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Karyotypes Among Various Morphological Populations of Hill Mynah, *Gracula religiosa,* from Different Areas in Thailand

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Abstract: The study on eleven characteristics including head, bill, body, wattle, wing, tail and tarsus of hill Mynahs from different populations inhabited throughout Thailand discriminated birds into five groups: Northern, Modified Northern, Intermediate, Modified Southern and Southern. Specifically, there were five characters of the yellow connection between the anterior and posterior wattles. The differences among the averages of these characteristics of five different groups were statistically significant. On the other hand the karyological study did not show any correlation between phenotypic variation and genetic divergence in chromosomal aspect. There was no geographical difference in karyotypes. The sizes and shapes of five different hill Mynah group chromosomes were similar. The diploid chromosome number (2n) was 80: ten pairs of macrochromosomes and thirty pairs of microchromosomes. There was a karyotypical invariance among various morphological populations of hill Mynahs from different areas in this study. Eventually, the role of microchromosomes or further study of DNA level is implicated.

Key words: *Gracula religiosa*, Hill Mynah, karyotypes, morphological variation

Introduction

It has been known for years that two subspecies of Hill Mynahs, *Gracula religiosa intermedia,* the northern race and *G. r. religiosa,* the southern race, were found in Thailand. Recent studies on eleven external morphological characteristics such as head, bill, body, wattle, wing, tail and tarsus of 749 live hill Mynahs which inhabited throughout Thailand indicated that there were three new Hill Mynah groups. The first group, named group MN (Modified Northern) had the yellow connection line approximately 90% of the length between the anterior and posterior wattles. The second group, named group I (Intermediate), had the yellow connection line half way between the anterior and posterior wattles. The third group, named group MS (Modified Southern), had the yellow connection line approximately 10% of the length between the anterior and posterior wattles (Fig. 1) (Archawaranon and Wongwasana, 1998). Morphological measurements of these three new groups showed the differences from the northern and the southern races. They were bigger than the northern race but smaller than the southern race, especially, head area, bill cone, bill curvature, posterior wattle length, body mass, body length, body circumference, wing length and tarsus length. The averages of most characteristics increased toward the south (Table 1). These morphological variation were significantly different (Archawaranon and Techatraisak, 2000 unpublished). The patterns of variation postulated that gene flow has gradually occurred, so the variation may be genetically determined. Studies of phenotypic and genetic covariation in bird species have been intensively investigated both in DNA (Zink, 1982; Baker *et al.*, 1990; Zink and Avise, 1990; Johnson and Martin, 1992; Triggs *et al.*, 1992; Rasmussen, 1994; Cicero and Johnson, 1995; Brumfield and Capparella, 1996; Foggo *et al.*, 1996, Rhodes *et al.*, 1996; Zink and Blackwell, 1996; Baker and Johnson, 1998) and in chromosomes (De Boer and Van Bocxstaele, 1981; Hobart *et al.*, 1982; Shields, 1982; Cox and James, 1984). Although the chromosomal morphology study is a basic aspect of genetic knowledge, intraspecific chromosomal polymorphism in bird specie has generated the idea that it may play an important role in speciation (White, 1978). Karyological study of Hill Mynahs of which morphological variation existed in different geographic populations in Thailand disclose the association between phenotypic variation and chromosomal morphology of this species.

Materials and Methods

Sample sources: Karyotypes of birds from different areas in Thailand were studied in 2000. Those three new Hill Mynah groups were found between 6° to 16°N whereas the northern race inhabited between 9° to 20° 30′ N and the southern race inhabited between 5° 30′ to 9° N. The tissues of the Northern group were obtained from birds inhabited between 16°- 20° 30′ N, the MN group between 14°- 16° N, the I group between 9°-14° N, the MS group between 6°- 9° N and the Southern group between 5° 30′- 6° N. Birds were trapped and released at the original places after the tissues were taken.

Karyological study: Karyological study was conducted at the Department of Anatomy, Faculty of Medicine, Chiangmai University, Chiangmai, Thailand. The leather was removed from the proximal part of bird leg before cutting 5 mm^2 skin. Sterile skin was rinsed with RPMI 1640 and antibiotics for 3–4 times. Before culturing, the skin was finely chopped and put in culture bottle. The media presently used were amniomax (Gibco) with 20% fetal calf serum. CO_2 incubator at 42°C was the most proper condition for culturing for three days. On the fourth day cultivation was inspected under inverted microscope and culture media were again changed. The fibroblasts were easily recognized and ready to be harvested in 7–10 days. The cells were washed with phosphate buffer and treated twice with 0.075 M. KCl hypotonic solution and fixed with methanol: acetic (3:1) three times. Supernatant was removed and cool fixative was added. The cell suspension was used for preparing metaphase chromosomes by modified air dried technique. Giemsa stain 10% in Sorensen's buffer was staining technique used in this method.

