

Early-life environmental effects on birds: epigenetics and microbiome as mechanisms underlying long-lasting phenotypic changes

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ABSTRACT

Although the long-lasting effects of variation in early-life environment have been well documented across organisms, the underlying causal mechanisms are only recently starting to be unraveled. Yet understanding the underlying mechanisms of long-lasting effects can help us predict how organisms will respond to changing environments. Birds offer a great system in which to study developmental plasticity and its underlying mechanisms owing to the production of large external eggs and variation in developmental trajectories, combined with a long tradition of applied, physiological, ecological and evolutionary research. Epigenetic changes (such as DNA methylation) have been suggested to be a key mechanism mediating long-lasting effects of the early-life environment across taxa. More recently, changes in the early-life gut microbiome have been identified as another potential mediator of developmental plasticity. As a first step in understanding whether these mechanisms contribute to developmental plasticity in birds, this Review summarizes how changes in early-life environment (both prenatal and postnatal) influence epigenetic markers and the gut microbiome. The literature shows how both early-life biotic (such as resources and social environment) and abiotic (thermal environment and various anthropogenic stressors) factors modify epigenetic markers and the gut microbiome in birds, yet data concerning many other environmental factors are limited. The causal links of these modifications to lasting phenotypic changes are still scarce, but changes in the hypothalamic–pituitary–adrenal axis have been identified as one putative pathway. This Review identifies several knowledge gaps, including data on the long-term effects, stability of the molecular changes, and lack of diversity in the systems studied, and provides directions for future research.

KEY WORDS: Developmental plasticity, Early-life environment, DNA methylation, Gut microbiome, Avian, Developmental programming

Developmental plasticity in birds

Developmental plasticity is defined as the early-life environment contributing to permanent, long-lasting effects on individual phenotype (such as physiology, morphology and behavior, e.g. Stearns, 1989). Adaptive developmental plasticity refers to changes in the phenotype that increase organismal fitness (e.g. Nettle and Bateson, 2015). Yet human-induced changes in the environment

may lead to non-adaptive developmental plasticity, such as permanent disruption of endocrine function (Ghalambor et al., 2007). Avian (and other egg-laying) species are excellent models for studying developmental plasticity for several reasons: (1) their embryos develop externally to the mother, and therefore prenatal resources and other environmental factors can easily be measured and experimentally manipulated; (2) for economic reasons, there is extensive knowledge about early-life effects of birds using poultry as a model system; and (3) there is a long history of research and broad knowledge on the physiology, ecology and evolution of wild bird species and populations, allowing fitness-related effects to be addressed.

In the past decade, a key challenge in ecology and evolution has been characterizing the molecular mechanisms mediating developmental plasticity, following findings in biomedical research (Gluckman et al., 2008; Laubach et al., 2018). A putative mechanism that has been suggested to mediate developmental plasticity is changes in epigenetic markers (Laubach et al., 2018). More recently, changes in the gut microbiome have been raised as another putative key mechanism underlying developmental plasticity (Desbonnet et al., 2015; Heijtz et al., 2011). The first step in exploring whether developmental plasticity in birds is mediated by these two molecular mechanisms is to understand whether early-life environmental variation alters epigenetic markers and/or the gut microbiome. Therefore, the main aim of this Review is to summarize the evidence on how and which early-life environmental factors influence these molecular mechanisms in birds (see [Tables 1 and 2](#)). This Review considers both microbiome and epigenetics as underlying mechanisms of developmental plasticity to provide a broad overview of this young scientific subfield in ecology and evolution. Early-life environment is considered broadly to give readers from different backgrounds an overview of the current knowledge. Both prenatal and early postnatal stages have been discussed, to detect whether there are potential differences in the developmental stage at which the environmental variation is occurring. Furthermore, to unravel the generality of the patterns, the Review aims to cover a large range of bird taxa, including captive species and wild species, and species that differ in their developmental modes; precocial species are well-developed at hatch (e.g. poultry), whereas altricial species are much less developed and more dependent on parental care at hatch (e.g. passerines). Whether the observed changes in molecular markers are linked to the phenotypic variation driven by early-life environment is thereafter discussed. Lastly, current key knowledge gaps and challenges, and future directions in the field are addressed.

