

Chapter IV

Result

4.1 Results

4.1.1 Genomic DNA extraction, visualization quality and determined concentration of extracted DNA

Quality of genomic DNA of all specimens of Green Peafowl was extracted from feather tip by using QIAamp[®] DNA Mini Kit. The quality of extracted DNA was visualized via 1% agarose gel. The result showed that the extracted DNA by using QIAamp[®] DNA Mini Kit in Figure 4.1.

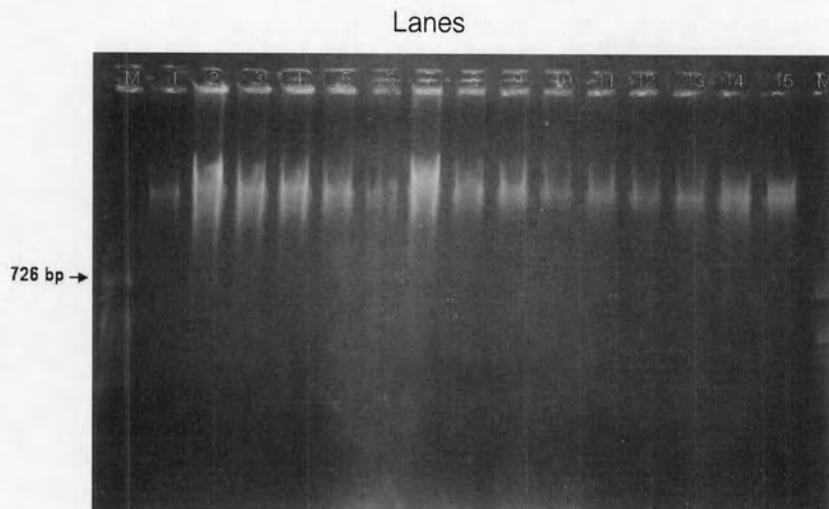


Figure 4.1 Ethidium bromide staining of 1% agarose gel showed the smear band of extracted DNA from 15 green peafowl feather tips.

Lane M : Phi X 174 / Hinf I standard marker

Lane 1-15 : genomic DNA of green peafowl (*Pavo muticus*) extract by QIAamp[®] DNA Mini Kit

The genomic DNA of all specimens was measured the DNA absorbant (OD) by UV spectrophotometer at OD 260 nm, and then the concentration of genomic DNA was

calculated. The concentration of extracted DNA by using QIAamp[®] DNA Mini Kit had ranged from 14.05 to 61.70 µg/mL.

4.1.2 Genetic variation of Green Peafowl

The genetic variations of Green Peafowl from northern and western part of Thailand were studied by eight microsatellite loci. Sample size of Green Peafowl from northern and western were 25 individual per site and Red Jungle Fowl (*Gallus gallus spadiceus*) (for outgroup) was 12 individual. All primers generated group of stutter bands, but the most intense band within group of stutter bands was scored and calculated allele frequencies. The number of alleles and allele frequency of Green Peafowl from northern, from western and Red Jungle Fowl of each microsatellite loci are shown in Table 4.1.

The polymorphic alleles were observed at microsatellite HUU002 locus. Green Peafowl from northern, Green Peafowl from western and Red Jungle Fowl showed 2 alleles.

Only 2 alleles were observed at microsatellite LEI166 locus from all investigated population (Green Peafowl from northern, Green Peafowl from western and Red Jungle Fowl).

The microsatellite MCW034 locus showed 4 alleles per locus. Green Peafowl from northern, Green Peafowl from western and Red Jungle Fowl were 2, 3 and 4 alleles, respectively.

The microsatellite MCW069 locus showed 2 alleles per locus. Green Peafowl from northern, Green Peafowl from western and Red Jungle Fowl were 2 alleles.

The polymorphic alleles were observed at microsatellite MCW080 locus. Green Peafowl from northern and from western showed 3 alleles, while Red Jungle Fowl showed 2 alleles.

Only 2 alleles were observed at microsatellite MCW098 locus from all investigated population. Green Peafowl from northern, Green Peafowl from western and Red Jungle Fowl showed 2 alleles.

The microsatellite MCW295 locus showed 4 alleles per locus. Green Peafowl from northern and western showed 2 alleles while, Red Jungle Fowl were 4 alleles.

The polymorphic alleles were observed at microsatellite MCW330 locus. Green Peafowl from northern, from western and Red Jungle Fowl showed 2 alleles.

Table 4.1 The allele number and allele frequency of Green Peafowl from northern and western and Red Jungle Fowl were analyzed by eight microsatellite loci.

Allele Number	Allele frequency - HUJ002 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.800	0.520	0.833
2	0.200	0.480	0.167
Allele Number	Allele frequency - LEI166 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.620	0.700	0.727
2	0.380	0.300	0.273
Allele Number	Allele frequency - MCW034 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.940	0.200	0.333
2	0.060	0.660	0.333
3	-	0.140	0.167
4	-	-	0.167

Allele Number	Allele frequency - MCW069 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.620	0.200	0.500
2	0.380	0.800	0.500
Allele Number	Allele frequency - MCW080 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.460	0.260	0.500
2	0.500	0.380	0.500
3	0.040	0.360	-
Allele Number	Allele frequency - MCW098 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.680	0.780	0.833
2	0.320	0.220	0.167
Allele Number	Allele frequency - MCW295 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.620	0.780	0.625
2	0.380	0.220	0.125
3	-	-	0.208
4	-	-	0.042
Allele Number	Allele frequency - MCW330 locus		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. gallus spadiceus</i>
1	0.780	0.840	0.500
2	0.220	0.160	0.500

Two varieties of Green Peafowl and Red Jungle Fowl were measured the mean number of alleles per locus. The highest of mean number of alleles per locus in Red Jungle Fowl was 2.50; while Green Peafowl from western was 2.25 and the lowest mean number of alleles per locus in Green Peafowl from northern was 2.13. The result is shown in Table 4.3.

Furthermore, mean number of alleles per locus of all varieties was compared by Wilcoxon sign rank test. The result showed no significant mean difference between varieties ($P>0.05$). Data are shown in Appendix V.

The allele distribution of eight investigated loci HJJ002, LEI166, MCW034, MCW069, MCW080, MCW098, MCW295 and MCW330 from two varieties of Green Peafowl and varieties of red jungle fowl are shown in Figure 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10 and 4.11, respectively. In addition, histograms of allele frequencies are shown in Figure 4.12, 4.13, 4.14 and 4.15, respectively.

