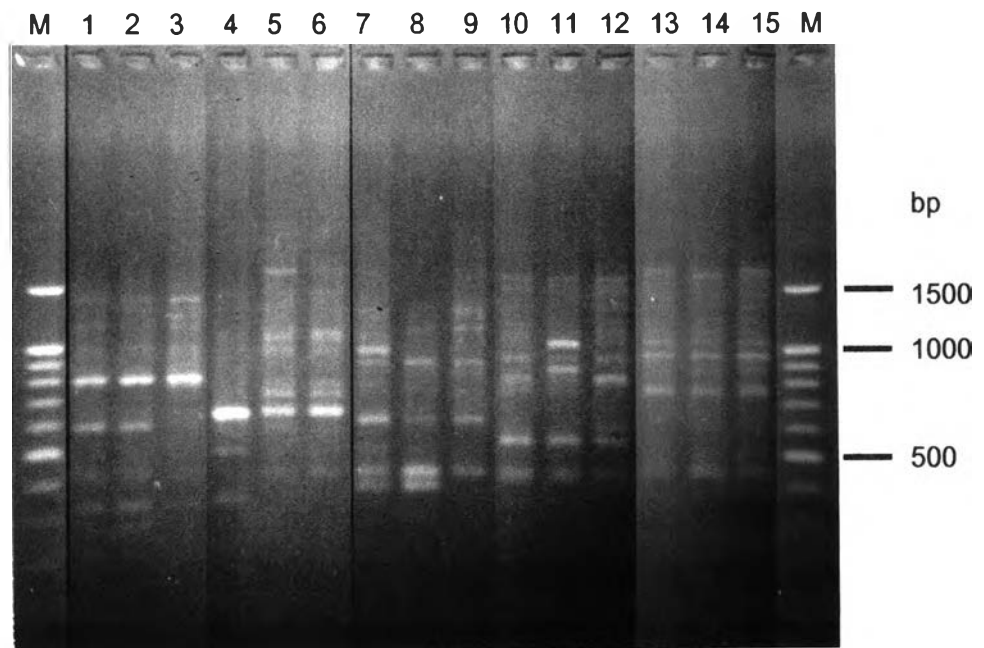


Figure 3.9 RAPD patterns of interspecies generated from primer UBC-133

Primer UBC-135

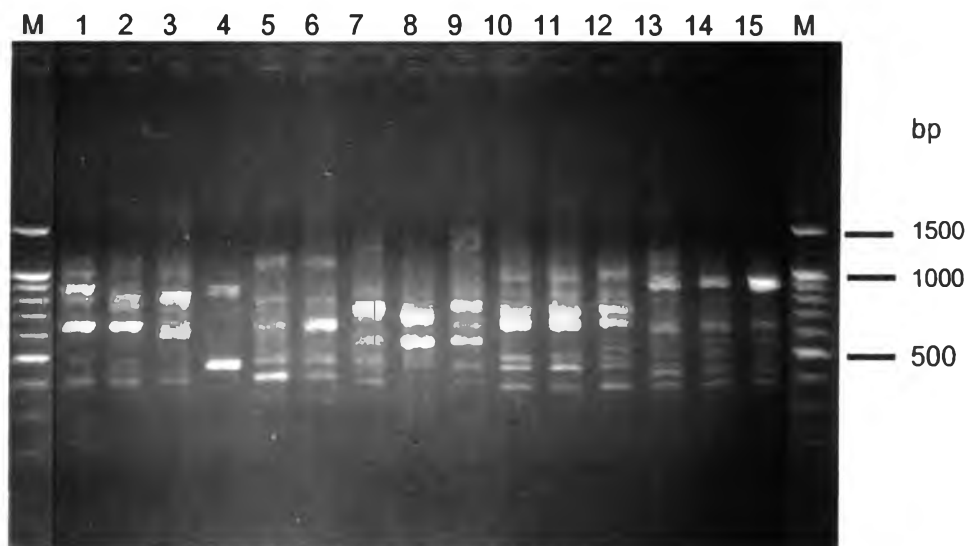


Figure 3.10 RAPD patterns of interspecies generated from primer UBC-135

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Gallus_HKK1, Gallus_HYP, Gallus_ST1
Lane 4-6	=	Argusianus_HLBL3, Argusianus_PTC1, Argusianus_STH1
Lane 7-9	=	Rollulus_KMR1, Rollulus_STH3, Rollulus_PTC2
Lane 10-12	=	Lophura_PK1, Lophura_STH1, Lophura_PTC1
Lane 13-15	=	Pavo_PTC1, Pavo_LP, Pavo_SD4

Sixteen RAPD fragments (bands) which were generated by OPA-18 primer among interspecies were scored 1500, 1400, 850, 800, 750, 720, 700, 680, 650, 580, 540, 520, 500, 450, 380 and 320 base pairs (Appendix B7). From this primer with 15 individuals of interspecies that could be amplified all individuals (100%). It is can be amplified in all 15 individuals of 5 gallopheasants. The scorable bands at 700 bp were specific band for Crested Wood Partridge while 850, 800 and 500 bp were specific bands for Siamese Fireback and 650 and 540 bp were specific bands for Red junglefowl. There is no specific band for Green Peafowl (Table 3.2, figure 3.6).

Sixteen RAPD fragments (bands) which were generated by OPC-02 primer were scored 1300, 1000, 850, 820, 800, 750, 720, 700, 620, 580, 550, 500, 420, 320, 300 and 250 base pairs (Appendix B7). From this primer with 15 individuals of interspecies that could be amplified only 14 individuals (93.33%). The scorable bands at 1000 and 620 bp were specific bands for Siamese Fireback while 320 bp was specific band for Crested Wood Partridge and 750 bp was specific band for Great Argus. There is no specific band for Red Junglefowl and Green Peafowl (Table 3.2, figure 3.7).

Twenty three RAPD fragments (bands) which were generated by OPP-03 primer were scored 1200, 980, 950, 850, 780, 720, 700, 680, 650, 620, 600, 580, 550, 520, 500, 450, 420, 400, 380, 350, 300, 280 and 250 base pairs (Appendix B7). From this primer with 15 individuals of interspecies that could be amplified all individuals (100%). The scorable bands at 680 bp was specific band for Red Junglefowl. At 520, 300 and 280

bp were specific bands for Great Argus while 780 bp was specific band for Green Peafowl and 700 bp was specific band for Crested Wood Partridge. There is no specific band for Red Junglefowl and Green Peafowl (Table 3.2, figure 3.8).

Twenty two RAPD fragments (bands) which were generated by UBC-133 primer were scored 1500, 1400, 1300, 1200, 1100, 1050, 950, 920, 900, 820, 800, 750, 680, 650, 600, 550, 500, 450, 400, 380 and 350 base pairs (Appendix B7). From this primer with 15 individuals of interspecies that could be amplified all individuals (100%). The scorable bands at 1200, 1050 and 900 bp were specific bands for Siamese Fireback while 1100 and 1000 bp were specific bands for Crested Wood Partridge and 500 and 750 bp were specific bands for Great Argus. There is no specific band for Red Junglefowl and Green Peafowl (Table 3.2, figure 3.9).

Twenty one RAPD fragments (bands) which were generated by UBC-135 primer were scored 1400, 1300, 1200, 1000, 950, 900, 820, 800, 750, 700, 650, 620, 600, 580, 520, 500, 480, 420, 400, 390 and 380 base pairs (Appendix B7). From this primer with 15 individuals of interspecies that could be amplified all individuals (100%). The scorable bands at 700, 650 and 400 bp were specific bands for Crested Wood Partridge while 600 bp was specific band for Red Junglefowl Crested Wood Partridge and 520 bp was specific band for Siamese Fireback. There is no specific band for Great Argus and Green Peafowl (Table 3.2, figure 3.10).



