이학박사학위논문

VARIATION AND ADAPTIVE FUNCTION OF IRIDESCENT COLORATION IN THE BLACK-BILLED MAGPIE (*PICA PICA*)

까치(*Pica pica*)의 깃털에서 금속성광택의 변이와 적응적 기능

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ABSTRACT

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Iridescent coloration, which is produced by coherent scattering of light from keratin nanostructures in feathers, has not been fully investigated in terms of its adaptive function and evolutionary significance. In particular, there have been no studies of the iridescent coloration of avian species that lack distinctive sexual dichromatism. Black-billed Magpies (*Pica pica*), which are common and abundant throughout Korea, have two types of iridescent coloration on their wings and tail. I examined variation in iridescent coloration of magpies in relation to individual social class, body conditions, and breeding success, at Seoul National University, Seoul, Korea. In addition, I measured the iridescent coloration of magpies from nine local areas in Korea in order to understand links between the coloration and the variation in local climatic factors, in particular, deviation in temperature, humidity, precipitation, and wind speed of the study year from normal years. Adult magpies had higher color scores, characterized by brighter, shorter wavelength-directed, more saturated colors, than did young birds. The results imply that plumage coloration in magpies may act as a signal that enables young magpies to avoid direct competition with strongly territorial breeding adults, or with more experienced non-breeding adults in the same flock. Iridescent coloration was significantly linked with indices of size and body condition in males only, particularly, with their tail characteristics, which are often regarded as quality indicators in breeding males. Breeding males with brighter iridescent coloration started breeding earlier and had better breeding success, even though brighter coloration did not guarantee the earlier fledging of their young. Iridescent tail coloration had a higher deviation value, estimated by the coefficient of variation, than that of other morphological traits, and the coloration of males was mainly related to variation in climatic factors. Based on these results, I suggest that the iridescent tail coloration of male magpies can be an honest signal of body condition and breeding success and is therefore probably a trait selected for by females. Further research on the effect of environmental variation (such as nutritional conditions and climate fluctuations) on the feather structures of magpies, and the relationship between variations in feather structure and consequential variation in iridescent coloration, is necessary.

Keywords: Black-billed Magpie (*Pica pica*), iridescent coloration, plumage coloration, sexual selection, structural coloration

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coloration among the local groups did not differ significantly (tail brightness - $F_{4,638} = 0.97$, $P = 0.42$; tail hue - $F_{4,644} = 1.39$, $P = 0.24$). Asterisks denote significant differences between the sexes: ** $P < 0.01$, * $P < 0.05$.

Figure 4-4. Differences in deviation indices (coefficient of variation adjusted by tarsus length) of morphological characters of females and males from nine local regions. Post-hoc tests were made using the Mann-Whitney test and P-values were Bonferroni adjusted. Asterisks denote significant differences between the sexes: ** $P < 0.01$, * $P < 0.05$.

Figure 4-5. Relationships between tail coloration and anomalies in local climatic factors. Climatic anomalies were calculated as the ratio of the difference between the mean value of a 30-year baseline period (1971-2000) and the value for 2004 divided by the mean value for the 1971-2000 period. Statistically significant relationships were noted as bold regression lines.

Figure 4-6. Relationships between deviations in color and morphological characters and deviations in local climatic factors. Deviations in climatic factors were calculated as the ratio of the difference between the mean values of the period 1971-2000 and the value for 2004 divided by the mean value for the period 1971-2000. Asterisks denote significant levels as tested using the Spearman Correlation: ** $P < 0.05$, * $P < 0.10$.

GENERAL INTRODUCTION

The elaborate avian visual system

The most important means of communication for birds is via visual signals. Unlike primates including *Homo sapiens*, which have trichromatic vision, birds have a more broadly sensitive visual system known as tetrachromacy, shared only with a few fish, and some reptiles and amphibians, capable of visualizing UV wavelengths (Bowmaker et al. 1997; Vorobyev et al. 1998). Birds also use oil droplets located between the cone cells and the optic nerves (Vorobyev et al. 1998; Vorobyev 2003) to reduce light scattering and thus enhance transmission of the visual signal. Diurnal raptors typically have more than five times higher resolution than humans (Lande and Nielsson 2004), and except for the densest part of the retina, birds generally have as many as ten times the number of cone cells that humans have (Walls 1942, cited by Bradbury and Vehrencamp 2011). In order to process all of this visual information, birds have a large area of the brain devoted to visual perception, cognition, and memory of visual signals (Waldvogel 1990).

In parallel with their elaborate visual system, birds use their plumage not only to enhance their chances of survival through concealment and crypsis, retaining or reflecting heat to maintain body temperature (Gill 1994), but also to send complex signals relating to age, social dominance, and sex. Most importantly, elaborate plumage coloration is commonly used in a sexual context.

Plumage coloration as an important sexual signal in birds

The bright colors and patterns of avian plumages have inspired aesthetic appreciation among people worldwide, and have also evoked scientific interest for centuries. However, not all birds have conspicuous plumage coloration. In some species only one sex has conspicuous plumage while the other is cryptic, while in other species both sexes may be dull and they are commonly indistinguishable to the human eye. Why do some bird species have colorful plumage, or more colorful plumage than others?

Among the earliest studies that attempting to interpret the adaptive significance of plumage coloration, were those of Darwin (1859, 1871). Darwin (1859) wrote that: “I can see no good reason to doubt that female birds, by selecting, during thousands of generations, the most melodious or beautiful males, according to their standard of beauty, might produce a marked effect.” Female choice of males is one of the main mechanisms of sexual selection. Since the male traits that females select are related to the quality of those males, which helps to maximize the breeding success of females, females indirectly determine the quality of males through the selection of ornaments (Fisher 1930). Traits, such as conspicuous plumage colors or color patterns and excessively elongated feathers, whether of the crown, the wing, or the tail, require more energy both in their development and in their maintenance than normal feathering, and are an additional burden making predator avoidance more complex and energy consuming. Therefore, these sexually selected adornments may be considered handicaps because such conspicuous traits may reduce the survival of the bearer (Zahavi 1975, 1977). From a female's perspective, when choosing among competing potential mates, these male traits are regarded as "honest signals" of their quality (reviewed by Andersson 1994).

Since Darwin's (1859, 1871) pioneering work, there have been numerous studies on the various colorations of birds and their implications for sexual selection. Plumage coloration in males (or sometimes in females) has been studied in relation to body condition (Slagsvold and Lifjeld 1992), parasite control (Hamilton and Zuk 1982), immunity to disease (Dufva and Allander 1995), intrasexual competition (Rohwer 1982), the ability to hold key resources such as territories or food (Keyser and Hill 2000), mate choice (Kirkpatrick 1982; Hill 1990; reviewed in Hill 2006b), assortative mating (Andersson et al. 1998), and reproductive success (reviewed by Andersson 1994) in order to establish how plumage coloration may act as an honest indicator of individual quality.

Various types of plumage coloration and their measurement

Although numerous studies on plumage coloration (Bowers 1956; Hamilton and

Barth 1962; Rohwer 1975; Baker and Parker 1979; Burtt Jr. 1981; Andersson 1986; Butcher and Rohwer 1989) were undertaken during the 20th century, it was the advent of the spectrophotometer, which has enabled quantitative measurements of the colors that cover the entire avian visual range (Bennett et al. 1994), that has led to rapid development of this field (Burkhardt and Finger 1991; Hunt et al. 1998, 1999; Hausmann et al. 2002). I was not possible to explain the adaptive function of UV plumage coloration through traditional methods relying on the human visible range of light. Contrary to previous findings (reviewed by Bennett et al. 1994), recent studies using spectrophotometers have revealed that more than 90% of avian species are sexually dichromatic (Eaton 2005, 2007), implying that almost all birds use plumage color as a sexual signal.

Generally, the score for the plumage color component is extracted from the reflectance curve of the light, particularly from the wavelength of interest (Montgomerie 2006); the "brightness" is a peak reflectance value of the curve (Andersson 1999), the "hue" is a wavelength value at the peak reflectance (Andersson 1999), and the "chroma" is the ratio between the integration area of the entire reflectance and the area of the wavelength range of interest (Andersson et al. 1998; Shawkey et al. 2003) (Figures I, II). The patterns of the reflectance curves are dependent on the mechanisms by which the colors are produced. Colored pigments, such as carotenoids or melanins produce yellow, red, brown, or black colors. The micro- and/or nanostructure of feathers produce white, blue, violet, ultraviolet, iridescent, or fluorescent colors according to various light scattering methods. While

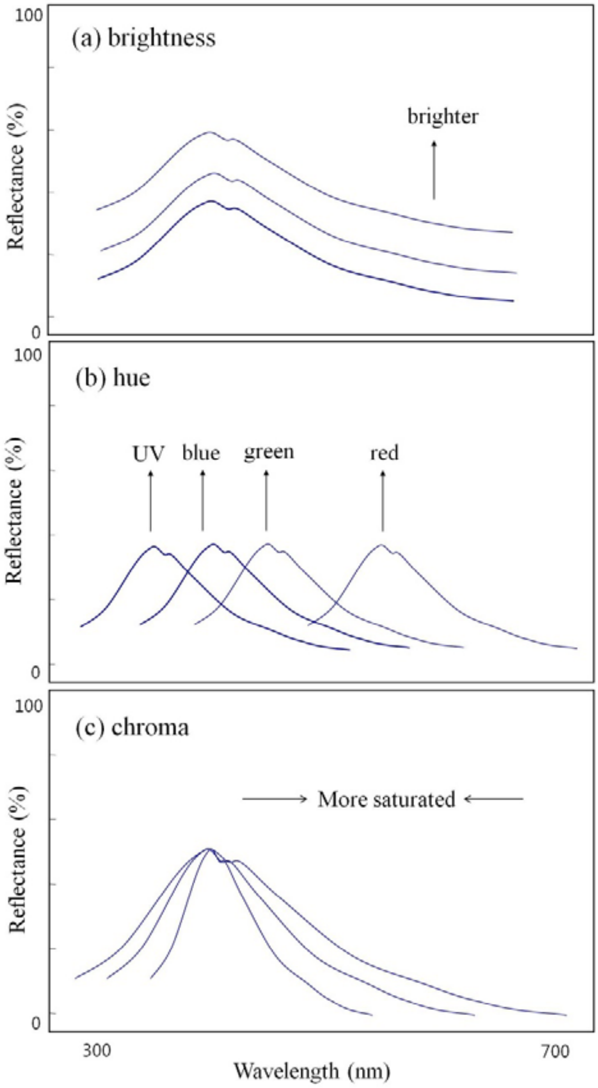

Figure I. Components of color: (a) brightness, peak value of the light reflectance curve, (b) hue, value of wavelength peaks, (c) chroma, ratio between the integration of curve area of a particular wavelength range and the entire wavelength range.

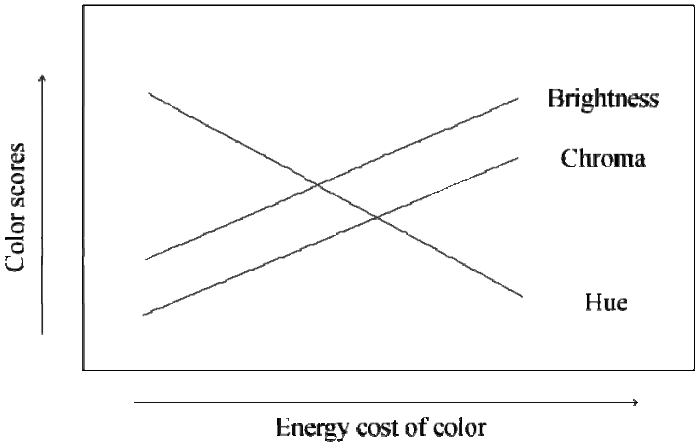

Figure II. A schematic diagram of the relationship between the energy cost of color production and color expression for general structural coloration.

the colors produced by pigmentation have been long studied, the research history into structural coloration is rather short because large parts of the ultraviolet region of the spectrum are not perceived as color by the human eye and because the color production mechanisms are very complex and depend on microscopic changes in feather structures (Prum 2006).

Aims and Objectives of the study

Despite there having been many studies on the ecological significance of non-iridescent structural coloration (Bennett et al. 1996; Andersson et al. 1998; Hunt et al. 1998, 1999, 2001; Johnsen et al. 1998; Keyser and Hill 1999; Banks 2001; Pearn et al. 2001; Siitari and Huhta 2002; Siitari et al. 2002; Mennill et al. 2003; Shawkey et al. 2003; Siefferman and Hill 2003; Alonso-Alvarez et al. 2004; Doucet et al. 2004; Woodcock et al. 2005; Delhey et al. 2007; Siefferman et al. 2007; Budden and Dickinson 2009), studies of iridescent coloration, which is produced by the interference effect of coherent scattered light from the hollow melanin granules within barbules (Durrer 1977, cited by Kinoshita 2008), are in their early stages because of the difficulty in quantifying aspect of colors that vary widely depending on the angles of the light emitted (Meadows et al. 2011). Consequently, previous studies of different species or even of the same species have led to inconsistent results concerning the relationships between colors and the traits of the bearers of those colors (Bennett et al. 1997; Cuthill et al. 1999; Doucet 2002; Perrier et al. 2002; Doucet and Montgomerie 2003; Costa and Macedo 2005; Hill et al. 2005; Doucet et al. 2006; Bitton and Dawson 2008; Bitton et al. 2008; Santos et al. 2009). Variation in feather structure results in variation in iridescence (Prum 2006). Therefore, in order to clarify whether iridescent coloration is adaptive, and whether it is selected as a sexual signal, I investigated the relationship between the variation in plumage coloration of the Black-billed Magpie (*Pica pica*), and the various internal and external environments that may affect variation in magpie feather structure. In Chapter 1, I examined whether three types of structural coloration (including two iridescent colors and one white non-iridescent color) yield information on an individual magpie's social status, such as age, sex, and territory ownership. In Chapters 2 and 3, the relationship between iridescent coloration and individual quality was investigated to test whether iridescent coloration acts as a sexually selected trait. In Chapter 2, I analyzed the relationships between magpie body condition (size, feather condition, hematological traits, and immunological traits) and iridescent coloration, and in Chapter 3 I looked at the link between iridescent coloration and reproductive success. In Chapter 4, I examined how the external environment affects variation in iridescent coloration, in order to determine the method for the adaptive variation of iridescent coloration as a signal.

Study Species

Birds in the family Corvidae, which includes the [1]Black-billed Magpie (*Pica pica*), are widely known to be sexually monochromatic (both sexes having the same coloration), with little size dimorphism (approximately 10%; Erpino 1968). No previous studies have investigated whether or not plumage characteristics are sexually selected factors that affect the evolution of mating systems among the Corvidae.

The Black-billed Magpie is a social, sexually monochromatic species, which commences breeding only after pair formation and the territorial acquisition. In densely populated areas, it usually takes several years for a pair to obtain a territory.

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[1] The suggested English name of the study population *Pica pica sericea* is the Eurasian Magpie (Gill and Donsker 2012). However, I have used the English name Black-billed Magpie following the Checklist of the Birds of Korea (The Ornithological Society of Korea 2009).

Both sexes show intensive territoriality, but males spend more time and energy in territorial defense (Birkhead 1991). Black-billed Magpies are boldly patterned and have both non-iridescent and iridescent colors in their plumages. The bright white scapular area and the white belly are non-iridescent, contrasting with the remainder of the plumage which is essentially black, however, the wings though appearing black also show blue iridescence, while the seemingly black tail has green iridescence. Furthermore, the magpie is a widely distributed, abundant member of the Corvidae in Korea, making it an ideal species in which to examine the potential role and function of plumage coloration as a signal.

Study area

The study area at the Seoul National University campus (37°27’N, 126°57’E) consists of buildings and small patches of bush and grass in a 4.7 km^2 sized semi-urban area near a forested mountain (Figure III). Annually, 50-60 breeding pairs of Black-billed Magpies hold territories in the area, and several flocks of non-breeding individuals reside on the periphery of the campus. In this area, intensive competition between breeding pairs, between territorial and non-territorial individuals, and among non-territorial individuals, has been observed over the years due to the high breeding density (Lee 2005).

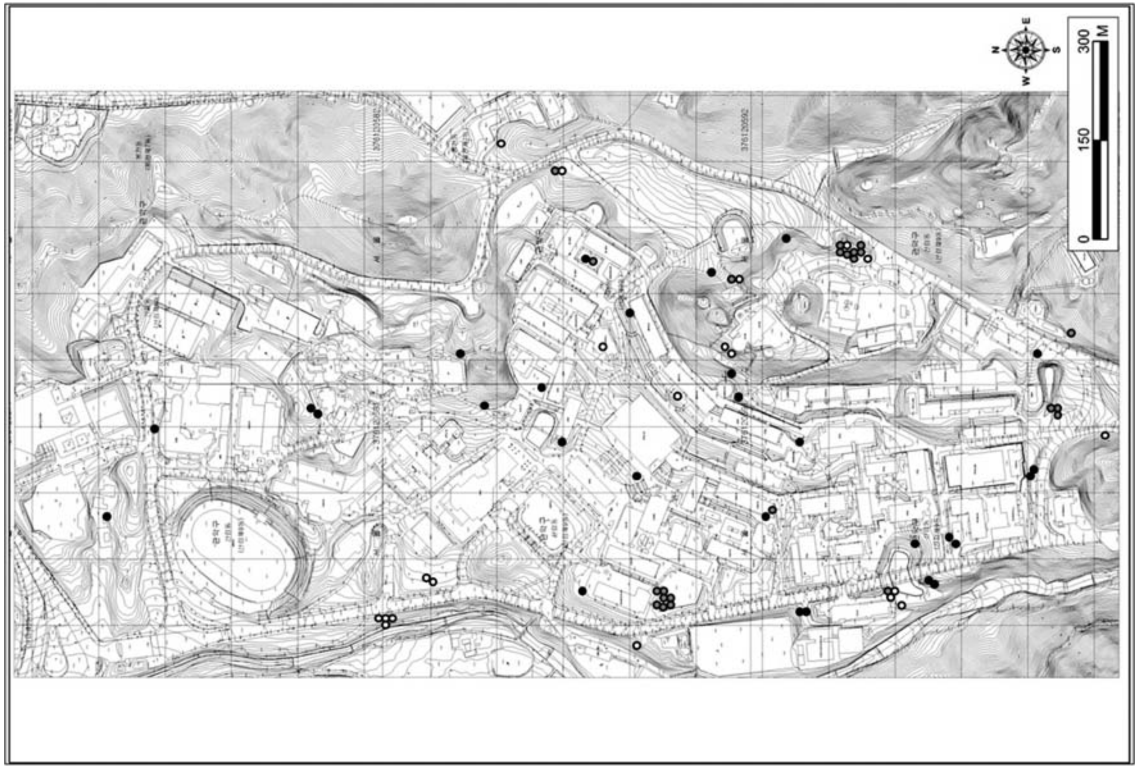


Figure III. The study site (Seoul National University campus) and locations where magpies were captured. Black circles: territorial AHYs (after-hatching-year); grey circles: non-territorial AHYs; white circles: HYs (hatching-year).

General methods

Magpies in the study area were caught using ladder traps (Bub 1991) in 2003, 2004, and 2006. Trapping trials were continued from October to December by when the autumn molt of adults (complete molt) and hatching-year young birds (most of the feathers except for a few primaries) has been completed (Ginn and Melville 1983). This was to avoid any effects from feather abrasion on plumage coloration. I measured the body size of magpies, and marked them with color rings and patagial wing tags engraved with two letters for future identification. I assessed their color, and took small samples of blood (0.3–0.5 mL) for hematological assay and molecular sex determination. To evaluate their reproductive success, I monitored breeding behaviors from the January following marking, and accessed active nests approximately twice per week throughout the breeding period to check breeding performance (i.e. egg number, chick number, time of hatching, mortality rate, and fledging rate). To examine variation in plumage coloration according to environmental variables, magpies that had been harvested during an ongoing damage control program by the Korea Electric Power Corporation were collected from nine regions in Korea. The relationship between the variation in coloration and local climatic factors was investigated using data from the Korea Meteorological Administration.

CHAPTER 1

MULTIPLE STRUCTURAL COLORS REFLECT SOCIAL STATUS SUCH AS AGE, SEX, AND TERRITORY OWNERSHIP IN THE BLACK-BILLED MAGPIE (*Pica pica*)

Abstract

I investigated whether structural colors represent class signals related to age, sex, and territory ownership in a social, sexually monochromatic species, the Black-billed Magpie (*Pica pica*). Among the measured reflectance spectra from the white scapulars, and iridescent wing and tail plumage, there were significant color differences between the age classes in all measured parts, with adults having higher color scores than young magpies. Color differences between males and females and between territorial owners and non-breeding adults were only detected in the greenish iridescence of the tail plumage. Adult male magpies had higher tail plumage color scores than did females. Territorial females tended to show lower tail color scores than non-breeding adult females, whereas there was no significant difference between territorial and non-breeding males. Color differences among

individuals belonging to different social classes may lessen agonistic confrontations between territorial and non-territorial individuals. Sex differences in coloration may enable prompt sex recognition and so facilitate pair formation. Moreover, the higher tail color scores of adults, particularly among males, supports previous suggestions that the tail characteristics of avian species with relatively long tails may represent a visual signal of the bearer's quality.

Introduction

Plumage coloration, as a signal indicative of the bearer's quality such as body condition (Hamilton and Zuk 1982) or social status (Rohwer 1975), has long been investigated. Many observational and experimental studies have revealed that the white and non-iridescent structural colors are indicative of an individual's quality, with individuals of higher quality having higher color scores. Non-iridescent colors serve to indicate individual quality because body condition and stress during molt are both believed to affect the development of feather nanostructures, the fineness and density of which affect the production of more or less intensive and/or saturated colors (Shawkey et al. 2003). The same hypothesis has been tested for iridescent coloration, but the results so far have been inconsistent. Several species, including European Starlings *Sturnus vulgaris* (Bennett et al. 1997), Satin Bowerbirds *Ptilonorhynchus violaceus* (Doucet and Montgomerie 2003; Doucet et al. 2006), Blue-black Grassquits *Volatinia jacarina* (Doucet 2002), Wild Turkeys *Meleagris gallopavo* (Hill et al. 2005), and Tree Swallows *Tachycineta bicolor* (Bitton and Dawson 2008; Bitton et al. 2008), have been shown to exhibit positive correlations between iridescent plumage color and body condition, social or sexual signals. However, contradictory results have been found in other species and even in the same species (Perrier et al. 2002; Costa and Macedo 2005; Santos et al. 2009). Such inconsistencies may result from the spatial organization of the arrays of keratin, melanin, and air layers, and may vary among species (Prum 2006), which may result in considerable variation in the iridescent color.

Whereas the quality-signaling function of structural coloration has been studied widely (Bennett et al. 1996; Hunt et al. 1999; Keyser and Hill 1999; Banks 2001; Pearn et al. 2001; Siitari and Huhta 2002; Siitari et al. 2002; Siefferman and Hill 2003; Alonso-Alvarez et al. 2004; Doucet et al. 2004; Woodcock et al. 2005; Delhey et al. 2007; Siefferman et al. 2007; Budden and Dickinson 2009), there have been few studies on the variation in structural colors, especially in iridescent colors, among different social classes (Hill 1988; Mennill et al. 2003; Morimoto et al. 2005). Some research has demonstrated that plumage coloration can serve as a status indicator that contributes to the reduction of severe agonistic confrontations between and within age or sex classes (e.g. Rohwer 1975; Senar 1999). Visible signals that indicate social status could be important in the Black-billed Magpie (*Pica pica*), because in this strongly territorial species there are frequent agonistic interactions between individuals (Birkhead 1991). Black-billed Magpies have both non-iridescent and iridescent colors in their plumage. Among the former, non-iridescent colors, they have bright white scapular and belly feathers, which serve to enhance the strong black-and-white contrast in this species. Contrasting black-and-white patterns have been reported as being related to individual quality in some species (Mennill et al. 2003; Doucet et al. 2004; Woodcock et al. 2005). Among the latter, iridescent colors, Black-billed Magpies exhibit bluish iridescence on their secondaries and greenish iridescence on their tail feathers. Vigneron et al. (2006) suggested that the color difference between the two iridescent plumage areas in magpies was caused only by a slight difference in the light-scattering process.