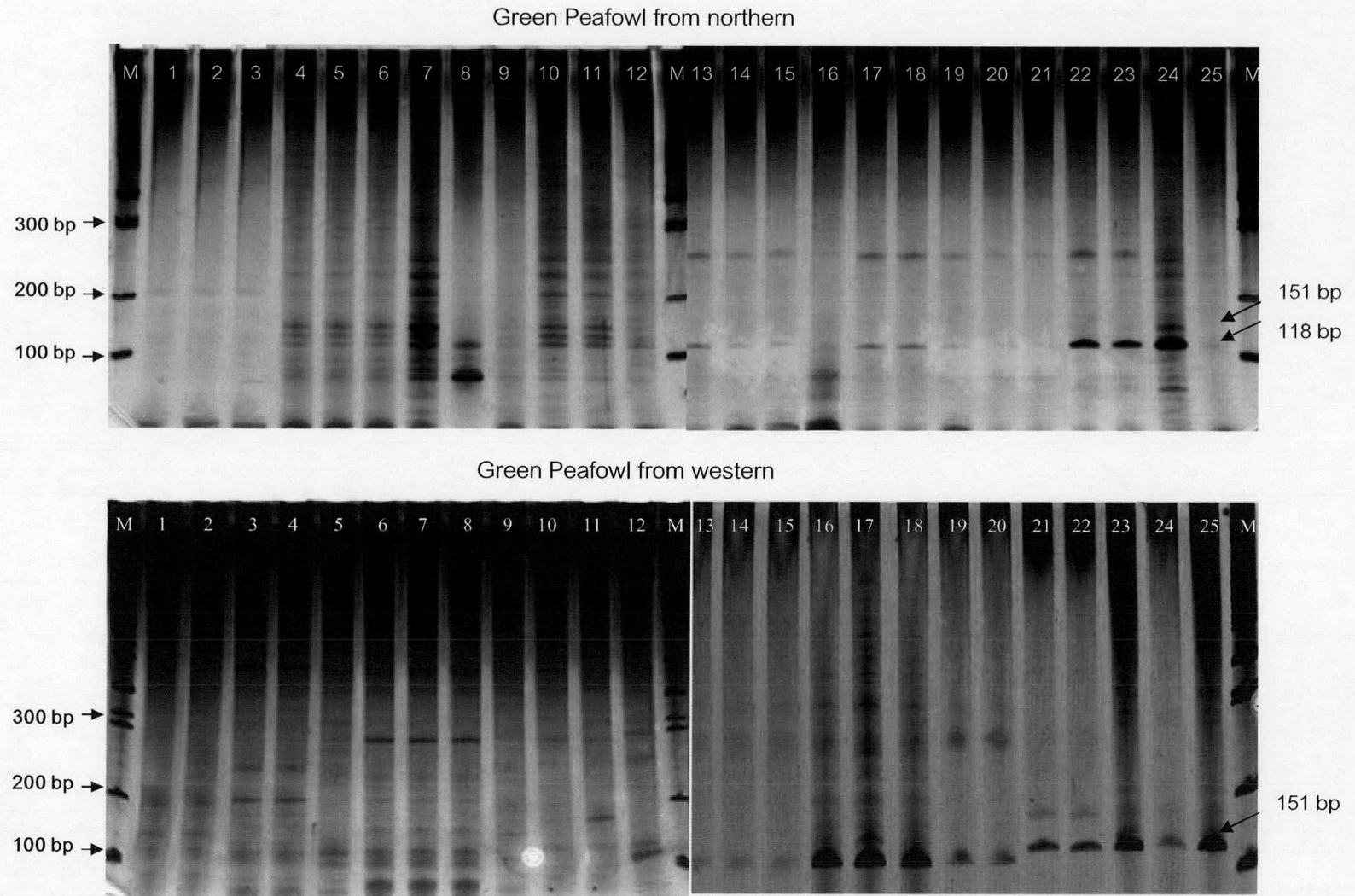


Figure 4.2 Silver staining of 8% polyacrylamide gel showing alleles distribution of HUJ002 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

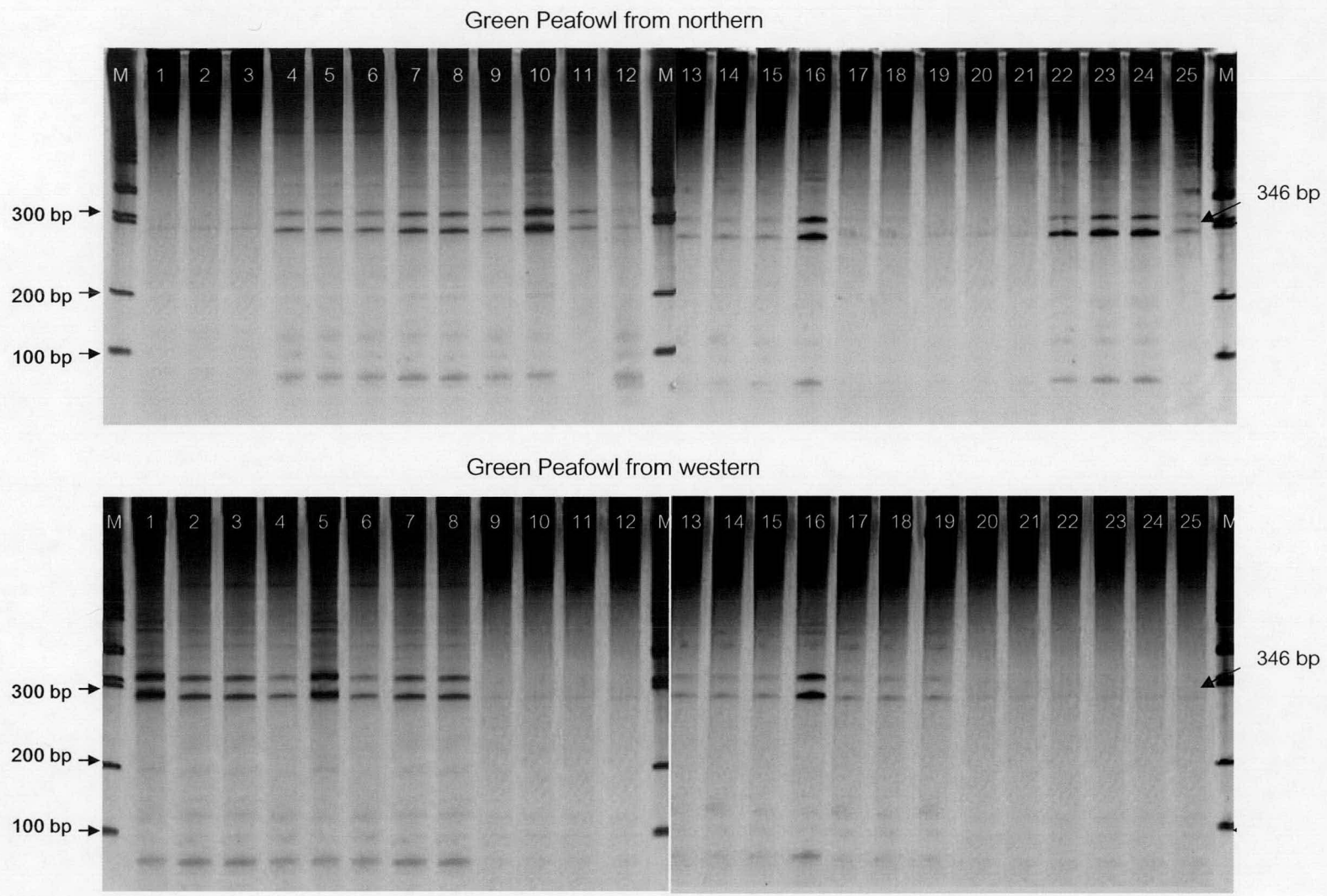


Figure 4.3 Silver staining of 8% polyacrylamide gel showing alleles distribution of LEI166 locus from 25 individual of Green Peafowl from northern and western (lenes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

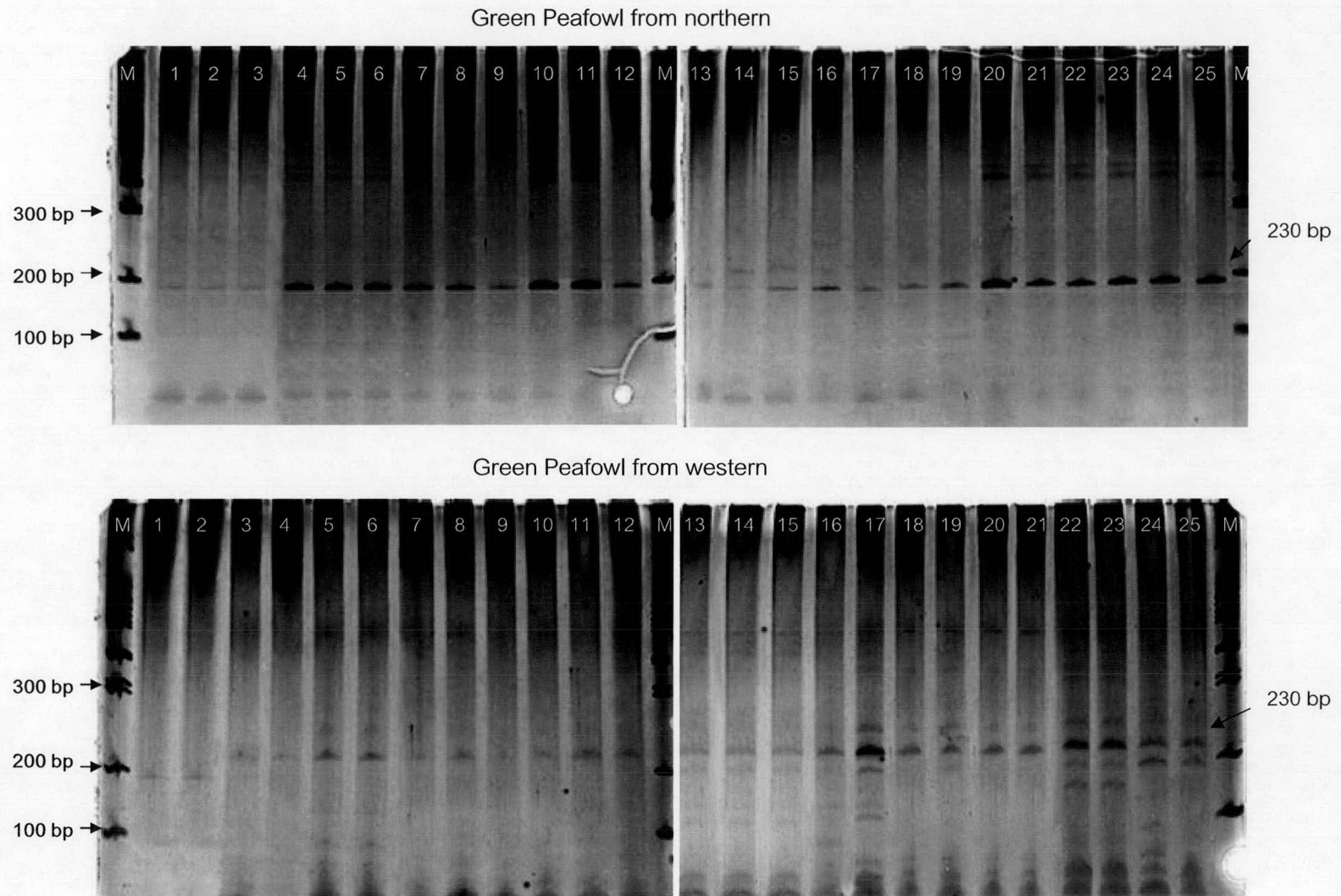


Figure 4.4 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW034 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