3.4.2 Analysis of genetic polymorphism in intraspecies

3.4.2.1 Analysis of genetic polymorphism of Great Argus (*Argusianus argus argus*) using the primer OPA-18

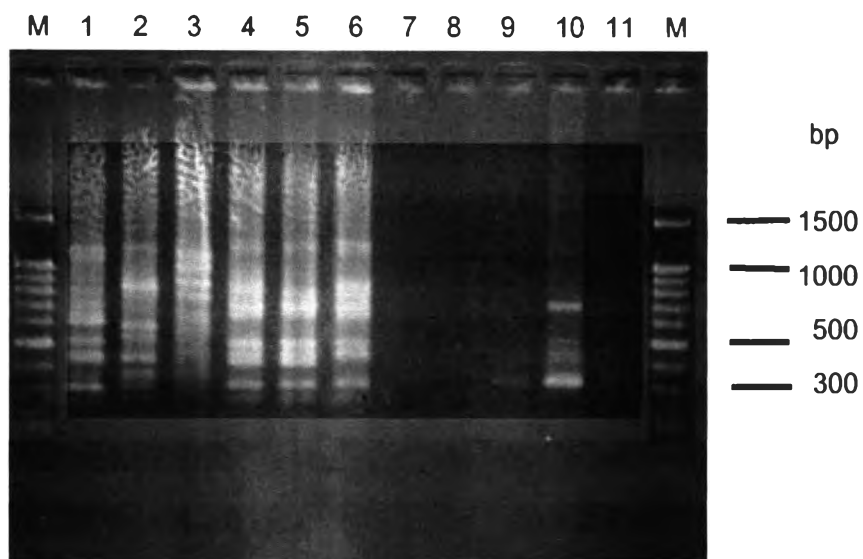


Figure 3.11 RAPD patterns of Great Argus generated from primer OPA-18

3.4.2.2 Analysis of genetic polymorphism of Great Argus (*Argusianus argus argus*) using the primer OPC-02

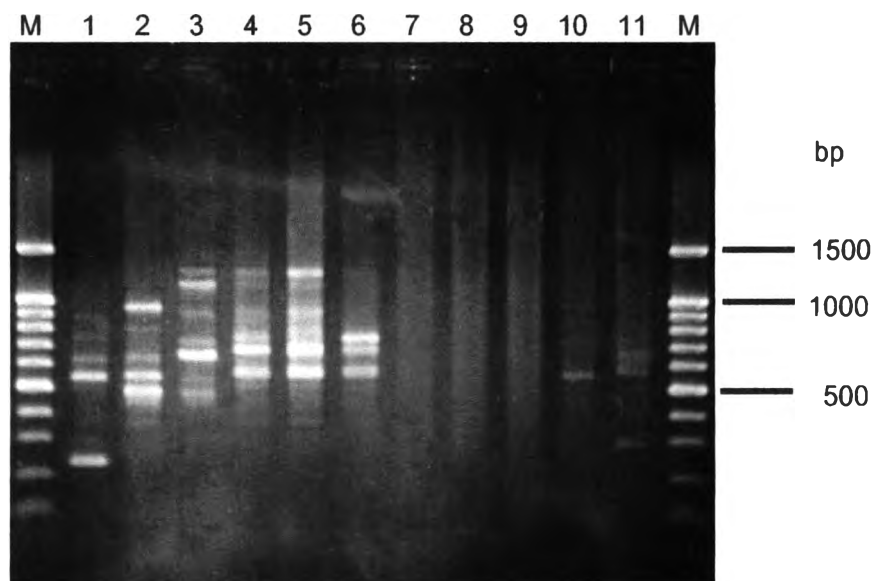


Figure 3.13 RAPD patterns of Great Argus generated from primer OPC-02

3.4.2.3 Analysis of genetic polymorphism of Great Argus (*Argusianus argus argus*) using the primer OPP-03

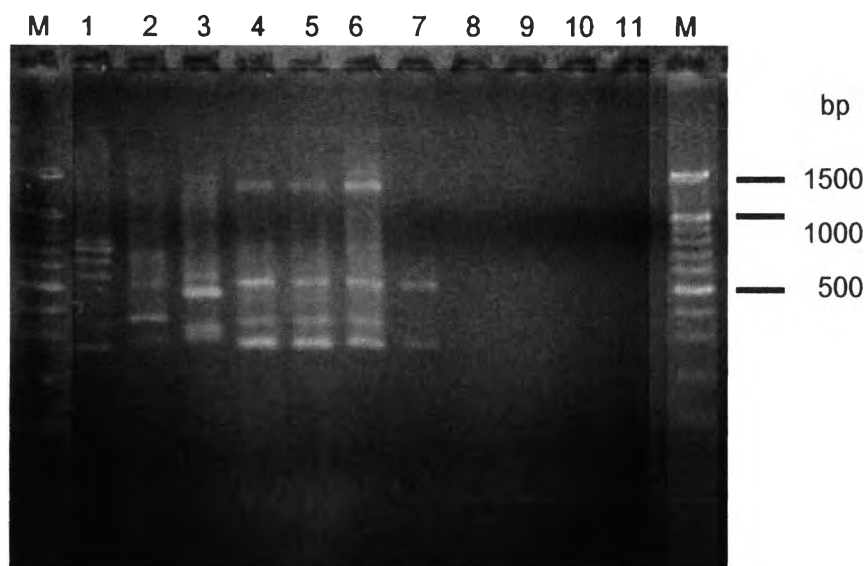


Figure 3.13 RAPD patterns of Great Argus generated from primer OPP-03

3.4.2.4 Analysis of genetic polymorphism of Great Argus (*Argusianus argus argus*) using the primer UBC-133

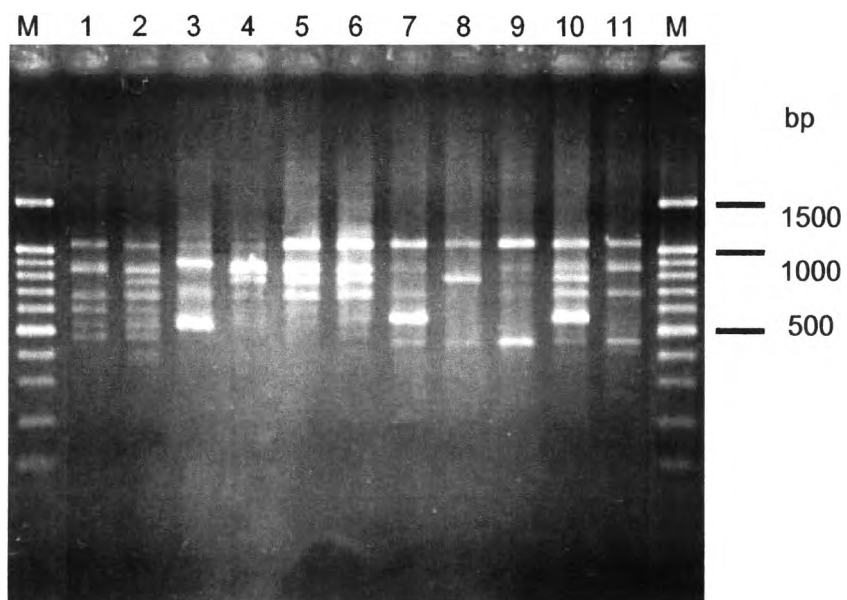


Figure 3.14 RAPD patterns of Great Argus generated from primer UBC-133

3.4.2.5 Analysis of genetic polymorphism of Great Argus (*Argusianus argus argus*) using the primer UBC-135

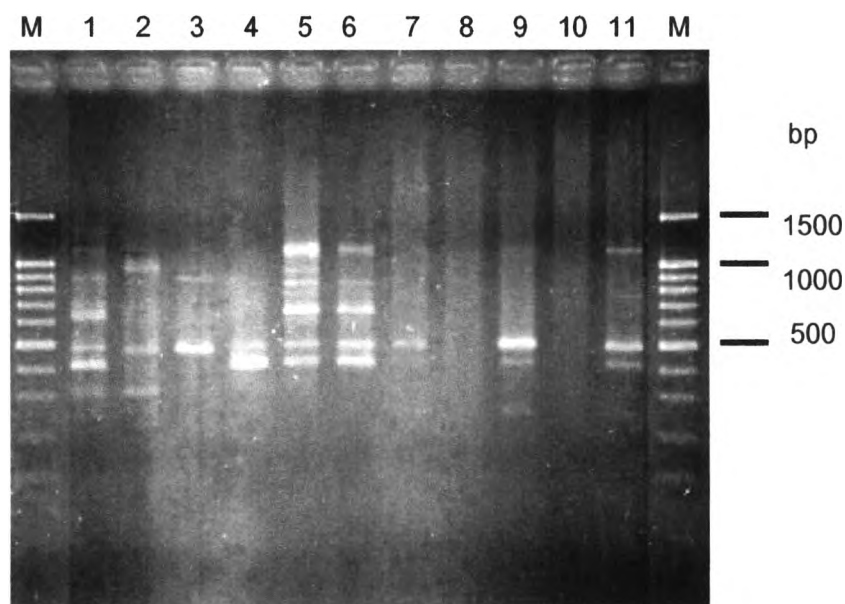


Figure 3.15 RAPD patterns of Great Argus generated from primer UBC-135

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Hala Bala wildlife sanctuary (HLBL)
Lane 4-6	=	Khao Pratabcharng wildlife research and breeding station (PTC)
Lane 7-9	=	Khao Soi Dao wildlife research and breeding station (SD)
Lane 10-11	=	Individual farm in Amphoe Sattaheep, Chonburi Province (STH)

Seventeen RAPD fragments (bands) of intraspecies in Great Argus which were generated by OPA-18 primer can be scored 1200, 1100, 1000, 900, 850, 800, 700, 600, 590, 580, 550, 500, 450, 410, 380, 300 and 280 base pairs (Appendix B1). From this primer with 11 individuals of Great Argus (*Argusianus argus argus*) that could be amplified only 9 individuals (81.82%). The only scorable band at 800 bp is specific band for Great Argus from Hala Bala wildlife sanctuary, Narathivas province. (Table 3.4, figure 3.11)

Seventeen RAPD fragments (bands) of intraspecies in Great Argus which were generated by OPC-02 primer can be scored 1300, 1200, 950, 900, 880, 800, 750, 720, 700, 650, 550, 500, 480, 450, 350, 300 and 250 base pairs (Appendix B1). From this primer with 11 individuals of Great Argus (*Argusianus argus argus*) that could be amplified only 8 individuals (72.73%). The scorable band at 700 and 500 bp are specific bands for Great Argus from Khao Pratabchang wildlife research and breeding station while 650 bp is specific band for Great Argus from Hala Bala wildlife sanctuary, Narathivas province. (Table 3.4, figure 3.12)

Fourteen RAPD fragments (bands) of intraspecies in Great Argus which were generated by OPP-03 primer can be scored (1500, 1400, 800, 720, 700, 650, 550, 500, 480, 450, 380, 340, 300 and 280 base pairs (Appendix B1). From this primer with 11 individuals of Great Argus (*Argusianus argus argus*) that could be amplified only 7 individuals (63.64%). The only scorable band at 1400 bp is specific band for Great Argus from Khao Pratabchang wildlife research and breeding station. (Table 3.4, figure 3.13)

Ten RAPD fragments (bands) of intraspecies in Great Argus which were generated by UBC-133 primer can be scored 1200, 1100, 900, 850, 780, 700, 690, 600, 580 and 450 base pairs (Appendix B1). From this primer with 11 individuals of Great Argus (*Argusianus argus argus*) that could be amplified all individuals (100%). There is no specific band for Great Argus (Table 3.4, figure 3.14)

Eleven RAPD fragments (bands) of intraspecies in Great Argus which were generated by UBC-135 primer can be scored 1200, 1000, 900, 850, 800, 680, 650, 500, 420, 320 and 250 base pairs (Appendix B1). From this primer with 11 individuals of Great Argus (*Argusianus argus argus*) that could be amplified only 9 individuals (81.82%). There is no specific band for Great Argus (Table 3.4, figure 3.15)