Götmark (1997), in the only study of the signaling function of the pied plumage of magpies, concluded the strong black and white contrast posed an increased predation risk, and thus served as a “handicap signal”. Although it is highly likely that plumage areas provide signal information about the bearer that can be used in social contexts (such as aggression and dominance), this possibility has not been examined so far in this species. Therefore, in this study I investigated whether the different plumage areas of Black-billed Magpies carry information concerning the age, sex, or territoriality of individuals. I examined correlations between structural coloration, iridescent or white plumage, and information on individual identity, in order to assess the signaling function of plumage coloration in Black-billed Magpies for intraspecific communication.

Materials and Methods

Trapping magpies

I trapped magpies from late October to early December in 2003, 2004 and 2006. This period of the year was selected because it corresponds to the end of the annual molting season (Ginn and Melville 1983; Nam pers. obs.) and so that the effect of abrasion on plumage coloration could be minimized (Örnborg et al. 2002). I placed hand-reared magpies, as decoys, into ladder traps (2 ×3 ×2 m, Bub 1991). Because of the high density of the study population compared with previously studied populations (Lee et al. 2011), and because magpies are very cautious even after becoming accustomed to the presence of people, I placed traps near nests at night and put in the decoy birds before sunrise. Newly caught magpies, and decoys, were immediately retrieved from traps in order to prevent nearby magpies from becoming trap shy. When trapping trials were made in adjacent territories, the trials were separated by at least two days and different decoy birds were used. After measuring captured birds, I collected blood samples for molecular sexing. For future identification, I marked the individual magpies with colored rings and patagial wing tags on which two letters had been written with acrylic paint.

Identification of age, sex and territory ownership

The extent of black on the tips of the primary feathers was used to discriminate between hatching-year (HY) birds and after-hatching-year (AHY) birds following Erpino (1968) and Lee et al. (2007). I measured the length of the black tip of the longest primary feather (generally P7) in order to determine age of trapped individuals. HY individuals generally have longer black tips than AHY birds, and the distribution of the length of the black tip is bimodal with a clear distinction between the two age classes. The mean black tip length of AHYs was 10.65 ± 2.27 mm, and that of HYs was 29.29 ± 5.38 mm (mean ± SD) in this study.

Male magpies are approximately 10% larger than females (Reese and Kadlec 1982; Scharf 1987; Kavanagh 1988; Lee et al. 2007), but there is considerable overlap in the size distributions of males and females, which hampers accurate sex determination based on morphology. To facilitate molecular sexing, and to further hematological research, I collected 0.3-0.5 mL of blood from a brachial vein of each captured bird. Two chromodomain-helicase-DNA-binding protein genes (CHD-W and CHD-Z) with different intron sizes were amplified using PCRs with P2 and P8 primers (Griffiths et al. 1998).

The breeding behavior of marked individuals was observed in the breeding season following capture, spanning the periods from January to June. AHYs were classified as “territorial AHYs” when they were observed to show nest-oriented behaviors, such as territorial behavior (giving alarm calls, tree-topping and chasing intruders) and breeding behavior (nest building, incubation and collecting food for nestlings). If AHYs did not show nest-oriented behaviors, or failed to maintain breeding territories throughout the breeding season, they were regarded as “non-territorial AHYs”. Although such birds showed occasional nest building or territorial behaviors around pre-occupied territories and in flocking areas, all of their nest-oriented behaviors were temporary and unsuccessful.

Color measurement and analysis

The scapular feathers of the Black-billed Magpie appear pure white; the secondary feathers and the wing coverts are iridescent with a bluish sheen, while the tail feathers are iridescent with a greenish sheen for most of their length but have a purple-bluish sheen at the tip. I measured the reflectance of the scapular, wing and tail plumage using a spectrophotometer (USB2000; Ocean Optics, Dunedin, FL, USA). Measurements include: (i) the middle part of the white scapular region (non-iridescent), (ii) the middle part of the outer vane of one of the 4-6th secondary remiges of the left wing (bluish iridescence), and (iii) the middle part of the vanes of the uppermost (1st) rectrix (greenish iridescence) (Figure 1-1). Light in the UV to visible wavelengths was emitted from a deuterium-tungsten-halogen light source, and a white standard was used for calibration prior to each reflectance measurement. From the entire measured spectra (250-800 nm), the bird-sensitive spectrum, which lies within the UV-A to the visible light range (320-700 nm), was selected for analysis using OOIBase software (Ocean Optics) (Figure 1-2). All data were measured using a reflectance probe perpendicular to the plane of the feathers. Each area was measured five times, and averaged (to test correlations between color components) or treated as repeated measures (to test color differences between groups). To measure the size of the white scapular patch, digital photographs were taken and the lengths of the two longest perpendicular axes of the oval-shaped scapular patch were measured with the wings in a naturally closed position, using vernier calipers. The relative area of the white patch was calculated by counting the number of white pixels using Adobe Photoshop 7.0 (Adobe System Inc., San Jose, CA, USA). Calculated patch areas were divided by wing lengths in order to adjust for the effects of body size on the size of the white scapular patch.

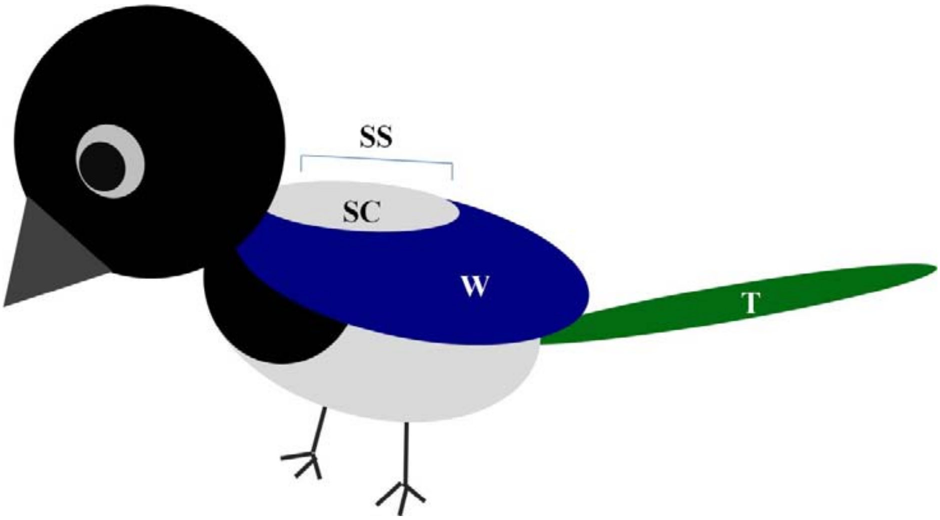


Figure 1-1. Plumage areas of the Black-billed Magpie (*Pica pica*) for which colors were measured. W: wing color (iridescence), T: tail color (iridescence), SC: scapular color (non-iridescence), and SS: scapular size (non-iridescence).

From the reflectance measured from iridescent wing and tail feathers, three color components were determined: brightness (peak reflectance; Andersson 1999), hue (wavelength at the peak reflectance; Andersson 1999) and chroma (proportion of integration of reflectance at hue ± 50 nm to 320-700 nm; Shawkey et al. 2003). Two color components were determined from white scapular plumage: brightness (mean reflectance from 400 nm to 700 nm; Delhey et al. 2003) and UV-chroma (proportion of integration of reflectance at 320-400 nm to integration of reflectance at 320-700 nm; Andersson et al. 1998).

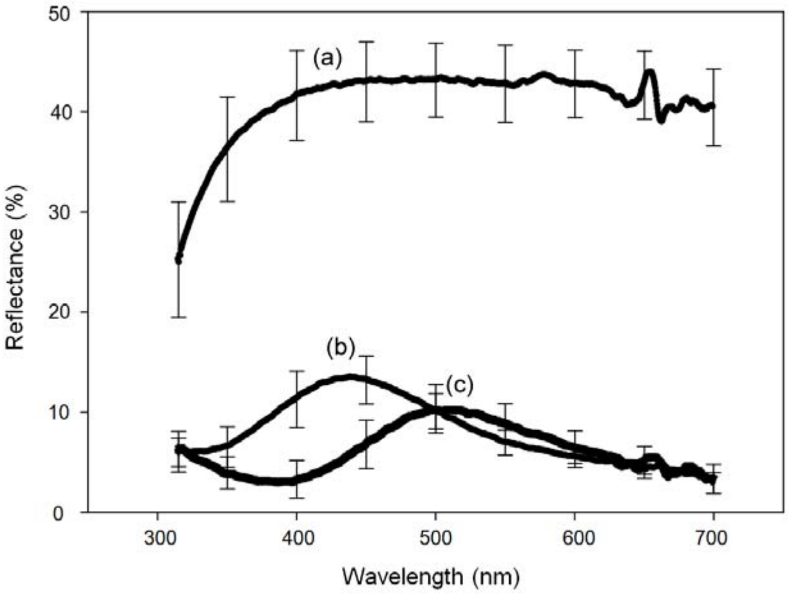


Figure 1-2. Reflectance spectra of (a) scapular, (b) wing, and (c) tail plumage of the Black-billed Magpie (*Pica pica*). Vertical bars represent standard deviations noted every 50 nm.

Statistical analyses

I examined the correlation of the color components from different plumage parts by conducting Pearson correlation analysis on the averages from five measurements of each color component. Neither wing and tail brightness, nor wing chroma were normally distributed. These were natural log and arcsine transformed respectively before the analysis. In order to test for differences in color components among individuals of different age, sex classes and territory holding status, I used a general linear mixed model for repeated measures. Since there was no effect of year on the color components, I included "year" as a random factor in all of the analyses. The entire dataset, including both HYs and AHYs, was examined to establish whether the color components differ depending on the age and sex of individuals. Differences in coloration according to territory ownership were examined for AHYs only, because many HYs disperse from their natal area in late autumn (Birkhead 1991; Nam pers. obs.), and none of them was observed to establish their own territories in the following year in the study area. Although HYs may disperse and establish their own territories outside the study area, this is unlikely to affect the main results because the chances of HYs establishing successful territories are known to be quite low (Birkhead 1991). Post-hoc analysis of color differences between the sex and territory ownership classes of AHYs was conducted and the significance of pairwise differences was adjusted by Bonferroni correction. All statistical procedures were conducted using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

Results

In total, 71 Black-billed Magpies were caught (43 in 2003, 19 in 2004, and 9 in 2006). Based on the results of molecular sexing, 34 individuals were females and 37 were males. It was also established that 20/71 (28.2%) were HYs and 51/71 (71.8%) were AHYs. Among HYs, nine were females and 11 were males. Among AHYs, 28 (54.9%; 13 females, 15 males) individuals established territories in the study area during the breeding season following capture.

Correlations among color components

Correlations between the color components of the white scapular patch and those of the iridescent parts of the wings and tail were low except for scapular brightness, which showed a positive correlation with wing and tail chroma (Table 1-1).

Color components were correlated between iridescent plumage parts. Brightness and chroma within and between wings and tails were positively correlated, with brighter plumage having greater chroma (Table 1-1). Hue was negatively correlated with brightness or chroma in both wings and tails (Table 1-1). Males tended to have higher correlation coefficients in brightness between wings and tails than females ($r = 0.54$, $P < 0.01$ in males; $r = 0.42$, $P = 0.01$ in females). Correlations between wing chroma and tail brightness ($r = 0.39$, $P = 0.02$), wing chroma and tail hue ($r = -0.41$, $P = 0.01$) were only detected in males. Correlation coefficients of color components between wings and tails were higher in AHYs than in HYs. Most of the color components in wings and tails were significantly correlated in AHYs (wing brightness – tail brightness: $r = 0.50$, $P < 0.01$; wing hue – tail hue: $r = 0.37$, $P = 0.01$; wing chroma – tail brightness: $r = 0.28$, $P = 0.05$; wing chroma – tail hue: $r = -0.30$, $P = 0.03$; wing chroma – tail chroma: $r = 0.27$, $P = 0.05$), whereas none of the same components between the wings and tails of HYs were significantly correlated.

Table 1-1. Correlation matrix among color components of the plumage of the Black-billed Magpie (*Pica pica*) based on two-tailed Pearson correlation analysis. Correlation coefficients are presented, with statistical significance indicated as * for $P < 0.05$ and ** for $P < 0.01$.

		Wing			Tail			Scapular		
		Brightness	Hue	Chroma	Brightness	Hue	Chroma	Brightness	UV-Chroma	Size
Wing	Brightness		-0.13	**0.29****	**0.46****	-0.12	-0.05	-0.05	0.11	0.06
	Hue			**-0.39****	-0.22	**0.32****	0.03	-0.18	0.07	0.11
	Chroma				**0.23***	**-0.34****	**0.26***	**0.40****	0.02	0.05
Tail	Brightness					-0.21	**0.39****	0.17	0.04	0.11
	Hue						0.11	-0.15	-0.10	-0.01
	Chroma							**0.35****	-0.04	0.08
Scapular	Brightness								-0.15	0.04
	UV-Chroma									-0.11
	Size									

Color differences among classes

Both iridescent and white structural colors showed differences in relation to sex, and/or age (Table 1-2). Age differences were found in all plumage parts. AHYs had brighter scapular patches, shorter wavelength hues in the wing and tail, and higher proportions of the peak area in wings and of the UV-A area in the scapulars (Table 1-2).

Sex differences in coloration were only detected in the tail feathers. Males had brighter and shorter wavelength directed tails than females (Table 1-2). Among AHYs, males also had brighter ($F = 9.97$, $P < 0.01$) and shorter wavelength directed ($F = 10.04$, $P < 0.01$) color in their tails than did AHY females. Sex differences in plumage color were observed among territorial AHYs ($t = -4.39$, $P < 0.01$ in brightness; $t = 2.82$, $P = 0.03$ in hue), but not among non-territorial AHYs (Figure 1-3). Tail brightness revealed significant interactions between territory ownership and sex ($F = 7.39$, $P < 0.01$), and this was due to lower brightness values in territorial AHY females than in non-territorial AHY females ($t = -2.95$, $P = 0.02$; Figure 1-3).

The area of the white scapular patch did not differ significantly in relation to the classes considered (Table 1-2).

Table 1-2. Color and colored patch size differences in the plumage of Black-billed Magpies (*Pica pica*) in relation to age and sex. Statistical significance levels are indicated as * for $P < 0.05$, ** for $P < 0.01$, and ^{ns} for non-significant differences. Values are noted as mean ± SD.

Plumage Part	Color Components	HY		AHY		F value	
		Female	Male	Female	Male	Age	Sex
Wing	Brightness	16.50 ± 2.47	15.36 ± 3.10	18.12 ± 3.39	18.23 ± 4.77	3.05^{ns}	1.42^{ns}
	Hue	426.17 ± 15.83	419.31 ± 12.86	414.38 ± 12.50	412.86 ± 11.61	19.64^{**}	2.60^{ns}
	Chroma	0.41 ± 0.04	0.43 ± 0.03	0.46 ± 0.03	0.46 ± 0.03	29.75^{**}	0.94^{ns}
Tail	Brightness	11.40 ± 2.37	12.22 ± 2.46	11.76 ± 2.27	14.00 ± 3.56	3.49^{ns}	6.87^{**}
	Hue	528.39 ± 11.74	509.56 ± 15.46	512.02 ± 16.35	502.58 ± 14.89	11.44^{**}	18.91^{**}
	Chroma	0.35 ± 0.04	0.37 ± 0.02	0.36 ± 0.03	0.37 ± 0.02	1.56^{ns}	3.03^{ns}
Scapular	Brightness	42.05 ± 4.33	45.26 ± 5.73	47.54 ± 4.07	47.50 ± 5.32	17.78^{**}	1.37^{ns}
	UV-Chroma	0.18 ± 0.03	0.18 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	4.47^{*}	0.73^{ns}
	Patch size	3.81 ± 0.83	4.40 ± 0.38	3.84 ± 0.69	3.93 ± 0.69	1.34^{ns}	3.81^{ns}

Discussion

Differences in coloration between age classes

The structural colors of Black-billed Magpies differ between age classes for all of the plumage areas measured: white scapular plumage and two iridescent colors in wings and tails. Black-billed Magpies are resident and territorial throughout the year (Birkhead 1991; Lee 2005). Although territorial breeders sometimes tolerate alien young as well as their own young during the dispersal period (Baeyens 1981a), young magpies are likely to elicit agonistic responses from territory holders as they pass through defended territories during their dispersal from their natal areas to flocking areas in late autumn. Furthermore, birds in their hatching year must also compete with older and more experienced individuals for food during their first winter. Having plumage colors that are distinct from those of older individuals may be helpful for young magpies in reducing aggressiveness directed at them by territorial adults and older individuals in the same flock, and in avoiding fatal competition (Conover et al. 2000; Morimoto et al. 2005; Mitrus 2007).

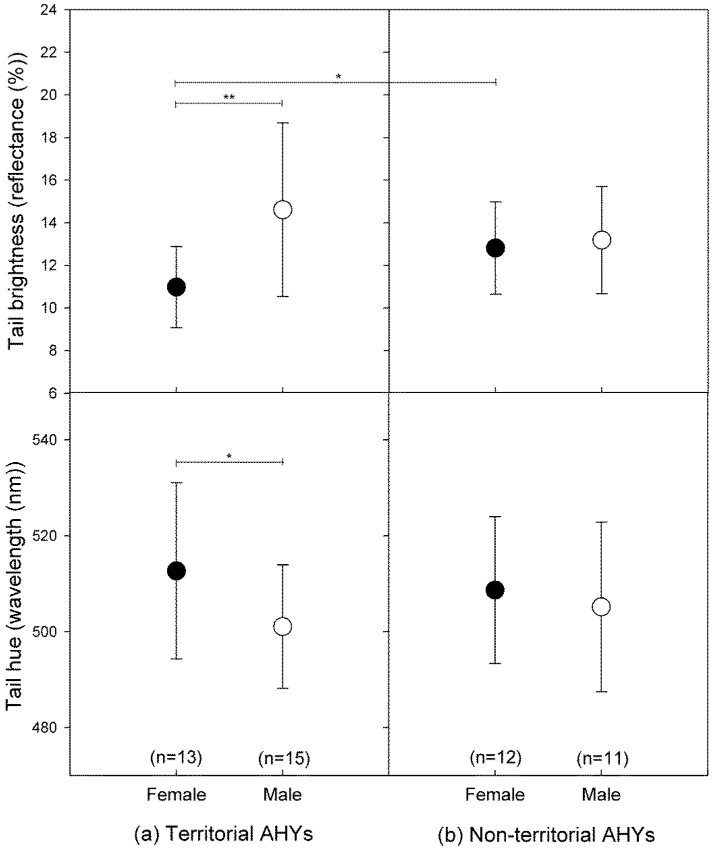


Figure 1-3. Sex differences among Black-billed Magpies (*Pica pica*) of tail brightness and hue, in: (a) territorial AHYs, and (b) non-territorial AHYs. Filled circles represent mean values for females, and open circles indicate mean values for males. Vertical bars represent the standard deviation.

Differences in coloration between the sexes

Sex differences were found only in the brightness and hue of the tail. In species such as magpies, with long and heavy tail feathers, maintenance of those feathers may represent an energetic cost or a time burden (Fitzpatrick and Price 1997; Aparicio et al. 2003), and the length and mass of the tail feathers, and the extent of damage to them can be correlated with individual quality (Blanco and de la Puente 2002). Considering that variation in brightness can be related to the number or condition of barbules (Shawkey et al. 2003; Doucet et al. 2006; Bitton and Dawson 2008), tail plumage brightness may be an honest signal of individual quality in Black-billed Magpies.

Differences in coloration according to territory ownership

I predicted that territorial and non-territorial AHY male magpies would show differences in tail color, as has been found in certain other species (e.g. the Galápagos Hawk *Buteo galapagoensis*; Whiteman and Parker 2004). Obtaining and defending a territory is costly and can be dangerous for magpies, particularly in areas of high competition (Shannon 1958). Individuals of higher quality are able to establish their territories earlier than individuals of lower quality (Birkhead 1991), thus territorial AHY male magpies should score higher for color in their tails than non-territorial males. However, I found no statistical differences in tail color of AHY males based on territory ownership, and territorial AHY females even had less bright tails than did non-territorial AHY females.

The reasons for these unexpected results are not clear, but I suggest two possibilities that are not mutually exclusive. Firstly, the quality of an individual when it develops its feathers may differ from its quality when it establishes a territory. Because territory acquisition is extremely difficult in this species (Birkhead 1991; Nam pers. obs.), territory owners spend much of their time and energy defending their territories. In other words, territory turnover rarely happens, and once a territory has been formed, ownership does not easily change and intruding males are rarely successful (Verbeek 1973; Buitron 1988; Veiga et al. 2001). Therefore, changes in the territory owner's quality may not affect territory turnover. Particularly, if the magpies in the study population have high mate fidelity in their occupied territories, and if a territorial female has proven her reproductive success, the female may remain territorial regardless of any seasonal changes in the quality and brightness of her tail. Secondly, it is likely that age is also correlated with individual quality, because the probability of acquiring good-quality territories or foraging skills is enhanced by age (Skórka and Wójcik 2008). Although older male magpies are more likely to be selected by females than younger ones, regardless of their social status in the flock (Komers and Dhindsa 1989), many older AHYs in this study area were also non-territorial, perhaps owing to the high magpie density in the limited area, low territory turnover rate, and high competition for territory acquisition. Therefore, the lack of any difference in age structure between territorial and non-territorial AHY males may lead to the absence of color differences in tails. Unfortunately, however, neither suggestion explains the lower brightness of the tails of territorial AHY females. Although older females, with more experienced breeding and foraging skills, may be duller in plumage than younger females in certain species (e.g. House Finch *Carpodacus mexicanus*; Hill 1993), species in which both sexes defend territories and are involved in parental care tend to mate assortatively (e.g. Merlin *Falco columbarius*, Warkentin et al. 1992; Blue Tit *Parus caeruleus*, Andersson et al. 1998; Black-capped Chickadee *Poecile atricapilla*, Ramsey and Ratcliff 2003; Northern Cardinal *Cardinalis cardinalis*, Jawor et al. 2003; Spanish Imperial Eagle *Aquila adalberti*, Ferrer and Penteriani 2003; Collared Flycatcher *Ficedula albicollis*, Laczi et al. 2011). A study on female iridescence in Tree Swallows (Bitton et al. 2008) and many studies on non-iridescent structural colors (reviewed by Amundsen and Pärn 2006) support the assortative mating theory, indicating that brighter males prefer brighter females. Therefore, the contradictory phenomenon found during this study among AHY females is interesting, but remains inexplicable.

Correlations among color components

The color components of the two iridescent plumage parts (wings and tail) seem to be more highly correlated with each other than with components of the white scapular patch. Research into the mechanism of color production in magpie remiges and rectrices (Vigneron et al. 2006) has revealed that although both have similar microstructures, the color production mechanism differs slightly. The greenish color of the tail is generated by photonic crystal first-gap reflection, whereas the bluish color of the wing is generated by the second-gap effect of first-gap infrared reflection. Although detailed color producing mechanisms may differ between remiges and rectrices, it is possible that iridescence in both sets of feathers is affected by the individual's body condition in a similar way, considering that the regularity and the thickness of the multi-layers, and the spacing of microstructures involved, influence iridescence (Bradbury and Vehrencamp 2011). Higher correlations between the wing and tail iridescent color variables in AHYs and males than in HYs and females may indicate that the two iridescent traits together enhance efficiency of signal transmission. However, considering that wing iridescence differed only in relation to age, whereas tail iridescence differed in relation to both age and sex, it is also possible that each colored part may act as a separate signal. Few studies have examined correlations among color traits or investigated the implication of multiple signaling among color traits between the sexes and/or age classes (Laczi et al. 2011). Therefore, further studies are needed on the production cost and the efficacy of the two iridescent plumage parts in order to clarify whether they form a backup signaling system or whether they send separate messages.