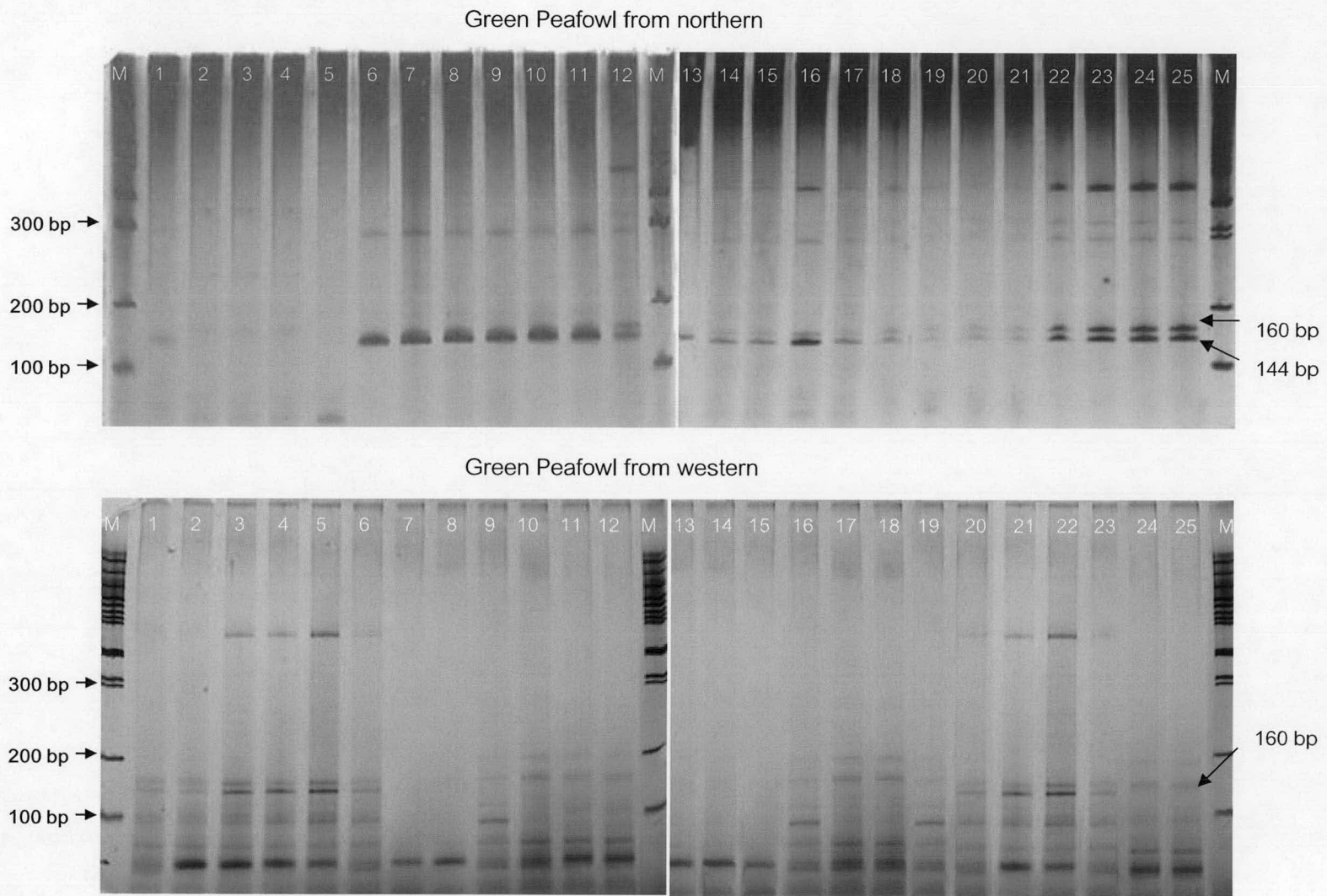


Figure 4.5 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW069 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

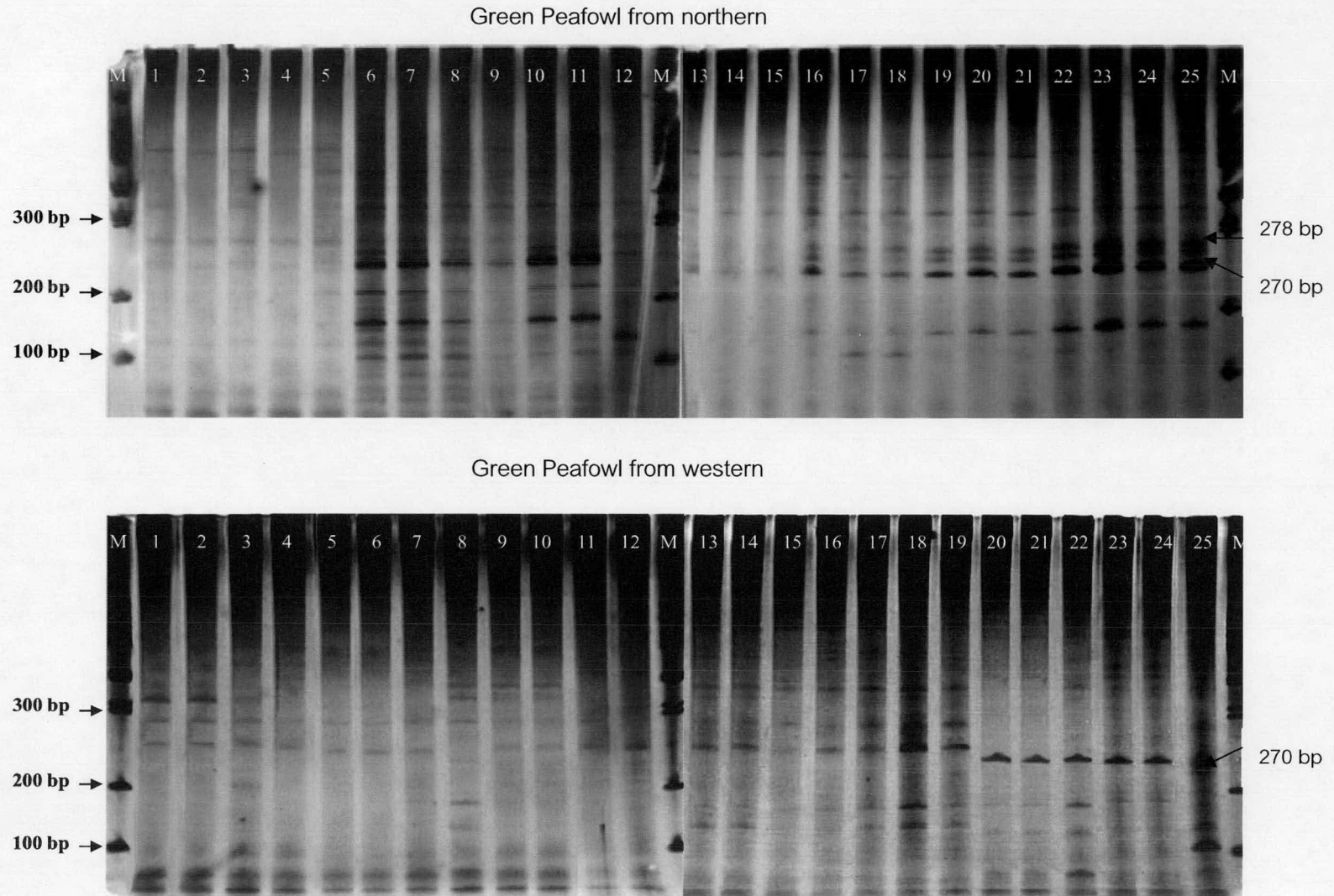


Figure 4.6 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW080 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