The metaphase chromosomes were checked under light microscope and photographed. Morphological and numerical analysis were based on the photographic prints. A total of 217 metaphases were used for morphological and number analysis. The characteristics nomenclature was made on the position of centromere (Levan *et al.*, 1964). Arm ratios were calculated by dividing the length of the long arm by that of the short arm of each chromosome. Chromosomes with arm ratios between 1–1.6 were named as metacentric, those between 1.7–3.0 as submetacentric and those with higher than 3.0 as subtelocentric.

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Results

The differences in morphology of hill Mynahs in Thailand showed that the northern birds had straight bill, small head and body, short tarsus and wing while birds from the southern part of the country had curved and thick bill, big head and body, long tarsus and wing (Table 1). Birds from the intervening area were intermediate in sizes and characters (Archawaranon and Techatraisak, 2000 unpublished).

Although tissue culture is rarely used in avian cytogenetics due to difficulties in initiating and obtaining good growth, this research presents the first success in using the technique to obtain avian karyotypes. The results from karyological study did not show the differences in diploid number (2n), the sizes and shapes of chromosomes among the five hill Mynah groups (Fig. 2). The most frequent diploid chromosome number of five different morphological groups was found to be 80 (Table 2). There were ten pairs of macrochromosomes, nine pairs of autosomes and one pair of sex chromosome (ZZ in male and ZW in female) and thirty pairs of microchromosomes. The arm ratios study showed that chromosome number 1, 2 and 5 were submetacentric; 3, 6, 7, 8 and 9 were subtelocentric; no. 4 was metacentric; Z chromosome was subtelocentric and W chromosome was submetacentric. In addition, the average arm ratios of each individual macrochromosome among five different Hill Mynah groups were similar (Table 3).

Discussion

There was an obvious distinction of morphological characteristics among five Hill Mynah groups in Thailand. The Northern and the Southern groups were unquestionably different. There were statistically significant differences in many characters between the Northern and the Modified Northern, and the Southern and the Modified Southern. The studies in other bird species showed that chromosomal polymorphism correlated with size variability. In Slate-colored juncos and White-throated sparrows (Rising and Shields, 1980), bill size and appendage size were shown to correlate with karyotypic differences. However, the differences in bill and limb sizes were more likely to affect the foraging behavior especially in winter flocks. Furthermore, it was showed that tan-stripe white-throated sparrow males with 22 karyotype had significantly longer bills than those white-stripe birds with 22^{m} karyotype. On the other hand, the study in red-winged blackbirds revealed no geographical differences in karyotypical morphology among four separated populations (Cox and James, 1984). Even among North American Blackbirds, no intraspecific polymorphism in karyotypes was found (Hobart *et al.*, 1982). These findings were identical to what we found in this Hill Mynah study. Karyotypes of five different morphological groups from five widely separated populations were same in sizes and shapes except for a slight but not significant difference of diploid chromosome numbers.

As a result of the general large number of small microchromosomes in avian species the diploid number was uncertain and underestimated because some might be misplaced or be covered by larger chromosomes in the preparation of dropping or spreading cells. A good karyotype preparation technique to get very clear microchromosomes for accurate counting was very difficult (Small *et al.*, 1993). Nevertheless, the repeated counting of large amount of metaphases as we did in this study should be acceptable so far. It is difficult at this point to decide whether the morphological variation of Hill Mynahs in Thailand has occurred because of microchromosomes or not. Rodionov (1996) reported that there were more than half of the mapped avian genes located on microchromosomes. Furthermore, crossing-over frequency was threefold higher in microchromosomes than in macrochromosomes due to the high GC content in microchromosomal euchromatin. Microchromosomes in avian species may play more a crucial role in some aspects than what is presently recognized. In particular, passeriformes was indicative of the variability of microchromosome numbers (Rodionov, 1997) which made the avian karyological study complicated.