Signaling aspects between iridescent and non-iridescent coloration

In contrast to differences in iridescence, only age differences were found in the brightness and UV-chroma of the white scapular patches of magpies. No difference was detected in the size of the white patch between age or sex classes. This is in contrast with earlier studies of chickadees and tits where color score (Mennill et al. 2003; Doucet et al. 2004; Woodcock et al. 2005) and size (Galván and Sanz 2008) of white patches were correlated with social status and individual quality. These results, in contrast to a previous suggestion that the white patches may serve as handicaps in magpies (Götmark 1997), indicate that the color and size of the scapular patch in magpies may not serve as signals of individual identity. Thus, I suggest that more information is conveyed by the iridescent colors of Black-billed Magpies than is contained by the white patches. These results pose an interesting question: Why don't the most conspicuous white wing patches contain information that would be useful for intraspecific communication? The answer to this question may lie in the fact that magpies use behavioral displays that potentially maximize the visual impact of their iridescent colors for intraspecific communication. They often perform tail and wing flicking displays in aggressive contexts as well as during greetings between mates (Birkhead 1991). During these displays, the magpies move the iridescent parts of their wings and tails so that the intensity of the colors may be maximized and the plumage colors may have a greater visual impact than those without behavioral displays or without iridescence. Whether magpies can “direct” their iridescence toward an intended receiver by means of behavioral displays remains to be examined (Doucet and Meadows 2009). Nevertheless, the presence of behavioral displays combined with iridescent colors in the very plumage parts that are emphasized during those displays, strongly suggests that the information content coded in iridescent colors can be delivered more efficiently to other individuals than that coded in white plumage. Magpies perform “lateral displays” (Siefferman et al. 2008) where they tilt the body and half-spread their tail feathers towards the individuals that they are confronting. The visibility of iridescent plumage, if it is coupled with behavioral displays wherein this part of the plumage is involved, may exceed that of white plumage. It is also possible that the presence of white scapulars surrounded by the bluish iridescence of the wing feathers may function to maximize the chromatic and achromatic contrast, without containing any information relevant to individual identity.

The Black-billed Magpie was previously regarded as sexually monochromatic. However, in this study, it was found to be cryptically dichromatic with multiple structural colored parts differing by age, sex, and territory ownership. In particular, the iridescent coloration of the tail differed between the sexes among breeding birds. The results suggest that the differences in the structural colors of the Black-billed Magpie can be used as signals that indicate an individual’s social status.

CHAPTER 2.

BODY CONDITION AFFECTS VARIATION IN THE IRIDESCENT COLORATION OF THE BLACK-BILLED MAGPIE (*Pica pica*)

Abstract

Many studies have revealed that the structural coloration produced by feather micro- or nanostructures signals honest information about an individual's quality because the structural density of feathers is affected by nutritional or stress conditions during feather formation. However, the quality information of iridescent coloration, which is a special type of structural coloration produced by the interference of coherent scattering of lights reflected from the feather, has not been fully documented. The hypothesis that the iridescent plumage coloration indicates an honest signal of the body condition in the Black-billed Magpies (*Pica pica*), which is represented by two different types of iridescent coloration on their wings and tail was tested. Among the quality variables tested, including body size, tail characteristics, and immunological indices, most of the significant correlations were

discovered for the iridescent tail coloration of males. No significant correlations were detected in females between iridescent coloration and quality indices. Males in better body condition had brighter iridescent tails, suggesting that the iridescent coloration of their tail signals their body condition. However, the correlation pattern between color and tail characteristics differed among the various classes of males. Tail brightness and total tail length were positively correlated, but only in breeding males, not in hatching-year males; perhaps young magpies do not produce the costly bright iridescent coloration.

Introduction

One of the most important signal transmission tools in avian communication is visual; in particular, plumage coloration serves an important role in advertising the quality of an individual. Various studies of pigment-based plumage coloration such as carotenoids and eumelanin, or structural coloration dependent on feather micro- or nanostructures, have shown that these colors can be an index of the bearer's condition when the feathers develop (reviewed by Hill 2006a). Therefore, an assessment of the relationship between the quality of birds and their plumage coloration can provide clues to their means of sexual selection and about the evolution of their species-specific mating system (Olson and Owens 1998; Lozano 1994, 2001; Hill 2002).

Conspicuous ornaments, such as bright plumage or long feathers, which are correlated with an individual's quality, act as "honest information" that enables females (the choosing sex) to assess the quality of their potential mate (Fisher 1930). These ornaments are considered as a "handicap" because they may decrease the bearer's chance of survival. Females are essentially choosing males with greater handicaps, which directly imply that such males have good enough genes to be able to survive whilst bearing such a handicap (Zahavi 1975, 1977). Hamilton and Zuk (1982) suggested that the bright plumage coloration of males is related to them carrying "good genes" for resistance against parasites, and the good genes are indirectly selected through the selection of bright plumage. More recently, several studies have reported a relationship between quality of immunocompetence against a range of factors and plumage coloration of hosts (Folstad and Karter 1992; Dufva and Allander 1995; Saino et al. 1999; Figuerola et al. 1999; Møller and Petrie 2002; Masello and Quillfeldt 2004; McGraw and Klasing 2006).

The long, heavy tails of magpies are known to serve as an index of body condition (Fitzpatrick 1998). A long tail may perhaps reduce maneuverability during foraging and make avoiding predators more difficult; in addition, holding and preening the long tail itself requires much energy (Wooley Jr. and Owen Jr. 1978; Yorinks and Atkinson 2000; Griggio et al. 2009; Maia and Macedo 2011). Therefore, both the length and the mass of the feathers are expected to be indices of handicaps (Blanco and de la Puente 2002). It is also known that the immunocompetence of individuals is related to some characteristics of the tail (Blanco and de la Puente 2002) as well as nutritional condition or breeding phenology (Sorci et al. 1997; Cucco et al. 2002; Soler et al. 2003) in magpies. In this study, the relationships between iridescent plumage coloration and quality indices such as body condition index, tail length, tail mass, and immune indices were investigated to test whether or not iridescent coloration signals honest information for body condition.

Materials and methods

Trapping magpies

I trapped magpies from late October to early December in 2003, 2004 and 2006. I used ladder traps (2 ×3 ×2 m, Bub 1991), and hand-reared magpies, as decoys, were put in the traps. Newly caught magpies, and decoys, were immediately retrieved from traps in order to prevent nearby magpies from becoming trap shy. When trapping, trials were made in adjacent territories; trials were separated by at least two days and different decoy birds were used.

Identification of age, sex and territory ownership.

The extent of black on the tips of the primary feathers was used to discriminate between hatching-year (HY) birds and after-hatching-year (AHY; older than second calendar year) birds following Erpino (1968). AHYs were classified as either "territorial AHYs" or "non-territorial AHYs" according to their territory ownership. Most of HYs in the study population did not acquire their own territory in the year following hatching owing to the intensive competition in the study area where the population density is high. Sex was recognized by molecular methods (Griffiths et al. 1998) to avoid inaccurate sex determination based on morphology, given the considerable morphometric overlap between the sexes (Lee et al. 2007).

Color measurement and analysis

I measured colored plumage features of magpies with a spectrophotometer (USB2000; Ocean Optics, Dunedin, FL, USA). Reflectance spectra curves of 320-700 nm were drawn using OOIBase software (Ocean Optics). The colored parts were measured with a reflectance probe perpendicular to the plane of the feathers, each area was measured five times. From the measured reflectance of iridescent feathers in the wing and tail, three color components were determined: brightness (peak reflectance; Andersson 1999), hue (wavelength at the peak reflectance; Andersson 1999) and chroma (proportion of integration of reflectance at hue ± 50 nm to at 320-700 nm; Shawkey et al. 2003).

Measurement of body size and tail characteristics

Captured magpies were immediately transported to the laboratory. Because of concerns that neighboring magpies or passer-bys might occupy the temporarily vacant territory of the captured magpie during the period of processing, all measurements were completed within one hour. I attached individually recognizable wing tags and color rings to each bird, and measured tarsus length and body mass. A body condition index (BCI: body mass/tarsus length) was calculated for each individual based on this data (Jakob et al. 1996). I also measured lengths of all twelve rectrices. Tail length was defined as the length of the longest rectrix (T1), and the lengths of all 12 rectrices were summed to provide “total tail length”.

Feathers exhibit fault bars (bands of void spaces caused by an absence of feather barbules), which are indicative of nutritional or stress conditions during feather formation (Murphy et al. 1989; Machmer et al. 1992), and these fault bars are related to an individual's quality (Slagsvold 1982). I counted the number of fault bars on each of the 12 rectrices and summed them, giving a total fault bar count for each individual (Table 2-1).

Hematological assay

The amount of plasma protein present in avian blood indicates both disease resistance and nutritive condition. A reduction in total plasma protein concentration occurs in almost all diseased birds, but it is also seen when birds are malnourished (Ots et al. 1998). The ratio of albumin and globulin, the two plasma protein components, is also used as a general indicator of immunity. A lower albumin/globulin ratio reflects lowered resistance against disease (Kawai 1973). An increase in white blood cells (WBCs) followed by stressors or inflammatory process symptoms, is usually due to an increase in heterophils (Dein 1986). The number of heterophils (analogous to neutrophils in mammals) is related to an acute response against antigens, whereas the number of lymphocytes is related to cell-specific immunity (Dein 1986). The ratio of heterophils to lymphocytes is widely used as an indicator of immunity (Gross and Siegel 1983). Parasites in blood also affect hematological contents (Valkiūnas 2005). Haemoproteus, one of the most common protozoan genera among avian blood parasites, was found in just one sample (female) during this study, and because of its rarity the sample was excluded from the statistical procedures.

Small amounts of blood (0.3-0.5ml, approximately 0.15~0.25% of total body mass) were taken from a brachial vein. I made three blood smears per bird so as to reduce errors in blood cell counts. The remainder of the blood sample was kept in an EDTA test tube without further processing. The tube and smears were sent to the hematology laboratory within three hours, and a qualified veterinarian (who did not have any information concerning the samples) completed the hematological analysis. The amount of total protein was calculated using the Biuret method with a spectrophotometer (Olympus AU400, Tokyo, Japan). Smear samples were stained with Eosin-Thiazine (Aerospray 7150 hematology, Wescor, USA). Then the veterinarian made a total WBC count, a differential count (heterophil, eosinophil, basophil, lymphocyte, monocyte), and analyzed blood fat and protein (albumin, globulin) (Table 2-1). Although a t-cell mediated immune response test is widely used in avian immunocompetence assays (Cheng and Lamont 1988), this test was not conducted in this study because it takes at least 24 hours and because I had observed several cases of takeover attempts within one hour during the absence of territory owners for processing.

Table 2-1. Explanations of various quality indices tested in this study.

	Indicators	Explanation
Body measurement	Size PC1 Size PC2	"Overall" body size generated by PCA using body measurements (Rising and Somers 1989)
	BCI	Body Condition Index calculated as body mass / tarsus length (Jakob et al. 1996)
Tail characteristics	Tail length	General indicators of quality
	Total tail length	Size (area) of stretched tail feathers, or a substitute for measurement of tail mass "Tail mass" is a known indicator of quality in magpies (Blanco and de la Puente 2002)
	Fault bars	General indicators of stress during molting (Murphy et al. 1989; Machmer et al. 1992) Known indicator of quality in magpies (Fitzpatrick 1998)
Immune characteristics	Total protein	Reduced under all disease conditions, and especially so during malnutrition (Ots et al. 1998)
	Alb/glb	Low albumin / globulin ratio indicates weak resistance against disease (Kawai 1973)
	WBC	Elevated white blood cell counts are symptomatic of stress, and the inflammatory process (Dein 1986)
	H/L	Increase with various stressors (infectious disease, starvation) (Gross and Siegel 1983)

Statistical analysis

The 71 magpies captured during the study were divided into three sub-groups of each sex: breeding AHY, non-breeding AHY, and HY, according to their ages and whether or not they owned territories. Since neither wing and tail brightness, nor wing chroma were normally distributed, these values were natural log and arcsine transformed before the analyses. The overall size of each magpie was calculated using principal component analysis (PCA) with the PROC PRINCOMP procedure (Table 2-2). Differences in all quality-related indices (see Table 2-1) by age and sex were tested using the PROC GLM procedure. The relationships among the coloration indices and the quality variables were analyzed by stepwise regression using the PROC REG STEPWISE procedure. Indices variables, which met the 0.15 significance level, were entered into the regression model. All statistical procedures were conducted using SAS 9.1 software (SAS Institute Inc. Cary, NC, USA).

Results

The molecular sexing procedure indicated that 34 of the 71 magpies captured were female and 37 were male. Among the 34 females, 13 individuals were territorial AHYs, 12 were non-territorial AHYs, and nine were HYs. Among the 37 males, 15 individuals were territorial AHYs, 11 were non-territorial AHYs, and 11 were HYs.

Table 2-2. Principal components of the body measurements of Black-billed Magpies (*Pica pica*).

Body measurement	Size PC1	Size PC2
Wing	0.38	-0.13
Tarsus	0.27	0.16
Total head (head to bill)	0.39	-0.15
Bill length (from bill base)	0.37	-0.26
Bill depth (at bill base)	0.20	0.66
Bill length (from nostril)	0.41	-0.24
Bill depth (at nostril)	0.22	0.59
Mass	0.38	0.07
Tail	0.31	-0.07
Eigenvalue	3.95	0.44
Proportion	1.25	0.14

Relationships between color components and body condition indices in females and males

In females, no body condition variable was related to iridescent plumage color (Table 2-3). In contrast, in males, both wing and tail iridescence were related to several body condition indices (Table 2-4). BCI was positively correlated with wing brightness (parameter estimate β = 4.13, F = 3.99, P = 0.05), tail brightness (β = 2.94, F = 4.21, P = 0.05), and tail chroma (β = 0.01, F = 6.96, P = 0.02). Overall body size PC1 was positively correlated with tail brightness (β = 9.80, F = 3.95, P =

0.01) and tail chroma ($\beta = 0.01$, $F = 9.80$, $P < 0.01$). Amount of total protein was positively correlated with tail brightness ($\beta = 3.79$, $F = 4.15$, $P = 0.05$), and negatively correlated with tail hue ($\beta = -17.22$, $F = 4.41$, $P = 0.05$). Total tail length was correlated only with tail brightness ($\beta = 1.81$, $F = 8.24$, $P = 0.01$) (Table 2-3).

Table 2-3. Correlation between iridescent color variables and body condition indices in male and female Black-billed Magpies (*Pica pica*). Variables tested were: PC1, size PC2, BCI, tail length, total tail length, total number of fault bars, total protein, albumin/globulin ratio, WBCs, and the heterophil/lymphocyte ratio. Only significant results are listed.

Sex	Plumage	Color variable	Significant condition variable	Estimate	F (P)
Females	Wing	Brightness			
		Hue	No significant correlation		
		Chroma			
	Tail	Brightness			
		Hue	No significant correlation		
		Chroma			
Males	Wing	Brightness	BCI	4.13	3.99 (0.05)
		Hue	No significant correlation		
		Chroma	No significant correlation		
	Tail	Brightness	BCI	2.94	4.21 (0.05)
			Total tail length	1.81	8.24 (0.01)
			Size PC1	9.80	3.95 (0.01)
			Total protein	3.79	4.15 (0.05)
		Hue	Total protein	-1.22	4.41 (0.05)
		Chroma	BCI	0.01	6.96 (0.02)
			Size PC1	0.01	9.80 (<0.01)

Relationships between color components and body condition indices and male status

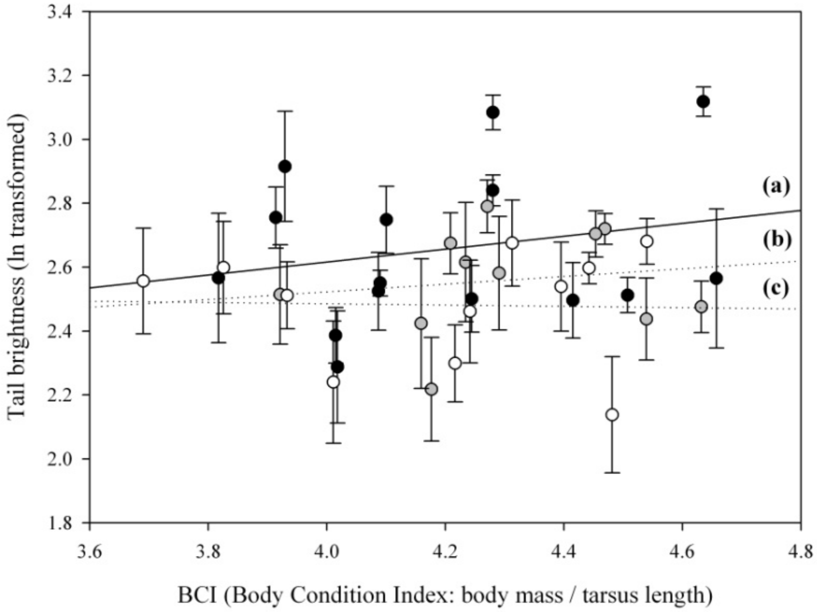
Many of the significant correlations were found in tail brightness (Table 2-4). Breeding males with higher body condition indices (BCI), larger body sizes (size PC1), and heavier tails (total tail length) had brighter tail coloration (BCI: $\beta = 2.00$, $F = 4.27$, $P = 0.05$; Size PC1: $\beta = 1.79$, $F = 9.85$, $P = 0.01$; total tail length: $\beta = 1.10$, $F = 5.12$, $P < 0.03$; Table 2-4, Figure 2-1). HY males also showed relationships between tail brightness and total tail length; however, individuals with longer and heavier tails had less bright tails (tail length: $\beta = -0.19$, $F = 9.42$, $P <0.01$; total tail length: $\beta = -0.07$, $F = 6.21$, $P = 0.02$; Table 2-4, Figure 2-1).

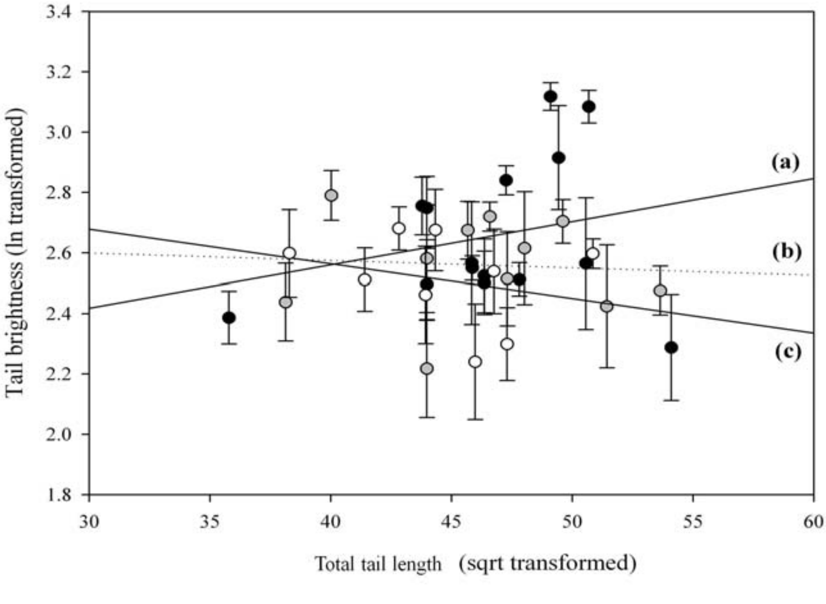
Table 2-4. Correlations between iridescent color variables and body condition indices of male Black-billed Magpies (*Pica pica*) of different statuses, based on stepwise regressions. Entered variables are: size PC1, size PC2, BCI, tail length, total tail length, total number of fault bars, total protein, albumin/globulin ratio, WBCs, and the heterophil/lymphocyte ratio.

Plumage	Color variable	Status	Variables entered	Estimate	F (P)
Wing	Brightness	Breeding AHY	BCI	1.35	2.83 (0.14)
		Non-breeding AHY	No variable selected		
		HY	No variable selected		
	Hue	Breeding AHY	No variable selected		
		Non-breeding AHY	No variable selected		
		HY	No variable selected		
	Chroma	Breeding AHY	No variable selected		
		Non-breeding AHY	No variable selected		
		HY	BCI	0.10	19.60 (0.02)

(Table 2-4 continued)

Plumage	Color variable	Status	Variables entered	Estimate	F (P)
Tail	Brightness	Breeding AHY	Model: $R^2 = 0.44$, $F = 6.81$, $p < 0.01$		
			BCI	2.00	4.27 (0.05)
			Tail length	-0.01	3.59 (0.07)
			Size PC1	1.79	9.85 (0.01)
			Total tail length	1.10	5.12 (0.03)
		Non-breeding AHY	No variable selected		
		HY	Model: R2 = 0.45, F = 8.88, p <0.01		
			Tail length	-0.19	9.42 (<0.01)
			Total tail length	-0.07	6.21 (0.02)
	Hue	Breeding AHY	Number of fault bars	1.13	9.14 (<0.01)
		Non-breeding AHY	No variable selected		
		HY	No variable selected		
	Chroma	Breeding AHY	No variable selected		
		Non-breeding AHY	No variable selected		
		HY	BCI	0.04	3.70 (0.07)


3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
Tail brightness (ln transformed)
(a)
(b)
(c)
3.6
3.8
4.0
4.2
4.4
4.6
4.8
BCI (Body Condition Index: body mass / tarsus length)


3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
Tail brightness (ln transformed)
(a)
(b)
(c)
30
35
40
45
50
55
60
Total tail length (sqrt transformed)

(Figure 2-1 continued)

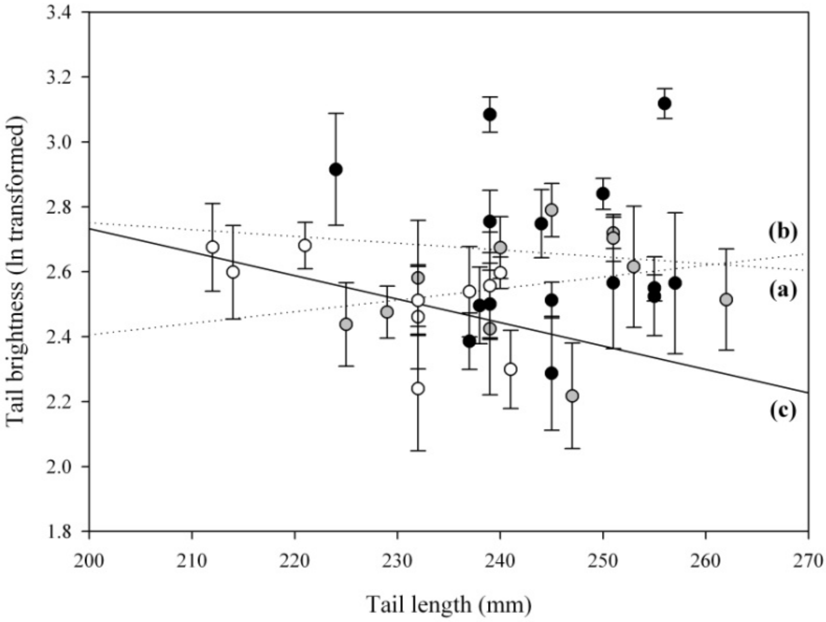


Figure 2-1. Relationships between tail brightness and quality variables in male Black-billed Magpies (*Pica pica*). (a) Black circles and regression line denote territorial AHYs, (b) grey circles and regression line indicate non-territorial AHYs, and (c) open circles and regression line denote HYs. Only statistically significant ($P < 0.05$) regression plots are shown with bold lines.

Discussion

Why does tail iridescence contain more information than wing iridescence?

Since magpies use both their wing and tail feathers during sexual displays by flicking and flirting with them (Birkhead 1991), and given that the iridescent colored parts are large in both the wings (secondary and greater coverts) and the tail (entire tail area), the strengths of the visual signals from the different colored areas were thought to be similar (Fitzpatrick 1998). However, on the basis of the results, it is likely that tail coloration carries more information relating to body condition than does wing coloration. Tail brightness, hue, and chroma were correlated with general size and condition (size PC1, BCI), tail characteristics (total tail length), and immune characteristics (total protein). Males in better body condition had higher brightness, shorter wavelength-directed hue, and more saturated chroma scores, whereas wing brightness was only correlated with BCI. This raises the question: Why does tail color contain more information than wing color?

