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Evolution of plumage coloration in the crow family (Corvidae) with a focus on the color-producing microstructures in the feathers: a comparison of eight species

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ABSTRACT

Plumage coloration has been the subject for a variety of questions that comprise the center of modern evolutionary biology. Unlike carotenoids that the concentration directly influences the intensity of the color, melanin, in addition to produce brown or black colors, is often involved in producing the structural coloration such as glossiness or iridescence. As the melanin granules can be located in the barbs or the barbules, we aim to (i) discern if the colors observed at macro scale comes from the barbs, the barbules or both in a series of related species and (ii) estimate the evolutionary history of the color-producing mechanisms in the family Corvidae that are known to have melanin-based coloration. From a preliminary comparative analysis on eight representative species, we found three coloration schemes in Corvidae; (1) matte colors of brown or black that were produced in barbs and barbules; (2) non-iridescent structural colors such as blue, bluish gray and white, that were produced in the barbs and (3) iridescent structural colors that were produced only in distal barbules. Comparative character analysis of these coloration schemes suggests that the ancestral state among these species were the colors produced in the barbs and that the color produced in the distal barbules is a derived character. The evolution of iridescence seems tightly linked to the evolution of the colors produced in the distal barbules. Data from more species should be incorporated in order to grasp a full picture on the evolutionary history of plumage coloration in this group of birds.

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Introduction

With such a diverse plumage colors, birds have been a model group for the biological studies of animal coloration since Darwin (1871). Specifically, plumage coloration has been investigated in terms of sexual selection (Darwin 1871; Andersson 1994), geographical differentiation and speciation (Mayr 1963), evolution of sexual dimorphism (Dunn et al. 2001) and evolution of polymorphisms (Roulin 2004). Researchers continue using plumage coloration as a model subject for a variety of questions that comprise the center of modern evolutionary biology (Greene et al. 2000; Palleroni et al. 2005; Rubenstein & Lovette 2009; Clarke et al. 2010).

Despite the long interest and intense research there are some areas that are relatively less explored. Bird coloration can be categorized as the relatively well-studied pigment-based coloration and the less studied structural coloration. During the last two decades, a system of pigment-based coloration, the carotenoids that produce yellow, orange and red coloration, has been extensively studied by biologists (McGraw 2006). Since many biologists are interested in the signaling function of the coloration, most studies concerned the variation in the carotenoid coloration among the species, populations and individuals and investigate the function of such variation. One of the characteristics of carotenoids is that the pigments cannot be synthesized but only extracted from the diet. The mechanism of inter-individual variation in the carotenoid-based coloration is simple; the more pigment content, the brighter or more intense the color (Lozano 1994; Olson & Owens 1998; McGraw 2006). In many species, the individuals with brighter carotenoid-based plumage color are known to signal their health status, because the carotenoid pigment can only be extracted from their diet, and individuals who have better access to the food can produce brighter yellow plumage. Since the mechanism is simple and the measurement is easy, such pigment-based coloration has long been studied.

Compared to the long history of research on carotenoids, the melanin-based coloration has not been well investigated. Melanin, producing brown and black

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colors, is a very common component of coloration in animals and is known to serve a variety of functions in birds, including physical protection (Barrowclough & Sibley 1980; Burtt 1986), protection from parasites (Goldstein et al. 2004), sequestering metals (Chatelain et al. 2014), camouflage and signaling (Bokony et al. 2003; Jawor & Breitwisch 2003; Roulin 2004). Unlike carotenoids that originate exogenously, melanin is endogenously synthesized in melanocytes and deposited as granules. One of the unique aspects of melanin is that it is often involved in producing the less-understood structural coloration of bird feathers. This is a coloration produced by the physical and optical interaction of light waves with the structure of an organism (Prum 2006). Structural coloration involves iridescent and non-iridescent colors. Although melanin may sometimes mask the structural colors (when the density of melanin granules is high; Doucet et al. 2006, Shawkey et al. 2006), it is known to contribute to them, especially to the iridescent coloration of feathers (when melanin granules are regularly arranged in the keratin-based feather tissue; Shawkey & Hill 2006, Shawkey et al. 2006, Lee et al. 2009, 2012). Iridescence is known to be produced by coherent scattering from laminar and crystal-like nanostructures and attracted interest of researchers relatively recently (Prum 2006). Melanin granules can be located in barbs or barbules and we were interested if iridescence observed at macro scale comes from barbs, barbules or both. However, the question of which parts of the feather microstructure, barbs or barbules, contribute to the color visible in macro scale have rarely been studied.