3.4.2.6 Analysis of genetic polymorphism of Red Junglefowl (*Gallus gallus spadiceus*) using the primer OPA-18

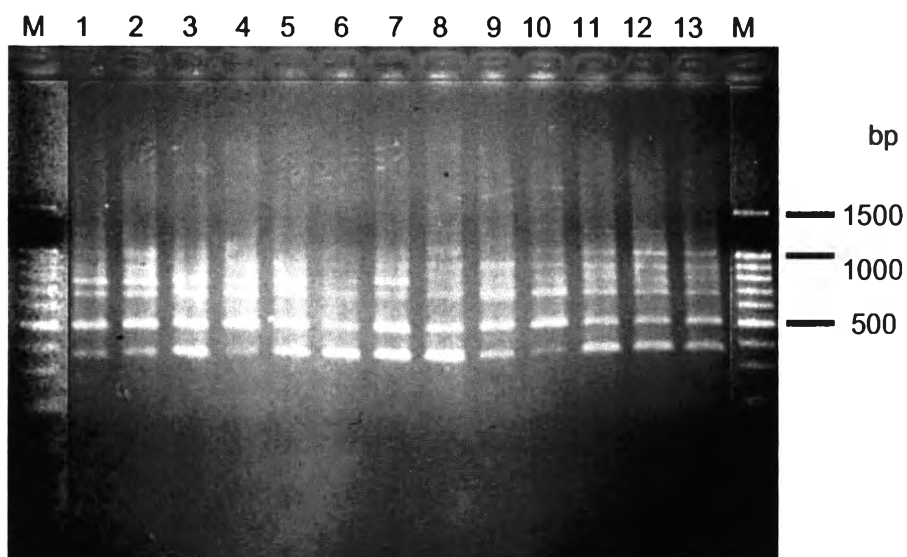


Figure 3.16 RAPD patterns of Red Junglefowl generated from primer OPA-18

3.4.2.7 Analysis of genetic polymorphism of Red Junglefowl (*Gallus gallus spadiceus*) using the primer OPC-02

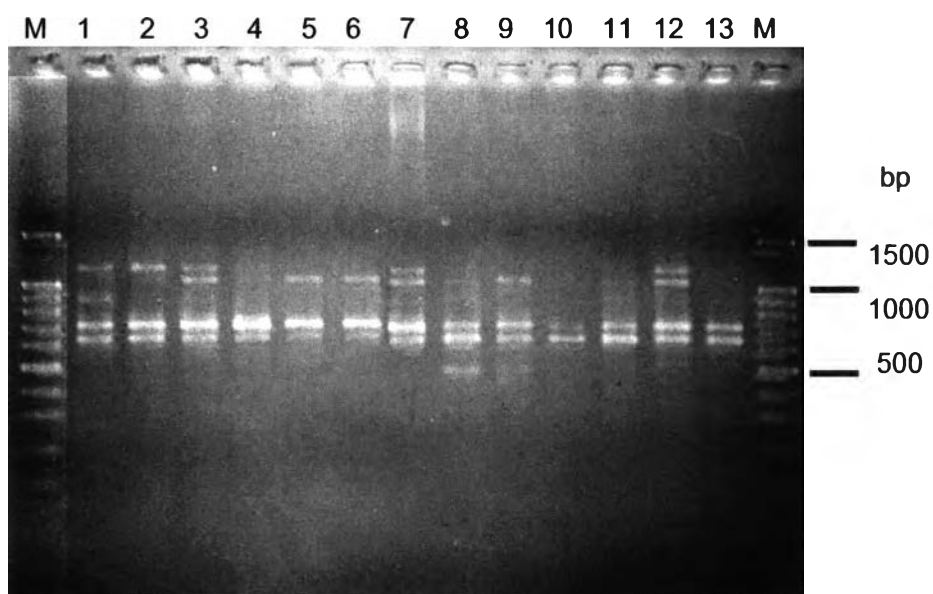


Figure 3.17 RAPD patterns of Red Junglefowl generated from primer OPC-02

3.4.2.8 Analysis of genetic polymorphism of Red Junglefowl (*Gallus gallus spadiceus*) using the primer OPP-03

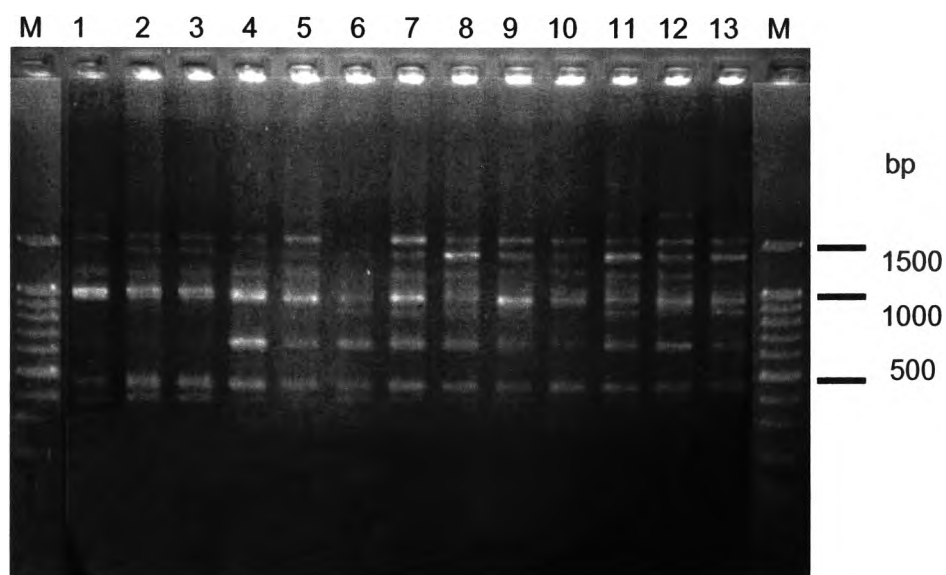


Figure 3.18 RAPD patterns of Red Junglefowl generated from primer OPP-03

3.4.2.9 Analysis of genetic polymorphism of Red Junglefowl (*Gallus gallus spadiceus*) using the primer UBC-133

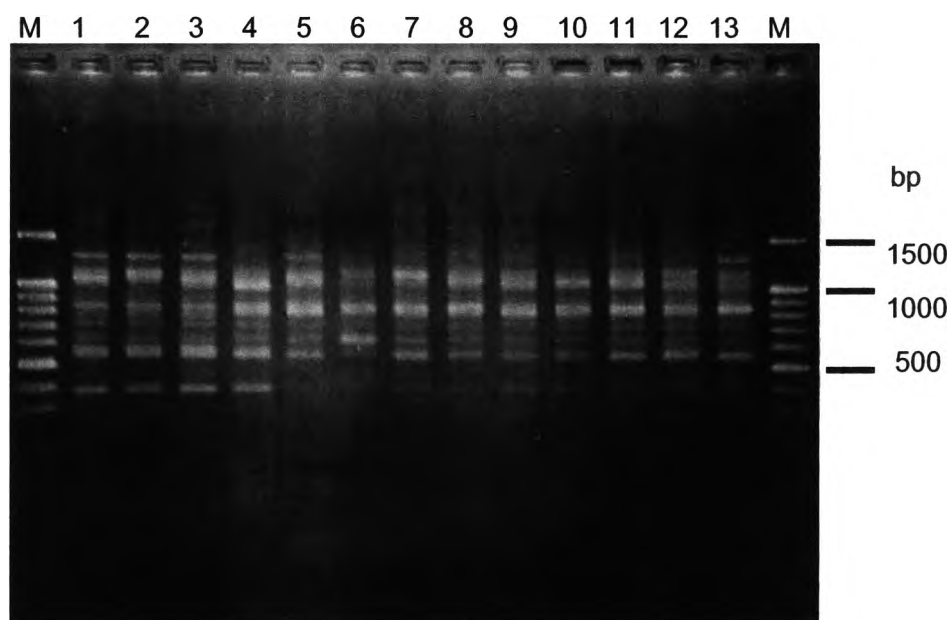


Figure 3.19 RAPD patterns of Red Junglefowl generated from primer UBC-133

3.4.2.10 Analysis of genetic polymorphism of Red Junglefowl (*Gallus gallus spadiceus*) using the primer UBC-135

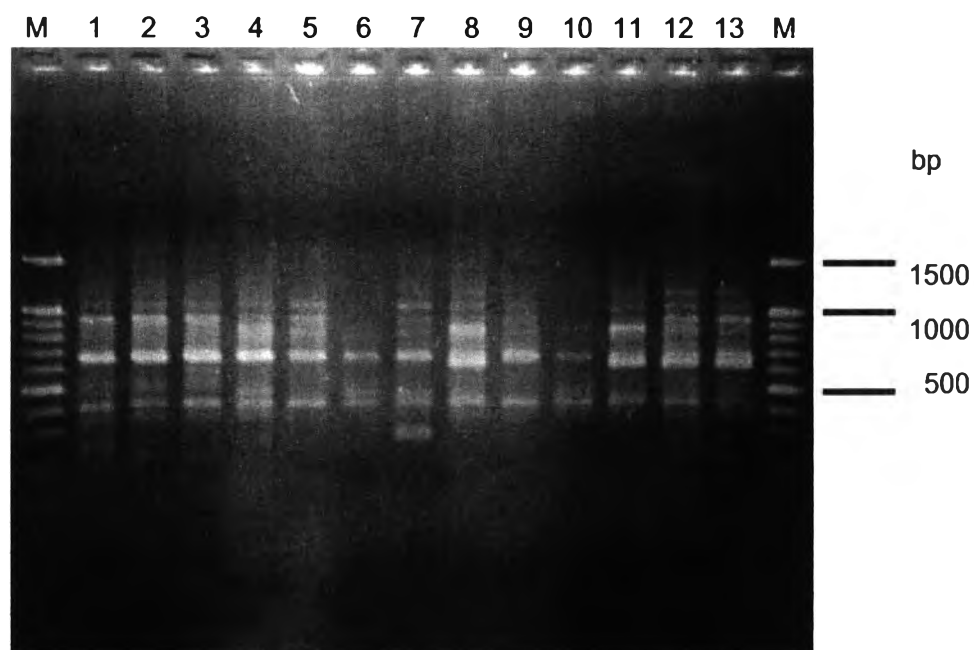


Figure 3.20 RAPD patterns of Red Junglefowl generated from primer UBC-135

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Huay Kha Kaeng Wildlife Sanctuary (HKK)
Lane 4	=	Huay Yang Pam wildlife research and breeding station (HYP)
Lane 5-7	=	Huay Kha Kaeng Wildlife Sanctuary (HKK)
Lane 8-10	=	Phatthalung wildlife research and breeding station (PTL)
Lane 11-13	=	Satun (ST)

Six RAPD fragments (bands) of intraspecies in Red Junglefowl which were generated by OPA-18 primer can be scored 1000, 900, 800, 700, 500 and 400 base pairs (shown in Appendix B2). From this primer with 13 individuals of Red Junglefowl (*Gallus gallus spadiceus*) that could be amplified all individuals (100%). There is no specific band for Red Junglefowl (Table 3.4, figure 3.16)

Six RAPD fragments (bands) of intraspecies in Red Junglefowl which were generated by OPC-02 primer can be scored 1200, 1100, 900, 720, 650 and 500 base pairs (Appendix B2). From this primer with 13 individuals of Red Junglefowl (*Gallus gallus spadiceus*) that could be amplified all individuals (100%). There is no specific band for Red Junglefowl (Table 3.4, figure 3.17)

Eight RAPD fragments (bands) of intraspecies in Red Junglefowl which were generated by OPP-03 primer can be scored 1500, 1400, 1200, 1000, 900, 650, 450 and 400 base pairs (Appendix B2). From this primer with 13 individuals of Red Junglefowl (*Gallus gallus spadiceus*) that could be amplified all individuals (100%). There is no specific band for Red Junglefowl (Table 3.4, figure 3.18)

Eight RAPD f fragments (bands) of intraspecies in Red Junglefowl which were generated by UBC-133 primer can be scored 1400, 1200, 1000, 850, 700, 600, 550 and 400 bp) (Appendix B2). From this primer with 13 individuals of Red Junglefowl (*Gallus gallus spadiceus*) that could be amplified all individuals (100%). There is no specific band for Red Junglefowl (Table 3.4, figure 3.19)

Nine RAPD fragments (bands) of intraspecies in Red Junglefowl which were generated by UBC-135 primer can be scored 1300, 1100, 950, 900, 700, 600, 500, 420 and 300 base pairs (Appendix B2). From this primer with 13 individuals of Red Junglefowl (*Gallus gallus spadiceus*) that could be amplified all individuals (100%). There is no specific band for Red Junglefowl (Table 3.4, figure 3.20)

