

Review

The mechanistic, genetic and evolutionary causes of bird eye colour variation

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Birds display a rainbow of eye colours, but this trait has been little studied compared with plumage coloration. Avian eye colour variation occurs at all phylogenetic scales: it can be conserved throughout whole families or vary within one species, yet the evolutionary importance of this eye colour variation is under-studied. Here, we summarize knowledge of the causes of eye colour variation at three primary levels: mechanistic, genetic and evolutionary. Mechanistically, we show that avian iris pigments include melanin and carotenoids, which also play major roles in plumage colour, as well as purines and pteridines, which are often found as pigments in non-avian taxa. Genetically, we survey classical breeding studies and recent genomic work on domestic birds that have identified potential ‘eye colour genes’, including one associated with pteridine pigmentation in pigeons. Finally, from an evolutionary standpoint, we present and discuss several hypotheses explaining the adaptive significance of eye colour variation. Many of these hypotheses suggest that bird eye colour plays an important role in intraspecific signalling, particularly as an indicator of age or mate quality, although the importance of eye colour may differ between species and few evolutionary hypotheses have been directly tested. We suggest that future studies of avian eye colour should consider all three levels, including broad-scale iris pigment analyses across bird species, genome sequencing studies to identify loci associated with eye colour variation, and behavioural experiments and comparative phylogenetic analyses to test adaptive hypotheses. By examining these proximate and ultimate causes of eye colour variation in birds, we hope that our review will encourage future research to understand the ecological and evolutionary significance of this striking avian trait.

Keywords: coloration, iris, pigments, pteridines, signalling.

Birds display a great variety of colours and patterns, which have given them a central role in the study of evolution (Wallace 1870, Darwin 1871, Mayr 1942). The focus of many studies has been the ecological and evolutionary underpinnings of bird feather colours. However, birds also display a myriad of eye colours, and this diversity has often been overlooked by researchers. As a result, the

causes of this diversity, from mechanistic to genetic to evolutionary, remain poorly known.

Here, we review the current state of knowledge on bird eye colour variation, including past advances and future directions. We begin by giving an overview of bird eye colours, describing their relative abundance and explaining how they vary within and between species. Then, we explore three distinct aspects of the question ‘why do birds have different eye colours?’ From a mechanistic perspective, we discuss the wide variety of pigments and structures that give bird irises their

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coloration. From a genetic perspective, we review the emerging field of avian eye colour genomics and offer hypotheses about the largely unknown set of loci that influence those pigments and structures. Finally, from an evolutionary perspective, we summarize ideas about what selective pressures may have shaped patterns of eye colour variation across the bird phylogeny. These causes of eye colour variation, from proximate to ultimate, are fully interlinked: the array of colours is the result of light interacting with pigments or structures in the iris, the development of these pigments and structures is controlled by genomic loci, and these loci evolve over time as the result of mutation, recombination, selection and drift. At all three levels, we summarize existing knowledge, identify unexplored questions, suggest new hypotheses and offer suggestions regarding future directions of research. The primary findings of each section, as well as the most promising avenues for future research, are summarized in Table 1.

METHODS

We conducted a broad survey of the literature using a forward and backward citation-chaining approach. Beginning with a small number of key references (Oliphant 1987a, Hudon & Muir 1996, Craig & Hulley 2004, Hill & McGraw 2006, Davidson *et al.* 2014, Negro *et al.* 2017, Passarotto *et al.* 2018), we identified studies that were cited by or that cited those papers. We also identified additional studies using searches of academic databases (Web of Science, JSTOR, Google Scholar) for keywords and keyword combinations including 'bird eye colo(u)r' and 'bird iris colo(u)ration'. To build appendices that catalogue patterns of bird eye colour variation, we included information from the studies we identified, as well as from the *Handbook of the Birds of the World Illustrated Checklists* (del Hoyo *et al.* 2014, 2016), the Birds of the World database (Billerman *et al.* 2022) and a variety of bird field guides (see Appendices S1 and S2 for citations).

BIRD EYE COLOUR VARIATION

The colours of bird eyes

The variety of iris colours found in birds is dazzling (Fig. 1). Billerman *et al.* (2022) include eye colour descriptions ranging from mundane ('dark

brown') to improbably colourful: including 'vivid emerald green' (Black-and-red Broadbill *Cymbirhynchus macrorhynchus*), 'sapphire-blue' (Wattled Broadbill *Sarcophanops steerii*) and 'pinkish scarlet, ruby, or spectrum red' (breeding Cattle Egret *Bubulcus ibis*) (Telfair 2020, Bruce 2021, Kirwan *et al.* 2021).

Several authors have attempted to catalogue and summarize bird eye colour diversity by dividing their continuous range of hues into discrete categories (see Fig. S1). Most bird species have dark eyes. Worthy (1997) categorized over 5000 species using literature descriptions and placed 52% in the darkest two categories (brown and black); 22% in an intermediate category (red and light brown); and 28% in the lightest two categories, which included yellow, orange, white, blue and light grey (see Appendix S1a for details).

Similar results were reported by Craig and Hulley (2004), who compiled two large datasets of passerine eye colours, one including species from five biogeographical regions ($n = 1654$) and the other species from 10 large passerine families ($n = 1143$). The prevalence of dark-eyed species in these datasets was approximately 82% and 71%, respectively, while the remaining 18–29% of species had light or colourful irises. After subdividing passerine species into those with red eyes and those with 'pale' eyes (e.g. yellow, white and blue), approximately 10% were red-eyed and 19% were pale-eyed. These findings match closely with Worthy (1997) for passerines (64% dark, 19% intermediate, 17% light), especially considering that species in Worthy's intermediate class would have been split between 'red' and 'dark' by Craig and Hulley (2004). Davis and Clarke (2022) used data from illustrated plates to show that 16.7% of non-passerines (671 of 4022 species) have yellow, orange or red eyes, which is broadly consistent with Worthy (1997) and Craig and Hulley (2004), but because Davis and Clarke's classification did not group white-eyed species with red- and yellow-eyed species, their results are not directly comparable.

Although most bird irises are shades of brown, white, yellow, orange or red, rarer colours account for some of the most striking bird eyes. For example, the eyes of Satin Bowerbirds *Ptilonorhynchus violaceus* are purple-blue (Fig. 1aa), those of Philippine Green-Pigeons *Treron axillaris* are a bright turquoise, and other pigeons and doves in the genera *Treron* and *Macropygia* have bicoloured irises

Table 1. Primary results and directions for further research for each main section of the paper.

	Main findings	Future research directions
Variation in bird eye colour	<ul style="list-style-type: none"> • Birds show a huge variety of eye colours (dark brown is most common). • Eye colour is variable in some clades and conserved in others. • Intraspecific differences due to age are common and colour sometimes varies with sex, geography, season and/or mood. 	<ul style="list-style-type: none"> • Develop standardized data collection methods for wild and captive birds. • Create computational methods to extract eye colour data from large photo databases like eBird.
Mechanistic causes of eye colour	<ul style="list-style-type: none"> • Many pigments and structures affect bird eye colours: the same colour can be the result of different mechanisms in different species. • Melanins, pteridines and purines are common iris pigments. • Carotenoids are only known from a few families. • Brightly coloured eyes can result from colourful pigment(s) over a reflective structure. 	<ul style="list-style-type: none"> • Conduct high-performance liquid chromatography analyses on most species. • Improve preservation of eyes and methods for chemical and histological studies.
Genetic causes	<ul style="list-style-type: none"> • A single gene controls the orange versus pearl eye colour in domestic pigeons; the same gene also affects coloration in fishes. • There is indirect evidence for a role of melanin genes in eye colour of pigeons and chickens. • Little is known about genetics of eye colour in wild birds. 	<ul style="list-style-type: none"> • Perform genomic studies of wild populations and across hybrid zones to identify genes affecting eye colour. • Combine genomic tools with captive breeding studies of domestic species.
Evolutionary causes	<ul style="list-style-type: none"> • Many hypotheses related to ecology or life history have been proposed but few have been directly tested. • Eye colour probably acts as a conspicuous signal of age, mate quality and/or species identity. • There is some evidence that eye colour may relate to camouflage, less evidence that it could affect vision. 	<ul style="list-style-type: none"> • Test associations between eye colour and ecological traits with phylogenetic comparative methods. • Conduct behavioural experiments in the field or laboratory to directly test promising hypotheses.

of magenta and cobalt (Fig. 1t) (Goodwin 1977, del Hoyo *et al.* 2020, Baptista *et al.* 2020a, 2020b, Frith & Frith 2020a). At the family level, cormorants (Phalacrocoracidae) are particularly notable for their colourful eyes, including shades of red, green and blue (e.g. Fig. 1c,s,z) (Orta 1992).

Birds can see shorter-wavelength light than humans (Cuthill *et al.* 2000), and many species have a cone cell type with peak sensitivity spanning ultraviolet wavelengths (Ödeen & Håstad 2003, Ödeen *et al.* 2011). Ultraviolet reflectance is common in bird feathers (Eaton &