Elucidating similarities and dissimilarities in color-production among related species with known phylogenies is necessary to expand and deepen our understanding of the evolutionary history of bird coloration. In this study, we focus on Corvidae. Family Corvidae, which contains jays, magpies and crows, provides an excellent model system for the evolution and diversification of coloration schemes that involve matt and iridescent structural colors. Corvidae includes 120 species, and some of them are highly cosmopolitan and abundant. Although phylogenetic relationship among the species is quite well described and resolved, the evolution of coloration and the color-production in Corvidae have not been studied. The aim is to present the initial step in the study of evolutionary history of plumage coloration in Corvidae, with a special focus on identifying the role of barbs and barbules in the production of colors perceived at a macro scale.

Materials and methods

Black feathers are commonly shared among many Corvid species: a species would possess at least some feathers, if not most of them, that are black (matt or iridescent). Different Corvid species also have plumage parts that contain species-specific colors of blue, gray and iridescence. Using Digital Microscope (Dino-Lite Pro AM413T5, Taiwan), we examined the plumage parts that show the common color (i.e. black) as well as the species-specific colors at 500X magnification. Using a variety of light conditions for each sample we determined whether the color/iridescence is produced in barbs, barbules, or in both structures. We selected eight species that represent several species-specific colors, and their tones, observed in Corvidae (blue, gray, blue-gray, brown, yellow and green). For this study we focused on the wing and tail feathers, but if the color looked similar between the wing and tail feathers we examined the wing feather only. For some specimens we confirmed that in cases of such a visible similarity between and tail and wing feathers the location (barb, barbule, or both) of color-producing tissue was the same between tail and wing. The terminology used in description of feathers and the color-producing microstructures is presented in Figure 1. We ignored contour and head feathers as these have different feather structures that are more difficult to study color-producing mechanisms. When the colors are present on the wing feathers, we examined the colors in the outer vanes only, since the inner vanes of all the species appeared similarly dull dark brownish gray. The Eurasian jay *Garrulus glandarius* has brown and black colors on the body and the wings (Figure 2(a)) but they also contain blue plumage on the wing (Figure 2(b)). Blue and gray coloration scheme can be found among New World jays and we chose the Mexican jay *Aphelocoma ultramarina* (Figure 2(c)) for our study. Similar blue and gray coloration can also be found in the Azure-winged magpie (*Cyanopica cyanus*; Figure 2(d)). Strong iridescence in blue or yellowish green can be found in the Black-billed magpie (*Pica pica;* Figure 2(e) and 2(f)). Weak iridescence in violet or navy can be found in the Spotted Nutcracker (*Nucifraga caryocatactes*; Figure 2(g)) and different species of Crows (*Corvus dauuricus*; Figure 2(h), *C. macrorhynchos*; Figure 2(i), and *C. frugeligus*; Figure 2(j)).

In order to conduct an analysis on the evolutionary history of plumage coloration among these species, we used the phylogenetic trees that were reported by Ericson et al. (2005). Ericson et al. reported two phylogenetic trees with slightly different topologies based on three genetic markers (cytochrome b, myoglobin and β-fibrinogen), and we used both trees to trace the evolutionary history of plumage coloration among the study species. We coded the colors into binary characters based on the location and the types of the colors