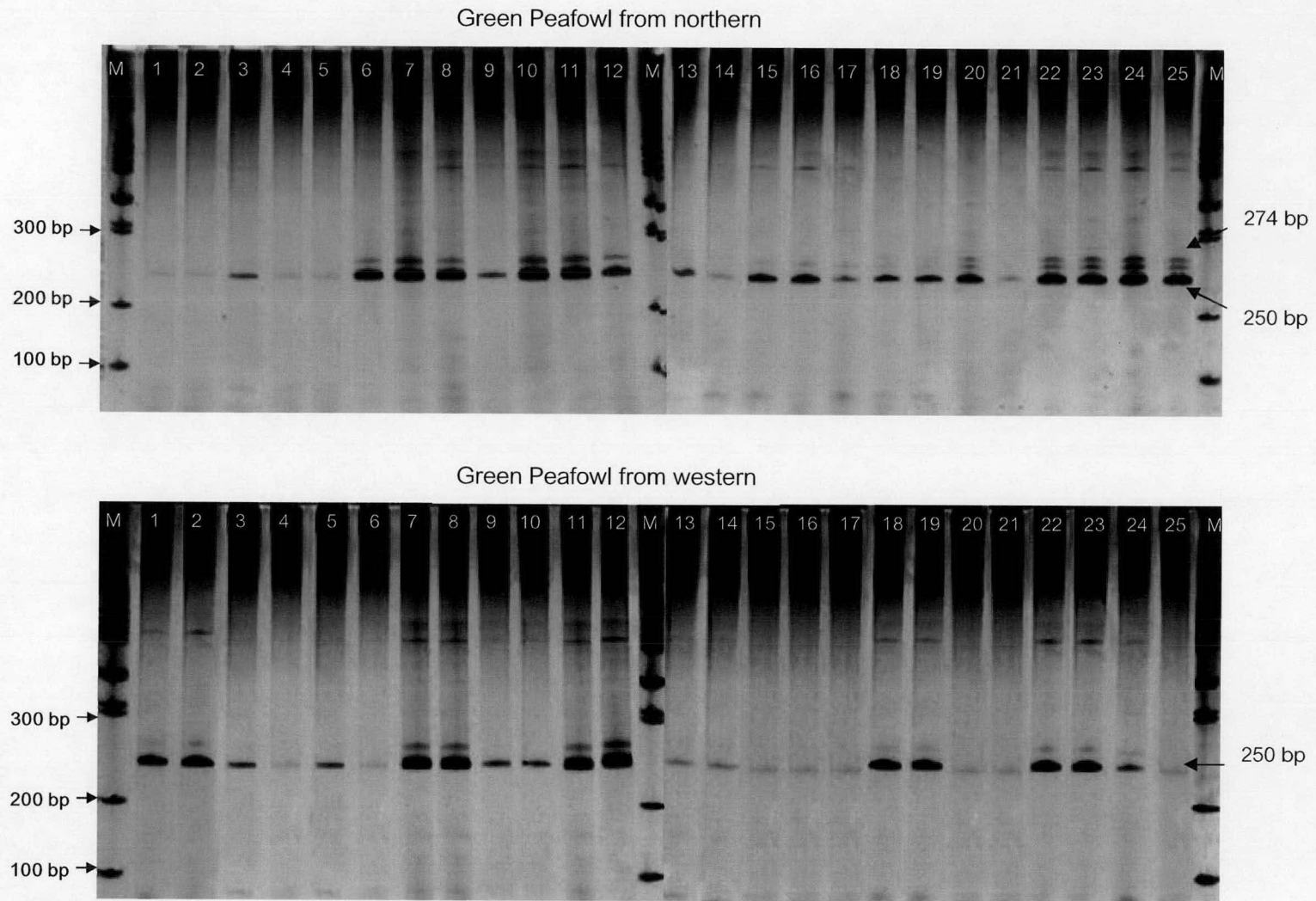


Figure 4.7 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW098 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

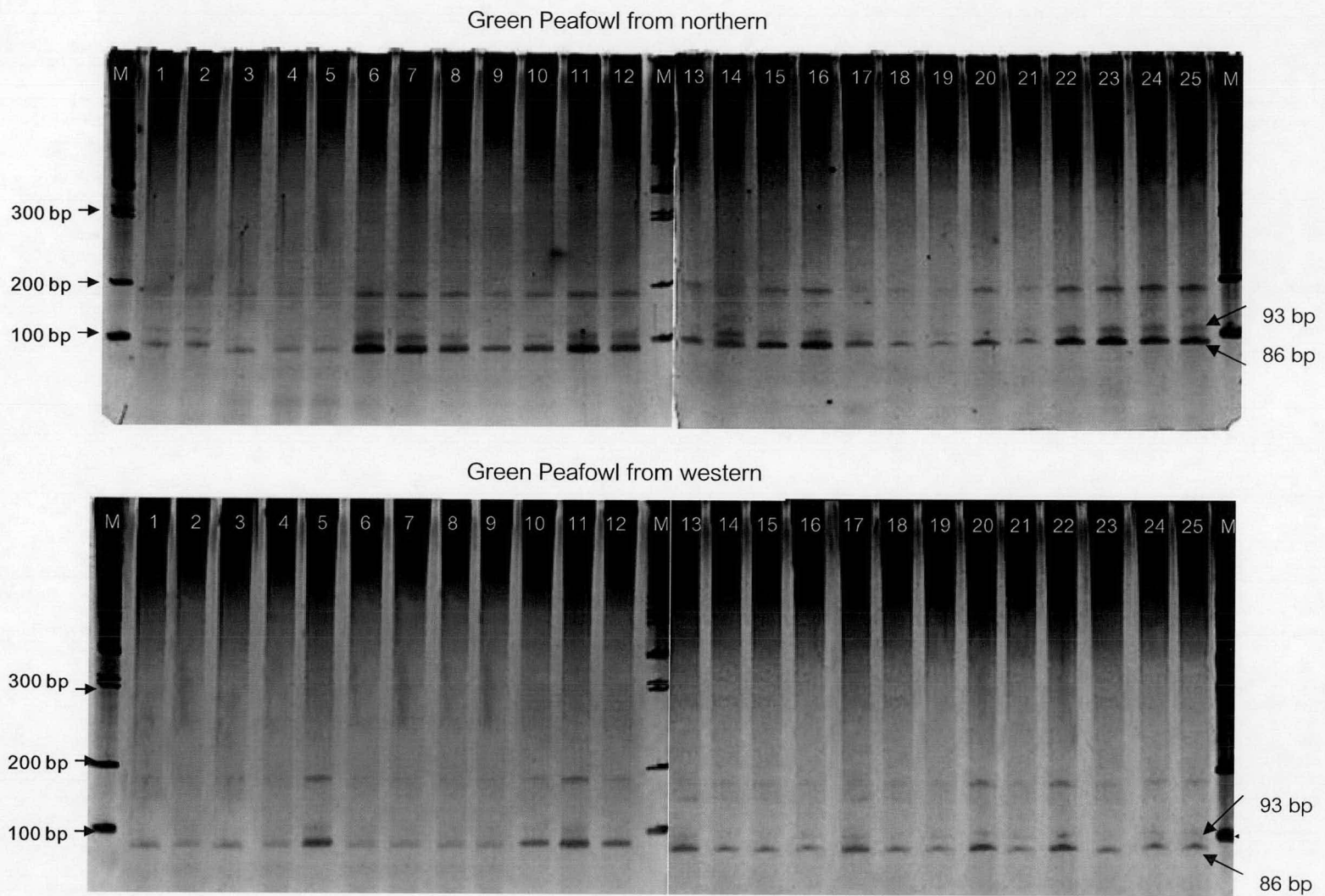


Figure 4.8 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW295 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