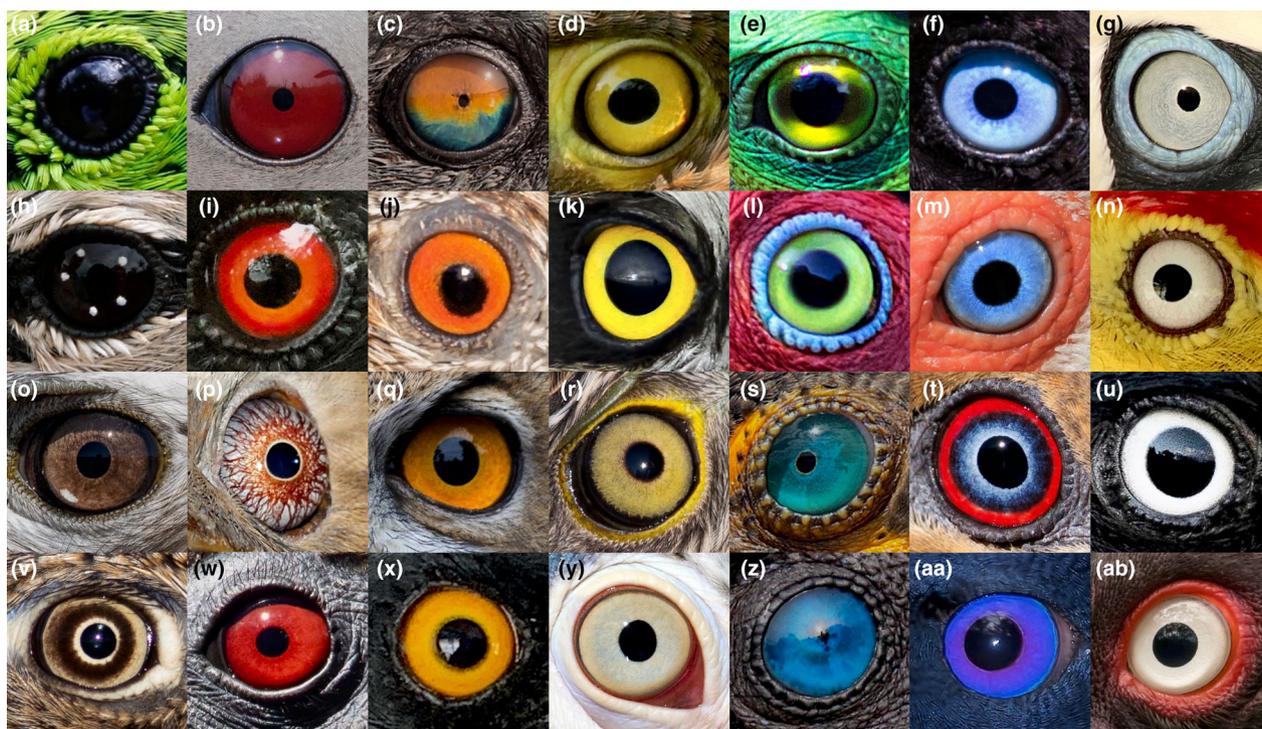


Figure 1. A sampling of the diversity of colours and patterns of bird irises. Species pictured are as follows. (a) Yellow-bellied Tanager *Ixothraupis xanthogastra*, (b) Red-throated Loon *Gavia stellata*, (c) Bank Cormorant *Phalacrocorax neglectus*, (d) Green Heron *Butorides virescens*, (e) Spot-billed Toucanet *Selenidera maculirostris*, (f) Crested Oropendola *Psarocolius decumanus*, (g) Northern Gannet *Morus bassanus*, (h) Three-streaked Tanager *Tchagra jamesi*, (i) Great Antshrike *Taraba major*, (j) Painted Buttonquail *Turnix varius*, (k) Short-eared Owl *Asio flammeus*, (l) Green-billed Toucan *Ramphastos dicolorus*, (m) American White Ibis *Eudocimus albus*, (n) Wire-tailed Manakin *Pipra filicauda*, (o) Red-tailed Hawk *Buteo jamaicensis*, (p) Philippine Frogmouth *Batrachostomus septimus*, (q) Eurasian Eagle-Owl *Bubo bubo*, (r) Sharp-shinned Hawk *Accipiter striatus*, (s) Double-crested Cormorant *Nannopterum auritum*, (t) Brown Cuckoo-Dove *Macropygia phasianella*, (u) Acorn Woodpecker *Melanerpes formicivorus*, (v) Greater Roadrunner *Geococcyx californianus*, (w) Crested Guan *Penelope purpurascens*, (x) Black-capped Donacobius *Donacobius atricapilla*, (y) Dalmatian Pelican *Pelecanus crispus*, (z) Brandt's Cormorant *Urile penicillatus*, (aa) Satin Bowerbird *Ptilonorhynchus violaceus*, (ab) King Vulture *Sarcorampus papa*. Photographs used with permission, by Oscar Johnson (a), Bryan Calk (b), Peter Ryan (c), Gregory Ashmore (d), Caio Brito (e, l), Nathan Rupert (f), Colin Dixon (g), Ngulia Ringing Project (h), Diego Cueva (i), Ramit Singal (j), Mark Schultz (k), Shannon O'Shea (m), Marquette Mutchler (n, u, w, x), Mason Maron (o, r), Rich Lindie (p), westshorewalk22 on Flickr (q), Timothy Krieder (s), David C. Simon (t), Nathan Dubrow (v), Brian Dandridge (y), Sharif Uddin (z), Terence Alexander (aa) and Daniel Parent (ab).

Lanyon 2003, Mullen & Pohland 2008), and some bird irises reflect ultraviolet light (Endler & Mielke 2005), although iris coloration has primarily been considered from the perspective of the human visual system. It is not known whether additional patterns or functions of bird eye colour would become apparent when ultraviolet reflectance is examined.

Multicoloured irises and heterochromia

Multiple colours can be present in a single bird iris. Radially multicoloured eyes (also known as 'central heterochromia') – in which colour differs between the centre of the iris (peripupillary zone)

and the periphery – are not uncommon, but they are often subtle. For example, the eyes of Rock Doves *Columba livia* shift peripherally from yellow to red-orange (Hollander & Owen 1939b, Oehme 1969, Maclary *et al.* 2021), and Boat-tailed Grackles *Quiscalus major major* often have irises that are brown around the pupil and transition to cream-yellow at the outer edge (Pratt 1974). Other species have more dramatically bicoloured eyes, including Greater Roadrunners *Geococcyx californianus*, with a light ring around the pupil set against an otherwise dark iris (Fig. 1v) (Hughes 2020), and Andean Cocks-of-the-Rock *Rupicola peruvianus sanguinolenta*, which

have contrasting yellow-and-red irises (Ridgely & Tudor 1994, Snow 2020). One unusual iris pattern observed in a few species – notably Common Mynas *Acridotheres tristis* and Three-streaked Tchagra *Tchagra jamesi* (Fig. 1h) – consists of a ring of light spots or ‘stars’ against a dark background (Feare *et al.* 2015, Craig 2021). Probably the most striking examples of multicoloured eyes in birds are the blue and yellow sharply radially bicoloured eyes of some birds-of-paradise in the genus *Parotia* (Bond 1919, Schodde & McKean 1973, Schodde & Mason 1974, Frith *et al.* 2020, Frith & Frith 2020b, 2020c).

Multicoloured eyes that are not radially symmetrical (also referred to as ‘sectoral heterochromia’) are uncommon in birds but have been observed in at least 10 families (Ryan 2013, Gutiérrez-Expósito 2019, Cardilini *et al.* 2022). Several species of *Selenidera* toucanets have chartrreuse eyes with darker sections on either side of the pupil (Fig. 1e) (Gutiérrez-Expósito 2019, Short 2020, del Hoyo *et al.* 2022) and *Turnix* buttonquails have a ‘dark crescent’ on the lower edge of the pupil (Fig. 1j) (Gutiérrez-Expósito 2019). Similarly, female *Haematopus* oystercatchers of multiple species have dark ‘eye flecks’ in the iris that make their pupils seem ‘keyhole-shaped’ (Mörrike 1955, Guzzetti *et al.* 2008, Gutiérrez-Expósito 2019). Despite their appearance, the shape of the pupil itself does not change, and the vertical-slit pupils of *Rynchops* skimmers (Zusi & Bridge 1981) and the square pinhole pupils of some penguins (Martin 1999) are the only known non-circular avian pupils (Ryan 2013, Banks *et al.* 2015). The Bank Cormorant *Phalacrocorax neglectus* shows a ‘sunset’ iris pattern with the eye horizontally bisected into an orange upper portion and a teal lower portion (Fig. 1c) (Cooper 1985, Ryan 2013, Orta *et al.* 2020).

Complete heterochromia iridum, a condition in which the left and right irises of a single individual are different colours, has also been reported in several species of birds (Poor 1946, David *et al.* 2013, Ryan 2013, Kinney *et al.* 2017, Ruiz-Esparza *et al.* 2017) but seems to be rare. However, Yoo *et al.* (2017) found that that the left eye of Common Cuckoos *Cuculus canorus* consistently had more extensive dark flecking than the right eye. The mechanism and significance of this phenomenon is unknown, as is whether other species show a similar pattern.

Interspecific eye colour variation

Interspecific variation in bird eye colour occurs at all taxonomic scales: in some cases, eye colour is preserved across large clades, whereas in others it varies between closely related species. Several families are composed entirely of species with dark eyes, but the reverse is not true: the only large family in which almost all species are light-eyed is the Ardeidae (Hancock & Eliot 1978). Worthy (1997) gives average darkness values for each family surveyed: the darkest-eyed families (minimum 15 species surveyed) are the Apodidae, Trochilidae, Scolopacidae, Hirundinidae and Caprimulgidae, whereas the lightest-eyed families are the Ardeidae, Otididae and Accipitridae (Appendix S1a). Overall, dark eyes are more prevalent among passerines, including largely or entirely dark-eyed families such as the Parulidae, Alaudidae and Rhinocryptidae (Worthy 1997, del Hoyo *et al.* 2016). The passerine families with the lightest average eye colours include the Sturnidae, Acanthizidae and Pipridae (Worthy 1997, del Hoyo *et al.* 2016).

Differences in eye colour are consistent with phylogenetic relationships in some groups. For example, the deepest splits within the Alcidae, Turnicidae, Platysteiridae and Hirundinidae largely divide them into light- and dark-eyed clades (Debus 1996, Nettleship 1996, Turner 2004, Sheldon *et al.* 2005, Pereira & Baker 2008). Similarly, the jay genus *Cyanocorax* is polyphyletic and should be split into two genera, one of which is mostly light-eyed and the other dark-eyed (Bonaccorso *et al.* 2010). At shallower phylogenetic levels, eye colour corresponds to genetic structuring in the Tawny-crowned Greenlet *Tunchiornis ochraceiceps* species complex (Buainain *et al.* 2021). It can even aid in the identification of cryptic species: what was once thought to be intraspecific variation in eye colour of Cream-vented Bulbuls *Pycnonotus simplex* was found to indicate the presence of the newly described Cream-eyed Bulbul *Pycnonotus pseudosimplex* (Shakya *et al.* 2019), and previously overlooked eye colour variation in bushshrikes led to the description of Willard’s Sooty Boubou *Laniarius willardi* (Voelker *et al.* 2010).

Conversely, within certain families and genera, eye colours change rapidly over evolutionary time and appear disconnected from phylogenetic relationships. Families that show frequent changes in eye colour, even among closely related species,

include the Anatidae (Carboneras 1992), Icteridae (Jaramillo & Burke 1999, Powell *et al.* 2014), Sturnidae (Sweijd & Craig 1991, Craig & Hulley 2004, Craig & Feare 2010) and Pycnonotidae (Fishpool & Tobias 2005). Within these families, genera with a high eye colour variability include *Aplonis* (Craig & Feare 2010) and *Aythya* (Carboneras 1992), which each contain species with white, yellow, red and brown eyes (see Appendix S1b for additional genera). Studies of such clades are likely to yield insights into factors affecting eye colour variation.

Intraspecific eye colour variation

Intraspecific eye colour variation can be attributed to differences in age, sex, geography, season, mood or individual variation within a population (Appendix S2). Negro *et al.* (2017) compiled a partial database of within-species eye colour variation in birds and found variation due to age to be most common. They noted 58 examples of age-related eye colour change within bird species, and others have identified additional cases (Wood & Wood 1972, Hardy 1973, Trauger 1974, Yunick 1977, Hudon & Muir 1996, Leukering 2000, David *et al.* 2013, Eitniear 2018, Passarotto *et al.* 2020, Polakowski *et al.* 2020). Collectively, these studies suggest that age-related eye colour variation is pervasive. Light-eyed species often have dark eyes as juveniles (Negro *et al.* 2017), whereas in species with dark eyes, the changes include shifts from chestnut to rufous or from grey to brown (Yunick 1977, Leukering 2000, Cueva 2018, Polakowski *et al.* 2020). Exceptions include Ospreys *Pandion haliaetus*, which have orange eyes as juveniles that turn yellow as adults (Bierregaard *et al.* 2020); many *Accipiter* species, which have yellow eyes that become red as they age (Snyder & Snyder 1974, Rosenfield *et al.* 2003); and Common Goldeneyes *Bucephala clangula*, which briefly have blue eyes between their dark juvenile and yellow adult eye colours (Nelson 1983). In some species, females acquire adult eye colour more slowly than males (Snyder & Snyder 1974, Picozzi 1981, Newton & Marquiss 1982, Scholten 1999). The mechanisms of age-related eye colour changes have only been examined in a few species (Bond 1919, Andrews & Naik 1965, Oehme 1969, Sweijd & Craig 1991).