3.4.2.11 Analysis of genetic polymorphism of Siamese Fireback (*Lophura diardi*) using the primer OPA-18

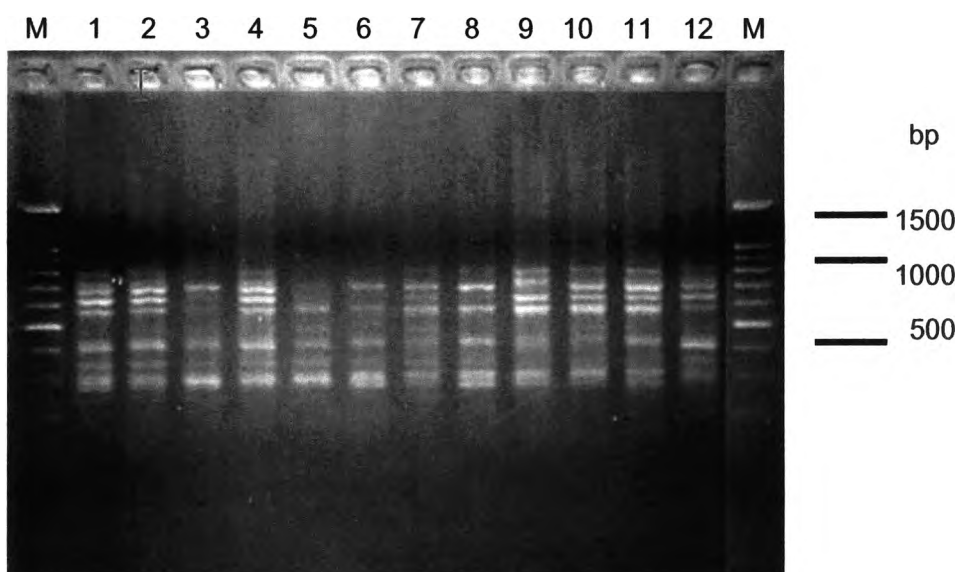


Figure 3.21 RAPD patterns of Siamese Fireback generated from primer OPA-18

3.4.2.12 Analysis of genetic polymorphism of Siamese Fireback (*Lophura diardi*) using the primer OPC-02

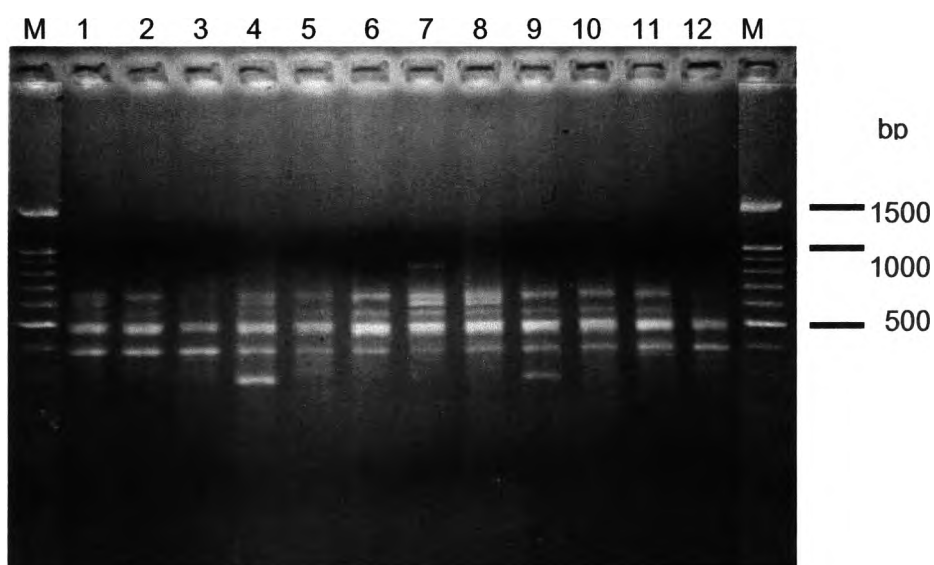


Figure 3.22 RAPD patterns of Siamese Fireback generated from primer OPC-02

3.4.2.13 Analysis of genetic polymorphism of Siamese Fireback (*Lophura diardi*) using the primer OPP-03

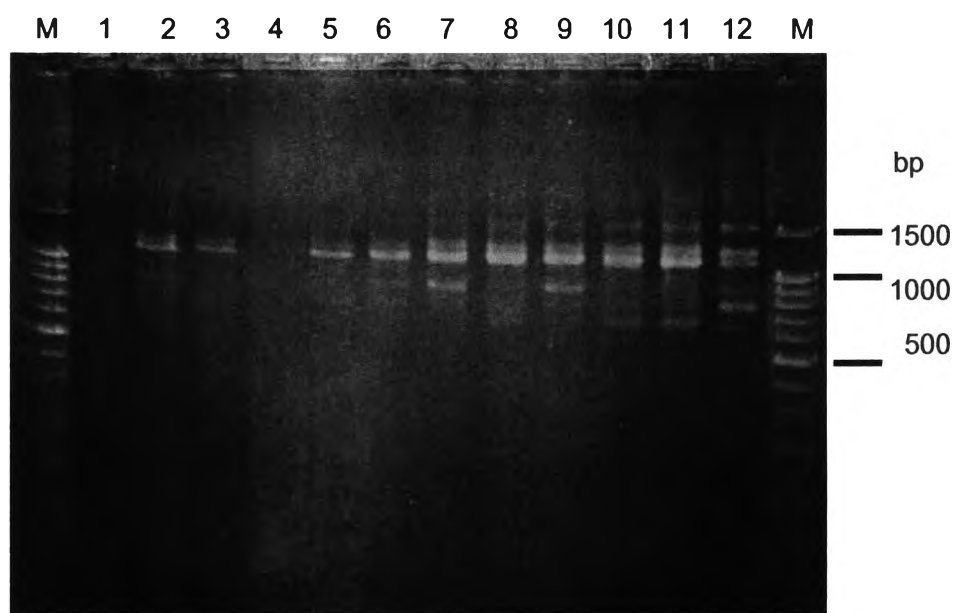


Figure 3.23 RAPD patterns of Siamese Fireback generated from primer OPP-03

3.4.2.14 Analysis of genetic polymorphism of Siamese Fireback (*Lophura diardi*) using the primer UBC-133

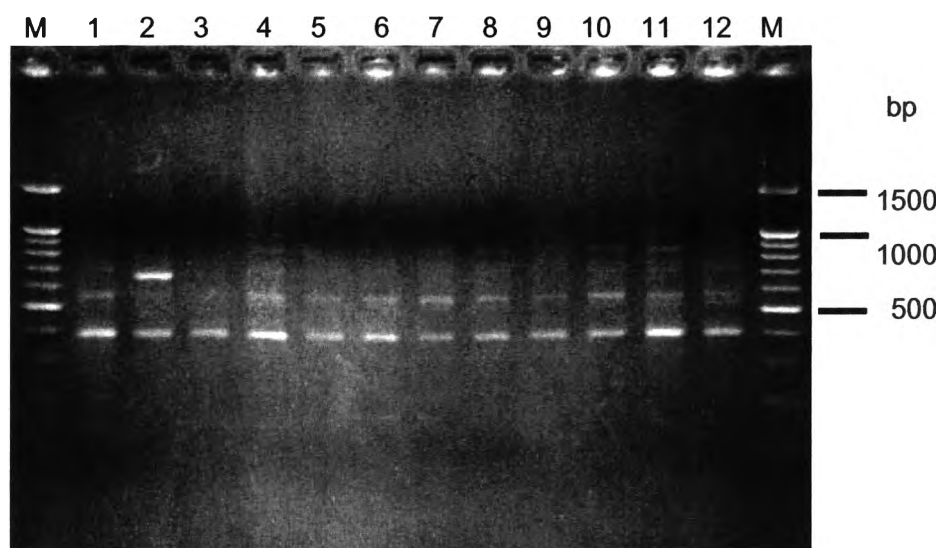


Figure 3.24 RAPD patterns of Siamese Fireback generated from primer UBC-133

3.4.2.15 Analysis of genetic polymorphism of Siamese Fireback (*Lophura diardi*) using the primer UBC-135

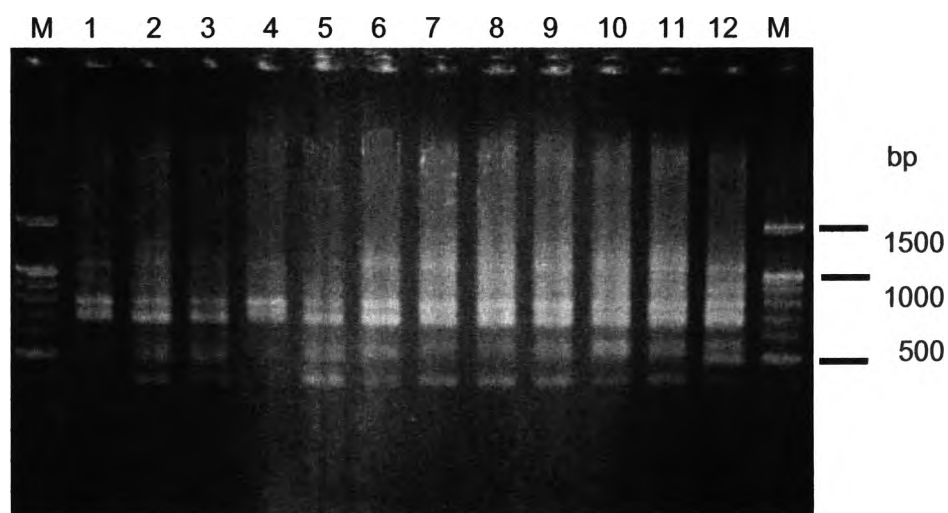


Figure 3.25 RAPD patterns of Siamese Fireback generated from primer UBC-135

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Phu Khieu wildlife research and breeding station (PK)
Lane 4-6	=	Individual farm in Amphoe Sattaheep, Chonburi Province (STH)
Lane 7-9	=	Khao Pratabcharng wildlife research and breeding station (PTC)
Lane 10-12	=	Khao Soi Dao wildlife research and breeding station (SD)

Ten RAPD fragments (bands) of intraspecies in Siamese Fireback which were generated by OPA-18 primer can be scored 850, 750, 700, 650, 580, 500, 420, 350, 300 and 280 base pairs (Appendix B3). From this primer with 12 individuals of Siamese Fireback (*Lophura diardi*) that could be amplified all individuals (100%). There is no specific band for Siamese Fireback (Table 3.4, figure 3.21)

Seven RAPD fragments (bands) of intraspecies in Siamese Fireback which were generated by OPC-02 primer can be scored 800, 650, 600, 550, 500, 400 and 300 base pairs (Appendix B3). From this primer with 12 individuals of Siamese Fireback (*Lophura diardi*) that could be amplified all individuals (100%). There is no specific band for Siamese Fireback (Table 3.4, figure 3.22)

Six RAPD fragments (bands) of intraspecies in Siamese Fireback which were generated by OPP-03 primer can be scored 1500, 1300, 1200, 900, 800 and 650 base pairs (Appendix B3). From this primer with 12 individuals of Siamese Fireback (*Lophura diardi*) that could be amplified only 10 individuals (83.33%). There is no specific band for Siamese Fireback (Table 3.4, figure 3.23)

Four RAPD fragments (bands) of intraspecies in Siamese Fireback which were generated by UBC-133 primer can be scored 900, 700, 550 and 400 base pairs (Appendix B3). From this primer with 12 individuals of Siamese Fireback (*Lophura diardi*) that could be amplified all individuals (100%). There is no specific band for Siamese Fireback (Table 3.4, figure 3.24)

Seven RAPD fragments (bands) of intraspecies in Siamese Fireback which were generated by UBC-133 primer can be scored 1100, 900, 800, 700, 550, 500 and 400 base pairs (Appendix B3). From this primer with 12 individuals of Siamese Fireback (*Lophura diardi*) that could be amplified all individuals (100%). There is no specific band for Siamese Fireback (Table 3.4, figure 3.25)