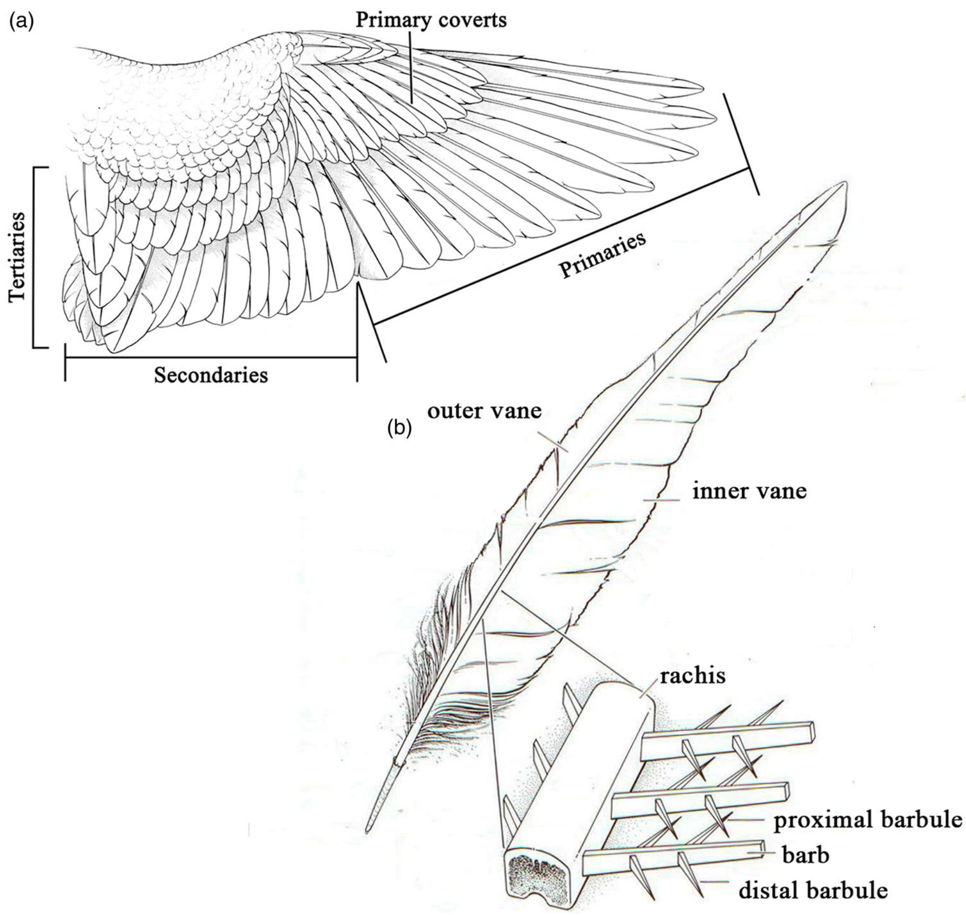

Figure 1. Names of the feathers (a) and feather microstructures (b) used in this study (adapted from Proctor & Lynch 1993).

being produced. Tracing evolutionary history of plumage colors were conducted using maximum parsimony principle with Mesquite ver. 2.75 (Maddison & Maddison 2011).

Results

All the eight species, listed in Table 1, contain black or dark gray colors in the proximal barbules or distal barbules in the inner vane where the colors are not visible. From the visible plumage, we found three types of coloration scheme among the eight representative species of Corvidae. First type exhibits colors that are produced from the barbs, not barbules, of the feathers. The barbules appear dark gray or black, which is the typical color produced by eumelanin. White and blue colors in the coverts of the Eurasian jay (Figure 3(b)), bluish gray colors of remiges and tail feathers in the Mexican jay and the Azure-winged magpie (Figure 3(c) and 3(d)) belong to this type. Whether the plumage appears blue or gray seems not only dependent on the colors of barbs and barbules (as in the Azure-winged magpie tail feather; the difference between white and bluish gray comes from the barbule color), but also dependent on the relative thickness of the barbs and the barbules; if the barbs are thick (as in the coverts of the Eurasian jay) the feather appears sky blue; if the barbs are thin (as in the remiges of the Mexican jay) the feather appears more bluish gray.

Second type shows matt color of brown and black, without any glossiness or iridescence. This can be found in the feathers of the Eurasian jay (Figure 3(a)) and the colors were produced in both the barbs and the barbules.

Third type of coloration scheme involves iridescence. Wing and tail plumage of the Black-billed magpie show very strong iridescence of blue and yellowish green (Figure 3(e) and 3(f)). Secondary feathers of the Spotted nutcracker, the Daurian jackdaw, the Jungle crow and the Rook show weak iridescence (Figure 3 (g)–(j)). Microscopic images show that the iridescence is produced in the distal barbules (Figure 3(g)–(j)).

Based on these microscopic observations, we coded the coloration schemes with binary characters: (i) the presence or absence of colors produced in barbs, (ii) the presence or absence of colors produced in barbules and (iii) the presence or absence of iridescence in distal

Figure 2. Eight representative species of the family Corvidae examined in this study and their feathers producing species-specific colors. (a), (b) Eurasian jay (*Garrulus glandarius*); (c) Mexican jay (*Apehlocoma ultramarina*); (d) Azure-winged magpie (*Cyanopica cyanus*); (e), (f) Black-billed magpie (*Pica pica*); (g) Spotted Nutcracker (*Nucifraga caryocatactes*); (h) Daurian Jackdaw (*Corvus dauuricus*); (i) Jungle crow (*Corvus macrorhynchos*) and (j) Rook (*Corvus frugeligus*). The light conditions varied to properly represent the colors that appear to naked eyes. Scale bars in the photos denote 1 cm.