Negro *et al.* (2017) also identified 24 species with sexual dimorphism in eye colour, and many

more have not been formally described or compiled (Appendix S2a). Based on that work, it does not seem that one sex has consistently darker or lighter eyes across bird species. For example, *Ephippiorhynchus* storks have yellow eyes in females and dark eyes in males (Elliott *et al.* 2020, Gula 2021), whereas the reverse is true in Brewer's Blackbirds *Euphagus cyanocephalus* (Hudon & Muir 1996, Jaramillo & Burke 1999).

Several species show brighter eye colours during the breeding season (Appendix S2b). For example, reddening of the iris during peak breeding season has been reported in herons and egrets (Hancock & Eliot 1978, Menkhorst *et al.* 2017). Similarly, Brown Pelican *Pelecanus occidentalis* irises change from brown during the non-breeding season to light blue during the pre-incubation breeding period (Schreiber *et al.* 1989). Seasonal changes in eye colour seem to occur primarily in groups that also show seasonal variation in the colours of other bare parts, such as facial skin or legs (Hancock & Eliot 1978, Elliott 1992, Martinez-Vilalta & Motis 1992). In herons, the increased redness of the iris during the breeding season may be caused mechanistically by changes in blood suffusion, but the mechanism underlying eye colour changes in pelicans is less clear. In chickens, eye colour may change in egg-laying females because the demands of yolk production reduce the carotenoids available for iris coloration (Smyth 1990). With closer study, seasonal variation in eye colour may prove to be more widespread in birds, particularly among species in which the changes are subtle (Kaufmann 1983, Filchagov 1993, Feare *et al.* 2015). Additionally, in some birds the degree of seasonal variation may differ between sexes or age classes, further complicating patterns of intra-specific variation.

Beyond seasonal changes, more rapid eye colour shifts are possible in some taxa. The eye colours of some species result from blood vessels in the iris, and individuals can change the shade of their iris according to mood or behaviour by altering the amount of blood flow to the region, as seen in Inca Doves *Columbina inca* (Chiasson & Ferris 1968, Chiasson *et al.* 1968, Ferris & Bagnara 1972), Black-bellied Starlings *Notopholia corusca* (McCulloch 1963, Britton & Britton 1970, Chittenden & Myburgh 1994, Craig & Feare 2020) and Red-billed Oxpeckers *Buphagus erythrorhynchus* (Raijmakers & Ellmer 2009). Other species can change the apparent colour of their iris by expanding or

contracting it to accentuate differently coloured regions (McIlhenny 1934, 1937, Craig 2021). This behaviour is most apparent in some *Parotia* species, which change the appearance of their radially bicoloured irises from entirely blue to yellowish at a specific moment during their mating display – a remarkable phenomenon that warrants greater study (Scholes 2008, Cordey 2019).

Geographical variation between populations of a single species (Appendix S2c) demonstrates that eye colours can also change on short evolutionary timescales. Plumage variation between subspecies has received substantial study in birds, but the same is not always true of eye colour differences, which may not be reflected in subspecies taxonomy. On the other hand, some closely related populations that differ in eye colour have recently been elevated to species rank (Appendix S1c).

Less common among birds are eye colour polymorphisms within populations. Negro *et al.* (2017) argued that these types of polymorphisms are essentially absent in wild birds, but several potential examples of population-level iris colour variation exist (Appendix S2d), including where populations hybridize (Williamson & Peyton 1963, Hoffman *et al.* 1978, Delpont *et al.* 2004, Gay *et al.* 2007, Canton 2014, Anderson 2015). However, it is often difficult to verify that eye colour variation does not result from differences in age, seasonality or other extrinsic factors, particularly given that some age-related variation in eye colour may be complex and can occur among adults of varying ages (Trauger 1974, Scholten 1999, Rosenfield *et al.* 2003). For example, age rather than population-level polymorphism has been used to explain the high degree of variation in eye colour among adult *Macronectes* giant petrels (Warham 1962, Carboneras *et al.* 2020). Likewise, it can be difficult to distinguish between discrete morphs and continuous variation when sample sizes are small, so further research on this subject is greatly needed.

Future research: Characterizing bird eye colour variation

The ease of preserving bird feathers and their colours by preparing study skins has been a tremendous boon to the study of avian coloration. Unfortunately, that advantage does not exist for studying eye colour, because irises generally lose their colour post-mortem and bird eyes are not typically preserved during the preparation of

traditional study skins (Erichsen 1985). Ornithologists interested in studying eye colour are in a situation more like that of museum ichthyologists and herpetologists, whose objects of study lose their colour on museum shelves. Fortunately, iris colour and the colours of other bare parts are now commonly noted on the labels of modern museum specimens – although these descriptions are difficult to compare because there is no standardized system used for describing eye colour (Joseph *et al.* 2023). For example, we examined the tags of 34 specimens of White-eyed Stipplethroats *Epinecrophylla leucophthalma dissita* in the collection of the Louisiana State University Museum of Natural Science and found 23 different eye colour descriptions, which would complicate any attempt to examine variation associated with age, sex or geography. Standardizing descriptions of these colours (Wood & Wood 1972) or collecting standardized photographs of irises and other bare parts and associating these with specimen records (Webster 2017) would make these observations more useful (Joseph *et al.* 2023).

The wealth of photographs available through eBird, iNaturalist, Wikiaves and other databases also holds great promise for helping to understand variation in eye colour among and within species. Even photos taken in highly heterogeneous light environments can provide usable data to understand geographical variation in eye colour, as demonstrated by a recent study on White-browed Scrubwrens *Sericornis frontalis* (Cake 2019). However, subtle intraspecific variation in eye colour is best studied using standardized photographs of birds in the hand (Cardilini *et al.* 2022), which can be taken during bird-ringing projects or using captive individuals. Comprehensive and standardized eye colour data across species will also facilitate the study of broad patterns of variation, such as whether eye colour varies with latitude or ecogeographical gradients.

There probably remains significant intraspecific variation in eye colour that has not yet been characterized. Careful investigations have revealed new patterns, such as consistent heterochromia and seasonal variation, even in common and well-studied species (Feare *et al.* 2015, Yoo *et al.* 2017). As demonstrated by the case of the Cream-eyed Bulbul (Shakya *et al.* 2019), in which an eye colour difference was a clue to the existence of a cryptic species, this underappreciated variation can be indicative of larger patterns. The question ‘what colour eyes does

this species have?’ may seem simplistic, but there is value in having a clear answer for all bird species and populations. Simply paying greater attention to this trait is likely to reveal new insights.

THE MECHANISTIC BASIS OF EYE COLOUR VARIATION

The proximate cause of eye colour variation in birds is the presence of different pigments and structures in the iris that reflect and absorb light. The mechanisms of eye coloration are perhaps better understood than more ultimate causes (the genetic and evolutionary drivers of variation), but even these mechanisms are complex and poorly known. The mechanisms that affect iris coloration in birds also differ markedly from those that affect feather coloration, which have been studied much more extensively. In addition to melanins and carotenoids, which can also affect plumage colour, purine and pteridine pigments are major components of bird iris coloration, and in some species blood vessels, colourless oil droplets, collagen fibres and other structural features also play a role (Oehme 1969, Oliphant 1987a). Several of these factors, particularly the role of purines and pteridines, were reviewed by Hill and McGraw (2006).

Beyond the distinct chemicals involved, iris coloration differs from feather coloration because the pigments are located within living cells, known as chromatophores, rather than deposited into non-living keratinous feathers (Oliphant *et al.* 1992). Diverse pigment cell types are found in the integument of fish, reptiles and amphibians, but in birds pigment cells are limited to the iris and the melanocytes of the dermis (Oliphant *et al.* 1992). The presence of non-melanocyte chromatophores makes the iris a unique and distinctive element of bird coloration. Some of the chromatophores found in bird irises resemble those in other vertebrate groups and may have been retained from birds’ distant evolutionary ancestors, whereas other novel chromatophore morphologies may have first evolved in bird irises (Oliphant *et al.* 1992). Chromatophores are often broadly categorized, based on their coloration, as xanthophores (yellow), erythrophores (red), leucophores (white), and iridophores or guanophores (reflective) (Bagnara 1966, Fujii 2000, Kimura *et al.* 2014). However, these categories do not distinguish between the pigment types present within each cell (Bagnara 1966, Oliphant & Hudon 1993), and additional terms exist to describe pigment cell

types that do not fit these traditional categories (e.g. ‘reflecting xanthophore’) (Tillotson & Oliphant 1990). Here, we will simply refer to bird irises and their pigment cells by the pigments or structures that they contain and the colours that these pigments and structures produce.

Histology of the iris

Bird eye colours are generated in the iris, the ring of muscle that controls the expansion and contraction of the pupil (Waldvogel 1990). Unlike humans, birds do not have a visible white sclera around the iris, so the visible portion of the eye consists of only the pupil and iris (Lord 1956, Ryan 2013, Polakowski *et al.* 2020). Bird irises consist of three layers (Fig. 2): the posterior (inner) layer of the iris is the highly melanated pigment epithelium; the stroma is a thick middle layer containing muscle, connective tissue, nerves and often pigment cells; and finally the anterior border layer includes pigment cells or blood vessels in some species (Rochon-Duvigneaud 1943, Oehme 1969, Ferris & Bagnara 1972, Oliphant 1981, 1987b, 1988, Sweijd & Craig 1991, Hudon & Oliphant 1995).

History of bird iris pigmentation research

During the early 20th century, researchers began to examine bird irises to identify the pigments and

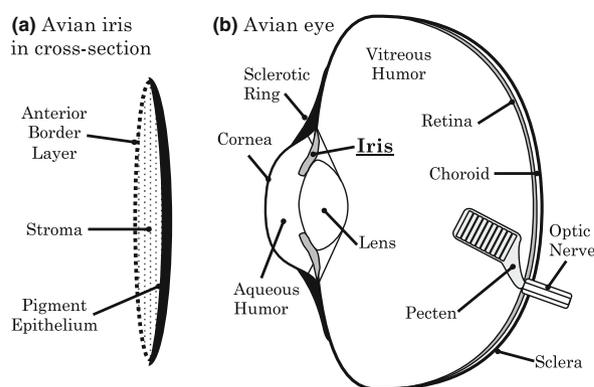


Figure 2. Simplified anatomy of the avian iris and eye. (a) Drawn using information from Oehme (1969), Oliphant (1987b, 1988), Sweijd and Craig (1991), Hudon and Oliphant (1995) and Van den Broeck (2022). (b) Drawn using information from Lord (1956), Meyer (1977), Waldvogel (1990) and Evans (1996), as well as images by Poultry Hub and jimbleak/Wikipedia.

structures generating coloration (e.g. Bond 1919). Hollander and Owen (1939a, 1939b) determined that the yellow coloration of chicken irises is caused by carotenoids, whereas the orange irises of domestic pigeons result from a different type of non-carotenoid pigment. In the 1960s, Oehme (1969) examined iris structures and pigments from approximately 150 bird species; identified melanins, carotenoids, blood vessels and multiple structural elements; and established that another class of pigments, pteridines, were common in light irises.