It takes relatively more energy to produce and maintain long tail feathers than short feathers; therefore, long feathers may serve as “handicap signals” with only high quality males able to produce and maintain good quality tails (Zahavi 1975, 1977). However, if female preference for males with long tails drives them to produce longer tails that are less dense and more fragile, in other words, drives them to produce a low energy tail, the trait does not indicate male quality (Fisher 1930).

Magpies are strongly territorial, both sexes participate in providing for their offspring, and mate fidelity based on territory is strong, thus the tail characteristics of this species are not expected to have undergone a Fisherian process. Therefore, it is not possible to state that tail length itself *cannot* be a preferred trait in choosing a mate even in a species with a relatively long tail. However, females cannot judge the mass (or density) of male tails merely by seeing them. Therefore, other information encoded in the tail, such as tail symmetry (Møller 1992), or the degree of tail damage (Blanco and de la Puente 2002) may serve as additional signals of male quality. Iridescent coloration may be an additional signal or enhancement of a signal indicating male quality. Black-billed Magpies, which are strictly resident (entirely non-migratory), have a relatively low level of variation in wing length compared to their variation in tail length (Lee et al. 2007), whereas migratory species exhibit more variation in wing length, which is usually a direct predictor of size and condition (Francis and Cooke 1986; Winkler and Leisler 1992). Therefore, the effect of variation in wing coloration, as well as wing size, may not be large and the effect of mate display also may be small.

Relationship between tail characteristics and tail brightness

There was an interesting pattern in the relationship between tail characteristics and tail coloration: tail brightness was positively correlated with total tail length (the sum of the lengths of all rectrices), but there was no relationship between tail brightness and tail length in territorial males. In contrast, both tail length and total tail length were negatively correlated in HY males. Since Black-billed Magpies are ground foragers, they invest more energy in structural growth than in feather growth (Birkhead 1991). The tarsus grows to over 90% of its full length before the young fledge (Birkhead 1991). The tail develops during the final stages of feather growth in chicks, and the young birds fledge before their tail feathers are fully grown. Young birds must allocate their energy to fledging, and to the full growth of the juvenile tail at the same time, and then subsequent post-fledging events, such as dispersal and winter survival, and first winter plumage molt also occur at the same time. In addition, HY magpies generally have no opportunity to acquire their own territory or breed in their first year following hatching (Birkhead 1991). Therefore, it is not likely that they invest more energy in tail development than in post-fledging survival. Consequently, young magpies have relatively short, thin, and more heavily damaged tails (with more fault bars and breaks) (Blanco and de la Puente 2002). Because of their limited energy, tail feathers that grow longer but more quickly, may have lower densities and hence be susceptible to greater damage. The negative correlations between tail iridescence and tail characteristics may result from trade-offs between tail growth and tail quality.

Female condition and iridescent coloration

It is believed that both parents in socially monogamous species use traits that convey signals relating to quality (Jones and Hunter 1993). However, in contrast to that hypothesis, I found no relationship between condition indices and iridescent coloration in female magpies during the current study. Such a result is in line with some previous research in which the tail characteristics of females were also found to be unrelated to other condition indices (Blanco and de la Puente 2002). In species in which both sexes possess the same colored parts, but where the color production mechanism and the degree of the coloration differ only slightly, there have been only a few studies showing that female coloration does not reflect body condition (reviewed by Amundsen and Pärn 2006). From the results shown in Chapter 1, that territorial AHY females had less bright tails than non-territorial AHY females, it might be expected that there is a trade-off in energy between breeding and molting just after the breeding season in territorial AHY females. However, this breeding-molting energy trade-off cannot explain the lack of a relationship between color and condition in the non-territorial AHY females. If females have any body condition indicator, whether it is coloration or some other physical characteristics, it would also be expected in non-territorial individuals, since they experience the same pressures of resource competition (food and potential territory) and mate competition in flocks. According to the results so far, female magpies do not seem to carry any condition-dependent visual signal, although their tails do signal the class distinction between AHY and HY age groups (see Table 1-2).

Does tail coloration reflect immune condition in magpies?

Among the immune characteristics tested, only the amount of total proteins, which is affected mainly by nutritional stress, was related to the brightness and hue of the tail in males. It is known that the level of immunity varies according to a range of seasonal events (Hegemann et al. 2012) such as elevation of the sex hormones that suppress immune expression (Grossman 1985), breeding (Moreno et al. 1999), seasonal changes in parasite loads (Møller et al. 2003), migration (Owen and Moore 2006; Machado-Filho et al. 2010), and food shortages (Machado-Filho et al. 2010). Molting is an important event in the life history of all birds, since individuals not only need considerable energy to produce the keratin required for the new feather coat, but they also require additional energy for vigilance against predators and for foraging. Molting can, therefore be considered as a stressful event in birds. However, the molting period takes place during a relatively favorable period after they have finished breeding, when they may experience less stress from food shortages, harsh weather, or strict territorial competition. Therefore, variation in immunity may not be great, and immune indices may not significantly reflect variation in plumage coloration. Consequently, the iridescent coloration of the magpie’s tail seems to reflect general condition, not the immune condition, at least for individuals after fledging.

The results of this study indicate that iridescent coloration in males reflects body condition; in particular, tail iridescence was highly correlated with condition indices. I suggest that the iridescent coloration of the tail is perhaps a “back-up signal” that serves to amplify the signal of the bearer’s condition conveyed by other tail feather characteristics, according to social class.

CHAPTER 3.

BRIGHTER TAIL IRIDESCENCE PREDICTS REPRODUCTIVE SUCCESS IN MALE BLACK-BILLED MAGPIES (*Pica pica*)

Abstract

The evolution of secondary sexual traits in monogamous birds has been explained by the Darwin-Fisher theory; sexual selection can drive the evolution of male ornaments when females with this ornament-preference breed earlier and more successfully. Whether or not tail iridescence, which is an indicator of body condition in breeding males, has any relationship with reproductive success in socially monogamous Black-billed Magpies (*Pica pica*) was investigated. Males with brighter tail iridescence initiated breeding earlier than less bright males, in terms of the estimated hatching date of the first chick; however, the offspring of brighter males did not fledge earlier. Males with brighter tails had better fledging success, than less bright males. The tail coloration of parental males was also related to the body condition index of their offspring, and the offspring of males

with brighter- and shorter wavelength-directed tails had better immune profiles at the end of the brooding periods. In this study, the earlier initiation of breeding and subsequent higher reproductive success of male magpies with brighter tails implies that bright tail iridescence may act as a selected trait at the very initial stage of pair and territory formation.

Introduction

Elaborate plumage features of male birds can be perpetuated when they are related to the preferences of females, even though they may be disadvantageous for male survival (Darwin 1871). For males, producing, maintaining and surviving with such ornaments is costly, therefore, the benefits of those ornaments should include more opportunities for mating and consequently greater numbers of surviving offspring. Although typically less extreme than those of polygynous species, sexual traits are also generally found among males of monogamous species (Kirkpatrick et al. 1990). Why have such costly traits evolved even when do not have more than one mate?

In support of a theoretical basis for the phenomenon of less conspicuous secondary sexual traits among males of monogamous species, Darwin (1871) and Fisher (1930) proposed that variation in the degree of ornamentation and in the breeding success of males are both related to female breeding success. When good-quality males with more elaborate ornaments arrive earlier on their breeding grounds and hence occupy better territories, they have greater chances of being chosen by females, and may thus start breeding earlier. Earlier breeders tend to have higher reproductive success (O’Donald 1972, 1980) because high quality males either contribute greater genetic quality to their offspring (Fisher 1930), or they acquire high quality territories thus they can afford to better provision their mate and offspring (Przybylo et al. 2001). The relationship between early breeding and the reproductive success of females (Price et al. 1988; Ilmonen et al. 2000), and the relationship between variation in male ornaments and breeding phenology in monogamous species have been widely studied and validated the Darwin-Fisher theory (O'Donald 1980; Møller 1988; Norris 1990; Møller 1994; Wolfenbarger 1999; McGraw et al. 2001; Dearborn and Ryan 2002; Siefferman and Hill 2003).

The Black-billed Magpie (*Pica pica*) is an example of a socially monogamous species. Both sexes appear to have the same plumage to the naked human eye, although males actually have brighter and shorter wavelength-directed colors than females (Table 1-2). Tail color in male magpies also reflects their body condition (Table 2-4, Figure 2-1), hence tail color may act as a visual indicator of male quality. As the Black-billed Magpie is a year-round territorial species, the original Darwin-Fisher theory (that arrival timing is related to the timing of initiating breeding) does not apply. However, male quality is positively correlated with breeding timing and success, even among sedentary and short-distance migratory species, has been well described (e.g. Nilsson and Smith 1988; Verhulst et al. 1995; Otter et al. 1999). The Korean study population also showed a distinctive relationship between early egg-laying and higher breeding success (Lee 2005). Therefore, the aim of the study was to test whether or not plumage iridescence in male magpies is under selection pressure from females, by investigating relationships between tail iridescence and the timing and success of breeding.

Materials and Methods

Trapping magpies

I trapped magpies from late October to early December in 2003, 2004 and 2006. I used ladder traps (2 ×3 ×2 m, Bub 1991), and hand-reared magpies, as decoys, were put in the traps. Newly caught magpies, and decoys, were immediately retrieved from traps in order to prevent nearby magpies from becoming trap shy. When trapping, trials were made in adjacent territories; trials were separated by at least two days and different decoy birds were used.

Identification of age, sex and territory ownership

Magpies were recognized as either "After-Hatching-Year (AHY)" that is older than second calendar year, or "Hatching-Year (HY)" according to the length of the longest primary (Erpino 1968). AHYs were categorized as either "territorial AHY" or "non-territorial AHY" according to their territory ownership. Sexing was done using the molecular method (Griffiths et al. 1998) to avoid inaccurate sex determination based on morphometrics, which overlap considerably (Lee et al. 2007). The sex of individually marked birds was also confirmed by observation of their breeding behavior in the season following captures.

Color measurement and analysis

I measured colored plumage features of magpies with a spectrophotometer (USB2000; Ocean Optics, Dunedin, FL, USA). Reflectance spectra curves of 320-700 nm were drawn using OOIBase software (Ocean Optics). All data were measured with a reflectance probe perpendicular to the plane of feathers, and each area was measured five times. From the reflectance of iridescent feathers in the wing and tail, three color components were determined: brightness (peak reflectance; Andersson 1999), hue (wavelength at the peak reflectance; Andersson 1999) and chroma (proportion of integration of reflectance at hue ± 50 nm to at 320-700 nm; Shawkey et al. 2003).

Breeding phenology

The magpie breeding season in the study area usually begins with intensive territorial behavior followed by nesting. From the January following trapping, newly built nests and individual breeding status (nest building, copulation, incubation) was monitored every three days. After incubation was observed to have begun (one individual of a pair remained in the nest for more than ten minutes without nest material and was sometimes fed by its partner), active nests were accessed by cargo crane. Nests were checked on average every 3.5 day, during which the following data were collected: number of eggs, hatching dates, number of hatchlings, size of chicks, and fledging dates. A droplet of blood (approximately 0.1

mL) was taken from the brachial vein of each chick for molecular sexing (Griffiths et al. 1998) and leukocyte counts. Observed or estimated hatching dates were used as the breeding initiation date, because actual initiation of nest building and egg laying were difficult to observe. If a hatching chick or a recently hatched chick was found when examining a nest, that date was considered to be the hatching date of the nest. If chicks older than one day were found, their hatching dates were estimated based on the growth curve of chicks 1-18 days old, for which exact hatching dates were known (Nam unpbl. data). The breeding period was defined as the period from the hatching date to the fledging date of the first chick in the nest. Condition of the chicks was calculated by dividing their body mass by their tarsus length (Jakob et al. 1996). Because the relationship between body mass and tarsus length is linear until day 18 (Nam unpbl. data) and because accessing nests containing chicks older than 18 days may affect the chicks' fledging schedule, I used only body condition information from chicks younger than 18 days. Chicks below the age of 18 days were sexed using Griffiths et al. (1998) molecular method. The offspring sex ratio was calculated as the number of males per total number of chicks in the nests. The degree of hatching asynchrony was calculated as the interval (in days) between first hatching and last hatching for each clutch size (Zach 1982). Males that produced more than one fledgling were regarded as having bred successfully.

Immunological assay

Half of the droplet of blood taken from chicks older than 18 days was smeared, because the number of leukocytes is dependent on age (Gladbach 2010). Smears were air-dried and stained with Eosin-Thiazine (Aerospray 7150 hematology, Wescor, USA), then a veterinarian unfamiliar with the objectives of the study made a total WBC count, and a differential count (heterophil, eosinophil, basophil, lymphocyte, and monocyte). The number of heterophils (which are analogous with neutrophils in mammals) is related to the acute response against antigens, whereas the number of lymphocytes is related to cell-specific immunity (Dein 1986). The ratio of heterophils to lymphocytes is a widely used indicator of immunity (Gross and Siegel 1983).

Statistical procedure

The tail brightness and tail hue of breeding males were not normally distributed, so data were natural log and square root transformed. Because the breeding data were from three different years, variation in the timing of breeding and the body condition of chicks (younger than 18 days) were compared. There were significant differences in the estimated hatching dates among the three years ($F_{2,111} = 9.244$, $P < 0.01$), but no difference was found in either the fledging dates ($F_{2,81} = 2.565$, $p = 0.11$) or body condition ($F_{2,201} = 0.804$, $P = 0.45$). I used residual values from the means of these variables to compensate for the year effect in the variation of hatching dates. Color differences between successful and unsuccessful breeding males were tested using the Mann-Whitney U-test owing to the small sample size. Relationships among tail coloration, and indices of breeding success and timing, were all tested using Spearman's correlation. All statistical procedures were conducted using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

Results

Of the 37 males studied, 15 males held their own territories and bred in the breeding season following trapping.

Differences in tail coloration among breeding males

Successful males (those that produced more than one fledged chick) had brighter tails than unsuccessful males ($z = -2.11$, $P = 0.03$), but there was no difference in tail hue between them ($z = -0.03$, $P = 0.98$; Figure 3-1).

Relationships between tail coloration and breeding indices

No correlation was found between clutch size and tail coloration (clutch size and brightness: $z = -0.26$, $P = 0.80$; hue: $z = -1.59$, $P = 0.11$), number of hatchlings and tail coloration (brightness: $z = -0.02$, $P = 0.99$; hue: -0.33, $P = 0.74$). Males with brighter tails produced more fledged chicks (ρ = 0.33, z = 2.73, P = 0.01), but no relationship was found between the number of fledglings and tail hue (z = -0.30, P = 0.76). Males with brighter tails also started breeding earlier (ρ = -0.31, z = -2.37, P = 0.02). None of the other indices relating to the timing of breeding were related to tail coloration (fledging date – brightness: z = -1.39, P = 0.16, fledging date – hue: z = 1.50, P = 0.13; breeding period – brightness: z = -1.94, P = 0.06, breeding period – hue: z = 1.28, P = 0.20; hatching asynchrony – brightness: z = 0.38, P = 0.70, hatching asynchrony – hue: z = 1.82, P = 0.07). Offspring quality was found to be related to their male parent's plumage color. The body condition index of chicks was positively correlated with parent male tail brightness (n = 53, ρ = 0.16, z = 2.62, P = 0.01). Chicks of males with brighter and shorter wavelength-directed tails had low H/L ratio values (n = 15; brightness: ρ = -0.27, z = -2.30, P = 0.03; hue: ρ = 0.38, z = 3.27, P < 0.01; Table 3-1).

Relationships between the timing and success of breeding

Hatching date was significantly correlated with the number of hatched and fledged offspring; earlier hatching led to more hatchlings (n = 11, ρ = -0.29, z = -2.42, P = 0.03), and also more fledglings surviving (n = 12, ρ = -0.47, z = -3.60, P < 0.01; Figure 3-2). I found no relationship between the fledging date and number of hatchlings (n = 7, ρ = -0.08, z = -0.39, P = 0.70) or fledglings (n = 8, ρ = -0.40, z = -1.93, P = 0.06).

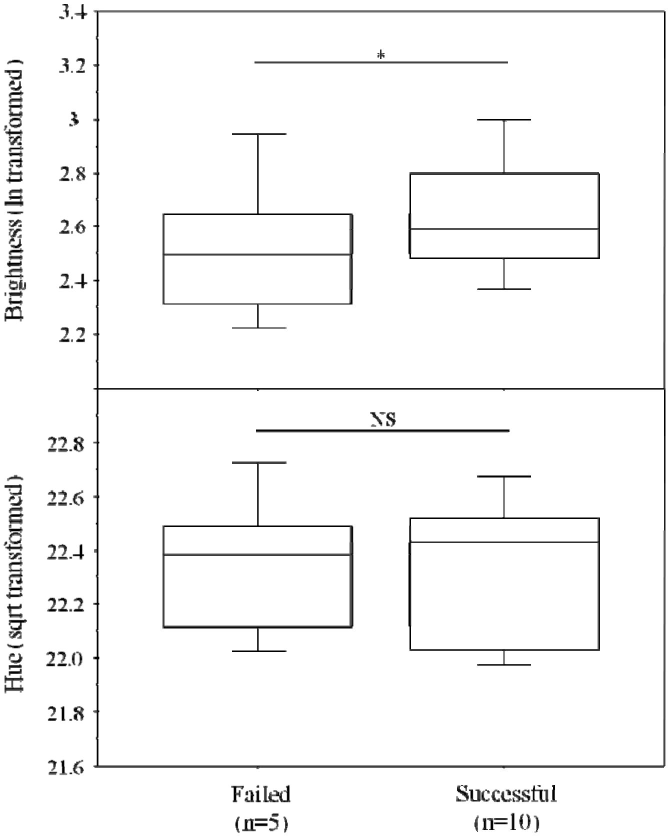


Figure 3-1. Differences in tail coloration between successful (fledged more than one chick) and unsuccessful breeding males. Statistically significant differences are indicated as * for $P < 0.05$ (Mann-Whitney U-test).

Table 3-1. Relationships between tail iridescence and indices of breeding estimated by Spearman's correlation. Correlation coefficients (ρ) are given. Statistically significant relationships are indicated as * for $P < 0.05$, ** for $P < 0.01$; NS indicates non-significant relationships.

		Tail brightness	Tail hue
		ρ	ρ
Breeding success	Clutch size	NS	NS
	Number of hatchlings	NS	NS
	Number of fledglings	0.33^{**}	NS
	Offspring sex ratio	NS	NS
	Offspring body condition	0.16^{**}	NS
	Offspring immunocompetence (H/L ratio)	-0.27^{*}	0.38^{**}
Breeding timing	Hatching date	-0.31^{*}	NS
	Fledging date	NS	NS
	Breeding period (fledging date - hatching date)	NS	NS
	Hatching asynchrony	NS	NS

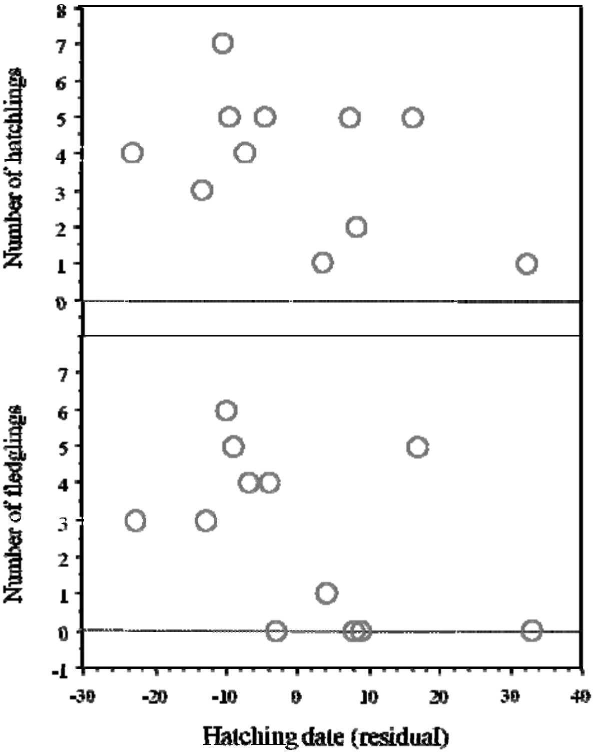


Figure 3-2. Relationships between hatching dates and numbers of hatchlings and fledglings. Both relationships are significant when tested using Spearman's Correlation.

Discussion

Modified Darwin-Fisher theory for species having long-term pair bonds

The Darwin–Fisher theory explains the presence of secondary sexual traits in monogamous species in which males with better traits are selected by females that are in good condition in the early part of the breeding season, and as a result, breed more successfully (Dearbon and Ryan 2002). However, in a wide range of avian species pairs tend to stay together for more than one season, therefore, mate choice by females does not occur in every breeding season although males retain their secondary sexual traits. This phenomenon is common among large, long-lived species with the opportunity to maintain a long time pair bond, including magpies (Dhondt and Adriaensen 1994; Choudhury 1995; Ens et al. 1996; Dubois and Cézilly 2002; Pampus et al. 2005), but it is also found among small, short-lived species with only a few seasons during which they can maintain their pair bond (Freeman-Gallant 1996; McGraw and Hill 2004). Two hypotheses have been proposed to explain why males of species with long-term pair bonds have secondary sexual traits. The first hypothesis suggests that prior breeding experience provides practical proof of partner quality. Male Savannah Sparrows *Passerculus sandwichensis*, for example, that provided high quality parental care to their first brood, receive greater paternity from their social mates in subsequent breeding attempts (Freeman-Gallant 1996). Furthermore, in an experimental study, when male Barn Swallows *Hirundo rustica* were given enhanced plumage coloration they received greater paternity from their social mates (Safran et al. 2005). These studies clearly show that signals of quality, especially those represented by plumage color, are linked to changes in female mating fidelity and pair bonding. Divorce has been reported in some populations of both Old World Eurasian Magpie and New World Black-billed Magpie *Pica hudsonia* (Baeyens 1981b; Birkhead et al. 1986; Buitron 1988; Dhindsa and Boag 1992), with a tendency for divorce to occur following low breeding success (Dubois and Cézilly 2002). Therefore, maintenance of bonds between magpie partners may imply that they have selected each other on the basis of having bred successfully in previous years, thus having demonstrated that they are of good quality. The second hypothesis suggests that, by maintaining their pair bond, females can reduce energy expended in seeking a new partner at the beginning of each breeding season. It may be critically important for a species that strongly defends resources from the early part of the breeding season onwards. Magpies of both sexes in the Korean study site defended their territories all year round. Since individuals without territories cannot even begin to breed, they may tend to maintain their pair bond for several seasons, or for life (Verbeek 1973; Buitron 1998).

Relationships between tail coloration and breeding phenology

The results of this study have shown that male tail brightness was related to earlier breeding (earlier hatching date), as well as higher breeding success (more fledged offspring). However, tail brightness was not related to the fledging dates of the offspring. Earlier fledging is advantageous not only in competition for food, but also with regards dominance when joining non-breeding flocks. Contradictory to my initial prediction, tail brightness was not related to offspring fledging date, and the date of fledging was not related to breeding success (number of fledged offspring). Two explanations can be posed for the lack of a relationship between hatching and fledging dates. Since magpies do not lay a second clutch after a first successful breeding attempt, date of fledging is not a pressure for second breeding (McGraw and Hill 2004). In addition, post-fledging parental care continues for a month or more after fledging (Nam pers. obs.). Because the fledged young generally stay within their natal territory until they form a flock and start to disperse, the quantity and quality of food available to fledged young may be largely dependent on the quality of their parents. Consequently, differences in fledging dates do not seem critically related to either post-fledging survival or dominance. In fact, fledging date seems to be related to the number of chicks, because large clutch sizes may lead to lower provisioning rates for each chick and hence delay the growth of the chicks. Thus it may take longer for chicks in nests with large clutches to fledge (Lee 2005).

Good parent hypothesis versus good territory hypothesis?