3.4.2.16 Analysis of genetic polymorphism of Green Peafowl (*Pavo muticus imperator*) using the primer OPA-18

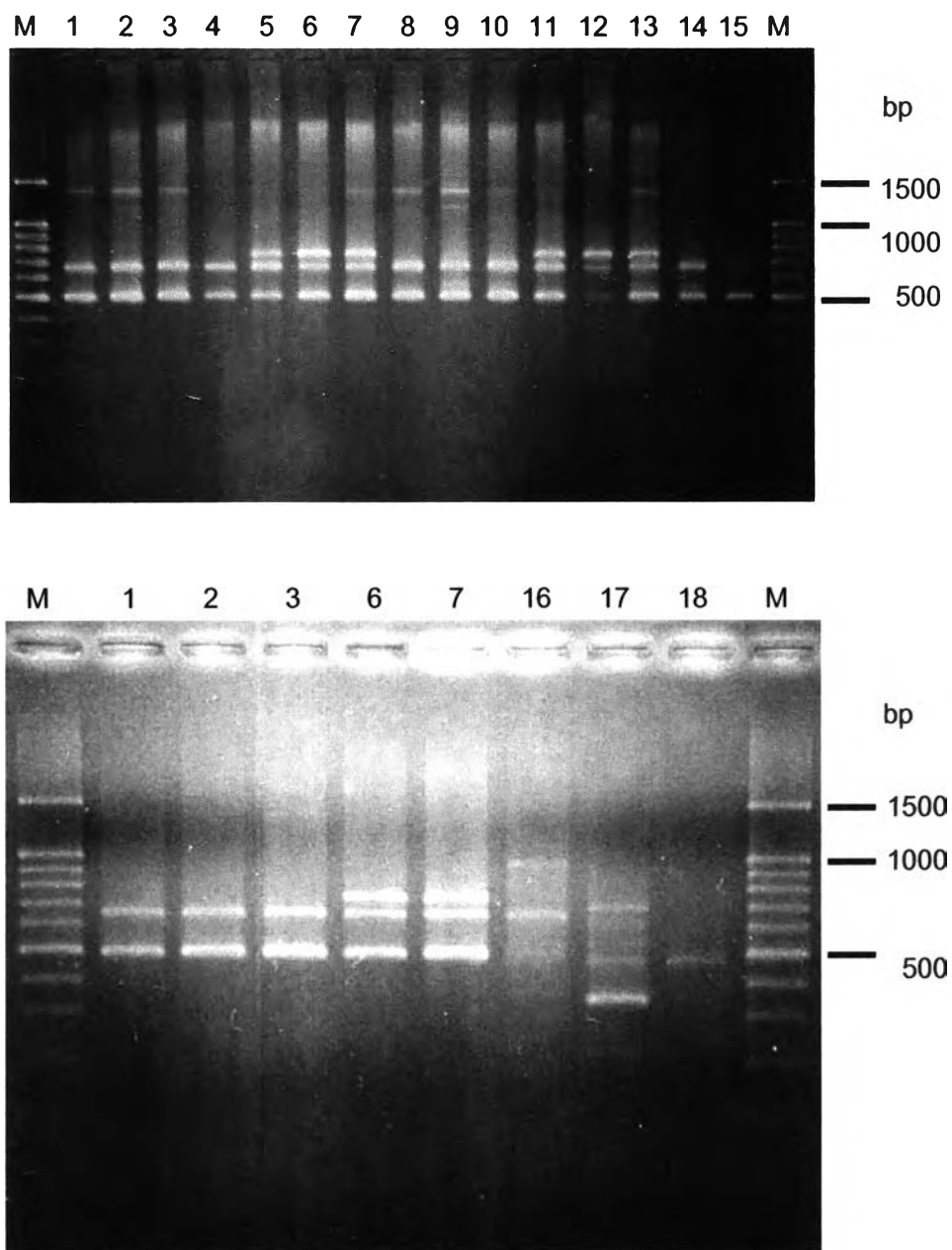


Figure 3.26 RAPD patterns of Green Peafowl generated from primer OPA-18

3.4.2.17 Analysis of genetic polymorphism of Green Peafowl (*Pavo muticus imperator*) using the primer OPC-02

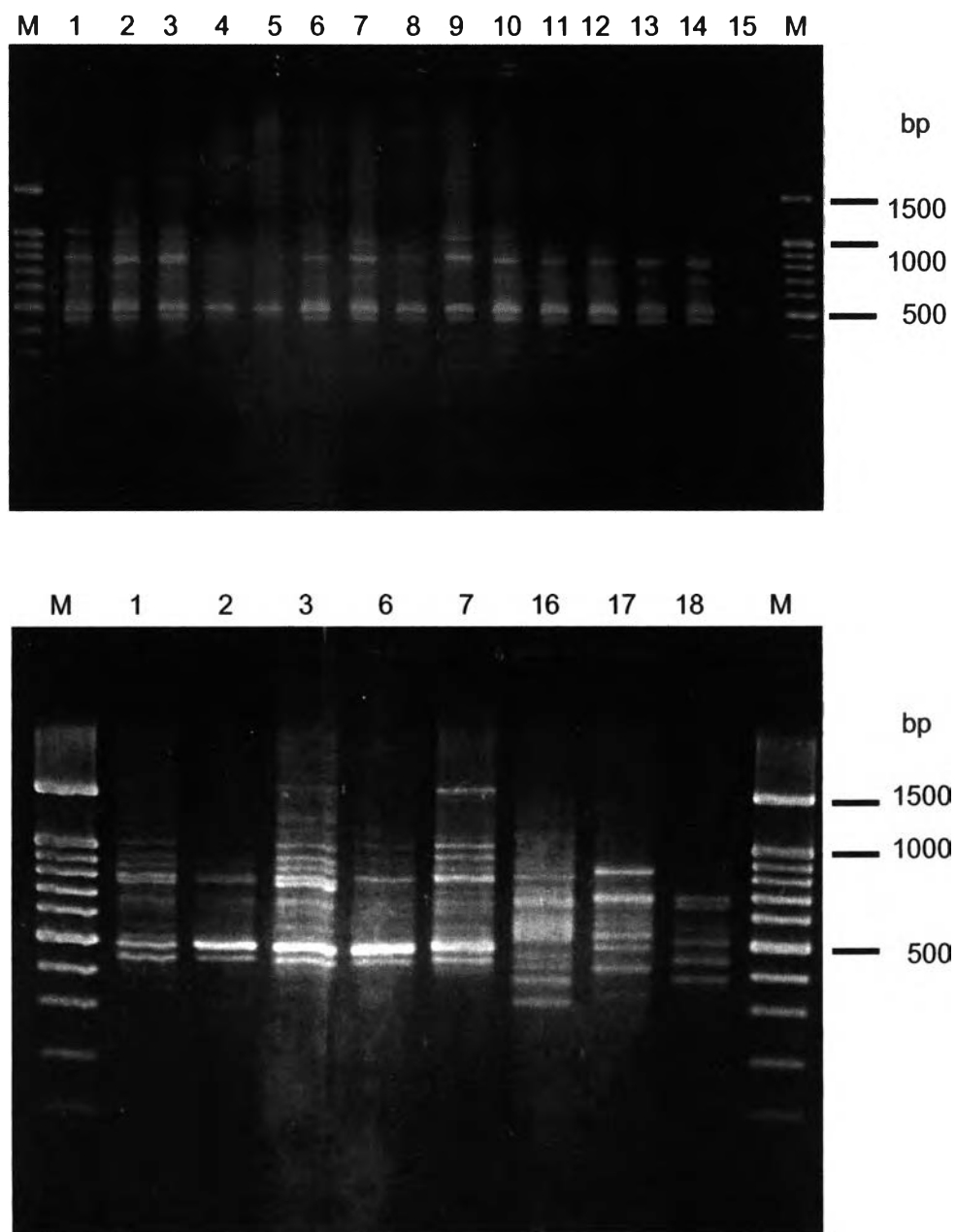


Figure 3.27 RAPD patterns of Green Peafowl generated from primer OPC-02

3.4.2.18 Analysis of genetic polymorphism of Green Peafowl (*Pavo muticus imperator*) using the primer OPP-03

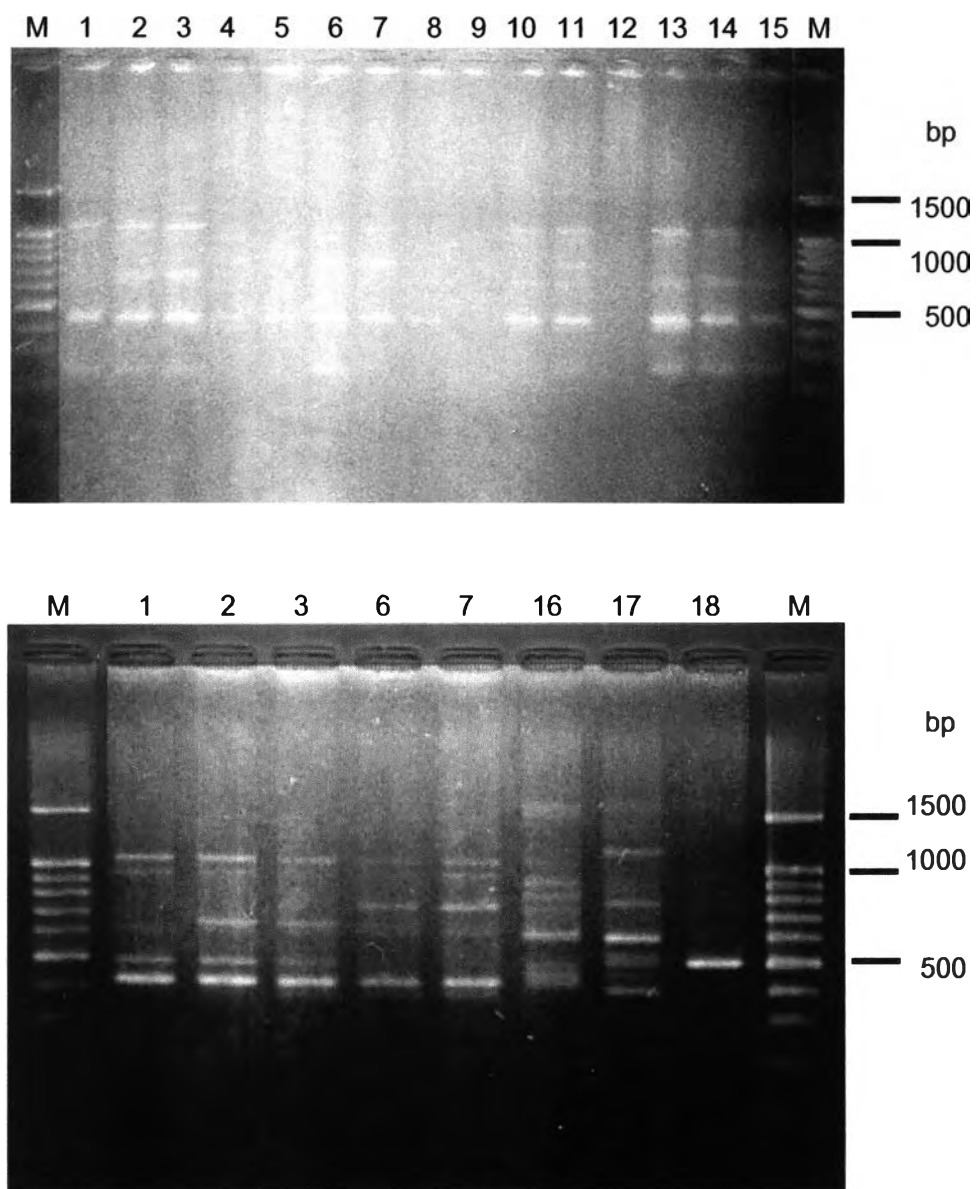


Figure 3.28 RAPD patterns of Green Peafowl generated from primer OPP-03

3.4.2.19 Analysis of genetic polymorphism of Green Peafowl (*Pavo muticus imperator*) using the primer UBC-133

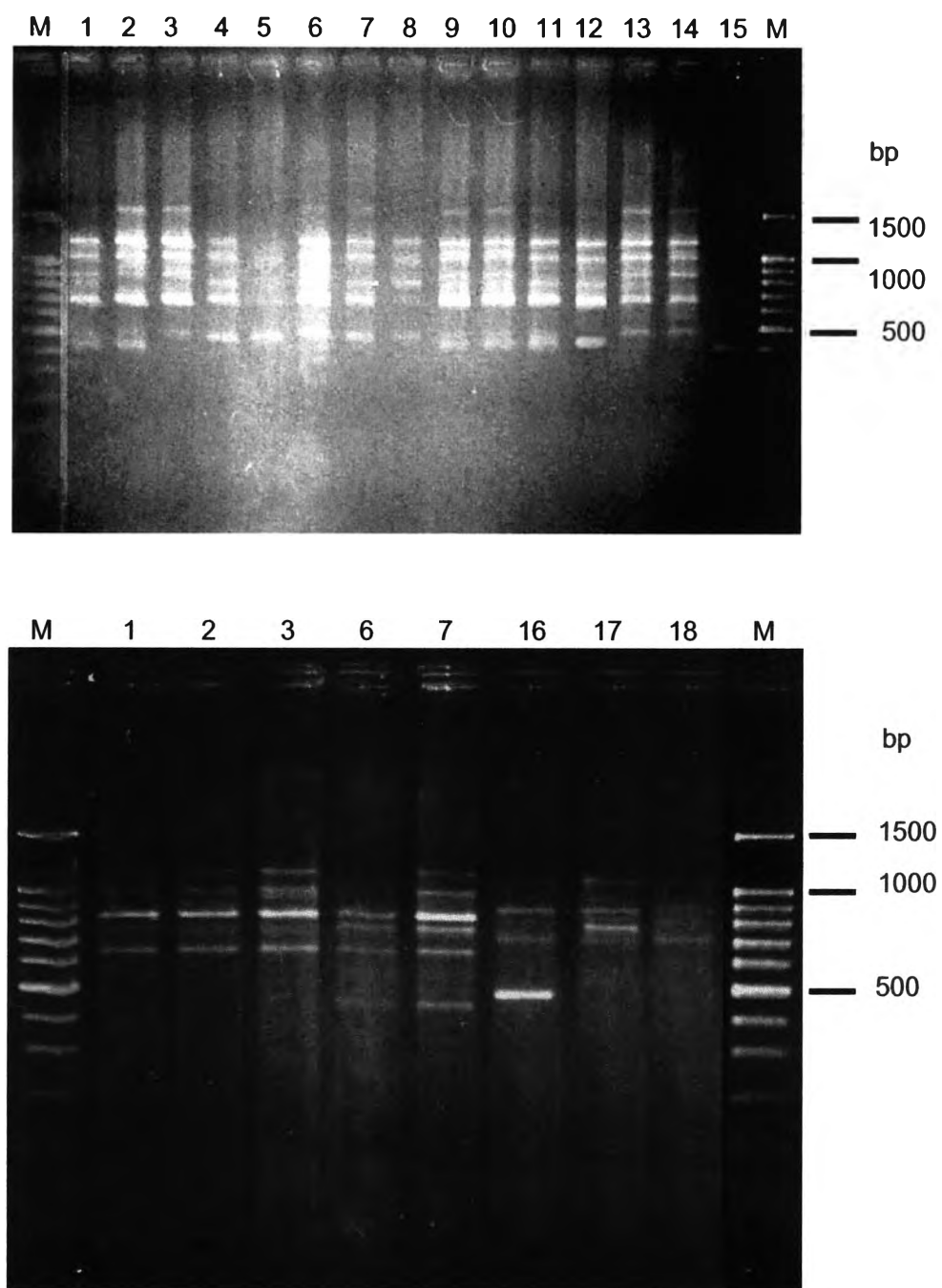


Figure 3.29 RAPD patterns of Green Peafowl generated from primer UBC-133

3.4.2.20 Analysis of genetic polymorphism of Green Peafowl (*Pavo muticus imperator*) using the primer UBC-135

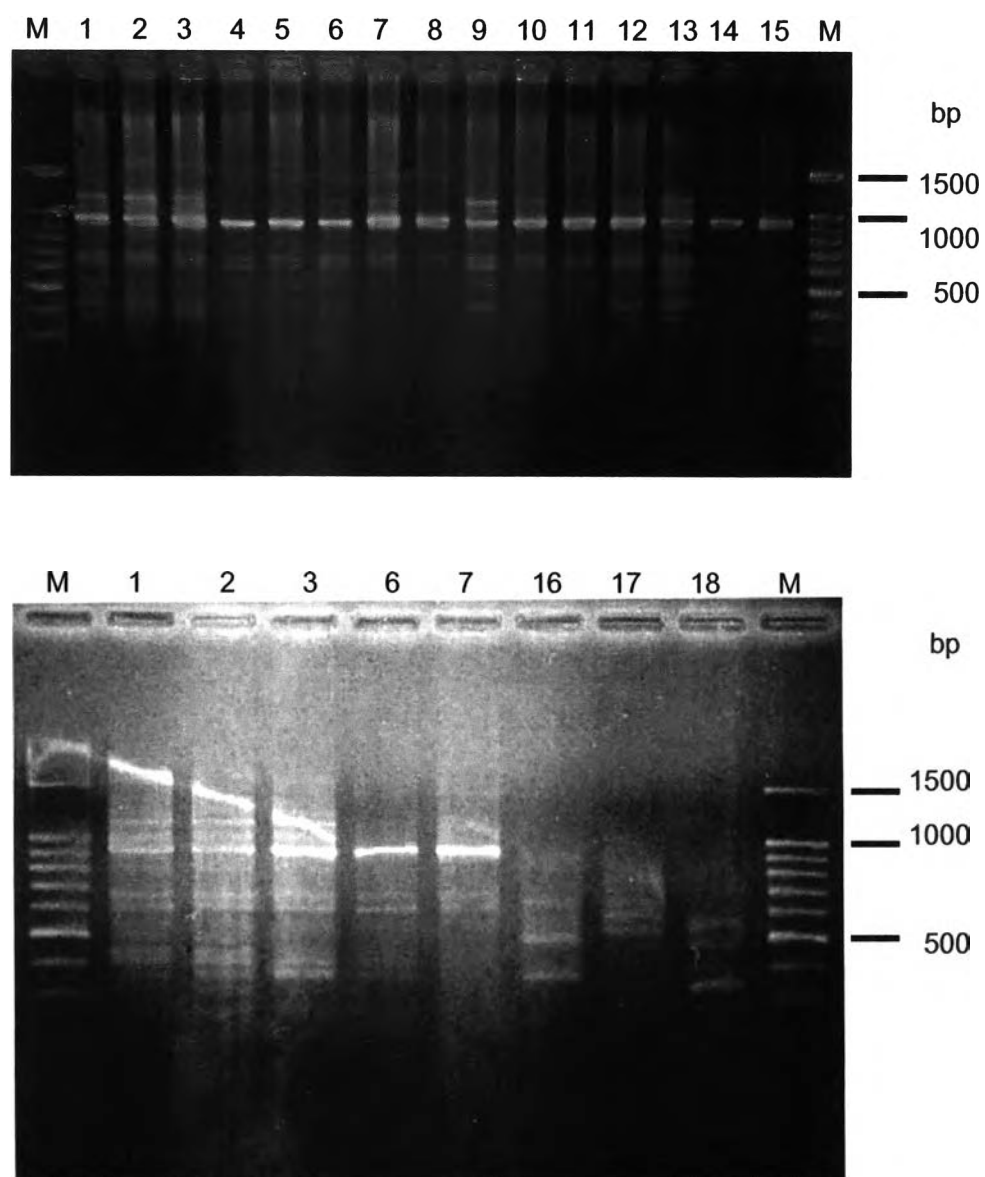


Figure 3.30 RAPD patterns of Green Peafowl generated from primer UBC-135

Lane M	=	1.5 kb+100 bp standard marker
Lane 1-3	=	Khao Pratabcharng wildlife research and breeding station (PTC)
Lane 4	=	Huay Yang Parn wildlife research and breeding station (HYP)
Lane 5	=	Pong Wild, Payao province (P)
Lane 6	=	Lumpang Wild, Lumpang province (LP)
Lane 7	=	Chiang Khong Wild, Chiang Rai province (CK)
Lane 8-10	=	Surin Wild (SR)
Lane 11-12	=	Phu Khieu wildlife research and breeding station (PK)
Lane 13-15	=	Khao Soi Dao wildlife research and breeding station (SD)
Lane 13-15	=	Khao Soi Dao wildlife research and breeding station (SD)
Lane 16-18	=	Ping watershed, Amphoe Li, Lumpun (Ping)