From this study, there was a karyotypical invariance among various morphological populations of Hill Mynahs from different areas. No differences appeared in karyotypes. It was possible that congeneric of bird species frequently showed identical karyotypes (Shields, 1982). Moreover, it was believed that there was little karyotypical diversity in the species of birds. Therefore, the role of

Table 1: Mean and standard deviation of morphological characteristics of five different hill group in Thailand

Characters	N	MN	I	MS	S
Head area (cm^2)	17.35 ± 0.95	17.52 ± 0.70	19.05 ± 0.92	20.82 ± 1.03	21.63 ± 2.01
Bill cone (cm^3)	6.70 ± 0.13	7.10 ± 0.15	8.30 ± 0.15	9.80 ± 0.25	11.50 ± 0.31
Bill curcature (degree)	5.68 ± 1.21	5.90 ± 0.96	7.15 ± 073	7.50 ± 0.80	8.19 ± 1.23
Anterior wattle area (cm^2)	0.94 ± 0.19	1.00 ± 0.21	1.06 ± 0.20	0.96 ± 0.23	0.96 ± 0.28
Posterior wattle length (cm)	2.86 ± 0.56	2.87 ± 0.40	3.13 ± 0.31	3.28 ± 0.23	3.34 ± 0.55
Body mass (gm)	189.37 ± 15.94	200.14 ± 13.33	231.00 ± 0.31	240.76 ± 11.55	280.80 ± 17.77
Body length (gm)	18.52 ± 0.74	18.69 ± 0.77	20.48 ± 0.71	21.23 ± 0.91	22.68 ± 1.22
Body circumference (cm)	18.04 ± 0.68	18.26 ± 0.96	20.11 ± 0.96	21.15 ± 1.03	22.02 ± 1.22
Wing length (cm)	16.49 ± 0.89	16.58 ± 0.96	17.58 ± 1.21	18.32 ± 1.26	18.18 ± 1.34
Tail length (cm)	7.51 ± 0.51	6.52 ± 0059	7.72 ± 0.16	6.10 ± 0.64	7.21 ± 0.55
Tarsus length (cm)	3.28 ± 0.21	4.19 ± 0.16	4.33 ± 0.16	4.59 ± 0.29	4.62 ± 0.29

Table 2: Percentage of various diploid chromosome number (2n) found in five different hill mynah group in Thailand

	Percentage of diploid chromosome number (2n)													
Group	<70	71	72	73	74	75	76	77	78	79	80	81	82	>83
N	-	-	-	-	5.00	-	5.00	10.00	15.00	10.00	**35.00**	5.00	5.00	10.00
MN	5.00	-	-	10.00	10.00	-	10.00	10.00	15.00	-	**30.00**	5.00	5.00	-
I	9.09	-	4.55	2.27	9.09	4.55	4.55	2.27	5.90	2.27	**34.09**	4.55	4.55	2.27
MS	4.76	-	-	9.52	9.52	-	9.52	4.76	9.52	4.76	**38.12**	4.76	4.76	-
S	12.00	8.0	8.00	4.00	-	-	4.00	8.00	4.00	16.00	**16.00**	16.00	4.00	-

N = Northern (n = 206), MN = Modified Northern (n = 126), I = Intermediate (n = 115), MS = Modified Southern (n = 139) and S = Southern (n = 163) group

Table 3: Average arm ratio of the nine macrochromosomes of five different hill mynah group in Thailand

	Chromosome number								
Hill Mynah group	1	2	3	4	5	6	7	8	9
N	2.25	2.67	5.67	1.13	1.80	3.33	3.13	3.50	3.50
MN	2.25	2.50	5.33	1.25	1.80	3.33	3.37	4.00	3.25
I	2.17	2.50	5.00	1.20	1.75	3.33	3.03	3.33	3.25
MS	2.13	2.60	5.67	1.14	1.75	3.67	3.23	4.00	3.25
S	2.22	2.80	6.00	1.14	1.80	3.33	3.10	3.50	3.25

N = Northern (n = 206), MN = Modified northern (n = 126), I = Intermediate (n = 115), MS = Modified Southern (n = 139) and S = Southern (n = 163) group