From the 1970s to the 1990s, bird eye colour research focused on these light-coloured pigments, beginning with the work by Ferris and Bagnara (1972) on doves and including many papers by Oliphant and collaborators in a variety of species (Oliphant 1981, 1987a, 1987b, 1988, Oliphant & Diocee 1982, Tillotson & Oliphant 1990, Oliphant *et al.* 1992, Hudon & Oliphant 1995). This work demonstrated that two related classes of pigments, pteridines and purines, are found together in many light-eyed bird species. The use of novel high-performance liquid chromatography (HPLC) techniques allowed Hudon and Muir (1996) to identify and quantify the concentrations of four specific purine and pteridine pigments in the irises of New World blackbirds (Icteridae), demonstrating that variation in the concentrations of these chemicals – of which guanine, a purine, was the most abundant – is associated with differences in eye colour. This remains the only HPLC study, and knowledge of the pigment composition of bird irises has remained static since the mid-1990s.

Types of bird iris pigments and structures

When studying bird coloration, it is risky to assume the pigments involved based on the colours observed (McGraw *et al.* 2004, Toral *et al.* 2008). This is especially true for iris coloration because similar colours are commonly produced by different mechanisms. For example, the irises of Canvasbacks *Aythya valisineria*, Red-eyed Vireos *Vireo olivaceus* and Bronzed Cowbirds *Molothrus aeneus* are each vivid red, but the colour is produced by carotenoids in Canvasbacks, pteridines in Red-eyed Vireos and the haemoglobin of blood cells in enlarged vessels on the surface of the iris in Bronzed Cowbirds (Oliphant 1987a,

Hudon & Muir 1996). Different mechanisms may also be combined, as in the iris of the Great Crested Grebe *Podiceps cristatus*, which appears red but which Oehme (1969) considered to be radially partitioned into four distinctly coloured zones, a subtle pattern produced by a complex combination of melanin, carotenoids, pteridines and enlarged blood vessels. Figure 3 gives examples of how different pigments and structures can produce the same eye colours – white, yellow and red – across species.

An additional key principle of bird iris coloration is that it is common for light-coloured irises to result from the interaction of colourful pigments with colourless light-reflecting substances, crystals or structures (Oehme 1969). These reflectors are not technically considered pigments, although they may be informally referred to as such (Oehme 1969, Oliphant 1988, Prum 2006). In these pigment–reflector systems, coloured pigments (often non-crystalline pteridines, carotenoids or haemoglobin) tint the light bouncing off a reflector (such as a crystalline pteridine or purine, a colourless oil droplet or a collagen bundle) to create a bright colour (Oehme 1969, Oliphant 1987a). In species where only pigments are present, the colour is often duller, as in Northern Flickers *Colaptes auratus* and Cactus Wrens *Campylorhynchus brunneicapillus* (Oliphant 1987a, 1988), and in species having only reflecting structures, the iris is white, as in Acorn Woodpeckers *Melanerpes formicivorus* (Fig. 1u; Oliphant 1987a, Oliphant & Hudon 1993).

We have summarized the known mechanisms of iris coloration for as many bird species as we could identify in Appendix S3, drawing in large part on Oehme (1969) and Oliphant (1987a). This table is intended as an accessible compendium of the available information for the species listed. In Figure 4, we explore some characteristics of these species, and emphasize how much work remains to attain a comprehensive view of avian iris coloration mechanisms. Below, we briefly discuss the main groups of pigments or structures involved in bird eye colour.

Melanins

Melanin pigments are found in the irises of all bird species that have been examined and often contribute to eye colour (Oehme 1969). Generally, melanin pigments (eumelanin and pheomelanin, though the former appears more common)

deposited in the stroma and anterior border layer are responsible for dark iris coloration. Melanocytes can vary from rounded to lumpy to coarsely or finely branched in shape, usually with anterior cells more rounded, posterior cells more ramified, and highly branched melanocytes covering blood vessels (Oehme 1969). Oehme also observed cells containing both eumelanin and colourless pteridines (melanopterocytes): when melanins and pteridines are present in the same iris, more melanin-rich cells tend to predominate near the pupil.

The posterior layer of the iris, the pigment epithelium, is always rich in melanin and very dark, but it is generally not involved in visible iris coloration: it is usually masked by pigments present in the anterior two layers (Oehme 1969, Hudon & Oliphant 1995). However, there are exceptions. Bond (1919) described the relatively unpigmented irises of some domestic pigeon breeds as being a 'simplex' eye, commonly referred to as a 'bull' iris by pigeon breeders (Bond 1919, Maclary *et al.* 2021). In 'bull-eyed' pigeons, pigment is not present in the stroma or anterior border layer, and the black eye colour observed in these breeds is caused by the melanin in the pigment epithelium. It is unclear whether this type of iris coloration occurs in adults of any wild species. Oehme (1969) specifically noted that he did not identify any species with this type of iris pigmentation, although he found that the irises of Eurasian Woodcocks *Scotopax rusticola* show a similar arrangement: they have relatively little melanin in the stroma and anterior border layer, and the remaining darkness of the iris can be attributed to melanin in the pigment epithelium. The dark eyes of juvenile Jungle Babblers *Turdoides striata* are also the result of the pigment epithelium viewed through the two unpigmented anterior layers. However, this condition is temporary, and adults are yellow-eyed (Andrews & Naik 1965).

Carotenoids

The carotenoids xanthophyll and carotene were among the first pigments to be identified in bird eyes (Hollander & Owen 1939a) and they give chicken irises their yellow colour. However, carotenoids are not responsible for all red and yellow irises in birds (Oehme 1969, Oliphant & Hudon 1993). Rather, carotenoids are responsible for bright eye colours in some bird taxa, but may be more phylogenetically restricted than other iris

pigments. Carotenoids are currently known from eight families: Phasianidae, Anatidae, Ardeidae, Strigidae, and a single species each in Podicipedidae, Phoenicopteridae, Jacanidae and Accipitridae (see Appendix S3) (Oehme 1969, Oliphant 1987a, 1988). Carotenoids have not yet been identified from the irises of any passerines. Within clades having carotenoid iris coloration, carotenoids are localized within lipid droplets of pigment cells with conserved structure: owls have spherical cells containing carotenoids and colourless pteridines or purines, whereas herons have branched cells containing carotenoids and yellow pteridines, overlain by blood vessels (Oehme 1969). Carotenoids are also notable – and distinct from other avian pigments – because they are derived from diet rather than synthesized endogenously (McGraw 2006).

Purines and pteridines

Purines and pteridines (also more specifically referred to as 'pterins') are usually responsible for light eye colours in birds (Oehme 1969, Oliphant 1987a, 1988). Here, we use the term 'pigment' broadly, because pteridines can absorb specific wavelengths of light and function as a 'true pigment' (Andrade & Carneiro 2021), but purines and pteridines can also exist in a crystalline form that reflects light: in effect, a form of structural white coloration (Oliphant & Hudon 1993, Prum 1999).

Cells containing pteridines and purines can range from rounded to branched and contain pigment granules of varied morphologies, among them 'reflecting crystals' and 'fibrous pterinosomes' (Oehme 1969, Ferris & Bagnara 1972, Oliphant 1981, 1987a, 1987b, 1988, Oliphant & Diocee 1982, Tillotson & Oliphant 1990, Sweijd & Craig 1991, Oliphant *et al.* 1992, Hudon & Oliphant 1995, Hudon & Muir 1996). Purines and pteridines often occur in conjunction, with a coloured non-crystalline pteridine and a colourless crystalline purine combining to form a 'coloured reflecting platelet' (Oliphant 1987a, 1987b, 1988). These platelets are a good example of a pigment-reflector combination, as discussed above, and reflecting purine (or, more rarely, pteridine) crystals can also serve as a backdrop that brightens the coloration of true pigments such as carotenoids or haemoglobin (Oliphant 1987a, 1988).

Oliphant (1987a) identified purines – always including guanine – in the irises of 20 species, all

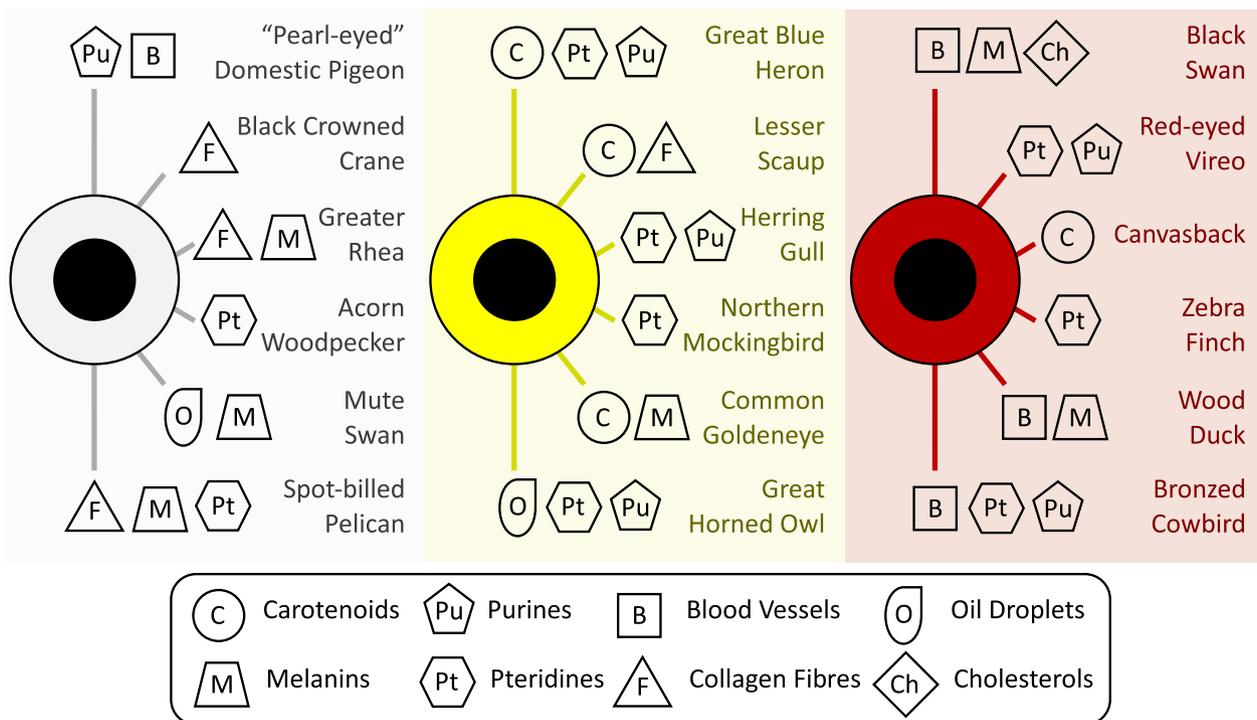


Figure 3. The same eye colours (white/grey, yellow and red) can be produced by a range of pigments, structures and their combinations. For each eye colour, examples of species are listed next to the iris coloration element(s) that give their eyes that colour. For sources, details and additional examples, see Appendix S3.

of which also contained pteridines. Conversely, there are cases in which pteridines are present and purines are absent (Oliphant 1987a, Oliphant & Hudon 1993). Due to the close association between pteridines and purines, Oehme (1969) did not recognize that two different classes of pigments were involved and referred only to pteridines in his work. However, pteridines and purines have subsequently been found together in the irises of several species he studied (Ferris & Bagnara 1972, Oliphant 1987a, 1987b, Hudon & Oliphant 1995).