Epigenetics in birds in a nutshell

Epigenetics broadly refers to changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change

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Table 1. Summary of studies on early-life environmental effects on avian epigenetic markers

Ontogeny	Specific factor	Molecular changes	Species	Age measured	Reference	
Resources						
Prenatal	Protein	Methylation of <i>UBC</i> gene	Chicken	1 year	Willems et al., 2014	
	Zinc	Global methylation, H3K9 acetylation	Chicken	E20	Sun et al., 2018	
	Vitamin C	Methylation of heat shock protein gene	Chicken	E20	Zhu et al., 2019	
	Folic acid	IGF methylation and DMNT expression	Chicken	42 days	Liu et al., 2016	
	Betaine	Methylation of cholesterol-related genes	Chicken	58 days	Hu et al., 2015	
	Egg corticosterone	Methylation of <i>GCR</i> and <i>CRH</i> genes	Chicken	113 days	Ahmed et al., 2014	
	Egg corticosterone	Methylation of <i>GCR</i> gene	Great tit	14–100 days	Hukkanen et al., 2023	
	Postnatal	Short-term food deprivation	Methylation of hypothalamus <i>CRF</i> gene	Chicken	7 days	Xiao et al., 2020
		Short-term food deprivation	Histone H3 methylation status	Chicken	3 days	Xu et al., 2012
		Betaine	Methylation of lipoprotein lipase gene	Chicken	66 days	Xing et al., 2011
Brood size (food quantity)		Overall DNA methylation	Zebra finch	10–11 days	Sheldon et al., 2018	
Brood size (food quantity)		Methylation of blood <i>GCR</i> gene	Zebra finch	2–4 years	Jimeno et al., 2019	
Brood size (food quantity)		Methylation of genes related to development, growth, metabolism, behavior and cognition	Great tit	14 days	Sepers et al., 2021	
Resource predictability		Methylation of <i>GCR</i> gene	Zebra finch	5 days	Rubenstein et al., 2016	
Social environment						
Prenatal	Con-/heterospecific acoustic cues	Overall DNA methylation	Zebra finch	E14	Antonson et al., 2021	
	Predator cues	Methylation of <i>GCR</i> gene	Yellow-legged gull	1 day	Ruiz-Raya et al., 2023	
	Competition	No effects on methylation of <i>GCR</i> gene	Yellow-legged gull	1 day	Ruiz-Raya et al., 2023	
Postnatal	Social isolation	Methylation of cancer-related genes	Chicken	28 days	Pertille et al., 2017	
Other biotic factors						
	Parasitism	No effects on methylation	Mockingbird	9–11 days	McNew et al., 2021	
	Viral infection (<i>Salmonella</i>)	Overall DNA methylation	Chicken	12 days	Wang et al., 2017	
	Prenatal microbiome via diet supplements	DNA methylation of immune genes	Chicken	42 days	Dunislawska et al., 2021	
Thermal environment						
Prenatal	Heat	Methylation of histone H3K4	Chicken	35 days	David et al., 2019	
	Heat	Methylation of myogenesis-related genes	Duck	E10–E27	Wang et al., 2019	
	Heat	Methylation of genes involved in heart developmental processes	Chicken	1 day	Corbett et al., 2020	
	Heat	Histone H3K9 modifications	Chicken	10 days	Kisliouk et al., 2010	
	Heat	Methylation of <i>HSP</i> genes	Chicken	42 days	Vinoth et al., 2018	
Postnatal	Heat	Methylation of <i>CRH</i> gene	Chicken	10 days	Cramer et al., 2019	
	Heat	Methylation of <i>BDNF</i> gene	Chicken	10 days	Yossifoff et al., 2008	
	Heat	Methylation of <i>HSP70</i> gene	Chicken	10 days	Kisliouk et al., 2017	
	Heat	MI-158 miRNA	Chicken	10 days	Kisliouk et al., 2011	
	Anthropogenic					
Prenatal	Urbanization	Overall methylation	House wren	1 day	von Holdt et al., 2023	
	PAH pesticides in eggs	Methylation of <i>CYP</i> genes	Chicken	E10–2 days	Brandenburg and Head, 2018	
	Methylmercury	No effects on methylation	Chicken	E19	Basu et al., 2013	
Postnatal	Urbanization	Methylation of genes related to metabolism	House wren		von Holdt et al., 2023	
	Metal pollution	Methylation of neural development genes	Great tit	14 days	Mäkinen et al., 2022	
	Lead pollution	Methylation of developmental genes	Great tit	14 days	Mäkinen et al., 2022	
	Arsenic pollution	Methylation of developmental genes	Great tit	14 days	Laine et al., 2021	
	Pesticides	Methylation of genes related to cellular signaling and endocrine function	Zebra finch		McNew et al., 2021	
	Pollution	Methylation of clock genes	Barn swallow	15 days	Romano et al., 2017	