Table 1. Plumage parts and their colors examined in this study.

Species	Location of the feather	Microstructure producing color	Color
Garrulus glandarius	Tertiary	Barb and barbule	Brown, black
	Primary covert	Barb	Blue
Aphelocoma ultramarina	Tail	Barb	Bluish gray
Cyanopica cyanus	Tail	Barb	Bluish gray, white
Pica pica	Secondary	Distal barbule	Iridescent blue
	Tail	Distal barbule	Iridescent yellowish green
Nucifraga caryocatactes	Secondary	Distal barbule	Weakly iridescent
Corvus dauuricus	Secondary	Distal barbule	Weakly iridescent
Corvus macrorhynchos	Secondary	Distal barbule	Weakly iridescent violet
Corvus frugeligus	Secondary	Distal barbule	Weakly iridescent violet

barbules, and traced the evolutionary changes on the phylogenetic trees.

The results are shown in Figure 4. Tracing the evolutionary history of these characters based on previously reported phylogenetic trees among the eight study species suggests that the ancestral state among these species were the colors produced in the barbs (tick mark (i) in Figure 4), as represented by the Mexican jay and the Azure-winged magpie (marked as gray circles in Figure 4). Crows (genus *Corvus*) are located at the crown of the tree and they all share weak iridescence produced in the distal barbules (marked as black circles in Figure 4). Thus, the analysis suggested that the iridescent colors produced in the distal barbules is a derived character (tick mark (iii) in Figure 4), while colors produced in the barbule in general is an intermediate stage (tick mark (ii) in Figure 4). The transition from the colors produced in the barbs to those produced in the barbules seems to have occurred in or before the Eurasian jay; unlike other species, the Eurasian jay exhibits colors from both the barbs and the barbules as well as the colors produced only in the barbs (marked as half-gray, half-black circle in Figure 4). The evolution of iridescence is tightly linked to the evolution of the colors produced in the distal barbules: except the Eurasian jay, all the species that have the colors produced in the distal barbules show iridescent colors. Interestingly, the species with iridescent colors did not have any distinct colors in the barbs, which means the basal feature of the Corvidae of the blue or white colors in the barbs (at least among our study species) was lost during the transition between ii and iii. This indicates that the processes responsible for the regular arrangements of the melanosomes within keratin matrix to produce iridescence occur only in the distal part of barbules, suggesting perhaps unique properties of these part of the feather that promote the evolution of iridescence.

Discussion

We found three coloration schemes in Corvidae; (1) matte colors of black that were produced in barbs and barbules; (2) non-iridescent structural colors such as blue, bluish gray and white, that were produced in the barbs and (3) iridescent structural colors that were produced only in distal barbules. Non-iridescent structural colors are known to be produced by the light scattering and refraction through the sponge layer that is composed of keratin and air (Prum et al. 1998, 1999, 2003; Shawkey et al. 2003; Doucet et al. 2004). In particular, blue coloration is known to be produced by the presence of spongy layer in the cortex of the barbs (as is shown in Steller's jays; Shawkey & Hill 2006). In the plumage part where non-iridescent structural colors were found, the barbule showed dark gray colors, which appeared to be similar to the typical color produced by eumelanin. Our results suggest that this is more ancestral state for the coloration among corvids, at least based on the eight species that were investigated in this study.

Iridescent colors are known to be produced by the orderly or quasi-orderly arrangements of melanin granules in the barbules (Prum 2006). If the melanin granules do not form densely packed layers, the iridescent color does not appear and the color becomes matte. According to our results, the iridescent colors appeared quite recently in Corvidae, and no evolutionary loss of iridescence had occurred, at least with the eight species that were considered. With regard to the evolution of iridescent colors among Corvidae, two intermediate species seem to be important; the Eurasian jay and the Black-billed magpie.