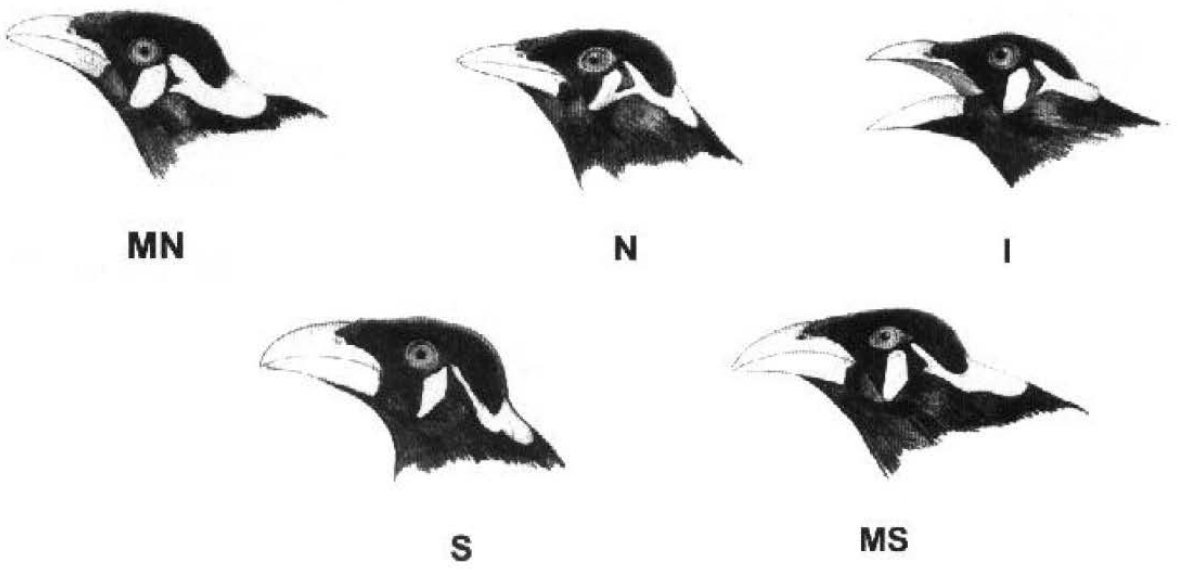

Fig.1: Characteristics of yellow connection between anterior and posterior wattles of five different hill mynah group in Thailand. (N = Northern, MN Modified Northern, I = Intermediate, MS = Modified Southern and S- Southern)

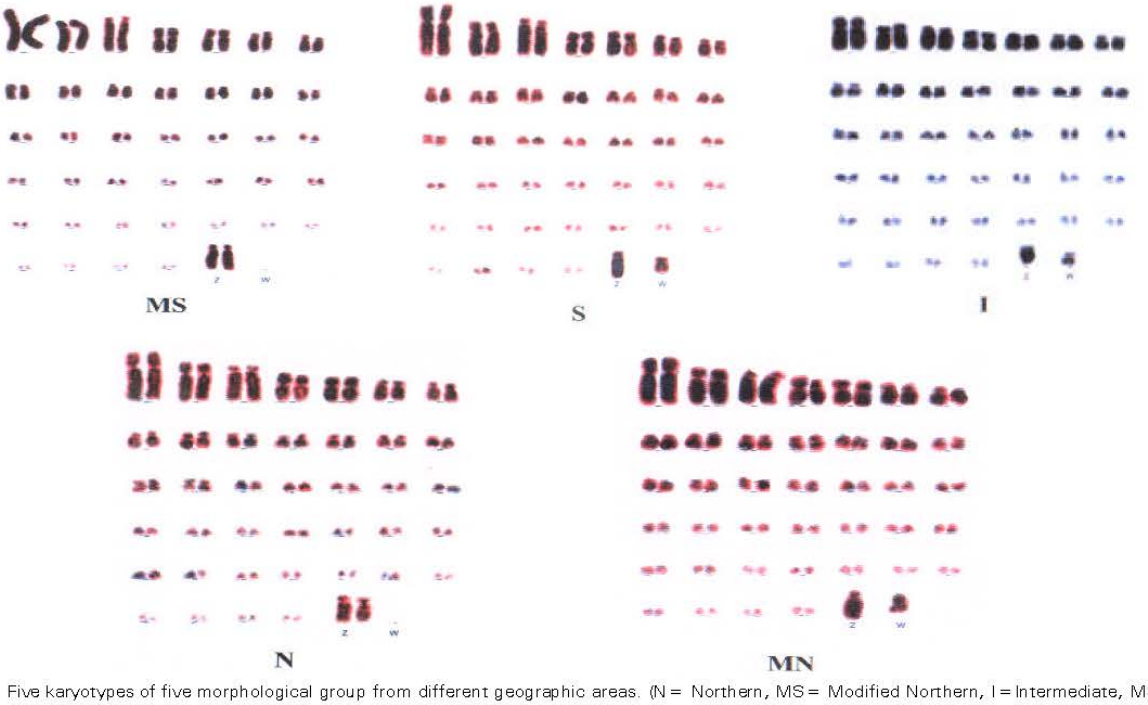

Fig. 2: Five karyotypes of five morphological group from different geographic areas. (N = Northern, MS = Modified Northern, I = Intermediate, MS = Modified Southern and S- Southern)

intraspecific variation of chromosomes on the speciation process in bird species is still questionable. In some aspects, phenotypical differences in Hill Mynahs may be not easily recognized by chromosomal level. A further study of DNA level is implicated.

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