The stage of ontogeny (pre/post-hatch) when the environmental factor was present, species, and age when measurements (i.e. responses) were taken are listed. day, days post-hatch; E, embryonic day.

to the DNA sequence (Wu and Morris, 2001). Because changes in gene expression are precursors or direct causes of changes in phenotypes, it is generally accepted that changes to epigenetic mechanisms alter phenotypic characteristics. In birds, epigenetic variation has been linked to various traits, such as cognition, reproduction, thermoregulation and immune function (reviewed by Sepers et al., 2019; Dunislawska et al., 2022; Bednarczyk et al., 2021). The key epigenetic mechanisms in vertebrates include DNA methylation (addition of a methyl group to cytosines, so called CpG sites, by methyltransferase enzymes), histone modifications (such as

acetylation and methylation) and small RNAs (Jaenisch and Bird, 2003); currently, in birds, most research concentrates on DNA methylation (Sepers et al., 2019; Laine et al., 2022). Epigenetic markers in birds are known to be influenced by both genetic background and the environment, yet the relative importance of each is not well understood (Sepers et al., 2019). Importantly, dynamic changes of DNA methylation in developing bird embryos have been observed (reviewed by Dunislawska et al., 2022, Sepers et al., 2021), with early prenatal stages especially susceptible to environmental influences, suggesting that changes in epigenetic markers could play

Table 2. Summary of studies on early-life environmental effects on avian gut microbiome

Ontogeny	Specific factor	Molecular changes	Species	Age measured	Reference
Resources					
Prenatal	Prebiotics	Diversity and composition	Chicken	Maximum 42 days	Siwek et al., 2018
Postnatal	Food quantity	No effect on diversity/ composition	Great tit	7 days	Liukkonen et al., 2022
	Macro and micronutrients (review)	Diversity and composition	Chicken	Various	Shehata et al., 2022
	Macro and micronutrients (review)	Diversity and composition	Chicken	Various	Gabriel et al., 2006
	Habitat/location	Diversity and composition	Greater flamingo	70 days	Gillingham et al., 2019
	Habitat/location	Diversity and composition	Great tit	14 days	Goossens et al., 2022
Social environment					
Prenatal	Con/heterospecific rearing	Composition	Zebra finch/ Bengalese finch	5–100 days	Maraci et al., 2022
Postnatal	Heterospecific rearing	Composition	Cuckoo and magpie	15–19 days	Lee et al., 2020
Thermal environment					
Prenatal	?				
Postnatal	Heat (reviewed)	Diversity and composition	Chicken	14–40 days	Cao et al., 2021
	Cold	Composition	Chicken	42 days	Yang et al., 2021
	Heat	Diversity	Tree swallow, Eastern bluebird	10 days	Ingala et al., 2021
Anthropogenic stressors					
Prenatal	?				
Postnatal	Herbicide	Composition	Japanese quail	56 days	Ruuskanen et al., 2020a
	Stress	Composition	Yellow-legged gull	3 days	Noguera et al., 2018
	Pesticide	Diversity	Chicken	18 days	Wu and Su, 2022
	Microplastics	Diversity and composition	Chicken	28 days	Li et al., 2023
	Mercury	Diversity and composition	Chicken	90 days	Zhou et al., 2020
	Chromium	Diversity and composition	Chicken	35 days	Li et al., 2021
	Copper	Diversity and composition	Chicken	90 days	Huang et al., 2021