Eurasian jays exhibited the two coloration schemes. They also seem to contain phaeomelanin in addition to eumelanin, based on the brown colors on the head and on the wings. Recently, the optical mechanism for white-blue-black patterns in the primary covert feather of the Eurasian jay was thoroughly studied (Parnell et al. 2015), and it appears that these multiple structural colors in one feather can be produced by rather simple mechanism of phase separation. This study concerns only one type of feather where the structural colors are produced in the barbs, and it is still currently unclear why only Eurasian jays, among the members of Corvidae, have such diverse coloration mechanisms (i.e. multiple

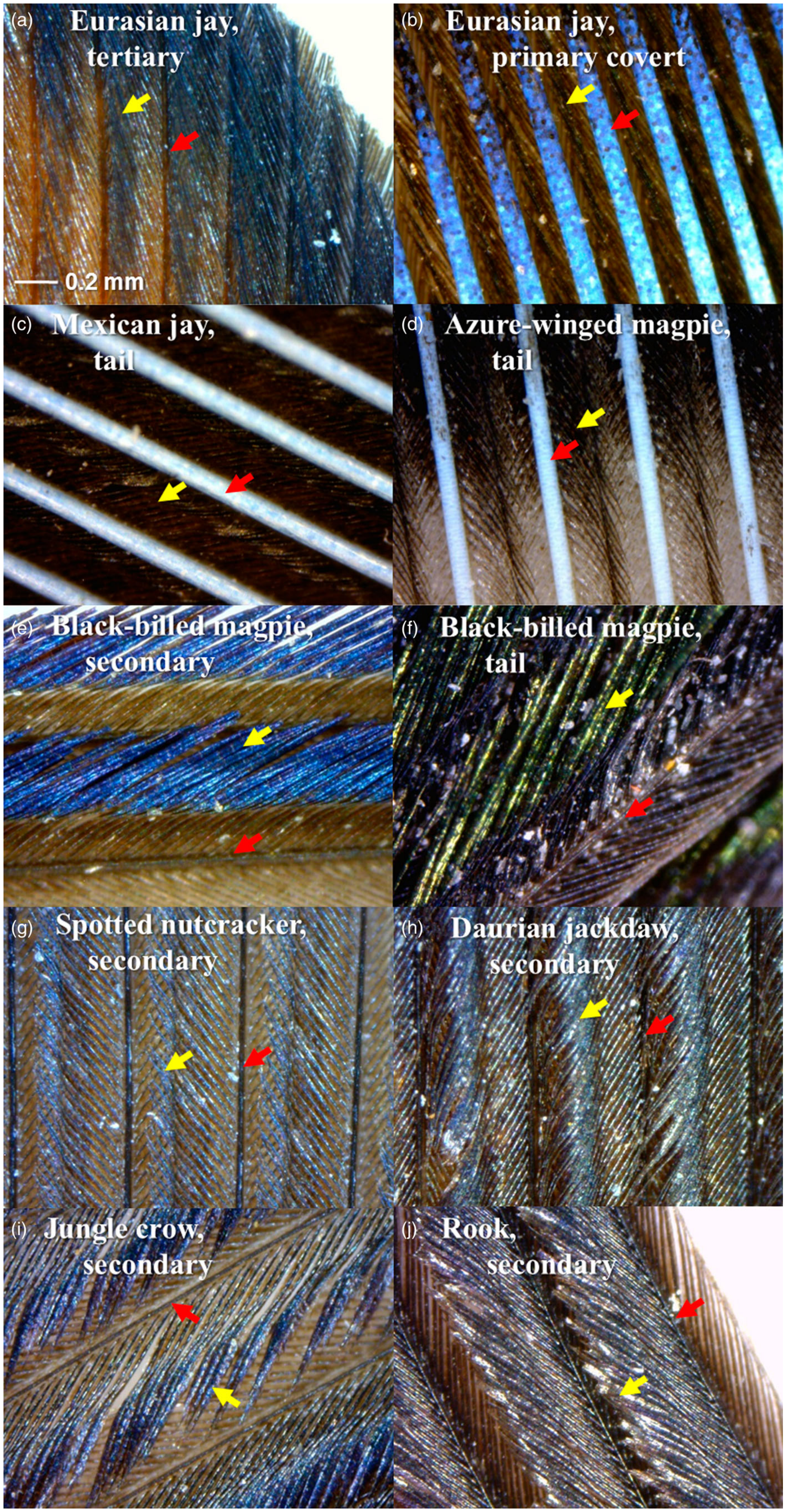

Figure 3. Microscopic photos (500X) of the feathers of the eight species showing the colors of the barbs (marked with red arrows) and the barbules (marked with yellow arrows). A scale bar is given in (a); same scale was used for the rest.