Because they were examined using HPLC techniques, the purines and pteridines of some Icteridae are probably the best-characterized iris pigments of any birds. Hudon and Muir (1996) discovered that the pteridines leucopterin and xanthopterin and the purines hypoxanthine and guanine were present in light-eyed icterids at concentrations that were orders of magnitude higher than in a dark-eyed control species. Guanine was particularly abundant in icterid irises, and it appeared to be the primary reflecting substance responsible for light eye colour (Oliphant 1987a,

Hudon & Muir 1996). Hudon and Muir (1996) also examined one non-icterid species (Red-eyed Vireo) and found evidence for a red pteridine pigment, which may have been pterorhodin. Additionally, the pteridine xanthopterin has been identified in the irises of Great Horned Owls *Bubo virginianus* (Oliphant 1981). Beyond these few examples, the types of pteridine and purine pigments found in bird irises are unknown, even for those species in which the general presence of pteridines and purines has been confirmed.

Blood vessels

Blood haemoglobin has been co-opted as a mechanism of iris coloration in some birds (Oehme 1969), and the size and arrangement of blood vessels in the iris vary widely between species (Mann 1931). Red colour can be produced by enlarged blood vessels on the anterior surface of the iris, as in male Wood Ducks *Aix sponsa* (Oehme 1969), and these colours can be brightened by a backdrop of purine and pteridine reflectors, as in Bronzed Cowbirds (Hudon & Muir 1996). In some species, haemoglobin present

in blood vessels adds a reddish tint to other pigments or structures in the iris, producing a range of oranges and pinks, as in domestic pigeons and Inca Doves (Oehme 1969, Ferris & Bagnara 1972). Seasonal or mood-related changes in the degree of redness often appear to be controlled by the amount of blood flow in the iris (Ferris & Bagnara 1972, Hancock & Eliot 1978), and the ability to control the amount of blood in vascularized tissues has similarly been implicated in the rapid 'flushing' of bare facial skin in some bird species (Negro *et al.* 2006). Immediate post-mortem colour change in birds with reddish irises could be one clue that blood vessels play a role in their eye coloration (Gheselin 1975, Oliphant 1988, David *et al.* 2013).

Structural blues and greens

The mechanisms of blue and green coloration in bird irises have not been studied in detail, although they are likely to be structural, because structural elements produce blue and green colours in bird feathers and bare parts (Prum & Torres 2003, Prum 2006) as well as human eyes (Sturm & Larsson 2009). Oehme (1969) attributed the blue and purple iris colour of a few species to 'Tyndall scattering' – the differential scattering of short wavelengths (Prum & Torres 2003, Prum 2006) – but did not elaborate on the specific structures responsible. Bond (1919) noted the presence of 'very finely fibrillated spindle cells of connective tissue type' that generated blue coloration in the iris of a Lawes's Parotia *Parotia lawesii*. Oliphant (1988) examined the velvety turquoise-green iris of a Double-crested Cormorant *Nannopterum auritum* (Fig. 1s) and found that the colour was produced by the presence of spindle-shaped cells containing membrane-bound granules with ordered arrays of filaments and rods. These studies lend evidence to the idea of a structural component to blue and green bird eye colours, but many of the specifics remain unknown and may differ between species.

Miscellaneous coloration elements

There are several uncommon elements that can also affect eye colour in a few bird species. For example, Oehme (1969) identified interwoven bundles of collagen fibres that generate grey or whitish structural coloration in a handful of species, including Greater Rheas *Rhea americana*, Black Crowned Cranes *Balearica pavonina*,

Western Jackdaws *Coloeus monedula* and Brown Pelicans. These fibres also serve as reflectors in conjunction with colourful pteridine or carotenoid pigments in Great White Pelicans *Pelecanus onocrotalus* and Lesser Scaup *Aythya affinis* (Oehme 1969, Oliphant 1988).

Oehme (1969) also noted a small number of species – Mute Swans *Cygnus olor*, Ferruginous Ducks *Aythya nyroca* and Velvet Scoters *Melanitta fusca* – whose irises contain oil droplets without carotenoids, which may produce a form of structural white coloration. Similarly, colourless lipid droplets can serve as light-reflecting structures to brighten coloration, as in the yellow irises of Great Horned Owls, which also contain purines and yellow pteridines (Oliphant 1981). This arrangement is the opposite of that observed in most owl species, in which the yellow iris colour is produced by carotenoids in lipid droplets and the purines and pteridines serve as colourless reflectors (Oehme 1969, Oliphant 1988). These colourless lipid droplets have only been identified from Anatidae and Strigidae, families in which carotenoid-bearing lipid droplets are also frequently present, so it seems possible that these structures are the result of a secondary loss of carotenoids.

Finally, in two species of waterfowl, Black Swans *Cygnus atratus* and Coscoroba Swans *Coscoroba coscoroba*, Oehme (1969) identified cells containing a reflecting crystalline structure composed of cholesterols. In both species, these crystals interacted with the red colour of blood vessels to produce pink irises, performing a reflecting function similar to that of pteridine and purine crystals in other species. These structures have not been examined by other authors or identified in other species.

Ontogeny of coloration mechanisms

The age-related development of pigments in bird irises is not well understood, except in a few species. Juvenile birds tend to have darker irises than adults, and changes in eye colour as birds age can result from simultaneous increases in light pigments and decreases in dark pigments. For example, in African Pied Starlings *Lamprolornis bicolor* dark brown juvenile irises become white in adults because of two distinct changes in pigmentation: an anterior layer of dark pigment (presumably melanin) is progressively lost, while the stroma fills with pigment cells containing reflecting platelets

(presumably purines or pteridines) (Sweijd & Craig 1991). Bond (1919) suggested similar age-based replacement of dark anterior pigment cells with light-coloured or reflective pigment cells in some breeds of domestic chickens and in Herring Gulls *Larus argentatus*, and Oehme (1969) observed a similar process in male Eurasian Marsh Harriers *Circus aeruginosus*. On the other hand, Jungle Babblers show a simpler mechanism to change from dark juvenile to yellowish-white adult eyes: light pigment and an increased density of muscle fibres in the iris stroma cover up the melanated pigment epithelium (Andrews & Naik 1965). In Northern Goshawks *Accipiter gentilis*, Oehme (1969) suggested that more and brighter pteridines are responsible for the age-related yellow-to-orange eye colour transition, and in Snowy Owls *Bubo scandiacus* he cited a greater concentration of carotenoids to account for the deeper yellow eyes of adults. In the single study that quantified age-related changes in pigmentation, Hudon and Muir (1996) found that a yellow-eyed adult male Brewer's Blackbird had 60 times more iris guanine than a juvenile male with a 'pale translucent' iris, and even a brown-eyed adult female had four times as much guanine as the juvenile. This suggests that the opaque yellow irises of adult males are a result of their high guanine content, but that the effects of moderately increased guanine in adult females might be counteracted by deposition of anterior melanin. As seen from these examples, the specific mechanisms of age-related variation in iris coloration vary between species, and colour transitions are often the result of several co-occurring changes in pigmentation.

The physiological factors that cause these pigmentary changes have yet to be directly studied. Hormone levels have been proposed to play a role (Trauger 1974, Scholten 1999, Passarotto *et al.* 2020), with androgens cited as a potential driver of ontogenetic eye colour changes (Trauger 1974).

Future research: Mechanistic basis of eye colour variation

Although the general structure and pigments involved in iris coloration have been characterized for approximately 188 bird species (Appendix S3), that total represents less than 2% of recognized bird species (Clements *et al.* 2021) (see Fig. 4) and there are no data on the mechanistic basis of

iris colour for 80% of bird families and 44% of bird orders. Beyond obscuring the mechanisms that cause different bird eye colours, this lack of data also masks potentially important phylogenetic patterns, and many questions remain, such as whether any passerines have carotenoids in their irises or what causes the bright red eyes of many antbirds (Thamnophilidae; e.g. Fig. 1i).

Basic research on the pigments and colour-producing structures of bird irises is therefore desperately needed. HPLC was used by Hudon and Muir (1996) to quantify specific iris pigments in a few songbirds. However, during the intervening 25 years, few published studies have performed similar analyses. Conducting these types of pigment analyses – as well as histological studies – on a diversity of bird irises would greatly expand our knowledge of bird iris coloration, more than 50 years after Oehme (1969). To facilitate this, collectors and collections should save eyes or irises when preparing new study specimens, and standard protocols should be developed to help preserve these samples so that they will be most useful.

GENETIC BASIS OF BIRD EYE COLOUR VARIATION

Bond (1919) wrote 'I am unable however to find that much work has been done on eye colour in birds from the genetic standpoint', and for the following century that statement held true, with our knowledge of the genetics of bird eye colour originating from breeding studies of domestic pigeons and chickens (Bond 1919, Hollander & Owen 1939a, 1939b). However, three recent papers have significantly advanced the field by identifying a specific gene (*SLC2A11B*) responsible for iris colour variation caused by pteridine pigmentation in domestic pigeons (Andrade *et al.* 2021, Maclary *et al.* 2021, Si *et al.* 2021).

Eye colour genetics of domestic birds

Domestic pigeons have a variety of eye colours, including the wild-type 'orange' and variants 'pearl' (a whitish colour) and 'bull' (black) (Staples-Browne 1908, Bond 1919, Hollander & Owen 1939b). It has long been recognized that the pearl eye colour of pigeons is a Mendelian trait and is autosomal recessive to orange (Staples-Browne 1908, Christie & Wriedt 1924,