In a territorial species, the quality of the breeding territory is as important as the quality of the parents (Bart and Earnst 1999). Two commonly proposed hypotheses, "the good parent hypothesis" in which females choose mates based on the quality of males, and the "good territory hypothesis" in which females choose mates based on the quality of the territory that the males hold, are not mutually exclusive. This is because better quality males tend to have better territories, but the relative importance between the two in choosing males differs depending on the species (Alatalo et al. 1988). That tail brightness was found in this study of magpies to be related to the breeding success of males, may support the good parent hypothesis as in an earlier study of the North American Black-billed Magpie (Dhindsa et al. 1989). Previous studies on magpies have suggested that the quality of males seems to be strongly positively correlated with territory quality, because high-ranking males (Baeyens 1981a; Reese 1982; Birkhead and Clarkson 1985) or older males (Komers and Dhindsa 1989) obtain better quality territories. However, the breeding density of the Korean study site is so high that breeding pairs already occupy almost every suitable area. In addition, magpies spend considerable energy defending their territories (Birkhead 1991). It is likely, therefore, that within the study area, acquisition of good-quality territories by pairs is opportunistic. This lends further support to the results of this study indicating that male quality is a better predictor of breeding success than is territory quality.

In this study, it was found that earlier breeding and higher reproductive success were related to plumage coloration in male magpies. The advantages of maintaining a long-term pair bond (such as that found in magpies), which may induce earlier initiation of breeding, can support the Darwin-Fisher theory, although present tail iridescence does not exactly reflect the color at the time when the female made her choice.

CHAPTER 4.

LOCAL CLIMATIC EFFECTS ON VARIATION IN TAIL IRIDESCENCE OF BLACK-BILLED MAGPIES (*Pica pica*) IN KOREA

Abstract

Ornamental coloration tends to be highly variable and phenotypically plastic compared with morphological traits that are not subject to sexual selection. Variations in plumage iridescence in Black-billed Magpies (*Pica pica*) collected from nine regions in Korea were examined to explore the possibility that local climatic factors might affect feather quality. No geographical variation in tail brightness was observed. The deviation index, as measured by the coefficient of variation (CV) for tail brightness, differed from the deviation indices of other quality variables and condition-independent variables such as wing length, whereas the CV of tail hue showed weak differences from other morphological or tail

characteristics. The differences in CVs between the sexes were not significant for either tail brightness or tail hue; however, the differences between tail brightness and other morphological variables tended to be greater in males than in females. Tail brightness and tail hue increased as the deviation in climate factors increased, and the relationship was stronger in males. The deviation in tail brightness was also positively correlated with anomalies in temperature and humidity of the study year. These results suggest that tail iridescence in male magpies is probably a trait that leads to directional selection, but aspects of the variation in tail brightness and tail hue seem to be different.

Introduction

Ornamental characters, such as bright colors or long tails of males, tend to have undergone a strong directional selection process, whereas ordinary morphological characters, such as tarsus or wing (of resident species) lengths, tend to have been under stabilizing selection, and they thus show less phenotypic variation (Endler 1986; Møller 1993; Hill 1995; Svensson and Gosden 2007). Therefore, it is generally believed that the expression of ornamental coloration tends to be highly variable and phenotypically plastic compared with morphological traits that are not subject to sexual selection (Hill 1995; Cuervo and Møller 2001; Figure 4-1). Ornamental characteristics have differential costs from other morphological characteristics (Andersson 1994). The costs for ornamental characteristics are either higher than for normal characteristics, such that they are regarded as an honest signal facilitating mate choice, as suggested by Zahavi (1975, 1977), or the relative costs for the expression of ornamental characteristics are smaller in larger individuals, such that larger individuals derive more benefit in the development of large ornaments (Petrie 1992).

The effects of environmental variation on avian plumage coloration has previously been studied in order to understand the signal content of avian color displays and the selective pressures that have led to their evolution (Hill 2006a; Botero and Rubenstein 2012). The effect of environmental variation (e.g. food

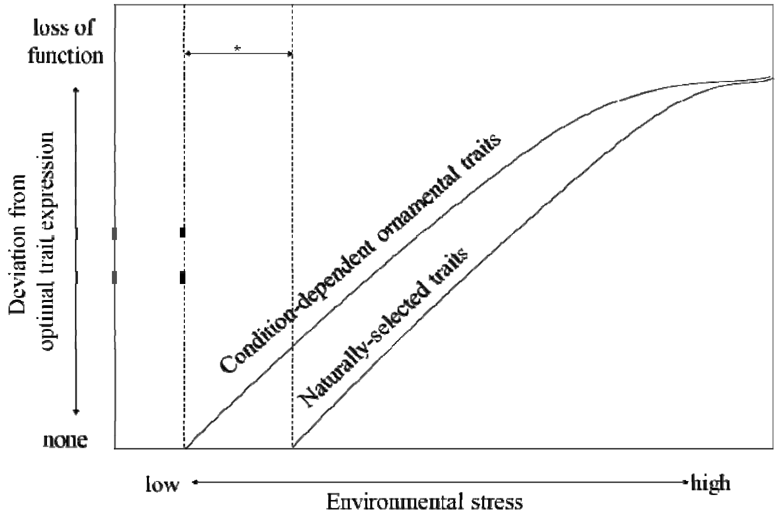


Figure 4-1. Differential effect of environmental stress on condition-dependent ornamental traits and naturally selected traits. The region marked * indicates that environmental stress is not so severe that variation of naturally-selected traits does not occur, but variation in ornamental traits is found (redrawn from Hill 1995).

shortages, higher precipitation, prolonged snow cover, unfavorable or unpredictable temperatures) on plumage coloration, particularly on carotenoid-based pigments has been much studied (see Hill 2006a for a review), because carotenoids, which produce yellow to red coloration, are derived from food. Unfavorable/unpredictable weather may causes food shortages, but weather events themselves may directly affect feather quality since unfavorable and unpredictable weather causes the release of stress hormones in birds (Romero et al. 2000; Wingfield and Kitaysky 2002); in particular, it instantly causes the release of stress hormones during the feather formation period (Romero et al. 2000). Specific weather conditions may also be related to the prevalence of feather-degrading bacteria that deleteriously affect feather quality (reviewed in Grubb 2006), and in turn affect structural coloration (Hill et al. 2005; Shawkey et al. 2007). How environmental variations affect the variation in plumage coloration in relation to sex is as yet unclear.

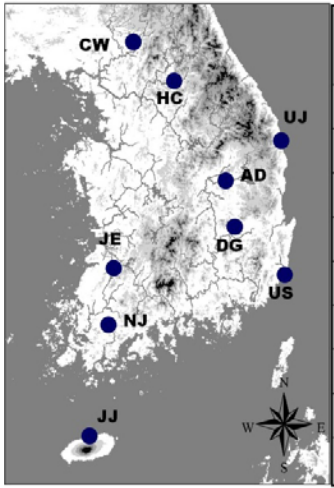
The aim of this study was to investigate whether or not the expression of tail iridescence in Black-billed Magpies (*Pica pica*) varies compared with other previously known quality indicators, such as the general body condition index, and naturally selected characteristics such as wing length. Whether or not environmental stress, such as unpredictable weather factors (measured by anomaly from the 30-year mean values), can affect the variation in tail coloration, or the difference in color between the sexes, was investigated, as well as the main causes of weather variables that affect color variation in magpies.

Prior to investigating the relationships between the tail iridescence and climatic factors, I tested the possibility that geographical barriers (such as mountains, rivers, and straits) have an effect on variation in iridescent coloration in magpies, in order to clarify whether the cause of variation in coloration is due to individual variation in the genes of favorable traits or a by-product of geographical isolation. Genetic variation among different geographical groups of magpies seems to affect distinctively the variation in plumage coloration including wing and tail iridescence (Birkhead 1991). The genetic structure of the magpie population in the Korean Peninsula is still unclear, as results so far contradict each other: Eo et al. (2002), studying variations of polymorphic DNA, found genetic differences between the eastern and western magpie populations separated by the Baekdudaegan mountains, whereas Jang (2004), studying mitochondrial DNA, found no such difference.

Materials and methods

Study species and sampling

In the early breeding season each year, the Korea Electric Power Corporation (KEPCO) controls the Black-billed Magpie population by harvesting them so as to reduce power blackouts caused by magpie nests built on electricity pylons. Magpie carcasses were collected within one or two days after harvest from 25 February to 9 April 2005 and refrigerated below -20°C for further analysis. After all samples had been collected, body and color measurements were made followed by dissections during July and August 2005.



Location	Temperature	Humidity	Wind speed	Precipitation
Andong (AD)	0.03	0.04	0.11	0.24
Cheorwon (CW)	0.01	0.02	0.18	0.06
Daegu (DG)	0.08	0.13	0.17	0.19
Hongcheon (HC)	0.08	0.02	0.11	0.21
Jeongeup (JE)	0.06	0.16	0.18	0.00
Jeju (JJ)	0.06	0.09	0.26	0.09
Naju (NJ)	0.06	0.15	0.14	0.27
Uljin (UJ)	0.08	0.09	0.03	0.16
Ulsan (US)	0.10	0.08	0.05	0.02

Figure 4-2. Sampling locations (left). The nine areas were categorized into five groups according to geographical barriers such as mountains (Baekdudaegan), rivers (Geumgang), and straits (Jeju strait). Climatic factor anomalies were calculated as the ratio of the difference between the mean value of the period 1971-2000 ("climatological standard normal" defined by KMA) and the value for 2004 divided by the mean value of the 1971-2000 period (right; climate data received from KMA).

A total of 377 magpies were collected from nine regions of Korea: Andong (N 36° 34', E 128° 43'), Cheorwon (N 38° 08', E 127° 18'), Daegu (N 35° 52', E 128° 36'), Hongcheon (N 37° 41', E 127° 53'), Jeongeup (N 35° 34', E 126° 51'), Jeju (N 33° 29', E 126° 30'), Naju (N 35° 00', E 126° 42'), Uljin (N 36° 59', E 129° 24'), and Ulsan (N 35° 32', E 129° 18') (Figure 4-2). The sample population of 377 birds was grouped into five sub-populations based on the assumption that certain mountains, rivers, and straits might represent significant enough geographical barriers to cause geographical variation at the genetic level: group 1 (Cheorwon and Hongcheon), group 2 (Jeongeup and Naju), group 3 (Jeju), group 4 (Andong and Daegu), and group 5 (Uljin and Ulsan).

Measurement and age/sex determination

Frozen samples were thawed at room temperature for approximately three hours. After they were completely thawed out, a range of measurements was made: wing chord, tail length, tarsus length, total head length, bill length, bill depth, and body mass. Individuals were aged based on the length of the black tip of the longest (usually 7th) primary feather (Erpino 1968; Lee et al. 2007). The mean length of the black tip was 11.24 ± 2.33 mm (mean ± SD, n = 132) in ASY (after-second-year) individuals and 27.11 ± 4.54 mm (n = 145) in SY (second-year) birds in this study. Because HY (hatching-year) individuals molt some of their primaries during autumn, the length of the black tip was not measured when SY magpies had replaced the longest primary, however it was still possible to age them based on their un-molted primaries. Males and females were identified during dissection based on the presence of either ovaries or testes. A total of 282 magpies of known age and sex were used in the analysis: 132 ASY (60 females and 72 males), and 150 SY (82 females and 68 males).

Morphometrics and condition-indicating traits

A general index for measuring body condition was calculated from the body mass divided by tarsus length, following Jakob et al. (1996). The lengths of each of the 12 rectrices was measured allowing tail length to be defined as the length of the longest rectrix (T1), and total tail length to be defined as the sum of the lengths of all 12 rectrices (see Table 2-1). To compensate for different deviations in measured variables owing to overall size differences (Eberhard et al. 1998; Green 2000), I divided all variables by tarsus length (a representative structural length). In this study, body mass was used as a general condition index instead of BCI (body mass / tarsus length). Wing size adjusted by tarsus length was selected as a condition-independent morphological characteristic, because variation in the wing size of a strictly sedentary species is unlikely under the selection pressure from the perspective of either sexual selection or natural selection relating to foraging or predator-avoidance behaviors (Copete et al. 1999).

Color measurement

The exposed parts of the closed wing (wing coverts, secondaries, parts of primaries) have a bluish iridescence, whereas all tail feathers, except for the tail tips, have a greenish iridescence. The colors of the centers of the 4-6th secondaries, and of the 1st tail feather were measured with all measurements repeated five times. A spectrophotometer (USB2000; Ocean Optics, Dunedin, FL, USA) with a deuterium-tungsten-halogen light source emitting light at 250-800 nm was used after being calibrated with a white standard prior to each measurement. From the entire wavelength measured, the reflectance value in the 320-700 nm range was selected for color analysis using OOIBase software (Ocean Optics). From the reflectance spectra, three color components were derived: brightness (peak reflectance; Andersson 1999), hue (wavelength at the peak reflectance; Andersson 1999), and chroma (proportion of integration of reflectance at hue ±50 nm to 320–700 nm; Shawkey et al. 2003).

Local climate information

I used the weather database of the Korea Meteorological Administration (KMA; http://www.kma.go.kr) for climatic information at each of the collecting locations. The anomaly of climate factors were calculated by the ratio of the difference between the mean value of common years (often determined as the last 30 years; in this study, 1971-2000) and the value in 2004 (the year in which the feathers being studied were grown) divided by the mean value for 1971-2000.

Statistical procedures

To test for differences in coloration between the sexes and among the five geographical groups, a mixed model using the PROC MIXED procedure was used. The compound symmetry (CS) type was selected as the covariance matrix structure because five random repeated measures were available. Each location was treated as a random factor. Differences between the coefficient of variables of coloration, condition-dependent variables, and condition-independent variables were tested using the Kruskal-Wallis test using the PROC NPAR1WAY procedure. For the post-hoc analysis, each pair of variables was tested using the Mann-Whitney test with the Bonferroni adjustment. Correlations between the color variables and the deviation of multiple climatic variables were estimated using the PROC REG procedure with backward elimination steps, and variables which met the significance level of 0.10 were retained in the regression procedure. In the correlation and regression procedure, the five repeated measures of the color variables were averaged and then analyzed. All statistical procedures were conducted using SAS 9.1 software (SAS Institute Inc. Cary, NC, USA).

Results

Color differences in relation to local geographical barriers

There were no differences between local geographical groups of magpies either in tail brightness ($F_{4,638}$ = 0.97, P = 0.42) or in tail hue ($F_{4,644}$ = 1.39, P = 0.24). No geographic differences in tail brightness were detected in either females ($F_{4,283}$ = 2.27, P = 0.06) or males ($F_{4,351}$ = 0.19, P = 0.94), and the results for tail hue were very similar (females: $F_{4,289}$ = 1.94, P = 0.10; males: $F_{4,351}$ = 0.81, P = 0.52; Figure 4-3). However, the coloration of each group showed significant differences between the sexes. Males had consistently brighter tails in every group, except for group 1 (group 1: $F_{1,85}$ = 0.03, P = 0.87; group 2: $F_{1,120}$ = 22.50, P < 0.01; group 3: $F_{1,44}$ = 25.46, P < 0.01; group 4: $F_{1,109}$ = 29.93, P < 0.01; group 5: $F_{1,179}$ = 28.33. P < 0.01; Figure 4-3). With regards tail hue, males had shorter wavelength-directed color than did females, except for group 2 (group 1: $F_{1,85}$ = 22.91, P < 0.01; group 2: $F_{1,120}$ = 3.71, P = 0.06; group 3: $F_{1,44}$ = 8.96, P < 0.01; group 4: $F_{1,114}$ = 6.68, P = 0.01; group 5: $F_{1,180}$ = 9.62, P < 0.01; Figure 4-3).

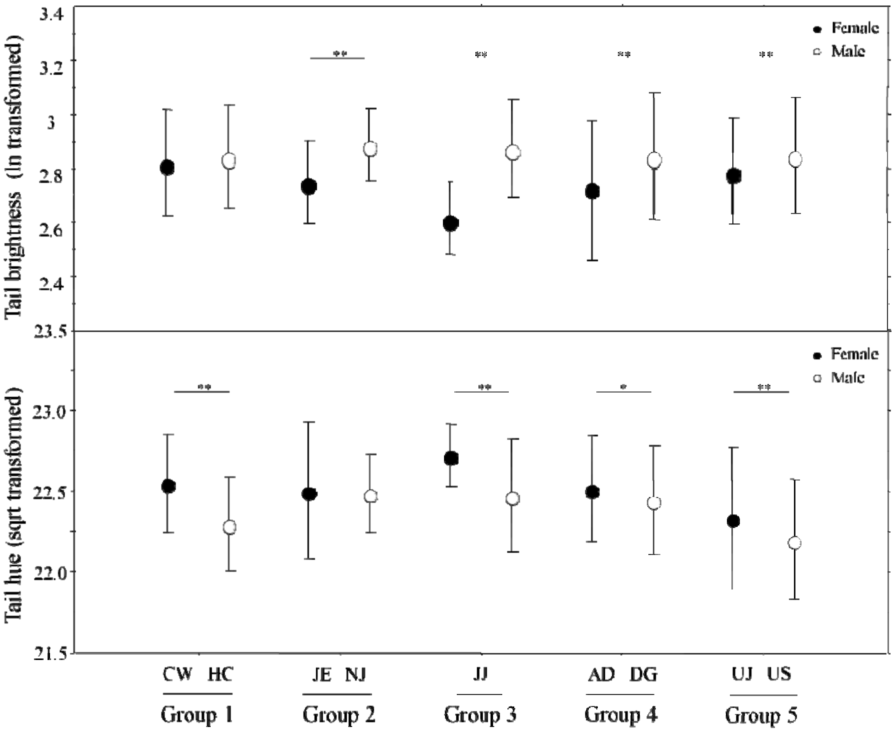


Figure 4-3. Local differences in tail brightness (top) and tail hue (bottom). Local groups were categorized by the geographical barriers (mountains, river, and straits) thought to affect them. CW: Cheorwon, HC: Hongcheon, NE: Jeongeup, NJ: Naju, JJ: Jeju, AD: Andong, Daegu: Daegu, UJ: Uljin, US: Ulsan. Tail coloration among the local groups did not differ significantly (tail brightness - $F_{4,638} = 0.97$, $P = 0.42$; tail hue - $F_{4,644} = 1.39$, $P = 0.24$). Asterisks denote significant differences between the sexes: ** $P < 0.01$, * $P < 0.05$.

Degree of deviations in coloration, condition indices, and condition-independent morphological characters

I tested to detect any difference in the coefficients of variation (CV) of given variables: tail coloration (brightness or hue), tail length, total tail length, body mass, wing length. There was no difference in tail brightness between the sexes ($z = -0.391$, $P = 0.69$). The CV of tail brightness was significantly higher than for any other variable in males (Kruskal-Wallis $H = 26.05$, $P < 0.01$; difference between tail brightness and tail length: $z = -3.58$, $P < 0.01$; tail brightness and total tail length: $z = -3.31$, $P < 0.01$; tail brightness and body mass: $z = -3.58$, $P <0.01$; tail brightness and wing length: $z = -3.58$, $P < 0.01$, non-significant relationships were not listed; post-hoc comparison using Mann-Whitney test were all adjusted using the Bonferroni correction; Figure 4-4), and a similar pattern was found among females ($H = 20.96$, $P < 0.01$; difference between tail brightness and tail length: $z = -3.22$, $P = 0.01$; tail brightness and body mass: $z = - 3.14$, $P = 0.01$; tail brightness and wing length: $z = -3.58$, $P < 0.01$; Figure 4-4). The sex difference was not significant for tail hue ($z = -0.97$, $P = 0.33$). Differences among the variables were detected in males ($H = 9.95$, $P = 0.04$), but post-hoc tests did not support differences between any variables after Bonferroni correction. No difference among the variables was detected among females ($H = 8.62$, $P = 0.07$).

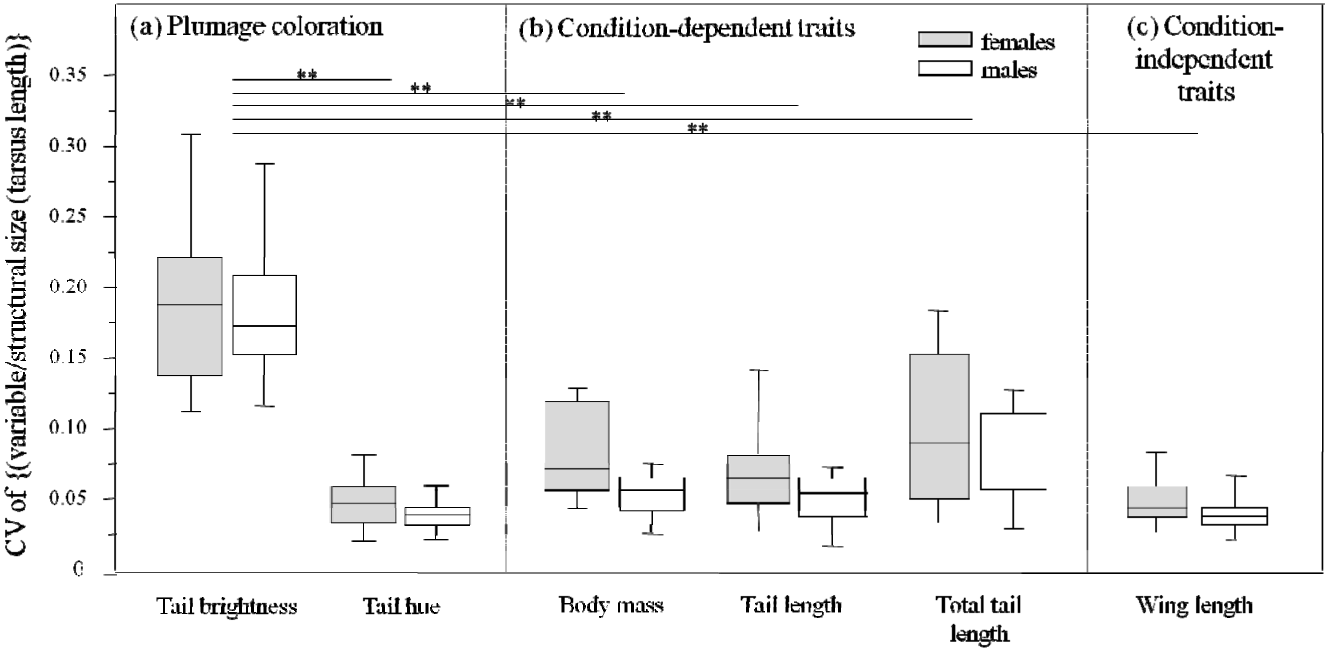


Figure 4-4. Differences in deviation indices (coefficient of variation adjusted by tarsus length) of morphological characters of females and males from nine local regions. Post-hoc tests were made using the Mann-Whitney test and P-values were Bonferroni adjusted. Asterisks denote significant differences between the sexes: ** P < 0.01, * P < 0.05.

Relationship between tail coloration and anomalies in the local climate

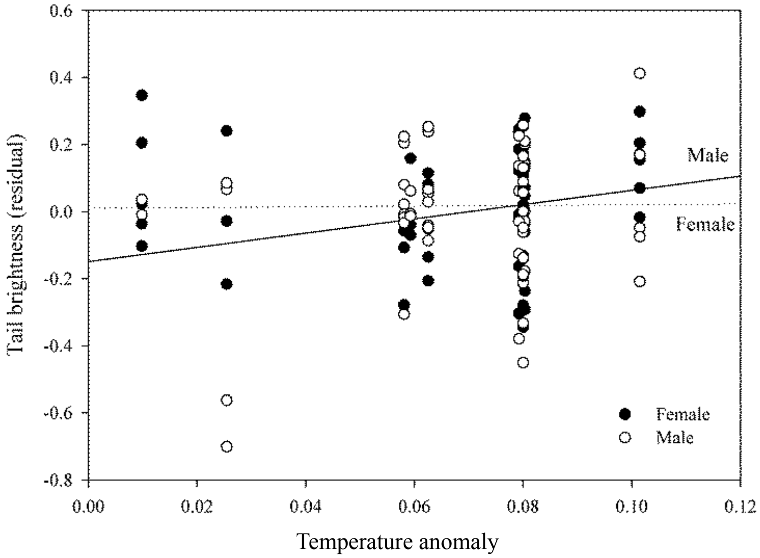
Only plumage coloration was related with anomalies of local climatic factors after a backward elimination procedure (Table 4-1). The relationship was stronger in males (Brightness: F = 3.81, P = 0.03 with temperature and wind speed; Hue: F = 1.65, P < 0.01 with humidity and wind speed) than in females (Brightness: no significant relationship with climate variables; Hue: F = 6.32, P = 0.02). Anomalies in climatic factors were related with both tail brightness (tail brightness – temperature in females: F = 1.18, P = 0.28; in males: F = 3.20, P = 0.08) and tail hue (tail hue – humidity in females: F = 0.10, P = 0.75, in males: F = 6.82, P = 0.01; tail hue – wind speed – females: F = 6.32, P = 0.02, males: F = 10.14, P < 0.01; Figure 4-5). There was a positive relationship between tail brightness and anomalies in temperature; when variation in temperature increased, brightness also increased. Tail hue and two climatic factors were also positively related, indicating that increases in anomalies of humidity and wind speed from the common years can reduce the quality of color (longer wavelength-directed hue).