The stage of ontogeny (pre/post-hatch) when the environmental factor was present, species, and age when measurements (i.e. responses) were taken are listed. The responses have been broadly categorized to changes in diversity or composition, but some studies also analyzed effects on particular microbial taxa. day, days post-hatch.

a role in mediating developmental effects in birds. Non-adaptive environmentally induced changes in epigenetic markers have been suggested to be linked to stress/pollution-related changes in reactive oxygen species, which may directly affect epigenetic markers (often decreasing methylation levels), or upregulated/increased antioxidant defenses could decrease the abundance of methyl groups (S-adenosylmethionine, SAM) needed for DNA methylation (Baccarelli and Bollati, 2009). However, the detailed molecular mechanisms are not well understood.

Microbiome in birds in a nutshell

The gut microbiome (hereafter, microbiome) refers to all microorganisms (bacteria, archaea, microbial eukaryotes and viruses), and their collective genes inhabiting the host's digestive tract, although most ecological microbiome studies focus on bacteria only (McFall-Ngai et al., 2013). In both poultry and wild birds, variation in gut microbiome diversity and composition has often been associated with growth (Davidson et al., 2021; Teyssier et al., 2018; Kohl et al., 2018; but see Liukkonen et al., 2022). Gut microbes play major roles in digestion, detoxification, and the synthesis of essential molecules, such as short-chain fatty acids (SCFAs), as well as influencing immune function (reviewed by Bodawatta et al., 2021, Broom and Kogut, 2018). In adult birds, the microbiome has recently been linked to variation in behavior and cognition (Florkowski and Yorzinski, 2023; Davidson et al., 2018; 2020; Kelly et al., 2022) and even survival (Worsley et al., 2021).

Both genetic and environmental variation influences microbiome diversity and composition in birds (Kers et al., 2018; Bodawatta et al., 2022; Teyssier et al., 2018), yet meta-analyses suggest that the

avian microbiome is highly flexible, and that environmental effects likely have a greater impact than genetic variation (Bodawatta et al., 2022; Trevelline et al., 2020). Establishment of the microbiome in birds mainly occurs just after hatching (but see Trevelline et al., 2018; van Veelen et al., 2018; Grizard et al., 2015 on egg microbiome), and seem to include a rapid diversification stage in early life, followed by stabilization later on, with nestling birds being more sensitive to environmental variation than adults (Somers et al., 2023).

A wealth of experimental studies (using pre- or postnatal probiotics or microbiome transplants) in poultry show how the early-life microbiome influences later-life traits (e.g. Wilkinson et al., 2020; reviewed by Krysiak et al., 2021), supporting the hypothesis that early-life environment contributes to developmental plasticity. The links between the microbiome and developmental plasticity are likely to be complex as the microbiome often has a bi-directional interaction with host physiology (McFall-Ngai et al., 2013). There are multiple hypothetical pathways through which changes in the microbiome could mediate developmental plasticity (summarized in Fig. 1). First, changes in the early-life environment (such as temperature, humidity and resources) could influence the presence of microbes in the environment, and, via horizontal transmission, cause shifts in the early-life microbiome. Second, some early-life environmental stressors could cause changes in early-life physiology (such as the stress response or gut physiology) with downstream consequences on the early-life microbiome. Lastly, exposure to antimicrobials (such as pollutants) could directly affect the microbes currently inhabiting the gut, thus modifying the early-life microbiome. Thereafter, any changes in the early-life gut microbiome could lead to long-lasting effects on the adult phenotype