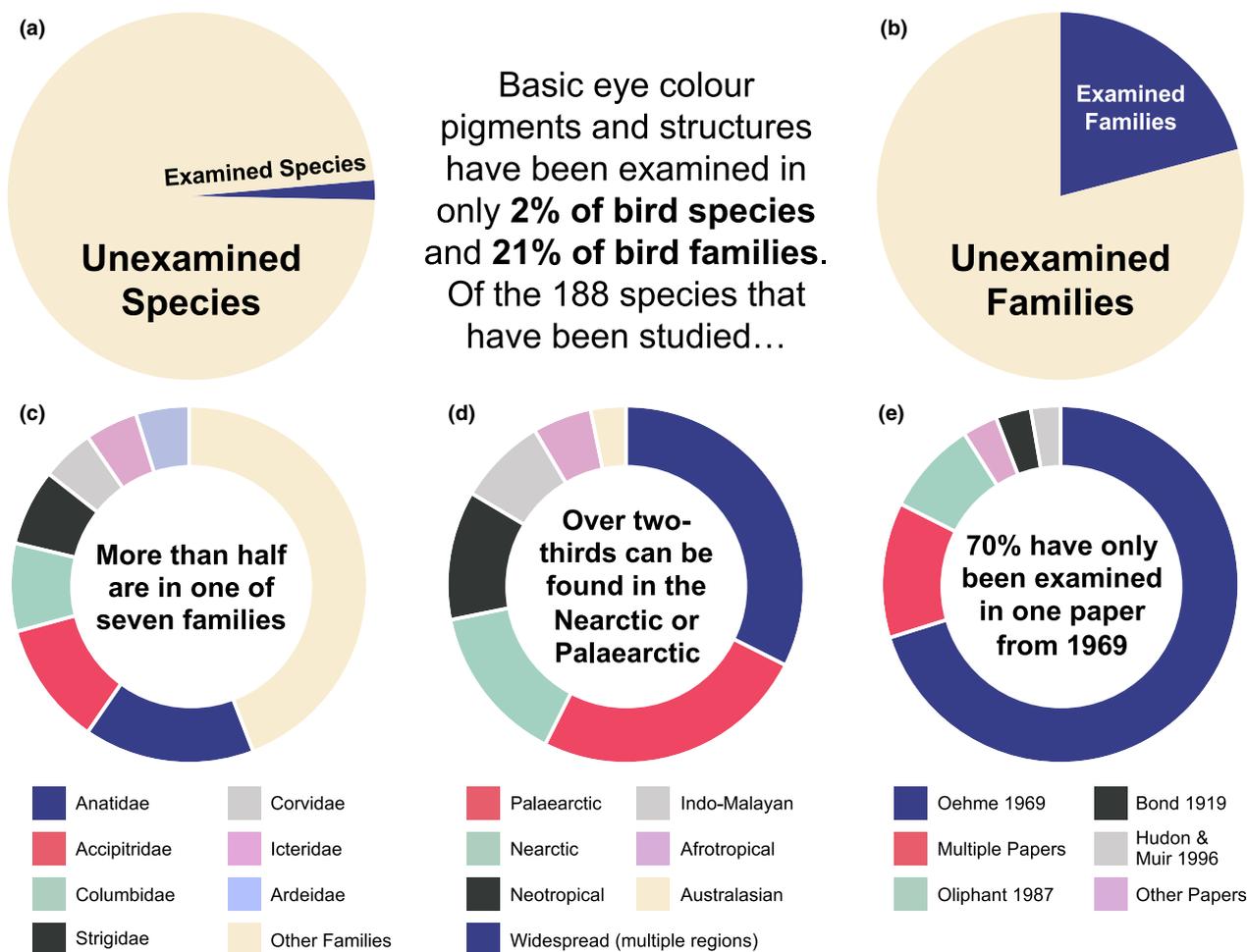


Figure 4. Previous studies of bird iris pigments and structures have examined only a few species (a) and families (b), and the distribution of effort across species, families and the globe has been uneven (c, d). Finally, most data that exist for eye pigments and structures derive from a small number of relatively old manuscripts (e). For a complete list of the species examined, see Appendix S3.

Bessmertnaja 1928, Hollander & Owen 1939b). Orange irises contain yellow pteridines and reflective purines whereas pearl irises lack yellow pteridines (Oehme 1969, Oliphant 1987a, 1987b), and these colour differences are associated with a locus referred to in older breeding literature as *Tr* (Bessmertnaja 1928, Hollander & Owen 1939b).

Three independent groups have recently used whole-genome sequencing of orange-eyed and pearl-eyed pigeons to pinpoint the location and identity of the *Tr* locus, and all came to an identical conclusion (Andrade *et al.* 2021, Maclary *et al.* 2021, Si *et al.* 2021). Using genomic analyses, each group found that a small (<10 kb) genomic region containing the membrane protein *SLC2A11B* (solute carrier family 2, facilitated

glucose transporter, member 11b) is strongly associated with orange/pearl iris colour variation, making it the first 'eye colour gene' to be identified in birds. They identified a nonsense mutation that dramatically truncates the *SLC2A11B* protein, alters expression of associated transcripts and probably causes pearl eyes in domestic pigeons (Fig. 5). The effects of *SLC2A11B* on the eye colour of domestic pigeons are supported by the association between the *SLC2A11B* gene and leucophore and xanthophore development in Medaka (Japanese rice fish *Oryzias latipes*), as well as loss-of-function experiments in Medaka that produced a similar loss of yellow pteridine pigment (Kimura *et al.* 2014). This suggests that at least some of the genes and pathways involved in bird iris

Phenotype	SLC2A11B Sequence	SLC2A11B Protein	SLC2A11B Expression	Pteridines	Other Colour Elements	Iris Colour
Orange	Wild-type ACGTGGAAT	Wild-type (504 residues) 	Normal Levels 	Yellow Pteridine Present 	Blood and Reflective Purines  	
Pearl	Nonsense Mutation ACGTGAAAT	Truncated (57 residues) 	Reduced Levels 	Yellow Pteridine Absent	Blood and Reflective Purines  	

Figure 5. The pathways to produce orange and pearl irises in domestic pigeons. Data from Si *et al.* (2021), Andrade *et al.* (2021) and Maclary *et al.* (2021). Iris photographs by Jian-Ou Gao, from Si *et al.* (2021), used with permission.

pigmentation are shared with other vertebrate groups that possess pteridine pigments.

In addition to the primary *Tr/SLC2A11B* eye colour locus, brown or chocolate-coloured plumage in domestic pigeons appears to be strongly associated with an alternative pathway to pale eyes, referred to as ‘false pearl’ (Levi 1957, Maclary *et al.* 2021). Because genetic work has shown that brown plumage results from the tyrosinase gene *TYRP1* (Domyan *et al.* 2014), the same gene or linkage group may also affect pigmentation of the iris.

Bull irises of domestic pigeons, which lack anterior pigmentation, are clearly associated with loci different from those involved in orange/pearl coloration. Unlike pearl irises, early crossing experiments involving bull irises did not produce consistent results (Staples-Browne 1908, Bond 1912, Hollander & Owen 1939b). However, these studies did note an association between bull irises and white or piebald plumage, which suggests that bull eyes are correlated with the deposition of feather melanin in addition to the deposition of iris pteridines and purines.

Maclary *et al.* (2021) examined bull eyes in domestic pigeons using quantitative trait locus (QTL) analyses and identified a large (~2 Mb) genomic region associated with the bull eye phenotype. However, their two experimental crosses showed different inheritance patterns, with incomplete penetrance and stochastic factors that affected observed phenotypes, and although both crosses implicated the same genomic region, the resulting bull phenotypes did not seem to be associated with a single genomic variant. Maclary

et al. (2021) also found a QTL for piebald plumage that overlaps the QTL associated with bull irises. These results suggest that bull irises and piebald plumage could result from multiple coding or regulatory changes in or near a pigmentation gene or group of genes. Although there were 60 genes in the region identified by the bull iris QTL crosses, one stands out as a potential candidate affecting the bull iris phenotype: *EDNRB2* (Maclary *et al.* 2021). *EDNRB2* is expressed at substantially lower levels in the embryos of bull-eyed individuals (Maclary *et al.* 2021), and *EDNRB2* and its homologues are associated with depigmentation of feathers, fur or irises of other birds (e.g. Miwa *et al.* 2007, Kinoshita *et al.* 2014, Xi *et al.* 2021) and mammals (Hosoda *et al.* 1994, Ceccherini *et al.* 1995, Metallinos *et al.* 1998), including humans (Puffenberger *et al.* 1994, Attié *et al.* 1995). *EDNRB2* is involved in the migration of neural crest-derived pigment cells (Lecoin *et al.* 1998, Pla *et al.* 2005), which may cause far-reaching effects on pigmentation in different tissue types.

Breeding studies involving domestic chickens have produced more equivocal results than those in pigeons. Davenport (1906) and Bond (1919) crossed chicken breeds of varying eye colours, and they noted an association between dark feathers and dark eyes, although MacArthur (1933) reported that different eye colours can occur with any plumage colour. These conflicting findings are likely to be a result of the role of multiple loci, some of which are pleiotropic. One locus, referred to as *Br*, primarily affects eye colour, with dark brown eyes recessive to light eyes

(MacArthur 1933, Hutt 1949, Smyth 1990), and linkage mapping has suggested that this locus is localized to the Z chromosome (MacArthur 1933, Hutt 1949). Although the *Br* locus directly affects iris coloration, *Br* is linked to the *Id* locus, which influences shank and eye colour, and Smyth (1990) reported that the melanin locus *E* can have additional effects on plumage and eye colour. More recent work has identified the genes *GRAMD3* and *MC1R* as corresponding to the *Id* and *E* loci, respectively (Dávila *et al.* 2014, Xu *et al.* 2017).

Eye colour genetics of wild birds

To date, essentially all research on bird eye colour genetics has been conducted using domesticated species, and the mutations that affect eye colour in domestic breeds may not be responsible for the variation seen in wild populations. Si *et al.* (2021) traced the origins of the pearl-eye-associated mutation in *SLC2A11B* to approximately 5400 years ago during the domestication process, and showed that it was under strong artificial selection at that time. Additionally, the depigmented 'bull' iris of some breeds of domestic pigeon is not homologous to the dark eyes of the Stock Dove *Columba oenas* or other wild species, which have melanin in the anterior border layer of their irises (Bond 1919), suggesting that the same genes are not necessarily involved. Still, the identification of specific loci involved with iris pigmentation in domesticated populations is a significant step forward for the field, and these discoveries have the potential to greatly facilitate similar studies of wild species.

Si *et al.* (2021) examined the *SLC2A11B* locus in whole genomes sequenced across the avian phylogeny and identified putatively deleterious mutations in *SLC2A11B* in Greater Rhea, Muscovy Duck *Cairina moschata*, Double-crested Cormorant, House Sparrow *Passer domesticus*, Anhinga *Anhinga anhinga* and African Grey Parrot *Psittacus erithacus* genomes. The first four species lack pteridines in their irises, but Anhinga and African Grey Parrot irises have yellow and colourless pteridines, respectively (Oehme 1969). Maclary *et al.* (2021) used a similar approach to show that a domain of *SLC2A11B* may be missing from the genomes of dark-eyed Hooded Crows *Corvus cornix* and the white-eyed Wire-tailed Manakins *Pipra filicauda* (Fig. 1n), neither of which have yellow pigments in the iris. Causal links between these potential

mutations and the eye colours of these species remain to be investigated.

We can also propose several hypotheses about the loci that may be involved in the iris colour of wild birds, building from knowledge about the underlying mechanisms. For example, a striking difference in guanine concentration in the irises of some Icteridae (Hudon & Muir 1996) is strongly associated with light/dark eye colour variation, and it is possible that a locus associated with a guanine-deposition pathway could be responsible for this variation. Other loci that are promising candidates for affecting eye colour include well-known genes such as *TYRP1* and *MC1R*, which have been indirectly shown to influence eye colour in domestic pigeons and chickens (Levi 1957, Smyth 1990, Dávila *et al.* 2014, Domyan *et al.* 2014); *HERC2* and *OCA2*, which account for most human eye colour variation (Duffy *et al.* 2007, Kayser *et al.* 2008) and are associated with melanin-based plumage coloration in *Sporophila* seedeaters (Campagna *et al.* 2017, Turbek *et al.* 2021); additional loci associated with melanin (Toews *et al.* 2016, Uy *et al.* 2016, Stryjewski & Sorenson 2017) and carotenoid (Brelsford *et al.* 2017, Toomey *et al.* 2017, Aguilon *et al.* 2021) coloration in bird feathers and bare parts (Mundy *et al.* 2016, Yu *et al.* 2017, Enbody *et al.* 2021); and two pteridine-associated genes, *GCH1* and *CSF1R*, that showed significant expression differences between orange-eyed and pearl-eyed pigeons (Si *et al.* 2021).