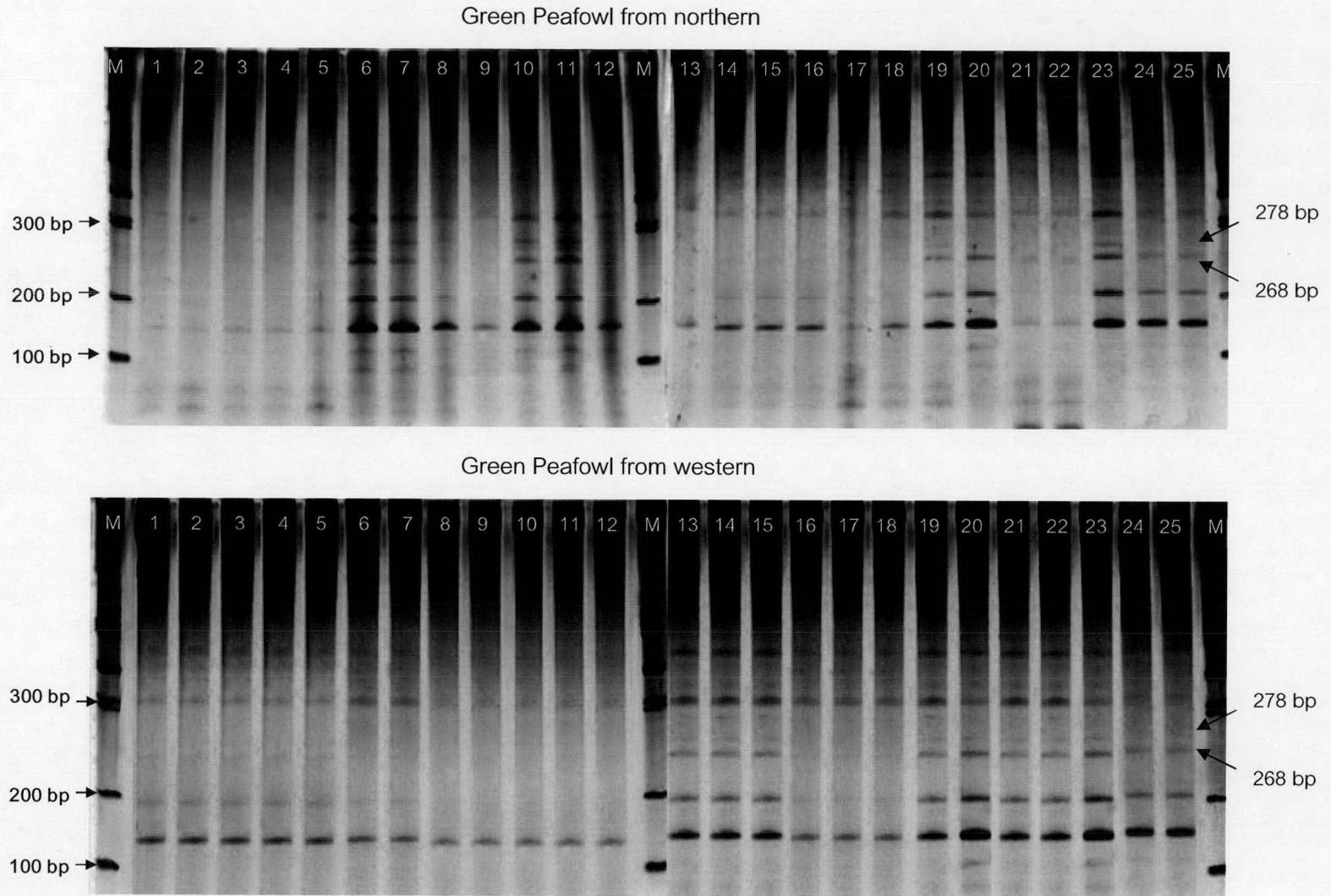


Figure 4.9 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW330 locus from 25 individual of Green Peafowl from northern and western (lanes 1-25). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

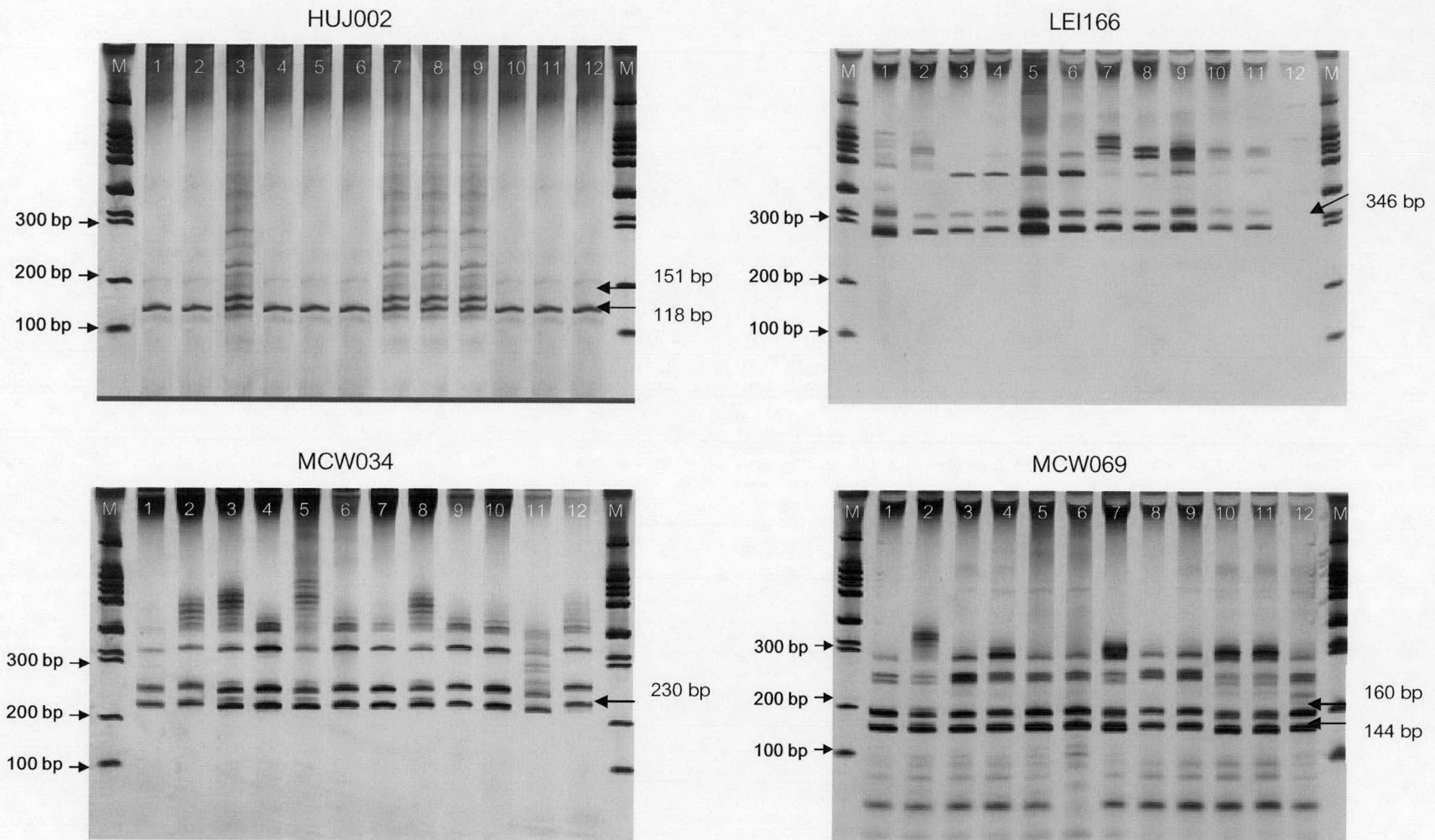


Figure 4.10 Silver staining of 8% polyacrylamide gel showing alleles distribution of HUJ002, LEI166, MCW034 and MCW069 locus from 12 individual of Red Jungle Fowl (lanes 1-12). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

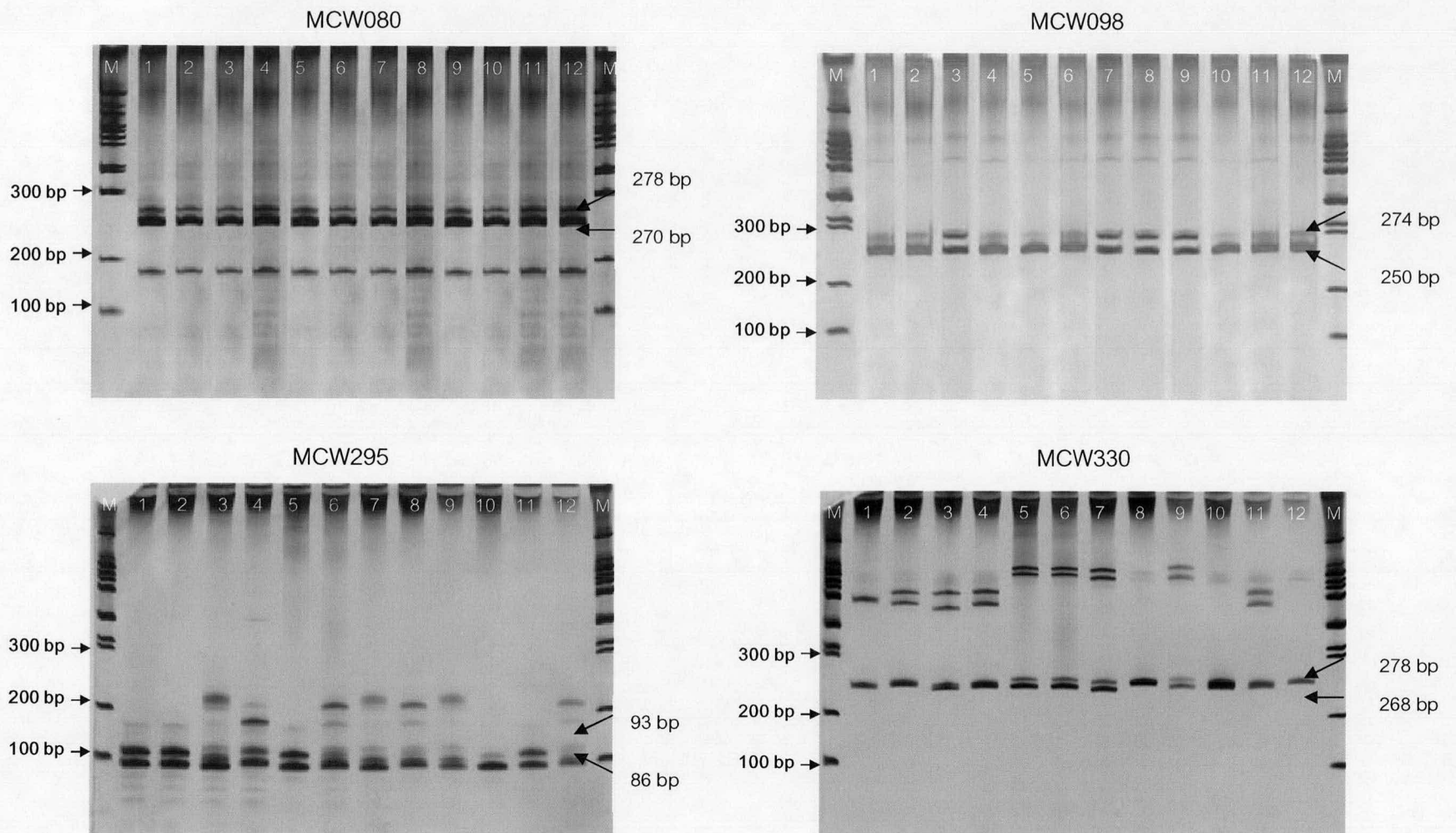


Figure 4.11 Silver staining of 8% polyacrylamide gel showing alleles distribution of MCW080, MCW098, MCW295 and MCW330 locus from 12 individual of Red Jungle Fowl (lanes 1-12). The size standard marker (M) is set of 100 bp + 1.5 Kb DNA Ladder.