Eleven RAPD fragments (bands) of intraspecies in Green Peafowl which were generated by OPA-18 primer can be scored 1400, 1300, 1000, 750, 700, 650, 600, 550, 500, 480 and 350 base pairs (Appendix B4). From this primer with 18 individuals of Green Peafowl (*Pavo muticus imperator*) that could be amplified all individuals (100%). There is no specific band for Green Peafowl (Table 3.4, figure 3.26).

Ten RAPD fragments (bands) of intraspecies in Green Peafowl which were generated by OPC-02 primer can be scored 1000, 950, 800, 700, 500, 450, 420, 400, 350 and 320 base pairs (Appendix B4). From this primer with 18 individuals of Green Peafowl (*Pavo muticus imperator*) that could be amplified only 16 individuals (88.89%). There is no specific band for Green Peafowl (Table 3.4, figure 3.27).

Ten RAPD fragments (bands) of intraspecies in Green Peafowl which were generated by OPP-03 primer can be scored 1100, 950, 900, 850, 700, 650, 600, 500, 450 and 400 base pairs (Appendix B4). From this primer with 18 individuals of Green Peafowl (*Pavo muticus imperator*) that could be amplified all individuals (100%). There is no specific band for Green Peafowl (Table 3.4, figure 3.28).

Nine RAPD fragments (bands) of intraspecies in Green Peafowl which were generated by UBC-133 primer can be scored 1300, 1100, 900, 800, 700, 650, 500, 450 and 400 base pairs (Appendix B4). From this primer with 18 individuals of Green Peafowl (*Pavo muticus imperator*) that could be amplified all individuals (100%). The only scorable band at 700 bp is specific band for Green Peafowl from Ping watershed, Amphoe Li, Lumpun province (Table 3.4, figure 3.29).

Nine RAPD fragments (bands) of intraspecies in Green Peafowl which were generated by UBC-133 primer can be scored 1200, 950, 680, 600, 550, 500, 450, 400 and 350 base pairs (Appendix B4). From this primer with 18 individuals of Green Peafowl (*Pavo muticus imperator*) that could be amplified all individuals (100%). There is no specific band for Green Peafowl (Table 3.4, figure 3.30)

3.4.2.21 Analysis of genetic polymorphism of Crested Wood Partridge (*Rollulus roulroul*) using the primer OPA-18

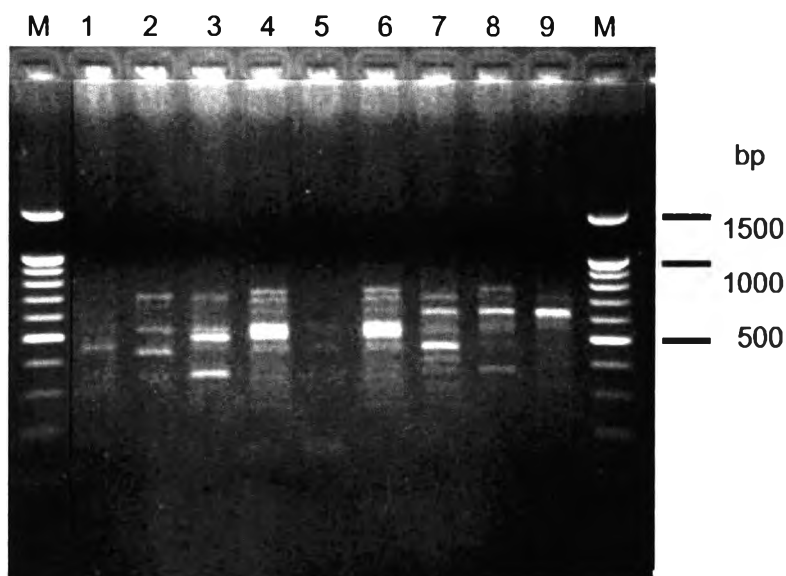


Figure 3.31 RAPD patterns of Crested Wood Partridge generated from primer OPA-18

3.4.2.22 Analysis of genetic polymorphism of Crested Wood Partridge (*Rollulus roulroul*) using the primer OPC-02

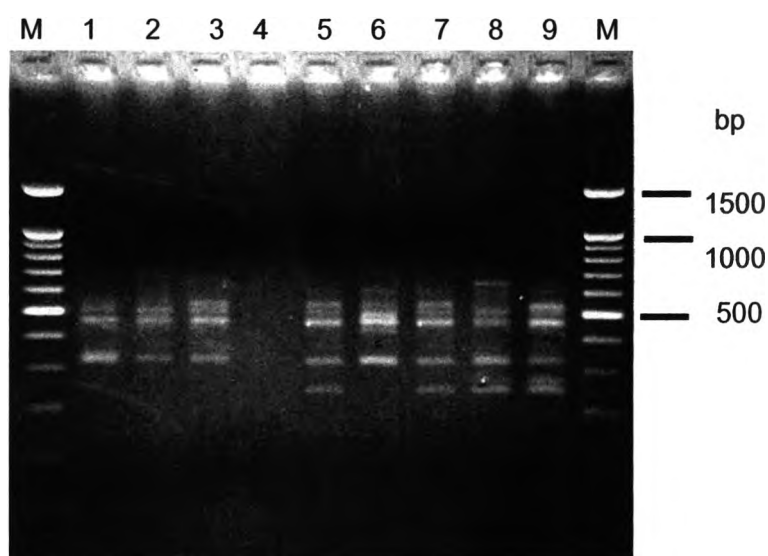


Figure 3.32 RAPD patterns of Crested Wood Partridge generated from primer OPC-02

3.4.2.23 Analysis of genetic polymorphism of Crested Wood Partridge (*Rollulus roulroul*) using the primer OPP-03

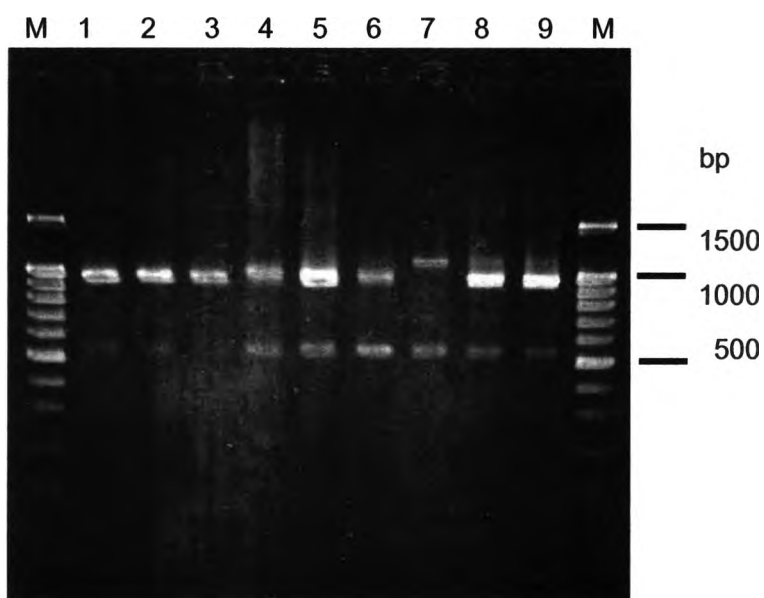


Figure 3.33 RAPD patterns of Crested Wood Partridge generated from primer OPP-03

3.4.2.24 Analysis of genetic polymorphism of Crested Wood Partridge (*Rollulus roulroul*) using the primer UBC-133

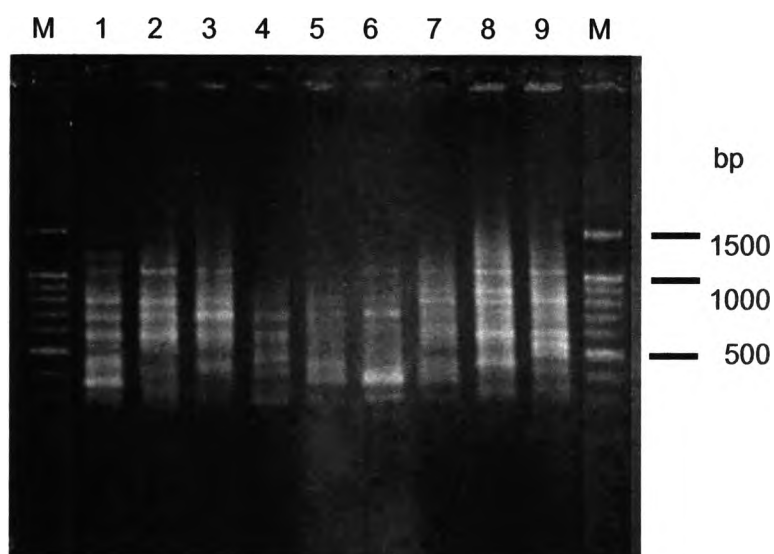


Figure 3.34 RAPD patterns of Crested Wood Partridge generated from primer UBC-133

3.4.2.25 Analysis of genetic polymorphism of Crested Wood Partridge (*Rollulus roulroul*) using the primer UBC-135

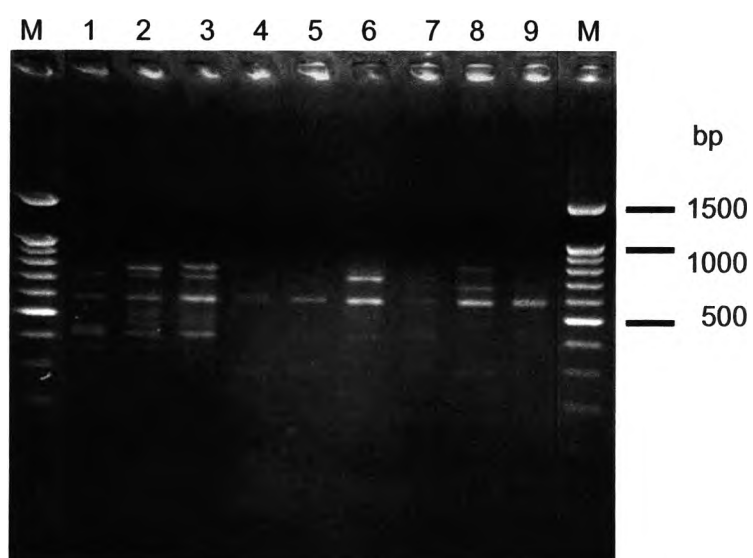


Figure 3.35 RAPD patterns of Crested Wood Partridge generated from primer UBC-135

Lane M	=	1.5 kb+100 bp standard marker
Lane 1	=	Individual Farm in Bangkok (BKK)
Lane 2-3	=	Khok Mai Rua wildlife research and breeding station (KMR)
Lane 4-6	=	Amphoe Sattaheep, Chonburi Province (STH)
Lane 7-9	=	Khao Pratabcharng wildlife research and breeding station (PTC)

Nine RAPD fragments (bands) of intraspecies in Crested Wood Partridge which were generated by OPA-18 primer can be scored 800, 700, 650, 550, 500, 480, 450, 420 and 380 base pairs (Appendix B5). From this primer with 9 individuals of Crested Wood Partridge (*Rollulus roulroul*) that could be amplified only 8 individuals (88.89%). There is no specific band for Crested Wood Partridge (Table 3.4, figure 3.31).