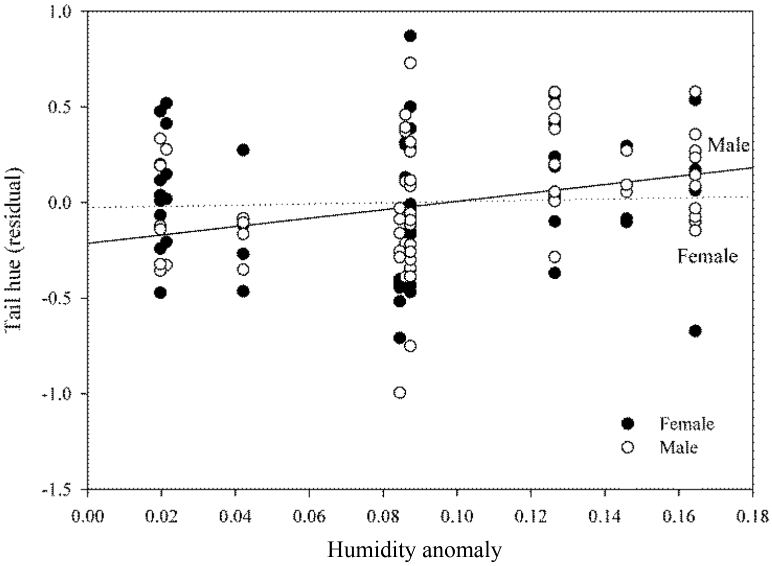
Relationship between deviations in tail coloration and deviations in local climate

Deviations in tail brightness and tail hue were related to anomalies several climatic factors (Figure 4-6), but the detailed patterns of response differed between variables. Deviation in the brightness of males increased as the anomaly of temperature and

Table 4-1. Relationships between tail iridescence and anomalies in local climatic factors (temperature, humidity, wind speed, and precipitation) conducted by multiple regression using backward elimination. Variables meeting a significance level of 0.10 were retained in the procedure.

Category	Variable	Sex	Variables retained	Estimate	F (P)
Tail coloration	Brightness	Female	All variables removed		
		Male	Model F = 3.77, P = 0.03		
			Temperature	3.42	6.65 (0.01)
			Wind speed	0.68	4.24 (0.04)
	Hue	Female	Wind speed	1.64	6.30 (0.02)
		Male	Model F = 6.95, P < 0.01		
			Humidity	1.50	3.38 (0.07)
			Wind speed	1.20	6.54 (0.01)
Condition-dependent variables	Body mass	Female	All variables removed		
		Male	All variables removed		
	Tail length	Female	All variables removed		
		Male	All variables removed		
	Total tail length	Female	All variables removed		
		Male	All variables removed		
Condition-independent variables	Wing length	Female	All variables removed		
		Male	All variables removed		


0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Tail brightness (residual)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Temperature anomaly
Male
Female
● Female
○ Male


1.0
0.5
0.0
-0.5
-1.0
-1.5
Tail hue (residual)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
Humidity anomaly
Male
Female
● Female
○ Male

(Figure 4-5 continued)

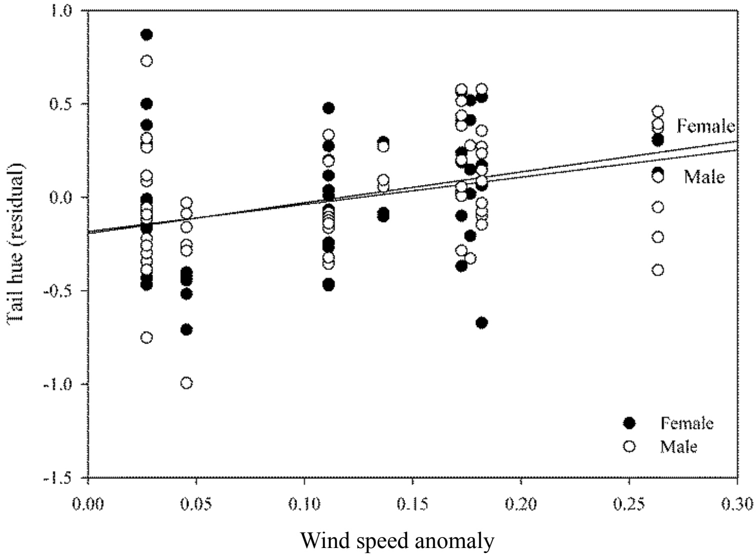


Figure 4-5. Relationships between tail coloration and anomalies in local climatic factors. Climatic anomalies were calculated as the ratio of the difference between the mean value of a 30-year baseline period (1971-2000) and the value for 2004 divided by the mean value for the 1971-2000 period. Statistically significant relationships were noted as bold regression lines.

humidity increased (brightness – temperature in males: ρ = 0.32, z = 2.04, P = 0.04, brightness – hue in males: ρ = 0.32, z = 2.31, P = 0.02). However, deviation in the hue of males tended to decrease with increased anomalies in humidity and precipitation (hue – humidity in males: ρ = - 0.03, z = -1.78, P = 0.07, hue – precipitation in males: ρ = -0.03, z = -1.71, P = 0.09) (Figure 4-6).

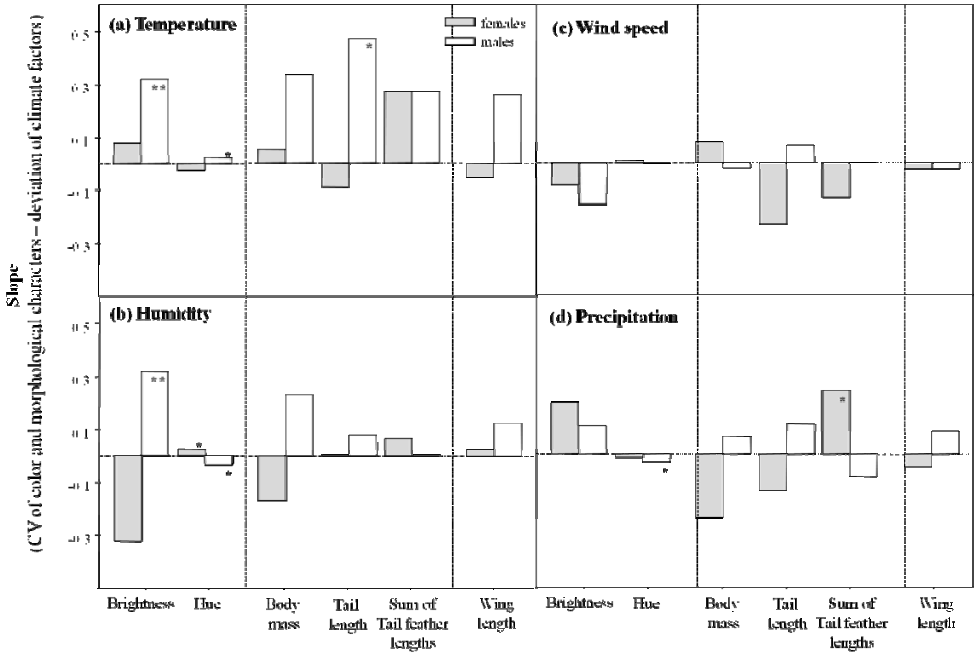


Figure 4-6. Relationships between deviations in color and morphological characters and deviations in local climatic factors. Deviations in climatic factors were calculated as the ratio of the difference between the mean values of the period 1971-2000 and the value for 2004 divided by the mean value for the period 1971-2000. Asterisks denote significant levels as tested using the Spearman Correlation: ** P < 0.05, * P < 0.10.

Discussion

Local adaptation and variation in iridescence

Jang (2004) showed that variation in the morphological characteristics of magpies is not related to geographical isolation on the Korean Peninsula. This was confirmed during the present study, which confirmed that there were no differences in plumage coloration in relation to potential geographical barriers. It is likely that no difference among the geographical groups is due to the greater variation of color within a population than that among populations. The results showed that the plumage coloration of magpie tails, especially in males, tended to be more affected by variation in the climate than other morphological characteristics (see Figures 4-4 and 4-5). Deviation in plumage coloration also seems to be greater than deviation in other morphological characteristics (see Figure 4-6). These results support previous studies in which sexually selected characteristics were considered to be more labile than naturally selected traits (Alatalo et al. 1988; Cuervo and Møller 1999, 2001), and thus deviate according to environmental changes (Hill 1995, 2006).

When anomalies in humidity and wind speed were greater than in the 30-year baseline period, tail hue was longer wavelength-directed, while tail brightness increased when anomalies in temperature were greater than in the 30-year baseline period. The relationship between greater anomalies in temperature and increased tail brightness, is contradictory to the prediction that anomalies in climatic factors from the 30-year baseline period increases unpredictability in the quantity and timing of emerging insect food or maintains the basal metabolic rate so that the anomaly acts as a stress factor. The mean temperature of the study year (2004) was higher than the mean temperature during the 30-year baseline period (1971-2000) in all nine areas from which specimens were collected. The increase in mean temperature may positively affect the quality of an individual owing to a decrease in basal metabolic energy requirements, increased food resources, and earlier food emergence for resident or short-distance migrant avian species (Hüppop and Hüppop 2003, 2005). However, the previously described condition indicators, such as body mass, tail length, and total tail length, were not correlated with the increase in temperature. Furthermore, tail brightness in males was only related to anomalies in temperature, while both sexes had a higher deviation value (CV) for tail brightness than for other morphological characteristics. Based on these results, variation in tail coloration may reflect the condition of the male bearer, but only to a very subtle degree.

Different aspects of the variation in brightness and hue

Tail brightness and tail hue varied according to anomalies in local climate; however, the effects of the climatic factors and the extent of their impacts on tail brightness and tail hue differed. For example, the deviation (CV) in tail brightness was significantly greater than for other morphological characteristics, whereas the deviation in tail hue did not differ from that of other characteristics. The relationship between color components and deviations in local climatic factors also followed differing patterns. Tail brightness was related to temperature anomalies, whereas tail hue was related to anomalies in humidity and wind speed. Also, deviation of tail brightness was highly correlated to anomalies in temperature and humidity, whereas deviation of tail hue was related to anomalies in temperature, humidity, and precipitation. This raises the question: How is it that two color components have different deviation patterns in relation to climatic deviation? There could be two explanations.

First, environmental stress may cause differential development of the feather structures that produce coloration, which in turn may affect the expression of brightness and hue differently. Consumption of more energy related to unpredictable climate may reduce the energy available for keratin formation, and that may affect the form or density of the feather nanostructure. However, the mechanism whereby environmental stress affects the form or density of the feather structure has not been elucidated. Second, climate factors may directly affect variation in color components. According to Eliason and Shawkey (2010) and Shawkey et al. (2011), the hue of iridescent coloration is affected instantly and reversibly by changes in ambient humidity via a change in the lattice size of the feather nanostructure. No such instantaneous color shift in relation to humidity was detected in this study, but that is not surprising as all measurements were made in a laboratory where both temperature and humidity were generally constant. Further information on the affects of ambient humidity on plumage coloration would be of value. In addition, feather-degrading bacteria easily proliferate in warm, humid conditions, and the amount of bacteria can affect changes in coloration (Shawkey et al. 2007, 2009).

Does tail hue signal condition in magpies?

Eliason and Shawkey (2010) mentioned that if hue is a condition-dependent characteristic, high-quality individuals may adapt in a humid environment by releasing more preening oil and forming a dense microstructure (Taylor et al. 2004) so that the reliability of the plumage visual signal will be maintained; conversely if all individuals within a population are exposed to similar humidity, any change in hue will be across the population and hence will not affect signal reliability (Eliason and Shawkey 2010). However, the results of this study revealed that tail hue had fewer relationships with quality indicators than did tail brightness (see Chapters 2 and 3). Moreover, deviation in hue, in response to anomalous climatic factors, was significantly less than the deviation in brightness, and even showed a negative relationship to anomalies in humidity and precipitation. Based on information available so far, it does not seem that tail hue provides an environmental condition-related signal. Tail hue differed significantly between the ages and sexes of magpies, so perhaps tail hue carries only a status class signal. Most studies of variation in iridescence have shown that the brightness and chroma of the UV range are related to the body condition of the particular sex being studied (Bennet et al. 1997; Doucet

2002; Shawkey et al. 2003; Doucet and Montgomerie 2003; Doucet et al. 2004; Bitton and Dawson 2008). Other research has shown, however, that hue is related only to age class (Bitton and Dawson 2008). Doucet et al. (2006) showed that variation in hue is related to the thickness of the feather keratin cortex, but there have been no studies on the relationship between cortex thickness and individual quality to date. In conclusion, brightness and hue may carry different information on quality, yet variation of thickness in keratin cortices is related to individual quality.

GENERAL DISCUSSION

My study has shown that the Black-billed Magpie (*Pica pica*), previously known as a monochromatic species, actually varies in color depending on age, sex, and territory ownership. In particular, the iridescent coloration of the tail was found to be an indicator of body condition and reproductive success among males. Tail coloration was also found to be related to climatic anomalies (variation in climatic factors from the means of a 30-year period) in males. Based on these results, I suggest that the iridescent coloration of the tail, along with previously known quality-indicating traits, such as tail length, mass, and structural defects in the tail, is a trait that honestly indicates the quality of male magpies.

Signaling aspects of plumage color in relation to status and condition

The three types of structural colors in magpies showed distinctive differences between age classes (Chapter 1, Table 1-2). In a high-density magpie population with strong territoriality throughout the year, differences in overall plumage coloration can serve as status class signals, and may permit young magpies to pass through adults' territories or to increase their chances of survival in non-territorial flocks during their first winter, by reducing the amount of aggressive behavior directed at them by adults.

Tail brightness did not differ significantly between adults and young birds, but it positively reflected condition in territorial adult males. In contrast, there was no relationship between tail brightness and body condition in non-territorial adult males, and there was even a negative relationship in young males (Figure 2-1). These results suggest that there may be different trade-off strategies between the full growth of the tail and body condition in male magpies depending on their age and territoriality; young males must spend more energy on their post-fledge survival than on developing full feather coloration, which is costly as well as risky in terms of attracting high rates of aggression from older competitors. Conversely, breeding males, which are territorial adults, should advertise their good body condition to potential intruders by means of their tail coloration. Further comparisons of each tail characteristic according to age class, are necessary to be able to fully understand the signaling aspects of the male magpie’s tail.

Tail brightness as an indicator of quality in male magpies

Contributing half of the total body length, the magpie's tail requires considerable energy for its growth and maintenance. Such a large, heavy tail also limits maneuverability, and the large contact area between the tail and ground vegetation may promote heavy infestations of ectoparasites such as ticks and mites. Thus, the tail of the magpie can be regarded as physical handicap, according to Zahavi (1975, 1977). Territorial males in better body condition had brighter and shorter wavelength-directed coloration in their tails, and the coloration was also related to other morphological characters of the tail such as total tail length (sum of the lengths of all rectrices) and the number of feather fault bars (Chapter 2). Tail coloration in males also had greater deviation than the other morphological characters. Moreover, under conditions of low-level environmental stress, such as small annual fluctuations in climatic conditions, the degree of deviation in color increased in relation to the increase in the anomalies in some climate variables. Therefore, these results support the prediction that tail iridescence is likely to be under directional selection (Chapter 4). Both the timing of breeding initiation, and breeding success, were found to be related to tail coloration (Chapter 3). Breeding success is an important factor supporting the maintenance of existing pair bonds in magpies and other species; therefore, good male breeders in a given year might have been in better quality when they were initially selected by females and formed their own territory.

The magpie is a socially monogamous species in which both sexes participate in territorial defense and parental care; both sexes display by flicking their tail feathers, which has long been suspected of being a signaling behavior (Birkhead 1991). Therefore, mutual selection is expected in the Black-billed Magpie. Interestingly, however, in my study the tail coloration of females was not found to be related to any factors that signal body condition. Contradictory evidence relating to quality indicators and assortative mating in magpies has previously been published, with Fitzpatrick and Price (1997) finding that individuals with similar extents of tail damage tended to form pairs, whereas Blanco and de la Puente (2002) showed that the tail characteristics of females carry much less information than do those of males. Murphy (2008) suggested two hypotheses to explain non-assortative mating in avian species in which both sexes have the same type of ornament: first, female plumage coloration may be a byproduct of genetic correlation (Lande 1980), and second, there may be another selection mechanism affecting plumage coloration other than mutual sexual selection (LeBas 2006; Murphy 2006). Though mutual selection in magpies is highly probable, considering their biparental care and territorial behaviors, the detailed process of mate choice is still unclear. Without knowing when and how mate selection occurs and the role of each sex in mate choice, conclusions concerning the effects of coloration on assortative mating in magpies are beyond the scope of my study. Although I have shown that tail brightness is a signal indicating quality in male magpies, future work on the relationships between the coloration of parents and offspring, other tail characteristics and female reproductive success, is necessary to provide further confirmation of assortative mating in this species.

Does tail coloration reflect the present quality of males?

Current plumage coloration of males does not necessarily reflect their color when mate choice took place, because of annual feather replacement during molt. However, males in better condition, or older males, are usually selected by females when the pair forms and occupies a new territory (Komers and Dhindsa 1989). Since magpies expend much energy and time throughout the year for defending territories, territory ownership is not easily changed and a pair holding its territory thus tends to maintain it for several years (Chapter 1). In addition, it may be advantageous for females to maintain their pair bonds from year to year because male quality tends to increase with age as their foraging skills improve, as they gain breeding experience, and as they improve in defending against potential predators and conspecific competitors. Despite possible changes in male quality between years and seasons, high rates of mate fidelity may deter recognition of male quality by researchers based on feather coloration.

Under some circumstances mate selection may occur even after territory acquisition and pair formation. Divorce has been reported in some magpie populations especially following low breeding success (Birkhead et al. 1986; Buitron 1988; Dhindsa and Boag 1992), and extra-pair copulation has also been observed (Buitron 1983; Birkhead and Biggins 1987). Therefore, males currently in good quality and in pairs, have presumably continued to perform well both in breeding and in territorial defense after initial mate selection occurred.

Considering all of these facts together, female magpies are unlikely to change mates due solely to reduced signal strength from the sexual traits of the male's feathers alone. Tail brightness may not directly reflect the present quality of male magpies, because feather coloration barely indicates short-term changes in male quality after previous molting. However, new tails are typically grown at the end of autumn, before wintering and the following breeding season, and the maintenance of good tails is closely related with the present quality of birds in general. Therefore, the brightness of tails that were replaced and maintained before wintering and breeding seasons, may signal body condition and expected future breeding performance. To validate the relationship between sexual traits and reproductive quality of males, further investigation of long-term reproductive success is needed.

Future studies

Link between environmental variation and color expression

Several studies, including mine, have investigated the impact of environmental effects on variation in plumage iridescence, but a new field of study to reveal how environmental variation affects the production of nanostructures of feather barbules has recently begun (Eliason and Shawkey 2010; Shawkey et al. 2011). The Black-billed Magpie exhibits two types of iridescent colors; interestingly and contrary to the results of the previous study on the anatomy of iridescence (Zi et al. 2003), the distance between air holes in the keratin cortex of the remiges, which has a short wavelength-directed hue (416.08 ± 13.40 nm in my study), is longer than that of the tail, which has a longer wavelength-directed hue (510.15 ± 17.14 nm in my study) (Vigneron et al. 2006). Such an inconsistency in the distances between the cortex air holes and the expected wavelengths reflected may be due to the different size and array of the melanin granules (Lee et al. 2009; Maia et al. 2011), or due to the second gap effect of the wing coloration followed by the reflectance spectrum from the first gap effect (Vigneron et al. 2006, Figure IV) as mentioned in Chapter 1. If the variation of color in remiges is dependent on the first-gap curve at 850 nm (Figure IV), the inconsistency between the color and anatomical structure of the wing keratin cortex can be explained. Based on the previous studies of feather structure, the effects of environmental variation (such as climatic variation and nutritional stress) on variation in the keratin cortex structure and in the array of melanin granules would make an interesting topic for future research. The effects of variation in the keratin cortex structure and the array of melanin granules on the first gap reflectance curve from the tail, and the first and second gap reflectance curves from the wings, also require further study in order to elucidate the mechanism of condition-dependent iridescence.

Role of melanin in the variation in iridescent coloration

Melanin is known to play a role in the background of reflected light from the feather keratin cortex (Maia et al. 2009), and the size and pattern of the array in the iridescent color is known to differ between the sexes (Lee et al. 2009; Maia et al. 2011). The quantity and distribution of melanin in magpie feathers, meanwhile, is complex (Lee et al. 2010) and not condition-dependent (Reed 1997); thus the role of melanin in the expression of iridescence is still vague. However, the presence of melanin is known to reinforce the feather structure preventing feather degradation by bacteria (Goldstein et al. 2004; Gunderson et al. 2008), and the quantity of melanin granules in feathers is related to the production of non-iridescent structural colors (Shawkey and Hill 2004; Shawkey et al. 2007; Gunderson 2008). The Black-billed Magpie is a ground forager (Birkhead 1991), and thus it is highly probable that their very long tail feathers are exposed to infections by feather degrading bacteria (Burtt Jr. and Ichida 1999). Therefore, a lack of melanin might affect the decline in coloration resulting from degradation of the keratin structure, though the melanin content is not directly related to variation in iridescence. For Chapters 1-3, color measurements were made just after the molt in order to control for any color change effects resulting from the action of feather degrading bacteria. However, for Chapter 4, samples were collected from late February to early April, thus it is not possible to completely exclude the potential effects of strong winter winds on feather wear (Jenni and Winkler 1994) and consequently on the expression of iridescent coloration. The combined effects of temperature, humidity, precipitation, and wind speed on feather wear differ between local areas where climatic conditions are different. Therefore, further research into variation in iridescence according to local climatic variation may provide valuable information on local adaptations and the varied strengths of sexual signals.

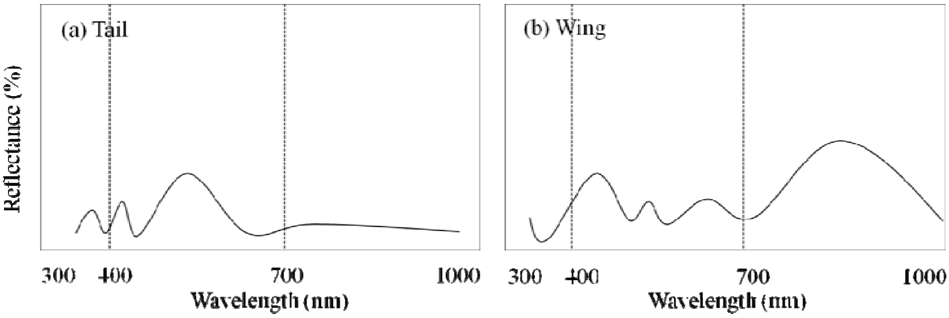


Figure IV. Reflectance spectra calculated from the photonic crystal model of (a) the tail and (b) the wing. Redrawn from Vigneron et al. (2006).