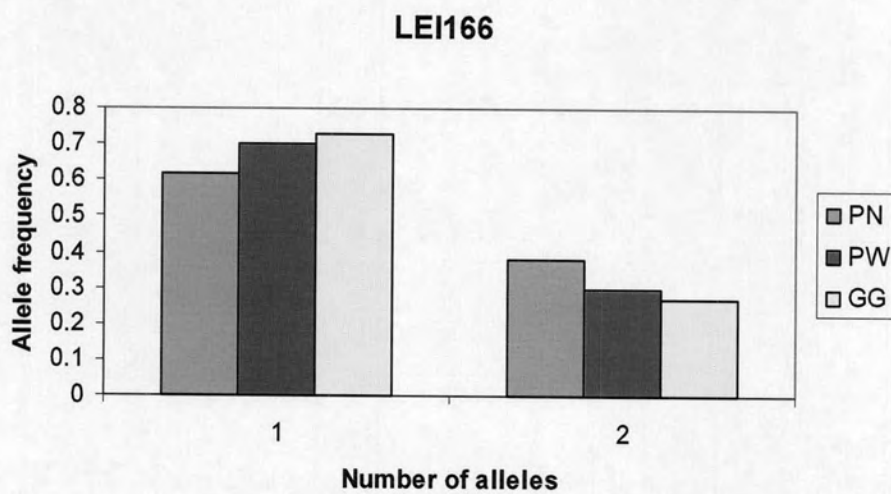
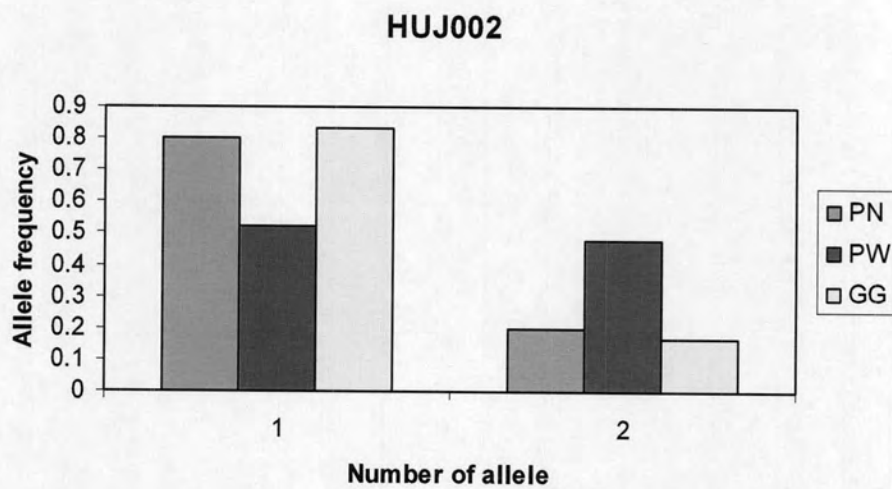


Figure 4.12 Histogram of allele frequency and number of allele of Green Peafowl from northern (PN), from western (PW) and Red Jungle Fowl (GG) at HUJ002 and LEI166 locus.

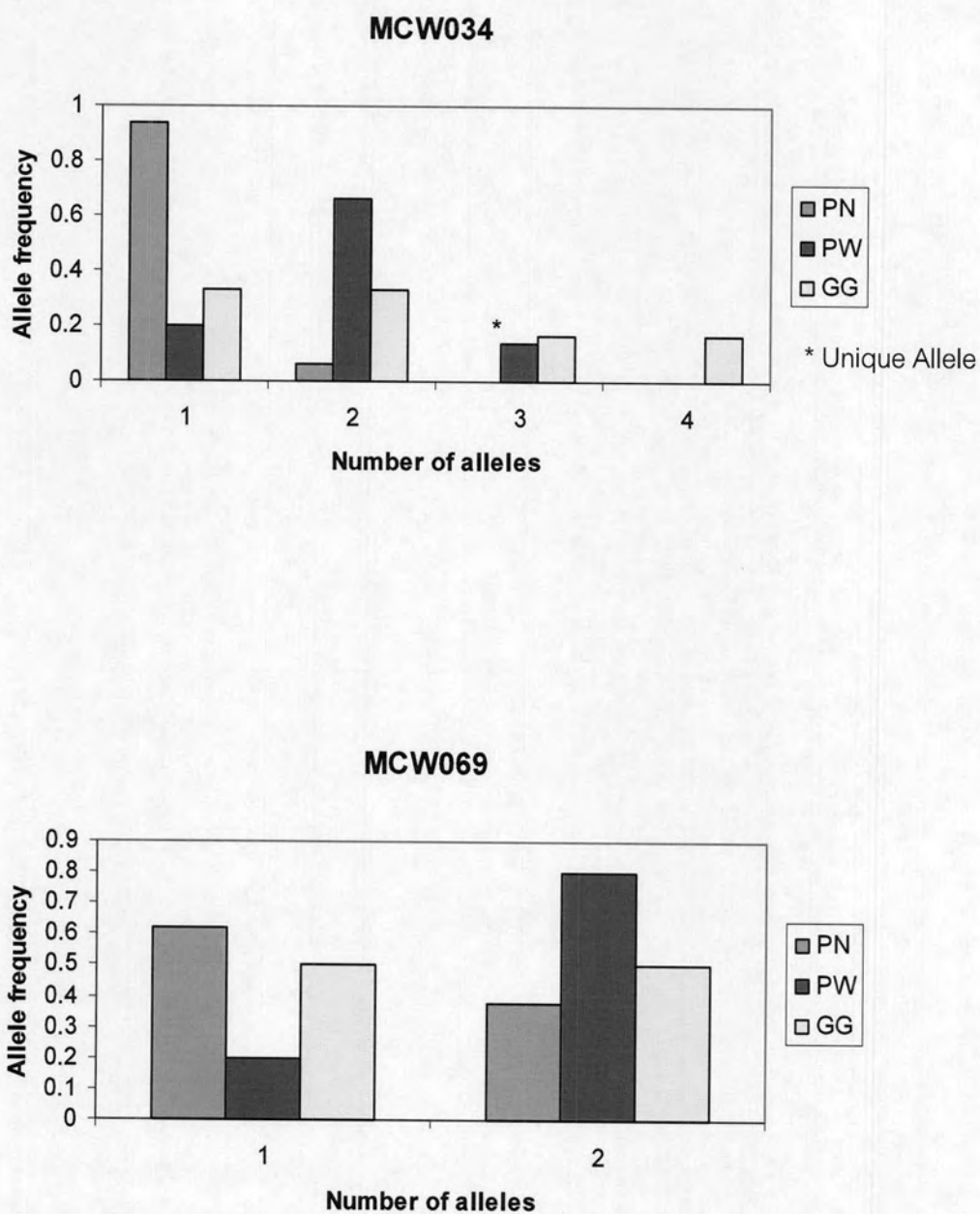


Figure 4.13 Histogram of allele frequency and number of allele of Green Peafowl from northern (PN), from western (PW) and Red Jungle Fowl (GG) at MCW034 and MCW069 locus.