Nine RAPD fragments (bands) of intraspecies in Crested Wood Partridge which were generated by OPC-02 primer can be scored 650, 600, 550, 500, 480, 450, 340, 280 and 250 base pairs (Appendix B5). From this primer with 9 individuals of Crested Wood Partridge (*Rollulus roulroul*) that could be amplified only 8 individuals (88.89%). There is no specific band for Crested Wood Partridge (Table 3.4, figure 3.32).

Four RAPD fragments (bands) of intraspecies in Crested Wood Partridge which were generated by OPP-03 primer can be scored 1200, 980, 950 and 550 base pairs (Appendix B5). From this primer with 9 individuals of Crested Wood Partridge (*Rollulus roulroul*) that could be amplified all individuals (100%). The only scorable band at 1200 bp is specific band for Crested Wood Partridge from Khao Pratabchang wildlife research and breeding station (Table 3.4, figure 3.33).

Twelve RAPD fragments (bands) of intraspecies in Crested Wood Partridge which were generated by UBC-133 primer can be scored 1100, 900, 800, 700, 600, 550, 500, 450, 420, 400, 350 and 300 base pairs (Appendix B5). From this primer with 9 individuals of Crested Wood Partridge (*Rollulus roulroul*) that could be amplified all individuals (100%). There is no specific band for Crested Wood Partridge (Table 3.4, figure 3.34).

Ten RAPD fragments (bands) of intraspecies in Crested Wood Partridge which were generated by UBC-133 primer can be scored 800, 700, 650, 600, 500, 450, 420, 410, 370 and 300 base pairs (Appendix B5). From this primer with 9 individuals of Crested Wood Partridge (*Rollulus roulroul*) that could be amplified all individuals (100%). The only scorable band at 500 bp is specific band for Crested Wood Partridge from Khok Mai Rua wildlife research and breeding station, Narathivas province (Table 3.4, figure 3.35).

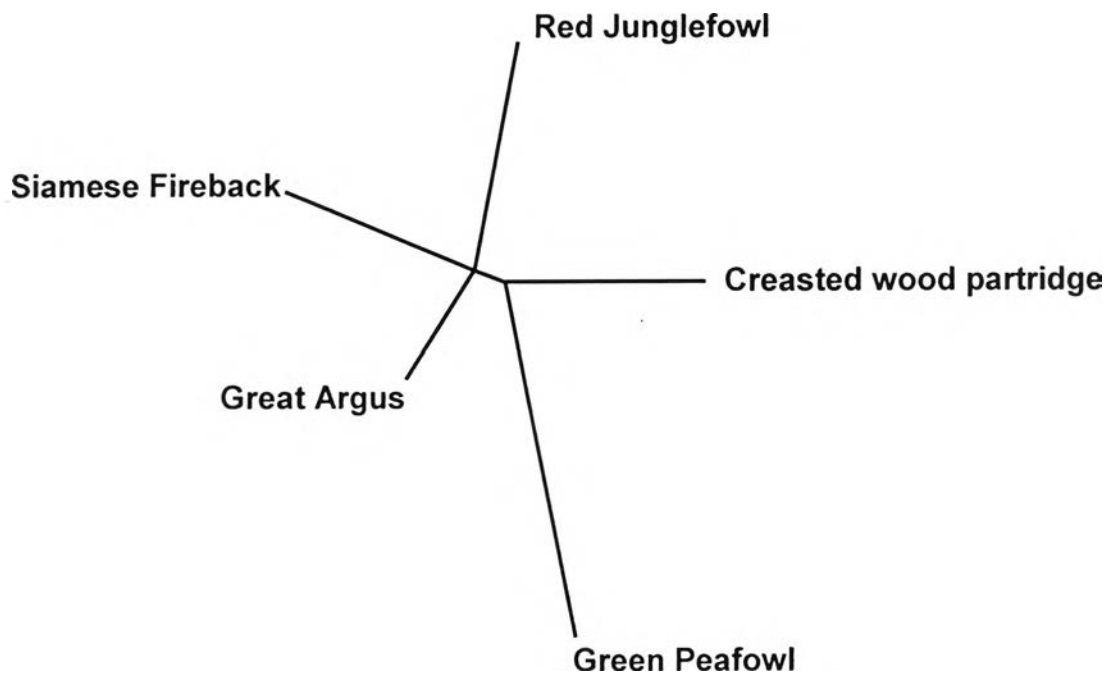
3.5 Genetic distance and Phylogenetic Relationships

Interspecies

The genetic distance of interspecies of five species in gallopheasants were shown in Table 3.5. Inferred phylogenetic tree by neighbor-joining method which were constructed from average genetic distances between pairs of each population indicated five separate groups (Figure 3.36). The average genetic distance was ranging from 0.2297-0.4519.

Table 3.5 Shown average genetic distance between interspecies which were analyzed by RAPD

Species	Red Junglefowl	Great Argus	Crested Wood Partridge	Siamese Fireback	Green Peafowl
Red Junglefowl	*****				
Great Argus	0.2521	*****			
Crested Wood Partridge	0.2742	0.2433	*****		
Siamese Fireback	0.2715	0.2297	0.3131	*****	
Green Peafowl	0.4519	0.3056	0.3493	0.3905	*****



0.1

Figure 3.36 A Neighbor-joining tree indicating relationship of interspecies based on genetic distance between pairs of each population by using RAPD-PCR analysis.

Intraspecies

The genetic distance of each taxon in intraspecies were shown in Table 3.6-3.10. A neighbor-joining tree constructed from average genetic distances between pairs of each population indicated three separated groups corresponding to Great Argus. The average genetic distance within Great Argus populations are ranging from 0.0706-0.2599. Between each population of *A. argus argus* shown genetic distance (0.2118-0.2824) but *A. argus argus* from Hala Bala Wildlife Sanctuary which were shown closely related to *A. argus argus* from Soi Dao wildlife research and breeding station have closely relationship in genetic distance which were less than other population (0.0706). (Figure 3.37)

Red Junglefowl are separated into three groups and the average genetic distance within Red Junglefowl populations are ranging from 0.0889-0.2283. Red Junglefowl from Huay Kha Kheang Wildlife Sanctuary is the intermediate population between Red Junglefowl from Huay Yang Parn and from the south population: Patthalung and Satun (0.1930-0.2283) while Red Junglefowl from Patthalung and Satun were the most closely related population. The average genetic distance of both population from the south was 0.0889 (Figure 3.38).

Siamese Fireback are separated into three different groups and the average genetic distance of Siamese Fireback population ranging from 0.0801-0.4500. The population from PK and STH were the most closely related population (0.0801) while Siamese Fireback from Pratabchang and Soi Dao population are the second closely related (0.1626) (Figure 3.39).

Green Peafowl are separated into two groups and the average genetic distance of among populations are ranging from 0.0710-0.5949. The Ping watershed population has genetic distance in large number than the others (Figure 3.40).



Crested Wood Partridge are separated into four different groups and the average genetic distance of Crested Wood Partridge populations are ranging from 0.2376-0.3990 (Figure 3.41).

Table 3.6 Average genetic distance between population in intraspecies of Great Argus (*Argusianus argus argus*) which were analyzed by RAPD using primer OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135

	HLBL	PTC	SD	STH
HLBL	*****			
PTC	0.2824	*****		
SD	0.0706	0.2118	*****	
STH	0.2599	0.2595	0.2196	*****

Table 3.7 average genetic distances between population in intraspecies of Red Junglefowl (*Gallus gallus spadiceus*) which was analyzed by RAPD using primer OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135

	HKK	HYP	PTL	ST
HKK	*****			
HYP	0.2060	*****		
PTL	0.0909	0.1930	*****	
ST	0.2002	0.2283	0.0889	*****

Table 3.8 average genetic distances between population in intraspecies of Siamese Fireback (*Lophura diardi*) which was analyzed by RAPD using primer OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135

	PK	STH	PTC	SD
PK	*****			
STH	0.0801	*****		
PTC	0.2726	0.2341	*****	
SD	0.4207	0.4500	0.1626	*****

Table 3.9 average genetic distances between population in intraspecies of Green Peafowl (*Pavo muticus imperator*) which were analyzed by RAPD using primer OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135

	PTC	HYP	PONG	LP	CK	SR	PK	SD	PING
PTC	*****								
HYP	0.1881	*****							
PONG	0.2933	0.0710	*****						
LP	0.3037	0.2560	0.3096	*****					
CK	0.1197	0.1982	0.2062	0.1715	*****				
SR	0.1349	0.1219	0.1734	0.1687	0.1343	*****			
PK	0.2722	0.1590	0.1108	0.0845	0.1787	0.0760	*****		
SD	0.1866	0.1430	0.0984	0.1955	0.2383	0.1211	0.1180	*****	
PING	0.5760	0.4186	0.5949	0.4608	0.5337	0.4203	0.4073	0.2881	*****

Table 3.10 average genetic distances between population in intraspecies of Crested Wood Partridge (*Rollulus roulroul*) which were analyzed by RAPD using primer OPA-18, OPC-02, OPP-03, UBC-133 and UBC-135

	BKK	KMR	STH	PTC
BKK	*****			
KMR	0.2638	*****		
STH	0.3236	0.3912	*****	
PTC	0.3990	0.2376	0.3571	*****

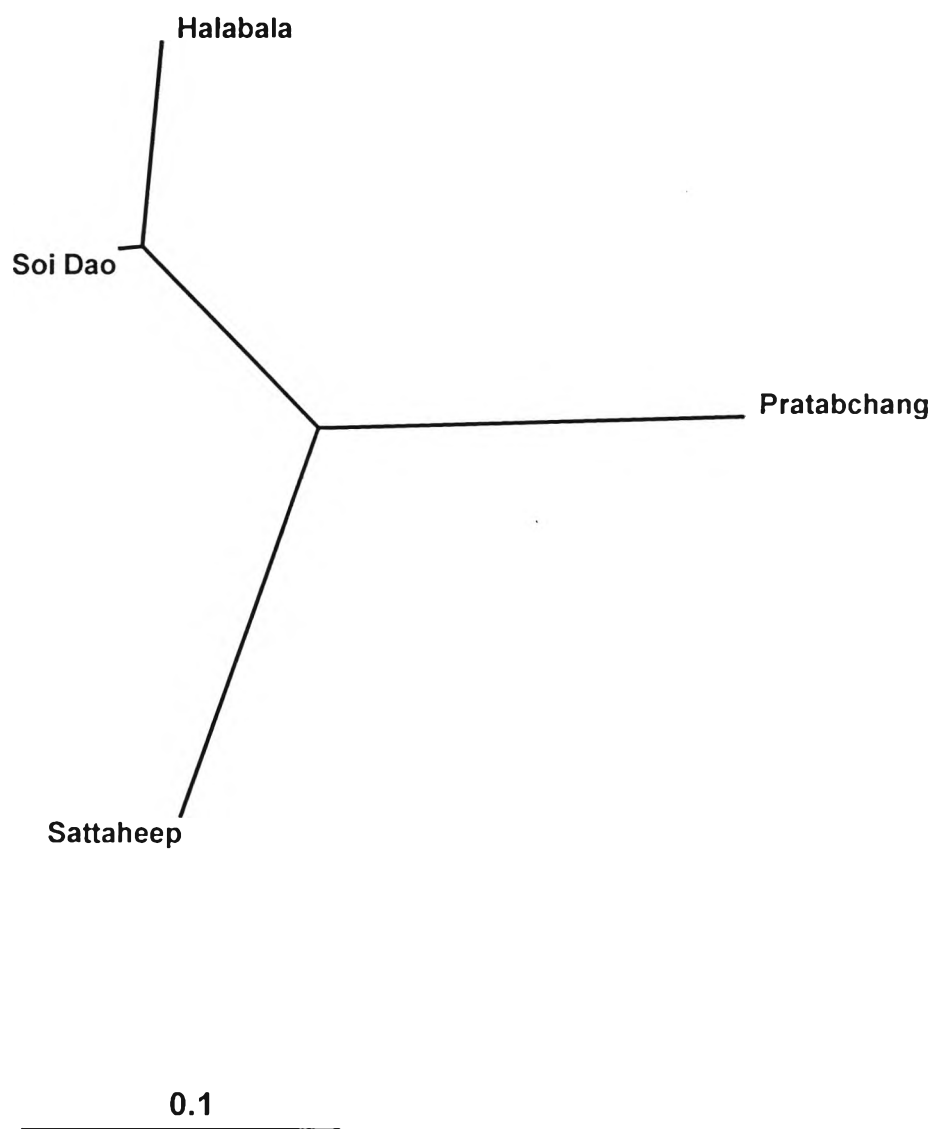


Figure 3.37 A neighbor-joining tree indicating relationships of intraspecies of Great Argus (*Argusianus argus argus*) based on genetic distance between pairs of each location by using RAPD-PCR analysis.