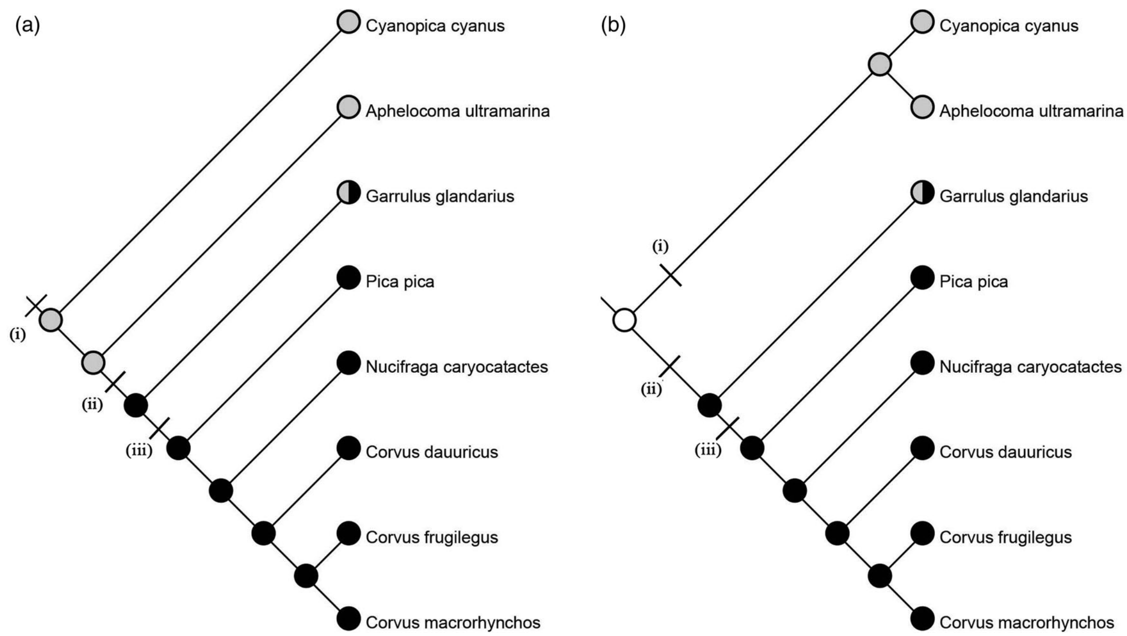

Figure 4. Evolution of plumage coloration in Corvidae inferred based on the known phylogenies of the eight species considered in this study. Phylogenetic trees were adapted from Ericson et al. 2005. Ericson et al. reported phylogenetic trees with two different topologies which are shown in this figure. The only difference between the two trees with regard to our study species is that *Cyanopica* (a) or the clade with *Cyanopica* and *Aphelocoma* (b) were the basal groups (outgroup not shown here). Gray and black circles denote the nodes with colors produced in the barbs and the barbules respectively, and white circle denotes the node with equivocal character states. Tick marks denote for the occurrence of the colors in the barbs only (i), the occurrence of the colors in the barbules (ii) and the occurrence of the iridescence in the distal barbules (iii).

structural colors and matt colors of brown and black, with possible involvement of both eu- and pheomelanin). However, such a versatility in the coloration like the one observed in the Eurasian jay might have provided the ancestral basis for the iridescent color to evolve in the lineage of Corvidae.

It is noticeable that the Black-billed magpie exhibits the strongest iridescent colors among Corvidae. Being located as the sister taxa to the crown *Corvus* group, the magpies have even stronger iridescent colors than the crows. Selective pressures leading to this strong iridescence in magpies have not been studied. They may include sexual or social selection for signaling. Although magpies appear sexually monochromatic, there seems to be a hidden sexual dichromatism (Nam et al., in review). In order to evaluate this possibility, sexual dichromatism in magpie structural iridescent coloration should be compared to those of other Corvid species.

In this preliminary study, we chose eight representative species for the analysis. Considering that Corvidae contains more than 120 species from over 20 genera, data from more species should be incorporated in order to grasp a full picture on the evolutionary history of plumage coloration in this group of birds. Particularly, investigating the plumage coloration in those species that correspond to the root group (e.g. *Cissa, Urocissa,* and *Temnurus*) is important for this purpose. Therefore, the future studies should sample the species from these South-East Asian genera, and any future research on feather developmental processes that create color-producing structures in the feathers should include these genera.

Disclosure statement

No potential conflict of interest was reported by the authors.

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