SUMMARY

Backgrounds

Following pioneering studies by Darwin (1871), plumage coloration has long been studied for its importance as an honest signal of quality in the chosen sex. There are various mechanisms whereby avian plumage colors are produced, the most recently explored of these is structural coloration, which is produced when light is scattered by micro- or nanostructures within feathers. This new field of study has revealed that good quality males have denser keratin structures in their plumage than low quality males. The feathers of good quality males are now known to scatter brighter, shorter wavelength-directed, and more saturated colors. However, the ecological significance of plumage iridescence, a unique form of structural coloration, has only rarely been studied owing to its unstable nature (Meadows et al. 2011) and complex structure (Prum et al. 2006).

The Black-billed Magpie (*Pica pica*) has three types of structural coloration: 1) non-iridescent white, made by incoherent scattering by the scapular feathers, 2)

bluish iridescence on the wings, and 3) greenish iridescence on the tail. Studying the function and significance of the various types of structural colors found in magpies (previously considered to be sexually monochromatic), will provide evidence supporting our understanding of the directional selection of iridescence.

Questions

The aim of the study was to examine whether or not the trait of iridescent coloration in male magpies contains information regarding quality that can be selected by females. The following questions were posed in order to clarify whether the structural coloration in magpies serves a signaling function or not (see Rohwer 1975), and whether or not there is a relationship with known “handicap” characteristics (Zahavi 1975, 1977), higher reproductive success followed by earlier breeding (Darwin 1871; Fisher 1930), and labile changes for directional selection when under environmental stress (Hill 1995).

* Are magpies sexually dichromatic?

* Does magpie plumage color vary in relation to age or territory ownership?

* Is the iridescent coloration dependent on body condition indices?

* Does the iridescent coloration indicate reproductive success among male magpies?

* Does variation in environmental conditions affect iridescent coloration?

Methods

The experimental phase of the study was conducted on the Seoul National University campus from mid October to December in 2003, 2004, and 2006. The breeding behavior of magpies captured for sampling in autumn or early winter was monitored in subsequent years. For the evaluation of climatic effects on plumage variation, magpies that had been harvested by the Korea Electric Power Corporation during an ongoing damage control program were collected from nine regions of Korea. Plumage color (of the white scapulars, bluish iridescent wings, and greenish iridescent tail) was measured using a spectrophotometer (USB2000; Ocean Optics, Dunedin, FL, USA). Color components such as brightness, hue, and chroma were all derived from the reflectance curve, and used in the analysis.

Findings

1) Differences in structural colors in relation to status

In each of the three areas of plumage for which colors were measured, after-hatching-year (AHY) individuals had brighter, shorter wavelength-directed, and more saturated colors than hatching-year (HY) individuals, making a clear visual distinction between adult and young birds. In late autumn, young birds dispersing from their natal territories to join wintering flocks must cross other defended territories and then must compete with other individuals for food during winter. Exhibiting a clear signal of age, i.e. a different plumage color, may serve to reduce aggressive behaviors directed at them by adults when crossing their territories, and also serve to reduce competition in wintering flocks, thereby allowing young magpies to experience increased chances of survival during their first winter.

In contrast to clear age-related difference in plumage coloration, the only differences found between the sexes were in the extent of tail iridescence: males had brighter, shorter wavelength-directed tail iridescence than did females. Fitzpatrick and Price (1997) and Blanco and de la Puente (2002) have suggested that a magpie’s long, heavy tail is related to its body condition. Therefore, the brighter and shorter wavelength-directed colors in male tail iridescence could signal higher quality.

Although the mean values of tail brightness and tail hue in territorial AHY males were higher than those of non-territorial AHY males, the differences were not statistically significant. In females, the brightness and hue scores of territorial AHY females were actually lower than those of non-territorial AHY females. These results, which contradict the prediction that only good quality individuals occupy territories, suggest two possibilities. Firstly, the age effect may be diluted in a dense population where many older individuals have no territories. Secondly, territorial individuals are more exhausted as a consequence of defending a territory and breeding; therefore, their present color may differ from their color at the time of territory formation.

2) Relationships between body condition and iridescent colors

The relationships between iridescent colors and body condition indices were statistically significant only in males. Whereas wing iridescence was rarely correlated with condition indices, tail color was dependent on various condition indices including body size, tail characteristics, and immunity both in AHY and HY males. The results support the hypothesis that tail iridescence provides an honest signal of body condition in males. The association between iridescence and other morphological characteristics of the tail, such as total tail length (the total length of all rectrices as a substitute for the measurement of tail mass) and the number of fault bars can serve to amplify quality signals in males.

Males of different age-related and territory-related statuses showed differing relationships between their colors and other indices of body condition. Color scores tended to be higher in territorial AHY males in better condition, whereas the opposite trend was found in HY males. There are several limiting factors to the full expression of tail color in HY males. The period from fledging to the end of the first winter is the critical period for their survival. In addition, there is little chance of HY birds breeding in their first year, especially for males. Therefore, the different relationship patterns according to age and territory ownership in males are thought to represent a trade-off in energy consumption between that used for survival and that used for full development of the sexual characteristics.

3) Relationships between reproductive success and male iridescent color

Males that bred successfully (fledged more than one chick) had brighter tail colors than males that failed to breed, although there was no difference between them in terms of hue. Males with brighter tail colors began breeding earlier, and produced more fledglings. Chick quality was also related to adult male tail brightness. Brighter breeding males produced chicks with higher body condition indices and low heterophil/lymphocyte (H/L) levels. Males whose nests had earlier hatching dates, produced more hatchlings and fledglings, but there was no relationship between fledging date and hatching or fledging success. Magpies are territorial and tend to have a long-term pair bond and thus they do not fit the parameters of the original Darwin-Fisher theory; however, the results still support the Darwin-Fisher theory. The advantages of maintaining partnerships from year to year may ensure earlier breeding, because females can save energy that they might otherwise expend in trying to find a partner, and previous experience is proof of the quality of partners.

4) Effects of local climate on iridescent coloration

The deviation of tail brightness was higher than that of tail morphological characteristics, regardless of their condition-dependency, whereas the deviation of tail hue did not differ from that of other characteristics. In males, deviation in tail brightness was significantly related to deviations in temperature and humidity. The deviation of tail hue was also significantly related to temperature, humidity, and precipitation, but the relationship was subtle compared with that of brightness. Therefore, tail brightness is more variable and plastic than tail morphological traits, and responsive to environmental changes, and thus probably a trait that leads to directional selection.

The low correlation between hue deviation and climatic deviation is mainly caused by the low level of deviation in hue, but there are also other possibilities. A decline in general body condition can lead to changes in brightness, hue, and chroma, but humidity alone, or an increase in feather-degrading bacteria, due to a combination of elevated humidity and temperature, can affect the color components in different ways (Eliason and Shawkey 2010; Shawkey et al. 2007, 2009, 2011). So far, tail brightness in magpies is the only known indicator of environmental variation, but carefully controlled experiments are needed to find the proximate mechanism by which changes in climatic condition affect feather structures.

Conclusion

The study found that the Black-billed Magpie (*Pica pica*), previously known as a monochromatic species, actually exhibits different coloration depending on age, sex, and territory ownership. In particular, in males, the iridescent coloration of the tail reflects body condition and reproductive success. Tail coloration of males was also related to anomalies (from the 30-year means) in climatic parameters. Based on these results, I suggest that the iridescent coloration of the tail is a trait that honestly signals the quality of male magpies.

REFERENCES

Alatalo, R., J. Hoglund, and A. Lundberg. 1988. Patterns of variation in tail ornament size in birds. Biological Journal of the Linnean Society 34: 363-374.

Alonso-Alvarez, C., C. Doutrelant, and G. Sorci. 2004. Ultraviolet reflectance affects male-male interactions in the blue-tit (*Parus caeruleus ultramarinus*). Behavioral Ecology 15: 805-809.

Amundsen, T., and H. Pärn. 2006. Female coloration: review of functional and nonfunctional hypothesis, p. 280-345. *In* G. E. Hill and K. J. McGraw [eds.], *Bird Coloration II: Function and Evolution*. Harvard University Press, Cambridge.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution 40: 804-816.

Andersson, M. 1994. *Sexual selection*. Princeton University Press, Princeton.

Andersson, S. 1999. Morphology of UV reflectance in a whistling-thrush: implications for the study of structural colour signaling in birds. Journal of

Avian biology 30: 193-204.

Andersson, S., J. Örnborg, and M. Andersson. 1998. Ultraviolet sexual dimorphism and assortative mating in Blue Tits. Proceedings of Royal Society of London series B 265: 445-450.

Aparicio, J. M., R. Bonal, and P. J. Cordero. 2003. Evolution of the structure of tail feathers: implications for the theory of sexual selection. Evolution 57: 397-405.

Baeyens, G. 1981a. The role of the sexes in territory defense in the magpie (*Pica pica*). Ardea 69: 69-82.

Baeyens, G. 1981b. Functional aspects of serial monogamy: the magpie pair-bond in relation to its territorial system. Ardea 69: 145-166.

Baker, R. R., and G. A. Parker. 1979. The evolution of bird coloration. Philosophical Transactions of the Royal Society London B 287: 63-130.

Banks, A. N. 2001. For your eyes only? The role of UV in mate choice. Trends in Ecology and Evolution 16: 473-474.

Bart, J., and S. L. Earnst. 1999. Relative importance of male and territory quality in pairing success of male rock ptarmigan (*Lagopus mutus*). Behavioral Ecology and Sociobiology 45: 355-359.

Bennett, A. T. D., I. C. Cuthill, and K. J. Norris. 1994. Sexual selection and the mismeasure of color. The American Naturalist 144: 848-860.

Bennett, A. T. D., I. C. Cuthill, J. C. Partridge, and E. S. Maier. 1996. Ultraviolet vision and mate choice in zebra finches. Nature 380: 433-435.

Bennett, A. T. D., I. C. Cuthill, J. C. Partridge, and K. Lunau. 1997. Ultraviolet plumage colors predict mate preferences in starlings. Proceedings of the National Academy of Sciences 94: 8618-8621.

Birkhead, T. R. 1991. *The Magpies*. T&AD Poyser, London.

Birkhead, T. R., and J. D. Biggins. 1987. Reproductive Synchrony and Extra-pair Copulation in Birds. Ethology 74: 320-344.

Birkhead, T. R., and K. Clarkson. 1985. Ceremonial gatherings of the magpie *Pica pica*: territory probing and acquisition. Behaviour 94: 324-332.

Birkhead, T. R., S. F. Eden, K. Clarkson, S. F. Goodburn, and J. Pellatt. 1986. Social organisation of a population of magpies *Pica pica* Ardea 74: 59-68.

Bitton, P.-P., and R. D. Dawson. 2008. Age-related differences in plumage characteristics of male tree swallows *Tachycineta bicolor*: hue and brightness signal different aspects of individual quality. Journal of Avian Biology 39: 446-452.

Bitton, P.-P., R. D. Dawson, and C. L. Ochs. 2008. Plumage characteristics, reproductive investment and assortative mating in tree swallows *Tachycineta bicolor*. Behavioral Ecology and Sociobiology 62: 1542-1550.

Blanco, G., and J. de la Puente. 2002. Multiple elements of the black-billed magpie's tail correlate with variable honest information on quality in different age/sex classes Animal Behaviour 63: 217-225.

Botero, C. A., and D. R. Rubenstein. 2012. Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. PLoS ONE 7: e32311.

Bowers, D. E. 1956. A study of methods of color determination. Systematic Biology 5: 147-160.

Bowmaker, J. K., L. A. Heath, S. E. Wilkie, and D. M. Hunt. 1997. Visual pigments and oil droplets from six classes of photoreceptor in the retina of birds. Vision Research 37: 2183-2194.

Bradbury, J. B., and S. L. Vehrencamp. 2011. *Principles of Animal Communication*. Sinauer Associates, MA.

Bub, H. 1991. *Bird Trapping & Bird Banding*. Cornell University Press, Ithaca.

Budden, A. E., and J. L. Dickinson. 2009. Signals of quality and age: the information content of multiple plumage ornaments in male western bluebirds *Sialia mexicana*. Journal of Avian Biology 40: 18-27.

Buitron, D. 1983. Extra-pair courtship in Black-billed Magpies Animal Behaviour 31: 211-220.

Buitron, D. 1988. Female and male specialization in parental care and its consequences in Black-billed Magpies. The Condor 90: 29-39.

Burkhardt, D., and E. Finger. 1991. Black, white, and UV: How birds see birds. Naturwissenschaften 78: 279-280.

Burtt Jr., E. H. 1981. The adaptiveness of animal colors. BioScience 31: 723-729.

Burtt Jr., E. H., and J. M. Ichida. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. The Auk 116: 364-372.

Butcher, G. S., and S. Rohwer. 1989. The evolution of conspicuous and distinctive coloration for communication in birds Current Ornithology 6: 51-108.

Cheng, S., and S. J. Lamont. 1988. Genetic analysis of immunocompetence measures in a White Leghorn chicken line. Poultry Science 67: 989-995.

Choudhury, S. 1995. Divorce in birds: a review of the hypotheses. Animal Behaviour 50: 413-429.

Conover, M. R., J. G. Reese, and A. D. Brown. 2000. Costs and benefits of subadult plumage in Mute Swans: testing hypotheses for the evolution of delayed plumage maturation. The American Naturalist 156: 193-200.

Copete, J. L., R. Mariné, D. Bigas, and A. Martínez-Vilalta. 1999. Difference in wing shape between sedentary and migratory reed buntings *Emberiza schoeniclus*. Bird Study 46: 100-103.

Costa, F. J. V., and R. H. Macedo. 2005. Coccidian oocyst parasitism in the blue-black grassquit: influence on secondary sex ornaments and body condition. Animal Behaviour 70: 1401-1409.

Cucco, M., R. Ottonelli, M. Raviola, and G. Malacarne. 2002. Variations of body mass and immune function in response to food unpredictability in magpies. Acta Oecologia 23: 271-276.

Cuervo, H. J., and A. P. Møller. 2001. Components of phenotypic variation in avian ornamental and non-ornamental feathers. Evolutionary Ecology 15: 53-72.

Cuervo, J. J., and A. P. Møller. 1999. Phenotypic variation and fluctuating asymmetry in sexually dimorphic feather ornaments in relation to sex and mating system. Biological Journal of the Linnean Society 68: 505-529.

Cuthill, I., A. T. D. Bennett, J. C. Partridge, and E. J. Maier. 1999. Plumage reflectance and the objective assessment of avian sexual dichromatism. The American Naturalist 153: 183-200.

Darwin, C. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London.

Darwin, C. 1871. *The descent of man, and selection in relation to sex*. Murray, London.

Dearborn, D. C., and M. J. Ryan. 2002. A test of the Darwin-Fisher theory for the evolution of male secondary sexual traits in monogamous birds. Journal of Evolutionary Biology 15: 307-313.

Dein, J. 1986. Hematology, p. 174-191. *In* G. J. Harrison and W. R. Harrison [eds.], *Clinical Avian Medicine*. Saunders, London.

Delhey, K., A. Johnsen, A. Peters, S. Andersson, and B. Kempenaer. 2003. Paternity analysis reveals opposing selection pressure on crown coloration in the Blue Tit (*Parus caeruleus*). Proceedings of Royal Society of London series B 270: 2057-2063.

Delhey, K., A. Peters, A. Johnsen, and B. Kempenaers. 2007. Fertilization success and UV ornamentation in bluetits *Cyanistes caeruleus*: correlational and experimental evidence. Behavioral Ecology 18: 399-409.

Dhindsa, M. S., and D. A. Boag. 1992. Patterns of nest site, territory and mate switching in Black-billed Magpies. Canadian Journal of Zoology 70: 633-640.

Dhindsa, M. S., P. E. Komers, and D. A. Boag. 1989. The effect of familiarity with an environment on the dominance relationships between juvenile and adult Black-billed Magpies. Ornis Scandinavica 20: 187-192.

Dhondt, A. A., and F. Adriaensen. 1994. Causes and effects of divorce in the Blue Tit *Parus caeruleus*. Journal of Animal Ecology 63: 979-987.

Doucet, S. M. 2002. Structural plumage coloration, male body size, and condition in the blue-black grassquit. The Condor 104: 30-38.

Doucet, S. M., and M. G. Meadows. 2009. Iridescence: a functional perspective. Journal of the Royal Society Interface 6: S115-S132.

Doucet, S. M., D. J. Mennill, R. Montgomerie, P. Boag, and L. M. Ratcliffe. 2004. Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. Behavioral Ecology 16: 218-222.

Doucet, S. M., and R. Montgomerie. 2003. Structural plumage colour and parasites in satin bowerbirds *Ptilonorhynchus violaceus*: implications for sexual selection. Journal of Avian Biology 34: 237-242.

Doucet, S. M., M. D. Shawkey, G. E. Hill, and R. Montgomerie. 2006. Iridescent plumage in satin bowerbirds: structure, mechanisms, and nanostructural predictors of individual variation in colour. Journal of Experimental Biology

290: 380-390.

Dubois, F., and F. Cézilly. 2002. Breeding success and mate retention in birds: a meta-analysis. Behavioral Ecology and Sociobiology 52: 357-364.

Dufva, R., and K. Allander. 1995. Intraspecific variation in plumage coloration reflects immune response in Great Tit (*Parus major*) males. Functional Ecology 9: 785-789.

Durrer, H. 1977. Schillerfarben der Vogelfeder als Evolutionsproblem. Denkschriften der Schweizerischen Naturforschenden Gesellschaft 14: 1-126.

Eaton, M. D. 2005. Human vision fails to distinguish widespread sexual dichromatism among sexually "monochromatic" birds. Proceedings of the National Academy of Sciences 102: 10942-10946.

Eaton, M. D. 2007. Avian visual perspective on plumage coloration confirms rarity of sexually monochromatic North American passerines. The Auk 124: 155-161.

Eberhard, W. G., B. A. Huber, R. L. Rodriguez S., R. D. Briceño, I. Salas, and V. Rodriguez. 1998. One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. Evolutionary Ecology 52: 415-431.

Eliason, C. M., and M. D. Shawkey. 2010. Rapid, reversible response of iridescent feather color to ambient humidity. Optic Express 18: 21284-21292.

Endler, J. A. 1986. *Natural selection in wild*. Princeton University Press, Princeton.

Ens, B. J., S. Choudhury, and J. M. Black. 1996. Mate fidelity and divorce in monogamous birds, p. 344-395. *In* J. M. Black [ed.], *Partnerships in birds*. Oxford University Press, Oxford.

Eo, S., J. Hyun, W.-S. Lee, T. Choi, S.-J. Rhim, and K. Eguchi. 2002. Effects of topography on dispersal of black-billed magpie *Pica pica sericea* revealed by population genetic analysis. Journal of Ethology 20: 43-47.

Erpino, M. J. 1968. Age determination in the black-billed magpie. Ibis 70: 91-92.

Ferrer, M., and V. Penteriani. 2003. A process of pair formation leading to assortative mating: passive age-assortative mating by habitat heterogeneity. Animal Behaviour 66: 137-143.

Figuerola, J., E. Munoz, R. Gutierrez, and D. Ferrer. 1999. Blood parasites, leucocytes and plumage brightness in the Cirl Bunting, *Emberiza cirlus*. Functional Ecology 13: 594-601.

Fisher, R. A. 1930. *The genetical theory of natural selection*. At The Clarendon Press, Oxford.

Fitzpatrick, S. 1998. Colour schemes for birds: structural coloration and signals of quality in feathers. Annales Zoologici Fennici 35: 67-77.

Fitzpatrick, S., and P. Price. 1997. Magpie's tails: damage as an indicator of quality. Behavioral Ecology and Sociobiology 40: 209-212.

Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. The American Naturalist 139: 603-622.

Francis, C. M., and F. Cooke. 1986. Differential timing of spring migration in wood warblers (Parulinae). The Auk 103: 548-556.

Freeman-Gallant, C. R. 1996. DNA fingerprinting reveals female preference for male parental care in Savannah sparrows. Proceedings of Royal Society of London series B 263: 157-160.

Galván, I., and J. J. Sanz. 2008. The cheek plumage patch is an amplifier of dominance in great tits. Biology Letters 4: 12-15.

Gill, F. 1994. *Ornithology*. W.H. Freeman and Company, New York.

Gill, F., and D. Donsker. (Eds.). 2012. *IOC World Bird Names* (v 3.2). Available at http://www.worldbirdnames.org

Ginn, H. B., and D. S. Melville. 1983. *Moult in Birds*. The British Trust for Ornithology, Thetford.

Gladbach, A., D. J. Gladbach, and P. Quillfeldt. 2010. Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese *Chloephaga picta leucoptera*. Comparative Biochemistry and Physiology Part A 156: 269-277.

Goldstein, G., K. R. Flory, B. A. Browne, S. Majid, J. M. Ichida, and E. H. Burtt Jr. 2004. Bacterial degradation of black and white feathers. The Auk 121: 656-659.

Götmark, F. 1997. Bright plumage in the magpie: does it increase or reduce the risk of predation? Behavioral Ecology and Sociobiology 40: 41-49.

Green, A. J. 2000. The scaling and selection of sexually dimorphic characters: an example using the marbled teal. Journal of Avian biology 31: 345-350.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7: 1071-1075.

Griggio, M., L. Serra, D. Licheri, C. Campomori, and A. Pilastro. 2009. Moult speed affects structural feather ornaments in the blue tit. Journal of Evolutionary Biology 22: 782-792.

Gross, W. B., and H. S. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Disease 27: 972-979.

Grossman, C. J. 1985. Interactions between the gonadal steroids and the immune system. Science 227: 257-261.

Grubb, T. C. 2006. *Ptilochronology: Feather Time and the Biology of Birds*. Oxford University Press, Oxford.

Gunderson, A. R. 2008. Feather-degrading bacteria: A new frontier in avian and host-parasite research? The Auk 125: 972-979.

Gunderson, A. R., A. M. Frame, J. P. Swaddle, and M. H. Forsyth. 2008. Resistance of melanized feathers to bacterial degradation: is it really so black and white? Journal of Avian Biology 39: 539-545.

Hamilton, T. H., and R. H. J. Barth. 1962. The biological significance of season change in male plumage appearance in some New World migratory bird species. The American Naturalist 96: 129-144.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218: 384-387.

Hausmann, F., K. E. Arnold, N. J. Marshall, and I. P. F. Owens. 2002. Ultraviolet signals in birds are special. Proceedings of Royal Society of London series B 270: 61-67.

Hegemann, A., K. D. Matson, C. Both, and B. I. Tieleman. 2012. Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. Oecologia DOI 10.1007/s00442-012-2339-3 (online published).

Hill, G. E. 1988. Age, plumage brightness, territory quality, and reproductive success in the Black-headed Grosbeak. The Condor 93: 379-388.

Hill, G. E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Animal Behaviour 40: 563-572.

Hill, G. E. 1993. Male mate choice and the evolution of female plumage coloration in the House Finch. Evolution 47: 1515-1525.

Hill, G. E. 1995. Ornamental traits as indicators of environmental health. BioScience 45: 25-31.

Hill, G. E. 2002. *A red bird in a brown bag: the function and evolution of colorful plumage in the house finch*. Oxford University Press, New York.

Hill, G. E. 2006a. Environmental regulation of ornamental coloration, p. 507-560. *In* G. E. Hill and K. McGraw [eds.], *Bird Coloration I: Mechanism and Measurements*. Harvard University Press, Cambridge.

Hill, G. E. 2006b. Female mate choice for ornamental coloration. *In* G. E. Hill and K. McGraw [eds.], *Bird Coloration II: Function and Evolution* Harvard

University Press, Cambridge.

Hill, G. E., S. M. Doucet, and R. Buchholz. 2005. The effect of coccidial infection on iridescent plumage coloration in wild turkeys. Animal Behaviour 69: 387-394.

Hunt, S., A. T. D. Bennett, I. C. Cuthill, and R. Griffiths. 1998. Blue tits are ultraviolet tits. Proceedings of Royal Society of London series B 265: 451-455.

Hunt, S., A. T. D. Bennett, I. C. Cuthill, and R. Griffiths. 1999. Preference for ultraviolet partners in the blue tit. Animal Behaviour 58: 809-815.

Hunt, S., I. C. Cuthill, A. T. D. Bennett, S. C. Church, and J. C. Partridge. 2001. Is the UV waveband a special communication channel in avian mate choice? Journal of Experimental Biology 204: 2499-2507.