Because pteridine and purine coloration have been more extensively studied among reptiles (McLean *et al.* 2017, Andrade *et al.* 2019), amphibians (Stuckert *et al.* 2019, Rodríguez *et al.* 2020), fish (Henning *et al.* 2014, Kimura *et al.* 2014, 2017) and insects (Grant *et al.* 2016, Pimslar *et al.* 2017, Vargas-Lowman *et al.* 2019, Woronik *et al.* 2019), the literature for these taxa may also be a source for identifying candidate genes associated with those types of coloration in bird irises (Andrade *et al.* 2021, Andrade & Carneiro 2021, Price-Waldman & Stoddard 2021), similar to the situation with *SLC2A11B* in medaka (Kimura *et al.* 2014).

Future research: The genetic basis of eye colour variation

The stage is set for a dramatic expansion of our knowledge of bird eye colour genetics, including

the identification of loci associated with eye colour differences in natural populations. Compared with recent papers examining the genetics of bird plumage colour (Funk & Taylor 2019), the genetic mechanisms affecting iris coloration have been under-studied and remain largely unknown in wild species (Price-Waldman & Stoddard 2021). The same tools and analyses that sparked a revolution in understanding bird plumage colour genetics – particularly the use of whole genome and transcriptome data in genome-wide association and gene expression studies – would be well-suited to answering questions about the genomic basis of eye colour variation. These techniques are most powerful when background genetic variation is low between individuals of varying phenotype, as in hybrid zones or polymorphic populations (Rieseberg & Buerkle 2002, Buerkle & Lexer 2008). As a result, systems of particular interest would be those in which eye colour varies within a population or between two closely related populations experiencing gene flow (see Appendices S1c and S2c,d). Basic descriptive studies of iris coloration can identify additional examples of promising natural systems for future study. At the same time, captive breeding experiments offer a potential avenue of study for some taxa, and domesticated species such as pigeons, chickens, parrots and finches will continue to provide ample opportunities for genomic research, as highlighted by the recent and impressive work to identify the *SLC2A11B* gene.

EVOLUTIONARY DRIVERS OF BIRD EYE COLOUR

The ultimate causes of bird eye colour variation are the selective pressures and stochastic processes that result in the appearance and fixation of new eye colours in lineages over evolutionary time. Evolutionary explanations for eye colour variation can be divided into two broad categories: those involving individual survival and those involving signalling. The former category includes effects of eye colour on vision, ultraviolet protection or camouflage, and the latter category includes the role of eye colour as a potential indicator to conspecific individuals of mate quality, age or sex. Associations between iris colour and habitat, light environment or foraging strategy could point towards a role for survival-based explanations, whereas associations with social structure or mating systems would strengthen arguments for the importance of

intraspecific signalling explanations (Craig & Hulley 2004). Additionally, a set of null hypotheses must be considered where iris coloration may not be under selection and is, instead, largely influenced by genetic drift or a by-product of other processes (Walls 1942, Oehme 1969). We consider these first.

Drift and pleiotropy

Drift frequently plays a significant role in trait evolution, so there may not be a direct causal explanation for each evolutionary shift in eye colour across the avian phylogeny. Conversely, conserved eye colours across a clade do not necessarily signify continued selection for that eye colour: this pattern could instead be a result of phylogenetic constraints on the pigments that can be produced by that group (Craig & Hulley 2004) or simply result from shared evolutionary history.

Additionally, through pleiotropic effects or linkage, selection on plumage coloration or other traits may indirectly shape the evolution of eye colour. A recent study of gulls (Laridae) found that dark-feathered heads are correlated with dark irises in the group, which the authors suggest could be the result of melanin pigmentation pathways shared between the two traits (Minias & Janiszewski 2020). However, selection on the amount of contrast between the eye and head could also explain the observed pattern, and dark irises in at least some adult gulls result from not just the presence of melanin but also the absence of light pigment (Bond 1919) – so a clearer mechanism would need to be identified to support the pleiotropy hypothesis. Nonetheless, several studies note a connection between plumage and eye colour in domestic pigeons, including the association between ‘bull’ irises and piebald plumage (Davenport 1906, Bond 1912, Hollander & Owen 1939b) and between ‘false pearl’ eyes and brown plumage (Levi 1957). The association between ‘bull’ eyes and piebald plumage is supported genetically (Maclary *et al.* 2021), and it is plausible that selection on other elements of bird coloration could have incidental effects on the evolution of eye colour.

Survival

To our knowledge, no studies have directly examined the association between eye colour and

individual survival or the effects of eye colour on traits that directly affect survival such as vision, foraging ability or crypsis. Rather, most studies have addressed these connections indirectly by identifying correlations between eye colour and life history characteristics that influence selection pressures such as habitat type, foraging behaviour and nocturnality.

Eye colour and vision

Eye colour could affect visual acuity or protect the retina against deleterious effects of ultraviolet radiation. However, Savalli (1995) characterized support for 'visual clarity' hypotheses as 'anecdotal'. The highly melanated pigment epithelium on the posterior side of the iris should have the greatest effect on the light passing through the iris (Walls 1942, Mörke 1955, Oehme 1969, Erichsen 1985). This layer is similarly pigmented in light- and dark-eyed species, with little or no effect on the visible colour of the iris, which is dictated by pigments in the stroma and anterior border layer (Oehme 1969). Asymmetric patches of dark pigmentation near the pupil in button-quail, pigeons and other species have been suggested to help reduce glare and improve vision (Gutiérrez-Expósito 2019), though like other visual hypotheses, this idea has not been tested experimentally.

Eye colour and camouflage

One of the few comprehensive, comparative phylogenetic studies of bird eye colour concluded that dark irises and nocturnal behaviour are associated in owls (Passarotto *et al.* 2018). They suggested that the evolution of dark eyes could result from selection for iris colours that make hunting owls less conspicuous to their prey. Experiments in chickens have shown that stuffed models with 'predator-like' yellow eyes are avoided by potential prey species to a greater degree than those with dark eyes (Scaife 1976). However, these experiments were not conducted in the dark, and the hypothesis that dark eyes provide nocturnal camouflage has yet to be tested.

A second comparative phylogenetic study suggests a different camouflage-related hypothesis, and both studies may point towards a more comprehensive model for the role of camouflage in shaping patterns of avian eye colour variation. Davidson *et al.* (2017) investigated the relationships between eye colour, parental care and cavity

nesting in passerines and found support for selection against bright eyes in non-cavity-nesting birds. They proposed a hypothesis in which dark eyes are selectively favoured among non-cavity nesters because they make individuals on the nest less conspicuous to predators, whereas selection may be relaxed in cavity-nesting species where incubating parents are already hidden from predators. Freed from natural selection for dark eyes, cavity-nesting species may then be under selection for bright eyes in a display or signalling context, as discussed below.

A variation of this hypothesis could also be applied to explain the results of Passarotto *et al.* (2018) with owls: perhaps dark eyes in nocturnal owls are under selection for camouflage from predators or mobbing songbirds at their daytime roosts. This is supported by findings that owl models with yellow eyes are mobbed at greater rates than models with blue eyes or no eyes (Hinde 1954, Curio 1975, Smith & Graves 1978, Kerlinger & Lehrer 1982). Also relevant is the presence of keyhole-like notches in the eyelids of potoos (Nyctibiidae) (Borrero 1974), which are extremely well-camouflaged nocturnal insectivores. These openings are thought to allow day-roosting potoos to see their surroundings without opening their eyelids (Borrero 1974, Cohn-Haft 1999), because doing so would expose their large and striking yellow eyes. The presence of this adaptation may suggest that there is selection pressure against exposed bright eyes in these cryptic nocturnal species. A related family of highly camouflaged nocturnal birds, the Caprimulgidae, is entirely dark-eyed.

Many patterns of bird eye colour variation are not explained by hypotheses related to crypsis (Walls 1942). However, the general principle that light eyes are selected against due to camouflage in many species, and that this constraint is stronger in some groups (day-roosting nocturnal species, songbirds with open nests) and weaker in others (diurnal predators, cavity-nesters, other species with enclosed nests), warrants further examination.

Eye colour and foraging behaviour

Worthy (1978, 1991, 1997) proposed that bird eye colour can be broadly correlated with foraging behaviour, stating that ambush predators tend to be light-eyed and 'pursuit' predators dark-eyed. Under this model, the largely light-eyed herons

and the wholly dark-eyed swifts would represent opposite ends of an ecological and eye colour continuum. Worthy also proposed multiple mechanisms explaining this correlation, including those relating to visual clarity and pleiotropic effects. However, the hypothesis has not been tested and empirical studies are needed to test whether a functional relationship between bird eye colour and foraging behaviour exists (Savalli 1995, Elias *et al.* 2008).

Eye colour and habitat

Associations between eye colour and foraging behaviour, visual acuity or camouflage could also produce an association between habitat and eye colour. Craig and Hulley (2004) identified several potential correlations in a global survey of bird eye colour variation, including a tendency for forest-dwelling species to be lighter-eyed in the Corvidae and South American Icteridae, but they acknowledged that the correlation may be a result of phylogenetic effects rather than habitat. Additionally, they found a higher-than-expected proportion of light-eyed passerines in Australia and South Africa: whether this is a result of habitat, phylogeny or another factor remains uncertain. A correlation between eye colour and habitat does seem to exist in frogs, with more arboreal species tending to be lighter-eyed (Glaw & Vences 1997, Amat *et al.* 2013), but the reasons for this pattern – and whether it could extend to birds or other vertebrates – are unknown.

Even genuine correlations between habitat and eye colour are not necessarily a result of a direct influence of iris coloration on survival: the utility of eye colour as an intraspecific signal could also be affected by the light environment, which is associated with habitat type (Endler 1993).

Signalling

Although few studies have directly examined how eye colour affects individual survival, there is stronger evidence that eye colour plays a role in mediating signalling among individuals. The most obvious examples of these types of interactions involve sexual selection (Darwin 1871), whereby eye colour influences mate choice, but bird eye colour may also play a role as a signal in a variety of additional social contexts (West-Eberhard 1979).

Sexual selection

The brightly coloured eyes of some species are involved in courtship and appear to be a result of sexual selection. For example, parotias and Flame Bowerbirds *Sericulus ardens* use their flashy irises in courtship dances (Scholes 2008, Gunton 2014, Cordey 2019). Male Satin Bowerbirds line their display bowers with blue objects and display purple-to-ultraviolet plumage (Borgia 1985, Doucet & Montgomerie 2003). Their brilliant purple eyes – which also reflect ultraviolet light (Endler & Mielke 2005) – may play a similar role in courtship, although eye colour is not sexually dimorphic in this species (Frith & Frith 2020a). Cormorants, which possess some of the most vividly coloured eyes of any bird group, are largely black-plumaged birds that often have patches of bare facial skin that become brightly coloured during the breeding season: their colourful eyes could be an extension of this phenomenon (Orta 1992). As mentioned above, some species of herons and pelicans have brighter or redder eyes during the breeding season, suggesting a role in courtship. On the other hand, sexual dichromatism in eye colour is atypical in birds (as discussed above), and in species having sexually dichromatic eyes, no obvious trends towards brighter male irises are evident (Negro *et al.* 2017).