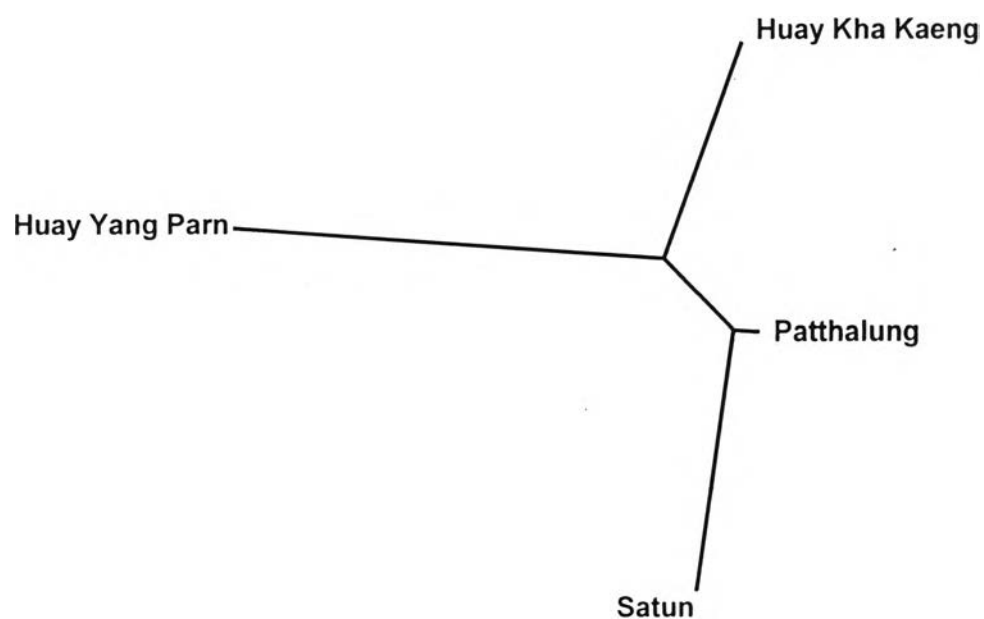


Figure 3.38 A neighbor-joining tree indicating relationship of intraspecies of Red Junglefowl (*Gallus gallus spadiceus*) based on genetic distance between pairs of each location by using RAPD-PCR analysis.

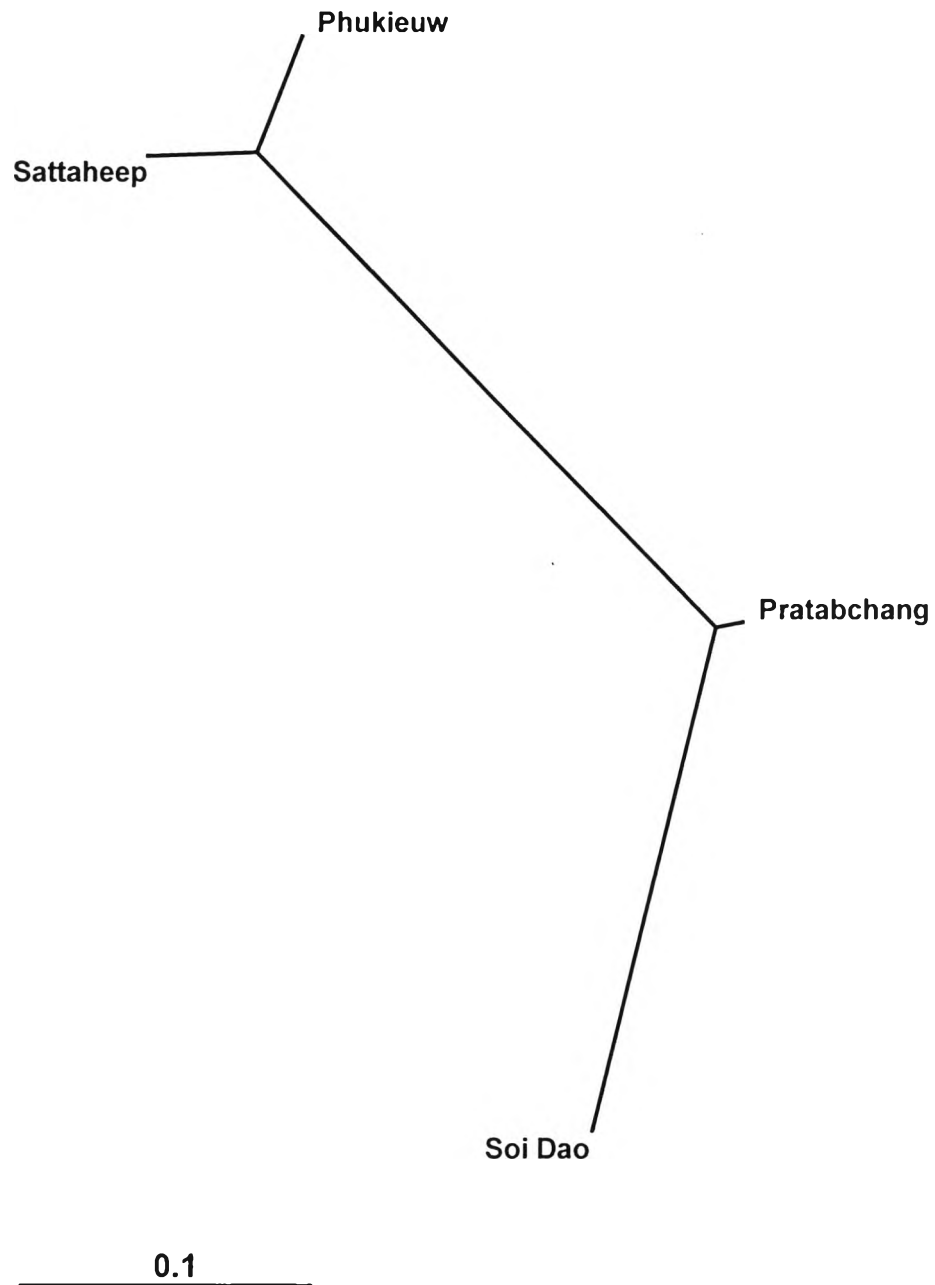


Figure 3.39 A neighbor-joining tree indicating relationship of intraspecies of Siamese Fireback (*Lophura diardi*) based on genetic distance between pairs of each location by using RAPD-PCR analysis.

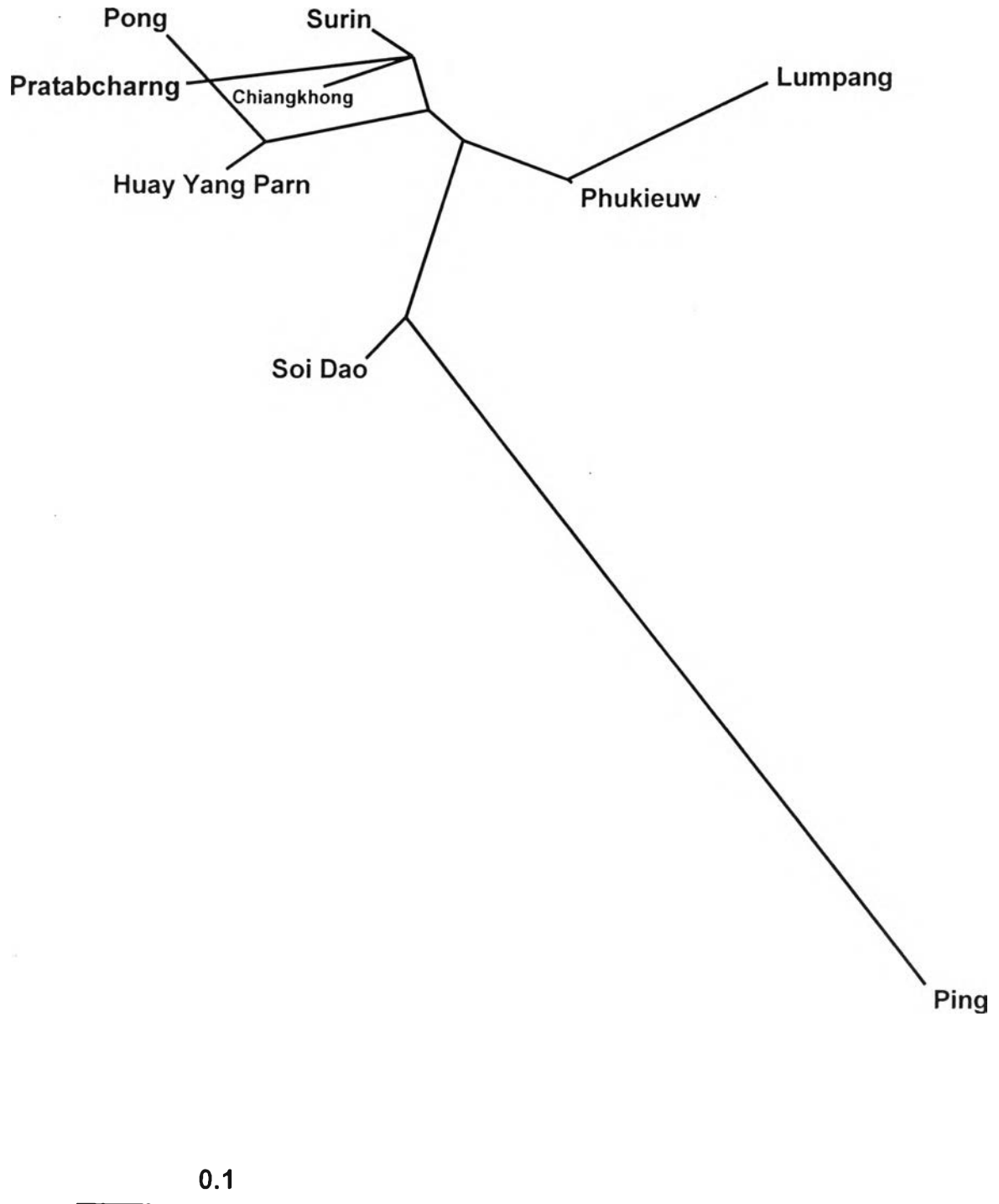
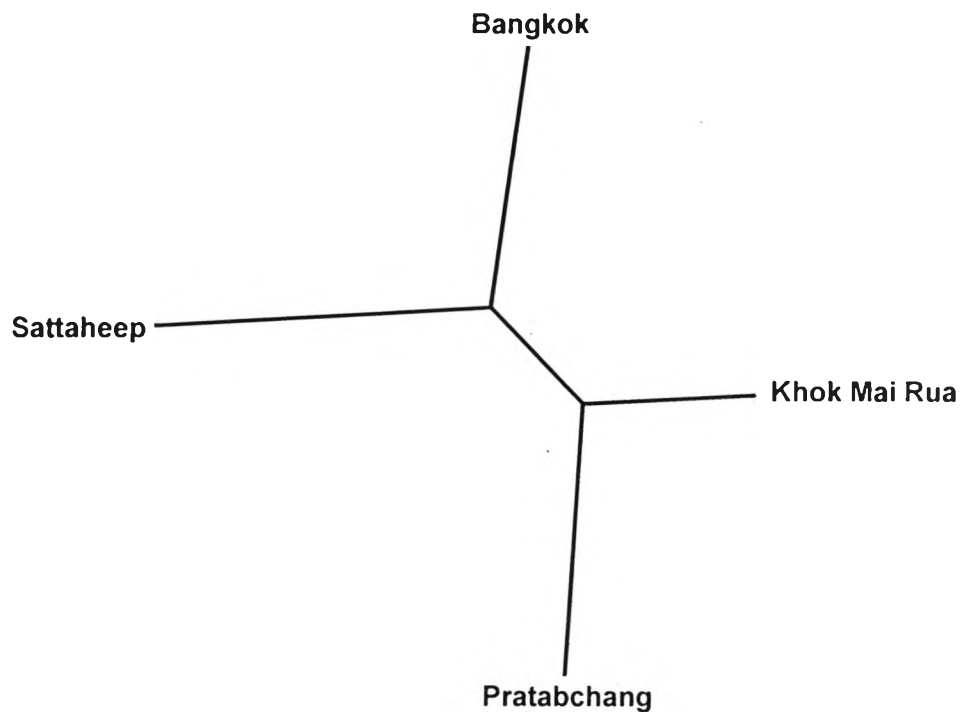


Figure 3.40 A neighbor-joining tree indicating relationship of intraspecies of Green Peafowl (*Pavo muticus imperator*) based on genetic distance between pairs of each location by using RAPD-PCR analysis.



0.1

Figure 3.41 A neighbor-joining tree indicating relationship of intra-species of crested wood partridge (*Rollulus roulroul*) based on genetic distance between pairs of each location by using RAPD-PCR analysis.