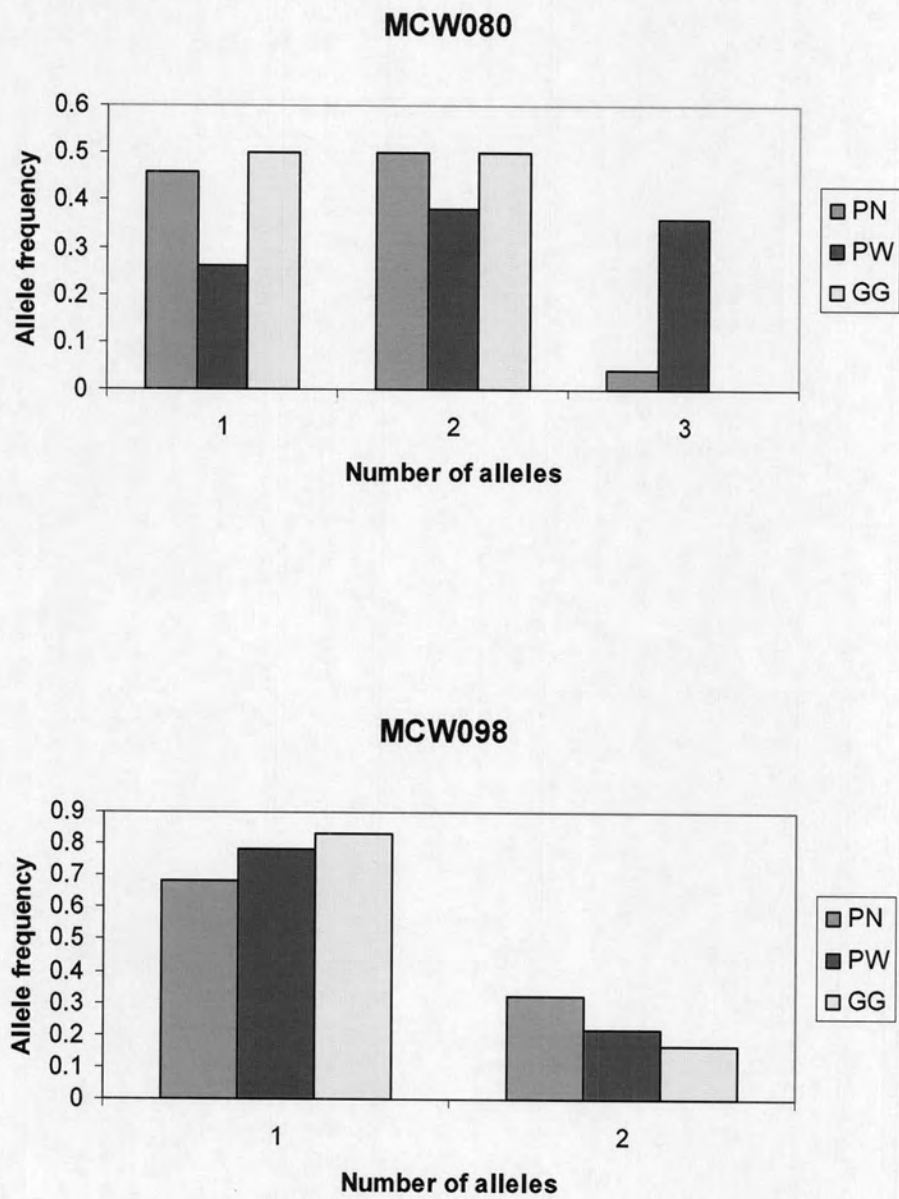


Figure 4.14 Histogram of allele frequency and number of allele of Green Peafowl from northern (PN), from western (PW) and Red Jungle Fowl (GG) at MCW080 and MCW098 locus.

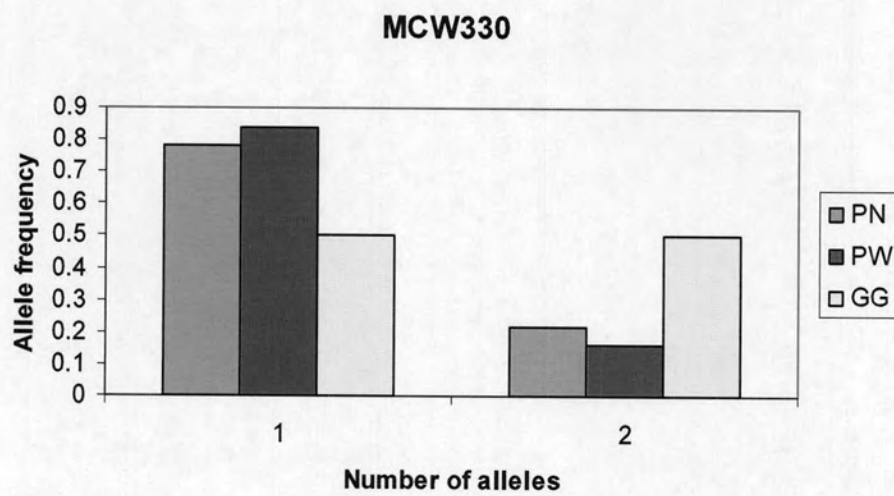
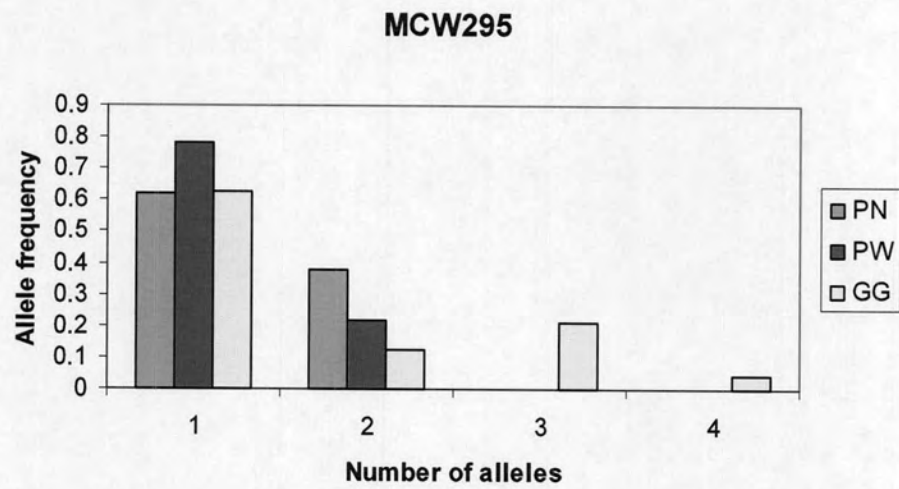


Figure 4.15 Histogram of allele frequency and number of allele of Green Peafowl from northern (PN), from western (PW) and Red Jungle Fowl (GG) at MCW295 and MCW330 locus.

The observed heterozygosity (h_{obs}) and that expected from Hardy-Wienberg assumption (h_{exp}) of all microsatellite loci of Green Peafowl (*Pavo muticus*) and Red Jungle Fowl (*Gallus gallus spadiceus*) were calculated in order to measure the genetic variation. Green Peafowl from northern at MCW080 locus showed the highest of observed heterozygosity ($h_{obs} = 1.000$), whereas, Green Peafowl from northern at MCW034 locus showed the lowest of observed heterozygosity ($h_{obs} = 0.120$). The result was shown in Table 4.2. Diverse distribution of allele frequency could be compared in terms of effective number of alleles (n_e). It was calculated for two varieties of Green Peafowl and Red Jungle Fowl was varied from 1.1 in Green Peafowl from northern to 2.9 in Green Peafowl from western. Data is shown in Table 4.2.

Table 4.2 The sample sizes, number of alleles, effective number of alleles (n_e), observed (h_{obs}) heterozygosity and expected from Hardy-Wienberg assumption (h_{exp}) of two investigated populations were analyzed using eight microsatellite loci

Microsatellite Locus	Varieties	Sample size	Number of allele/variety	h_{obs}	h_{exp}	n_e
HUJ002	<i>P. muticus</i> – northern	25	2	0.400	0.320	1.5
	<i>P. muticus</i> – western	25	2	0.480	0.499	2.0
	<i>G. g. spadiceus</i>	12	2	0.333	0.278	1.4
LEI166	<i>P. muticus</i> – northern	25	2	0.760	0.471	1.9
	<i>P. muticus</i> – western	25	2	0.600	0.420	1.7
	<i>G. g. spadiceus</i>	12	2	0.500	0.397	1.7
MCW034	<i>P. muticus</i> – northern	25	2	0.120	0.113	1.1
	<i>P. muticus</i> – western	25	3	0.520	0.516	2.0
	<i>G. g. spadiceus</i>	12	4	1.000	0.899	3.6
MCW069	<i>P. muticus</i> – northern	25	2	0.520	0.471	1.9
	<i>P. muticus</i> – western	25	2	0.470	0.320	1.5
	<i>G. g. spadiceus</i>	12	2	1.000	0.500	2.0

MCW080	<i>P. muticus</i> – northern	25	3	1.000	0.548	2.2
	<i>P. muticus</i> – western	25	3	0.760	0.672	2.9
	<i>G. g. spadiceus</i>	12	2	1.000	0.500	2.0
MCW098	<i>P. muticus</i> – northern	25	2	0.640	0.435	1.8
	<i>P. muticus</i> – western	25	2	0.440	0.343	1.5
	<i>G. g. spadiceus</i>	12	2	0.333	0.278	1.4
MCW295	<i>P. muticus</i> – northern	25	2	0.760	0.470	1.9
	<i>P. muticus</i> – western	25	2	0.440	0.343	1.5
	<i>G. g. spadiceus</i>	12	4	0.333	0.573	2.2
MCW330	<i>P. muticus</i> – northern	25	2	0.440	0.343	1.5
	<i>P. muticus</i> – western	25	2	0.320	0.269	1.4
	<i>G. g. spadiceus</i>	12	2	0.333	0.500	2.0