Eye colour variation is also involved in mate choice, and there is some evidence that it functions as an honest signal of mate quality (Zahavi 1975, Endler 1980, Hamilton & Zuk 1982, Lozano 1994). In Yellow-eyed Penguins *Megadyptes antipodes*, the saturation of their yellow eyes is positively correlated with breeding success in males and females, even when controlling for age (Massaro *et al.* 2003). The same study also found evidence of assortative mating by iris hue (but not saturation), while acknowledging the need for explicit mate-choice experiments. A similar correlation between yellowness of irises and nest success (but not mass, clutch size or egg volume) was found in female Little Owls *Athene noctua*, but not in Eurasian Scops Owls *Otus scops* (Passarotto *et al.* 2020) – observations that warrant additional experimental investigation. Male eye colour is associated with nesting success in Eurasian Sparrowhawks *Accipiter nisus*, though whether eye colour results in corresponding changes in female mate choice is uncertain (Newton & Marquiss 1982). Additional associations between eye colour and individual health have

been found in Northern Saw-whet Owls *Aegolius acadicus* (Wails *et al.* 2018) and American Kestrels *Falco sparverius* (Bortolotti *et al.* 2003) but not in Cooper's Hawks *Accipiter cooperii* (Rosenfield *et al.* 2003).

Carotenoid-based colours are often cited as honest signals of quality due to the cost of obtaining them and the possibility that carotenoids directly provide immunological and oxidative benefits (Endler 1980, Lozano 1994, von Schantz *et al.* 1999, McGraw & Ardia 2003). The yellow iris colour of Little Owls is generated by carotenoids (Oehme 1969), though this is probably not the case for most light-eyed bird species mentioned above, including penguins (McGraw *et al.* 2004), gulls and raptors (Oehme 1969). The role of pteridine-based colours as signals of mate quality is largely unstudied in birds (McGraw 2005, Andrade & Carneiro 2021). Eye colour in some species could also serve as an indirect signal of mate quality by indicating age, with older birds likely to be of higher quality (Snyder & Snyder 1974, Picozzi 1981, Newton & Marquiss 1982).

Differing eye colours may also play a role in species recognition (Rodríguez-Rodríguez & Negro 2021) and assortative mating in contact zones, which would affect the speciation process. Gulls have been shown to mate assortatively with respect to bare part coloration in the hybrid zones of Glaucous-winged Gulls *Larus glaucescens* and Western Gulls *Larus occidentalis* (Hoffman *et al.* 1978, Bell 1997, Moncrieff *et al.* 2013, Megna *et al.* 2014) and Herring Gulls and Caspian Gulls *Larus cachinnans* (Gay *et al.* 2007, Neubauer *et al.* 2009). The specific factors that affect mate choice in gulls have been the subject of controversy (Smith 1966, Pierotti 1987, Snell 1989, 1991), but these well-studied populations would be good systems for examining the relative contribution of eye colour to reproductive isolation.

Social signalling

Behavioural observations and patterns of interspecific variation suggest a role for eye colour in many social contexts, including nest defence, intraspecific competition, flock vigilance and cooperative breeding. Many types of intraspecific visual communication in birds tend to occur face-to-face, including antagonistic displays (Kenyon & Martin 2022), so the eye may be a prominent element

in close-range signalling between individuals (Rodríguez-Rodríguez & Negro 2021).

The most significant behavioural experiment on the consequences of bright eyes in birds was conducted in Western Jackdaws, a light-eyed, cavity-nesting corvid. Davidson *et al.* (2014) demonstrated that placing an image of a bright-eyed jackdaw, or even just a pair of bright eyes, in a nest cavity dissuaded other jackdaws from approaching, relative to a dark-eyed control image. They suggested that bright eyes function as a signal that a nestbox is defended and deter potential usurpers.

Birds can dilate and contract their pupils voluntarily, and in species with light irises the amount of colour displayed can change dramatically depending on pupil size (Hardy 1963, Craig 2022). African Pied Starlings have been observed contracting and dilating their pupils during food exchanges, with the donor widening its pale iris and the recipient narrowing theirs (Craig 2022). Similarly, contraction of the pupil to accentuate the iris ('eye-blazing' or 'eye-pinning') is common in parrots, and this behaviour is associated with excited states including social dominance interactions and courtship displays (Hardy 1963, Smith 1975, Moore *et al.* 2022). Similar behaviours have been observed in other families and may prove to be widespread across light-eyed birds (McIlhenny 1937, Hardy 1974, Craig & Hulley 2004).

Eye colour varies with age in many bird species, and could allow individuals to signal their age and social rank and to easily discern the age of conspecifics (Snyder & Snyder 1974, Newton & Marquiss 1982). As discussed above, eye colour as an age indicator could also play a role in mate choice and sexual selection. Additionally, the ability to evaluate age via eye colour could be advantageous in social species, particularly those with cooperative breeding, where reproductive roles are highly structured by age. For younger birds in such species, having an eye colour that marks them as a non-threatening juvenile might protect them from intraspecific aggression (Hardy 1974, Lawton & Lawton 1986). Bright-eyed adults and dark-eyed juveniles can be observed in cooperatively breeding starlings (Sweijd & Craig 1991, Craig & Hulley 2004), jays (Hardy 1973, Peterson 1991), Jungle Babblers (Andrews & Naik 1965), Grey-crowned Babblers *Pomatostomus temporalis* (Matthew & Christie 2020) and Acorn Woodpeckers (Koenig & Walters 2015). The age at which adult

eye colour is acquired was not found to be directly correlated with sociality in one genus of jays (Peterson 1991). However, experimental manipulation of bill colour to appear juvenile-like was shown to dramatically alter social interactions in Purplish-backed Jays *Cyanocorax beecheii* (Hardy 1974), so it seems plausible that age-based eye colour variation could function similarly.

Iris coloration could also relate to 'gaze sensitivity', the awareness of and response to where other individuals are looking (Davidson & Clayton 2016). In humans, white sclerae may have evolved to facilitate gaze perception (Kobayashi & Kohshima 2001, Tomasello *et al.* 2007, Yorzinski & Miller 2020, but see Caspar *et al.* 2021), and across primates lighter sclerae are correlated with increased cooperative social behaviour (Mearing *et al.* 2022). Whether there is an association between pale bird irises and intraspecific gaze sensitivity has not been explicitly studied, but in Great-tailed Grackles *Quiscalus mexicanus* paler irises can be distinguished relative to the surrounding feathers at greater distances than darker irises (Yorzinski *et al.* 2022). Additionally, Guillemain *et al.* (2012) showed that ducks consistently have different coloured eyelids and irises; these differences may function as signals of vigilance to other members of their flock by indicating whether the eye is open or closed.

The frequency of colourful bare eye-rings or facial skin in birds may also give insight into the factors affecting evolution of bright irises, especially because bare part coloration has been implicated in social signalling (Iverson & Karubian 2017). Willis (1969) suggested that the bright skin around the eye of some antbirds may give the illusion of a large eye (akin to an eyespot) to deter predators or competitors. Whether the occurrence of brightly coloured eye-rings and irises are correlated is an open question: in some families (Platysteiridae and Thamnophilidae), they appear to be negatively associated, but in other clades (*Prionops* spp.), bright eyes and bright eye-rings occur together (del Hoyo *et al.* 2016). More generally, the relationship between iris coloration and the coloration of the bare or feathered area surrounding the eye should not be overlooked: the same colour iris could contrast conspicuously in one species but blend in with the rest of the face in a different species.

Future research: Evolutionary drivers of eye colour variation

Two categories of future study would increase knowledge of evolution of bird eye colour variation. The first is phylogenetically informed comparative analyses of correlations between eye colour and ecological factors (Davidson *et al.* 2017, Passarotto *et al.* 2018). Moving beyond anecdotal impressions of correlations between eye colour and an ecological or life history trait of interest, these studies account for phylogenetic autocorrelation (Felsenstein 1985) to assess whether the trait is genuinely associated with observed eye colours across a phylogeny. These models require a robust and densely sampled phylogenetic hypothesis of the clade of interest, but the dramatic increase in the number of large (hundreds or thousands of species) bird phylogenies inferred with genomic data (McCullough *et al.* 2019, Oliveros *et al.* 2019, Harvey *et al.* 2020) make such studies feasible.

Secondly, behavioural experiments, such as those conducted by Davidson *et al.* (2014), will greatly advance understanding of bird eye colour evolution. These will be more difficult to perform than correlative analyses, but they are extremely valuable and provide many possible directions for study. By testing the response of wild conspecifics, predators or prey to models of varying eye colour, we can gain insight into how a hypothesized function for eye colour variation operates in a natural environment. Developing methods to alter the apparent colour of the iris of juvenile or adult birds to appear younger or older (akin to Hardy's (1974) painting of jay bills) could help to test hypotheses of how eye colour affects intraspecific social dynamics. Additionally, directly testing visual acuity (Caves *et al.* 2020), measuring the pupillary light reflex and sequencing genes associated with vision (White *et al.* 2022) in species of varying eye colour would facilitate testing hypotheses involving eye colour and visual traits.

It would be impractical to experimentally assess the array of evolutionary hypotheses suggested to explain elements of avian eye colour: a practical path forward would be to first conduct rigorous analyses of correlation in a clade of interest, then validate promising associations with experiments.

CONCLUSION

Despite their conspicuousness and variability, much remains unknown about the colour of bird eyes. Furthermore, previous work has been scattered across disciplines as diverse as pigment chemistry, game bird breeding, genomics and behavioural ecology. We hope that bringing together the literature on the patterns of bird eye colour variation and its mechanistic, genetic and evolutionary drivers, combined with the data compiled in Appendices S1, S2 and S3, will spur new advances in our understanding of the rainbow of eye colours in birds. The study of bird coloration has been central to our understanding of speciation and the evolutionary process, and greater knowledge of the drivers of bird eye colour variation may lead to similar insights and discoveries.

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AUTHOR CONTRIBUTIONS

Eamon C. Corbett: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; visualization; writing – original draft; writing – review and editing. **Robb T. Brumfield:** Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing – review and editing. **Brant C. Faircloth:** Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing – review and editing.

ETHICAL NOTE

None.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

Data Availability Statement

Data used in this review are included in the supplemental appendices.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. (a–d) Phylogenetic patterns of variation.

Appendix S2. (a–e) Intraspecific variation.

Appendix S3. Mechanisms of iris coloration.

Figure S1. A visualization of different authors' attempts to categorize the frequency of bird eye colours. Worthy's (1997) overall findings are shown at top and broken down between non-passerines (left) and passerines (right). Davis and Clarke (2022) just examined non-passerines, while two datasets compiled by Craig and Hulley (2004) contained only passerines. Each author categorized colours somewhat differently, so icons representing bird eye colours (white, grey, green, blue, yellow, orange, red, light brown, brown and black) are placed next to the category they would be sorted into in each dataset. Pie chart areas are scaled by the number of species surveyed.