Hunt, S., I. C. Cuthill, A. T. D. Bennett, and R. Griffiths. 1999. Preferences for ultraviolet partners in the bluetit. Animal Behaviour 58: 809-815.

Hüppop, K., and O. Hüppop. 2005. Atlas zur Vogelberingung auf Helgoland. Vogelwarte 43: 217-248.

Hüppop, O., and K. Hüppop. 2003. North Atlantic Oscillation and timing of the spring migration in birds. Proceedings of Royal Society of London series B 270: 233-240.

Ilmonen, P., T. Taarna, and D. Hasselquist. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proceedings of Royal Society of London series B 267: 665-670.

Jakob, E. M., S. D. Marshall, and G. W. Uetz. 1996. Estimating fitness: a comparison of body condition indices. Oikos 77: 61-67.

Jang, H. 2004. Genetic differentiation of Korean Black-billed Magpie (*Pica pica sericea* Gould), Ms.D. thesis. Seoul National University, Seoul.

Jawor, J. M., S. U. Linville, S. M. Beall, and R. Breitwisch. 2003. Assortative mating by multiple ornaments in northern cardinals (*Cardinalis cardinalis*). Behavioral Ecology 14: 515-520.

Jenni, L., and R. Winkler. 1994. *Moult and ageing of European passerines*. Academic Press, San Diego.

Johnsen, A., S. Andersson, J. Önborg, and J. T. Lifjeld. 1998. Ultraviolet plumage ornamentation affects social mate choice and sperm competition in bluethroats (Aves: *Luscinia s. svecica*): a field experiment. Proceedings of Royal Society of London series B 265: 1313-1318.

Jones, I. L., and F. M. Hunter. 1993. Mutual sexual selection in a monogamous seabird. Nature 362: 238-239.

Kavanagh, B. 1988. Discriminating the sex of magpies *Pica pica* from morphological data. Ringing and Migration 9: 83-90.

Kawai, T. 1973. *Clinical aspects of the plasma proteins*. Igaku Shoin, Tokyo.

Keyser, A. J., and G. E. Hill. 1999. Condition-dependent variation in the blue-ultraviolet coloration of a structurally based ornament. Proceedings of Royal Society of London series B 266: 771-777.

Keyser, A. J., and G. E. Hill. 2000. Structurally based plumage coloration is an honest signal of quality in male blue grosbeak. Behavioral Ecology 11: 202-209.

Kinoshita, S. 2008. *Structural colors in the realm of nature*. World Scientific, Singapore.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36: 1-12.

Kirkpatrick, M., T. Price, and S. J. Arnold. 1990. The Darwin-Fisher theory of sexual selection in monogamous birds. Evolution 44: 180-193.

Komers, P. E., and M. S. Dhindsa. 1989. Influence of dominance and age on mate choice in Black-billed Magpies: an experimental study. Animal Behaviour 37: 645-655.

Laczi, M., J. Török, B. Rosivall, and G. Hegyi. 2011. Integration of spectral reflectance across the plumage: Implications for mating patterns. PLoS ONE 6: e23201.

Land, M. F., and D.-E. Nilsson. 2004. *Animal Eyes*. Oxford University Press, Oxford.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34: 292-305.

LeBas, N. R. 2006. Female finery is not for males. Trends in Ecology and Evolution 21: 170-173.

Lee, E., H. Lee, J. Kimura, and S. Sugita. 2010. Feather microstructure of the black-billed magpie (*Pica pica sericea*) and jungle crow (*Corvus macrorhynchos*). The Journal of Veterinary Medical Science 72: 1047-1050.

Lee, E., H. Tanaka, K. Wakamatsu, and S. Sugita. 2009. Melanin-based iridescent feather color in the Jungle Crow. The Journal of Veterinary Medical Science 71: 1261-1263.

Lee, S.-I. 2005. Annual variation on breeding success and offspring sex ratio of the Black-billed Magpie (*Pica pica sericea*), Ph.D. thesis. Seoul National University, Seoul.

Lee, S.-I., H. Jang, S. Eo, and J. C. Choe. 2007. Sexual size dimorphism and morphological sex determination in the Black-billed Magpie in South Korea (*Pica pica sericea*). Journal of Ecology and Field Biology 30: 195-199.

Lee, S.-I., K. Seo, W. Lee, W. Kim, J. C. Choe, and P. Jabłoński. 2011. Non-parental infanticide in a dense population of the Black-billed Magpie (*Pica pica*). Journal of Ethology 29: 401-407.

Lozano, G. A. 1994. Carotenoids, parasites, and sexual selection. Oikos 70: 309-311.

Lozano, G. A. 2001. Carotenoids, immunity, and sexual selection: Comparing apples and oranges? The American Naturalist 258: 200-203.

Machado-Filho, R. A. N., G. M. Balsamão, and M. Â. Marini. 2010. Seasonal differences in immune profiles and body conditions of migratory and permanent resident neotropical flycatchers. The Condor 112: 579-590.

Machmer, M. M., H. Esselink, C. Steeger, and R. C. Ydenberg. 1992. The occurrence of fault bars in the plumage of nestling ospreys. Ardea 80: 261-272.

Maia, R., J. V. O. Caetano, S. N. Báo, and R. H. Macedo. 2009. Iridescent structural colour production in male blue-black grassquit feather barbules: the role of keratin and melanin. Journal of Royal Society Interface 6: S203-S211.

Maia, R., and R. H. Macedo. 2011. Achieving luster: prenuptial molt pattern predicts iridescent structural coloration in Blue-black Grassquits. Journal of Ornithology 152: 243-252.

Maia, R., R. H. F. Macedo, and M. D. Shawkey. 2011. Nanostructural self-assembly of iridescent feather barbules through depletion attraction of melanosomes during keratinization. Journal of Royal Society Interface: doi: 10.1098/rsif.2011.0456

Masello, J. F., and P. Quillfeldt. 2004. Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots *Cyanoliseus patagonus*? Journal of Avian Biology 35: 445-454.

McGraw, K. J., and G. E. Hill. 2004. Mate attentiveness, seasonal timing of breeding and long term pair bonding in the house finch (*Carpodacus mexicanus*). Behaviour 141: 1-13.

McGraw, K. J., and K. Klasing. 2006. Carotenoids, immunity, and integumentary coloration in red junglefowl (*Gallus gallus*). The Auk 123: 1161-1171.

McGraw, K. J., A. M. Stoehr, P. M. Nolan, and G. Hill. 2001. Plumage redness predicts breeding onset and reproductive success in the house finch: a validation of Darwin's theory. Journal of Avian Biology 32: 90-94.

Meadows, M. G., N. I. Morehouse, R. L. Rutowski, J. M. Douglas, and K. J. McGraw. 2011. Quantifying iridescent coloration in animals: a method for improving repeatability. Behavioral Ecology and Sociobiology 65: 1317-1327

Mennill, D. J., S. Doucet, R. Montgomerie, and L. M. Ratcliffe. 2003. Achromatic color variation in black-capped chickadees, *Poecile atricapilla*: black and white signals of sex and rank. Behavioral Ecology and Sociobiology 53: 350-357.

Mitrus, C. 2007. Male aggressive behaviour and the role of delayed plumage maturation in the red-breasted flycatcher *Ficedula parva* (Bechstein, 1792) during the breeding season. Biological Letters 44: 51-59.

Møller, A. P. 1988. Female choice selects for male sexual tail ornaments in the monogamous swallow. Nature 332: 640-642.

Møller, A. P. 1992. Female swallow preference for symmertrical male sexual ornaments. Nature 357: 238-240.

Møller, A. P. 1993. Patterns of fluctuating asymmetry in sexual ornaments predict female choice. Journal of Evolutionary Biology 6: 481-491.

Møller, A. P. 1994. Phenotype-dependent arrival time and its consequences in a migratory bird. Behavioral Ecology and Sociobiology 35: 115-122.

Møller, A. P., J. Erritzøe, and N. Saino. 2003. Seasonal changes in immune response and parasite impact on hosts. The American Naturalist 161: 657-671.

Møller, A. P., and M. Petrie. 2002. Condition dependence, multiple sexual signals, and immunocompetence in peacocks Behavioral Ecology 13: 248-253.

Montgomerie, R. 2006. Analyzing colors, p. 90-147. *In* G. E. Hill and K. McGraw [eds.], *Bird Coloration I: Mechanism and Measurements*. Harvard University Press, Cambridge.

Moreno, H., J. J. Sanz, and E. Arriero. 1999. Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. Proceedings of Royal Society of London series B 266: 1105-1109.

Morimoto, G., N. Yamaguchi, and K. Ueda. 2005. Plumage color as a status signal in male–male interaction in the red-flanked bushrobin, *Tarsiger cyanurus*. Journal of Ethology 24: 261-266.

Murphy, M. E., B. T. Miller, and J. R. King. 1989. A structural comparison of fault bars with feather defects known to be nutritionally induced. Canadian Journal of Zoology 67: 1311-1317.

Murphy, T. G. 2006. Predator-elicited visual signal: why the turquoise-browed motmot wag-displays its racketed tail. Behavioral Ecology 17: 547-553.

Murphy, T. G. 2008. Lack of assortative mating for tail, body size, or condition in the elaborate monomorphic Turquoise-Browed Motmot (*Eumomota Superciliosa*). The Auk 125: 11-19.

Nilsson, J.-Å., and H. G. Smith. 1988. Incubation feeding as a male tactic for early hatching. Animal Behaviour 36: 641-647.

Norris, K. J. 1990. Female choice and the quality of parental care in the great tit *Parus major*. Behavioral Ecology and Sociobiology 27: 275-281.

O'Donald, P. 1972. Sexual selection by variations in fitness at breeding time. Nature 237: 349-351.

O'Donald, P. 1980. Sexual selection by female choice in a monogamous bird: Darwin's theory corroborated. Heredity 45: 201-217.

Olson, V. A., and I. P. F. Owens. 1998. Costly sexual signals: Are carotenoids rare, risky, or required? Trends in Ecology and Evolution 13: 510-514.

Örnborg, J., S. Andersson, S. C. Griffith, and B. C. Sheldon. 2002. Seasonal changes in a ultraviolet structural colour signal in blue tits, *Parus caeruleus*. Biological Journal of the Linnean Society 76: 237-245.

Ots, I., A. Murumägi, and P. Hõrak. 1998. Haematological health state indices of reproducing Great Tits: methodology and sources of natural variation. Functional Ecology 12: 700-707.

Otter, K., S. M. Ramsay, and L. M. Ratcliffe. 1999. Enhanced reproductive success of female black-capped chickadees mated to high-ranking males. The Auk 116: 345-354.

Owen, J. C., and F. R. Moore. 2006. Seasonal differences in immunological condition of three species of thrushes. The Condor 108: 389-398.

Pampus, M., K. H. Schmidt, and W. Wiltschko. 2005. Pair bond and breeding success in Blue Tits *Parus caeruleus* and Great Tits *Parus major*. Ibis 147: 92-108.

Pearn, S. M., A. T. D. Bennett, and I. C. Cuthill. 2001. Ultraviolet vision, fluorescence and mate choice in a parrot, the budgerigar *Melopsittacus undulates*. Proceedings of Royal Society of London series B 268: 2273-2279.

Perrier, C., F. de Lope, A. P. Møller, and P. Ninni. 2002. Structural coloration and sexual selection in the barn swallow *Hirundo rustica*. Behavioral Ecology 13: 728-736.

Petrie, M. 1992. Are all secondary sexual display structures positively allometric and, if so, why? Animal Behaviour 43: 173-175.

Price, T., M. Kirkpatrick, and S. J. Arnold. 1988. Directional selection and the evolution of breeding date in birds. Science 240: 798-799.

Prum, R. O. 2006. Anatomy, physics, and evolution of structural colors, p. 295-353. *In* G. E. Hill and K. J. McGraw [eds.], *Bird Coloration I: Mechanism and Measurements*. Harvard University Press, Cambridge.

Przybylo, R., D. A. Wiggins, and J. Merilä. 2001. Breeding success in Blue Tits: good territories or good parents? Journal of Avian Biology 32: 214-218.

Ramsay, S. M., and L. M. Ratcliffe. 2003. Determinants of social rank in female black-capped chickadees (*Poecile atricapilla*). Canadian Journal of Zoology 81:117-121.

Reed, L. M., X. Wang, and C. H. Trost. 1997. Variation in the wing patches of juvenile Black-billed Magpies *Pica pica hudsonia*. Acta Ornithologica 32: 93-97.

Reese, K. P. 1982. The influence of winter social behavior on the habitat selection and reproductive success of the black-billed magpie, Ph.D. thesis. Utah State University, Logan.

Reese, K. P., and J. A. Kadlec. 1982. Determining the sex of black-billed magpies by external measurements. Journal of Field Ornithology 53: 417-420.

Rising, J. D., and K. M. Somers. 1989. The measurement of overall body size in birds. The Auk 106: 666-674.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution 29: 593-610.

Rohwer, S. 1982. The evolution of reliable and unreliable badges of fighting ability. American Zoology 22: 531-546.

Romero, L. M., J. M. Reed, and J. C. Wingfield. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. General and Comparative Endocrinology 118: 113-122.

Safran, R. J., C. R. Neuman, K. J. McGraw, and I. J. Lovette. 2005. Dynamic Paternity Allocation as a Function of Male Plumage Color in Barn Swallows. Science 309: 2210-2212.

Saino, N., R. Stradi, P. Ninni, E. Pini, and A. P. Møller. 1999. Carotenoid plasma concentration, immune profile, and plumage ornamentation of male barn swallows (*Hirundo rustica*). The American Naturalist 154: 441-448.

Santos, E. S. A., R. Maia, and R. H. Macedo. 2009. Condition-dependent resource value affects male-male competition in the blue-black grassquit. Behavioral Ecology 20: 553-559.

Scharf, C. S. 1987. Sex determination of the black-billed magpie, *Pica pica*. Canadian Field-Naturalist 101: 111-114.

Senar, J. C. 1999. Plumage colouration as a signal of social status, p. 1669-1686. *In* N. J. Adams and R. H. Slotow [eds.], 22nd International Ornithological Congress. Birdlife South Africa, Johannesburg.

Shannon, G. R. 1958. Magpie's rapid replacement of dead mate. British Birds 51: 401-402.

Shawkey, M. D., A. M. Estes, L. M. Siefferman, and G. E. Hill. 2003. Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. Proceedings of Royal Society of London series B 270: 1455-1460.

Shawkey, M. D., and G. E. Hill. 2004. Feathers at a Fine Scale. The Auk 121: 652-655.

Shawkey, M. D., S. R. Pillai, G. E. Hill, L. M. Siefferman, and S. R. Roberts. 2007. Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. The American Naturalist 169: S112-S121.

Shawkey, M. D., S. R. Pillai, and G. E. Hill. 2009. Do feather-degrading bacteria affect sexually selected plumage color? Naturwissenschaften 96: 123-128.

Shawkey, M. D., L. D'Alba, J. Wozny, C. Eliason, J. A. H. Koop, and L. Jia. 2011. Structural color change following hydration and dehydration of iridescent mourning dove (*Zenaida macroura*) feathers. Zoology 114: 59-68.

Siefferman, L., and G. E. Hill. 2003. Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds. Behavioral Ecology 14: 855-861.

Siefferman, L. M., M. D. Shawkey, R. Bowman, and G. E. Woolfenden. 2008. Juvenile coloration of Florida Scrub-Jays (*Aphelocoma coerulescens*) is sexually dichromatic and correlated with condition. Journal of Ornithology 149: 357-363.

Siefferman, L., Y.-J. Wang, Y.-P. Wang, and H.-W. Yuan. 2007. Sexual dichromatism, dimorphism, and condition-dependent coloration in blue-tailed bee-eaters. The Condor 109: 577-584.

Siitari, H., and E. Huhta. 2002. Individual color variation and male quality in pied flycatchers (*Ficedula hypoleuca*): a role of ultraviolet reflectance. Behavioral Ecology 13: 737-741.

Siitari, H., J. Honkavaara, E. Huhta, and J. Viitala. 2002. Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. Animal Behaviour 63: 97-102.

Skórka, P., and J. D. Wójcik. 2008. Habitat utilisation, feeding tactics and age related feeding efficiency in the Caspian Gull *Larus cachinnans*. Journal of Ornithology 149: 31-39.

Slagsvold, T. 1982. Sex, size, and natural selection in the Hooded Crow *Corvus corone cornix*. Ornis Scandinavica 13: 165-175.

Slagsvold, T., and J. T. Lifjeld. 1992. Plumage color is a condition-dependent sexual trait in male Pied Flycatchers. Evolution 46: 825-828.

Soler, J. J., L. de Neve, T. Perez-Contreras, M. Soler, and G. Sorci. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. Proceedings of Royal Society of London series B 270: 241-248.

Sorci, G., J. J. Soler, and A. P. Møller. 1997. Reduced immunocompetence of nestlings in replacement clutches of the European magpie (*Pica pica*). Proceedings of Royal Society of London series B 264: 1593-1598.

Svensson, E. I., and T. P. Gosden. 2007. Contemporary evolution of secondary sexual traits in the wild. Functional Ecology 21: 422-433.

Taylor, A. M., R. H. C. Bonser, and J. W. Farrent. 2004. The influence of hydration on the tensile and compressive properties of avian keratinous tissues. Journal of Materials Science 39: 939-942.

The Ornithological Society of Korea. 2009. *Checklist of the Birds of Korea*. Hanrimwon, Seoul.

Valkiūnas, G. 2005. *Avian malaria parasites and other haemosporidia*. CRC Press, New York.

Veiga, J. P., J. Moreno, P. J. Cordero, and E. Mínguez. 2001. Territory size and polygyny in the spotless starling: resource-holding potential or social inertia? Canadian Journal of Zoology 79: 1951-1956.

Verbeek, N. A. M. 1973. The exploitation system of the yellow-billed magpie. University of California Publications in Zoology 99: 1-58.

Verhulst, S., J. H. van Balen, and J. M. Tinbergen. 1995. Seasonal decline in reproductive success of the Great Tit: variation in time or quality? Ecology 76: 2392-2403.

Vigneron, J. P., J.-F. Colomer, M. Rassart, A. L. Ingram, and V. Lousse. 2006. Structural origin of the colored reflections from the black-billed magpie feathers. Physical Review E 73: 021914_1-7.

Vorobyev, M. 2003. Coloured oil droplets enhance colour discrimination. Proceedings of Royal Society of London series B 270: 1255-1261.

Vorobyev, M., D. Osorio, A. T. D. Bennett, N. J. Marshall, and I. C. Cuthill. 1998. Tetrachromacy, oil droplets and bird plumage colours. Journal of Comparative Physiology A 183: 621-633.

Waldvogel, J. A. 1990. The bird's eye view. American Scientist 78: 342-353.

Walls, G. L. 1942. *The vertebrate eye and its adaptive radiation*. Cranbrook Institute of Science, Michigan.

Warkentin, I. G., P. C. James, and L. W. Oliphant. 1992. Assortative mating in urban-breeding Merlins. The Condor 94: 418-426.

Whiteman, N. K., and P. G. Parker. 2004. Body condition and parasite load predict territory ownership in the Galápagos Hawk. The Condor 106: 915-921.

Wingfield, J. C., and A. S. Kitaysky. 2002. Endocrine responses to unpredictable environmental events: Stress or Anti-Stress Hormones? Integrative and Comparative Biology 42: 600-609.

Winkler, H., and B. Leisler. 1992. On the ecomorphology of migrants. Ibis 134: 21-28.

Wolfenbarger, L. L. 1999. Red coloration of male northern cardinals correlates with mate quality and territory quality Behavioral Ecology 10: 80-90.

Woodcock, E. A., M. K. Rathburn, and L. M. Ratcliffe. 2005. Achromatic plumage reflectance, social dominance and female mate preference in black-capped chickadees (*Poecile atricapillus*). Ethology 111: 891-900.

Wooley Jr., J. B., and R. B. Owen Jr. 1978. Energy costs of activity and daily energy expenditure in the black duck. Journal of Wildlife Management 42: 739-745.

Yorinks, N., and C. T. Atkinson. 2000. Effects of malaria on activity budgets of experimentally infected juvenile apapane (*Himatione sanguinea*). The Auk 117: 731-738.

Zach, R. 1982. Hatching asynchrony, egg size, growth, and fledging in tree swallows. The Auk 99: 695-700.

Zahavi, A. 1975. Mate selection - a selection for a handicap. Journal of Theoretical

Biology 53: 205-214.

Zahavi, A. 1977. The cost of honesty (Further remarks on the handicap principle). Journal of Theoretical Biology 67: 603-605.

Zi, J., X. Yu, Y. Li, X. Hu, C. Xu, X. Wang, X. Liu, and R. Fu. 2003. Coloration strategies in peacock feathers. Proceedings of the National Academy of Sciences 100: 12576-12578.

국문초록

조류의 금속성광택 깃털색은 깃털의 미세구조로부터 빛이 간섭현상을 일으켜 발생되며, 아직 성선택의 관점에서 어떠한 적응적 기능을 지니고 있는지 연구가 충분히 수행되어 있지 않다. 특히 암수의 외형이 인간의 시각으로 뚜렷하게 구분이 되지 않는 분류군에 대해서는 아직 종합적인 연구가 전무하다. 본 연구에서는 암수 모두 두 종류의 금속성광택의 깃털을 갖고 있으며 국내 전역에서 많은 개체가 서식하고 있는 까치(*Pica pica*)를 대상으로 성별, 연령, 영역 보유 유무 등의 사회적 지위에 따른 깃털색의 차이, 깃털색과 개체의 건강상태, 번식지표와의 상관관계, 국내 아홉 개 지역에서 평년과 비교한 기후변동이 깃털색의 변이에 미치는 영향을 조사하였다. 성조, 수컷의 금속성광택 깃털색은 유조, 암컷보다 높은 값의 색을 갖고 있는 것으로 나타났으며, 이는 강한 영역성을 보이는 까치에게서 금속성광택 깃털색은 어린 개체들이 성조와의 직접적인 경쟁을 피하고 수컷이 개체의 건강상태를 알리는 신호로서 작용할 가능성을 의미한다. 또한 금속성광택 깃털색은 수컷에게서만 크기, 건강상태를 나타

내는 지표들과 연관성을 갖고 있었으며, 특히 길고 무겁기 때문에 수컷의 질을 판단할 수 있는 지표라고 알려진 꼬리의 특성과 꼬리의 금속성광택 깃털색이 연관된 것으로 나타났다. 연구 대상 개체 중 다음 해에 번식에 참여한 수컷을 대상으로 까치의 금속성광택 깃털색이 번식 시기와 성공률, 새끼의 질과의 연관성을 조사한 결과, 높은 금속성광택 깃털색을 가진 수컷일수록 번식성공률과 새끼의 질이 높았으며 번식을 일찍 시작하였으나, 번식 종료시기에는 차이가 없었다. 꼬리의 금속성광택 깃털색은 신체의 다른 부위보다 개체간 변이 정도가 컸으며, 기온, 습도, 풍속 등의 기후인자의 변동이 주로 수컷의 깃털색 변이와 연관관계를 갖고 있었다. 본 연구들을 종합해 볼 때, 까치의 금속성광택 깃털색은 수컷에게서 건강상태나 번식성공과 같은 개체의 질(quality)을 평가할 수 있는 신호로서 암컷에게 선호되는 특질일 가능성을 내포하는 것으로 생각된다. 향후에 영양상태, 기상변동과 같은 외부 환경의 변화가 깃털의 구조적 특성에 미치는 영향과 깃털의 구조적 특성과 깃털색 변이의 상관관계에 대한 연구가 필요하다.

주요어: 구조색, 금속성광택, 깃털색, 까치, 성선택

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곁에서 수많은 영감과 위안을 주었던 동물들에게 마음속으로 고마움 전합니다. 복돌이, 까돌이, 상도, 꼬리, 순돌이, 딸기, 쵸코, 튼이, 얌이, 보라보라, 까복이, 매기, 장수, 만수, 새똥이, 보솜이, 하기스, 홍도, 홍이, 홍삼, 생굴이, 생조기, 하늘이, 행운이, 그리고 미처 이름도 만들어주지 못하고 헤어진 많은 동물들아 고마워! 어느 곳에서든 늘 행복하길!

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