Table 4.3 The mean number of sample size (N), mean number of alleles per locus (A), mean effective number of alleles per locus (a_e), mean observed Heterozygosity (H_o) and expected Hardy-Wienberg assumption (H_E) of Green Peafowl from northern and western were analyzed by eight microsatellite loci

Population	N	A	a_e	H_o	H_E
<i>P. muticus</i> – northern	25	2.13	1.73	0.58	0.40
<i>P. muticus</i> – western	25	2.25	1.81	0.50	0.42
<i>G. g. spadiceus</i>	12	2.50	2.00	0.60	0.49

The mean of observed heterozygosity (H_o) and expected from Hardy-Wienberg assumption (H_E) of each population for all loci were calculated. Mean observed heterozygosity of Green Peafowl from northern and western were 0.58 and 0.50, respectively. However, mean expected heterozygosity of Green Peafowl from western showed the highest value ($H_E = 0.42$) and followed Green Peafowl from northern ($H_E = 0.40$). This result is shown in Table 4.3. The difference of mean expected heterozygosity (H_E) was compared between varieties. It was assessed by a Wilcoxon sign rank test. The result showed no significant difference of H_E between varieties (Appendix V).

The mean of effective number of alleles per locus (a_e) was calculated in each variety. The result of effective number of alleles per locus of Green Peafowl from northern, Green Peafowl from western and Red Jungle Fowl were 1.73, 1.81 and 2.00, respectively. The result is shown in Table 4.3. Furthermore, mean effective number of alleles of all varieties was compared by Wilcoxon sign rank test. The result showed no significant mean difference between varieties (Appendix V).

The Hardy-Wienberg assumption was carried out using an exact test. Green Peafowl from northern and western conformed the Hardy-Wienberg assumption to the all of microsatellite loci; HUI002, LEI166, MCW034 MCW069, MCW080, MCW098, MCW295 and MCW330. The result of Hardy-Wienberg equilibrium test is shown in Table 4.4.

Table 4.4 The estimation under Hardy-Wienberg assumption of Green Peafowl from northern and western were analyzed by eight microsatellite loci (HUI002, LEI166, MCW034, MCW069, MCW080, MCW068, MCW295 and MCW330) when H_1 = heterozygote deficit.

Locus	P - value		
	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. g. spadiceus</i>
HUI002	1 ^{ns}	0.2418 ^{ns}	1 ^{ns}
LEI166	1 ^{ns}	1 ^{ns}	1 ^{ns}
MCW034	1 ^{ns}	0.4190 ^{ns}	1 ^{ns}
MCW069	0.8011 ^{ns}	1 ^{ns}	1 ^{ns}
MCW080	1 ^{ns}	0.5235 ^{ns}	1 ^{ns}
MCW098	1 ^{ns}	1 ^{ns}	1 ^{ns}
MCW295	1 ^{ns}	1 ^{ns}	0.0672 ^{ns}
MCW330	1 ^{ns}	1 ^{ns}	0.4486 ^{ns}

* Significant level was further adjusted by using a Bonferoni method, ^{ns} no significant

The exact test for genotypic linkage disequilibrium showed significance between locus pair of HUJ002 - LEI166, HUJ002 - MCW080, HUJ002 - MCW098, HUJ002 - MCW330, LEI166 - MCW080 and MCW034 - MCW330, whereas other locus pairs conformed to the genetic linkage equilibrium. This result is shown in Table 4.5.

Table 4.5 The pair wise comparison of genetic linkage disequilibrium between eight microsatellite loci of HUJ002, LEI166, MCW034, MCW069, MCW080, MCW098, MCW295 and MCW330 of two investigated Green Peafowl populations.

Locus pairs	P-value
HUJ002&LEI166	0.001*
HUJ002&MCW034	0.059 ^{ns}
HUJ002&MCW069	0.087 ^{ns}
HUJ002&MCW080	0.003*
HUJ002&MCW098	0.026*
HUJ002&MCW295	0.458 ^{ns}
HUJ002&MCW330	0.007*
LEI166&MCW034	0.916 ^{ns}
LEI166&MCW069	0.451 ^{ns}
LEI166&MCW080	0.008*
LEI166&MCW098	0.439 ^{ns}
LEI166&MCW295	0.570 ^{ns}
LEI166&MCW330	0.403 ^{ns}
MCW034&MCW069	0.080 ^{ns}
MCW034&MCW080	0.242 ^{ns}
MCW034&MCW098	0.636 ^{ns}
MCW034&MCW295	0.332 ^{ns}
MCW034&MCW330	0.018*
MCW069&MCW080	0.597 ^{ns}
MCW069&MCW098	0.087 ^{ns}

MCW069&MCW295	0.531 ^{ns}
MCW069&MCW330	0.219 ^{ns}
MCW080&MCW098	0.836 ^{ns}
MCW080&MCW295	0.114 ^{ns}
MCW080&MCW330	0.129 ^{ns}
MCW098&MCW295	0.423 ^{ns}
MCW098&MCW330	0.941 ^{ns}
MCW295&MCW330	0.414 ^{ns}

* Significant level was further adjusted by using a Bonferoni method, ^{ns} no significant

Green Peafowl from northern showed no significant difference from Green Peafowl from western at LEI166, MCW098, MCW295 and MCW330 locus whereas other loci showed significant difference (Table 4.6). Green Peafowl from northern showed no significant difference from Red Jungle Fowl in six loci excepted MCW034 and MCW295 locus. Green Peafowl from western showed significant difference from Red Jungle Fowl at LEI166 and MCW098 locus.

Table 4.6 The pair wise comparison of contingency tests of differentiation between Green Peafowl populations from northern and western by 8 microsatellite loci.

Varieties pairs	P-value							
	HUJ002	LEI166	MCW034	MCW069	MCW080	MCW098	MCW295	MCW330
PN&PW	0.0055*	0.5304 ^{ns}	0.0000*	0.0000*	0.0002*	0.3582 ^{ns}	0.1224 ^{ns}	0.4574 ^{ns}
PN&GG	1.0000 ^{ns}	0.4330 ^{ns}	0.0000*	0.4493 ^{ns}	1.0000 ^{ns}	0.2597 ^{ns}	0.0006*	0.0660 ^{ns}
PW&GG	0.0112*	1.0000 ^{ns}	0.0050*	0.0147*	0.0006*	0.7596 ^{ns}	0.0016*	0.0108*

* Significant level was further adjusted by using a Bonferoni method, ^{ns} no significant

PN = *P. muticus* from northern

PW = *P. muticus* from western

GG = *G. g. spadiceus*

The allele frequency at eight microsatellite loci in each pair of varieties was used to calculate the genetic distance. The genetic distance between each pair wise comparison of varieties was observed by using GENDIST (Felsenstein, 1993). The

lowest genetic distance was found between Green Peafowl from northern and Green Peafowl from western (0.1956), whereas the highest was found between Green Peafowl from western and Red Jungle Fowl (0.1974). The result is shown in Table 4.7. The resulting genetic distance was subjected to phylogenetic reconstruction based on the Neighbor-joining approach (Saitou and Nei, 1987).

Table 4.7 The pair wise comparison of genetic distance between Green Peafowl population from northern and western by using GENDIST.

	<i>P. muticus</i> - northern	<i>P. muticus</i> - western	<i>G. g. spadiceus</i>
<i>P. muticus</i> - northern	-		
<i>P. muticus</i> - western	0.1956	-	
<i>G. g. spadiceus</i>	0.1705	0.1974	-

The Neighbor-joining tree allocated all investigated varieties to different groups consisting of Green Peafowl from northern and western (group 1) whereas Red Jungle Fowl was outgroup (see in Figure 4.16).

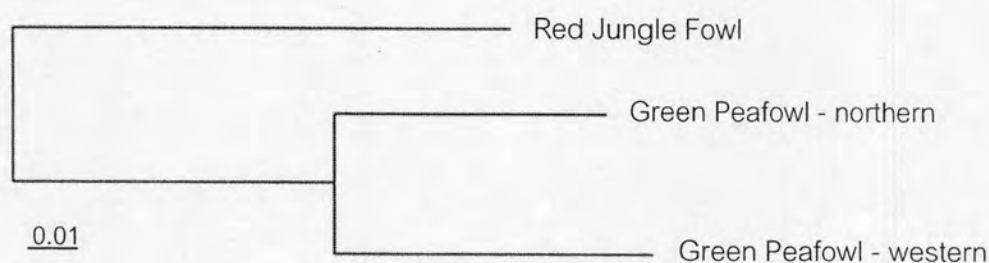


Figure 4.16 Phylogenetic tree of Green Peafowl (*Pavo muticus*) and out group Red Jungle Fowl (*Gallus gallus spadiceus*) by Neighbor-joining method.