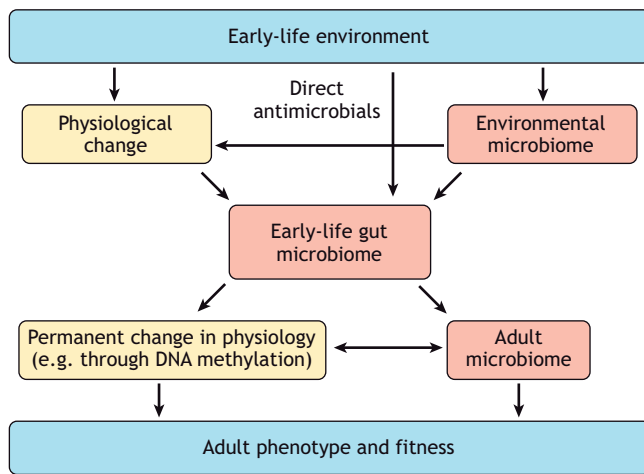


Fig. 1. Potential pathways of gut microbiome-mediated developmental plasticity.

if changes in the early-life microbiome cause permanent changes to molecular markers or physiology. For example, there is evidence for a link between the microbiome and epigenetic markers (Pan et al., 2018; D'Aquila et al., 2020; Dunislawaska et al., 2021). Alternatively, changes in the early-life microbiome could lead to permanent changes in the adult microbiome, for example if the founder microbial community influences later colonization in the gut (so called priority effects; Sprockett et al., 2018). The altered adult microbiome would then directly maintain phenotypic differences during adulthood.

Avian early-life environment and developmental plasticity

In the last decade, knowledge about biotic and abiotic factors shaping avian development has diversified: Starck (2009) considered food quantity, nutrient composition and temperature as the major environmental factors influencing offspring development, whereas recent studies have shown how a diverse combination of abiotic and biotic factors influence avian development, with long-lasting consequences on adult phenotypes. Abiotic factors range from humidity and light (seen in both natural and human-made contexts), to anthropogenic factors, such as noise and various chemical exposures. Biotic factors encountered during early-life development are also broad, ranging from parental care and other resources to social environment (including siblings and predator-prey associations), parasites and pathogens. In this Review, environmental factors are broadly categorized as biotic, including early life resources and the social environment, or abiotic, particularly thermal and anthropogenic stressors (see Tables 1 and 2), as we still have limited data on how molecular markers are associated with specific environmental factors. The following sections will discuss evidence of changes in epigenetic markers and the microbiome in relation to each of these environmental factors.

Early-life resource availability and developmental plasticity in birds

Limited or unpredictable resource availability, via both macro- and micronutrients, in early life have long-lasting consequences on avian phenotypes (e.g. Awad et al., 2009; Brenes and Roura, 2010; Raza et al., 2019). Limited resource availability can cause developmental stress, which has been linked to long-lasting effects (recently reviewed by Wada and Coutts, 2021) on physiology (e.g. reproductive axis Farrell et al., 2015; Schmidt et al., 2014), behavior, learning and memory (e.g. Schoech et al., 2011; Pakkala et al., 2016), and fitness

(e.g. Freeman et al., 2021). In addition to nutrients, birds are also exposed to various maternal developmental cues, such as hormones (e.g. steroid and thyroid hormones), that coordinate and direct development, crucially influencing later-life behavior, physiology and fitness (Ruuskanen, 2015; Ruuskanen and Hsu, 2018; Groothuis et al., 2019). Below, the changes in epigenetic markers and the microbiome in relation to these factors are discussed in more detail.

Early-life resource availability and epigenetics

Prenatal macronutrient resources, such as poor protein availability (via albumen removal), has been shown to increase methylation and expression of the ubiquitin gene, and expression of the glucocorticoid (GC) receptor in chickens (Willems et al., 2014). Similarly, the availability of micronutrients has been associated with changes in epigenetic markers in poultry: prenatal supplementation of zinc increases histone H3K9 acetylation (Sun et al., 2018), and folic acid supplementation influences IGF gene methylation and expression of methyltransferase enzymes that are essential for DNA methylation (Liu et al., 2016). Prenatal supplementation of vitamin C also increases expression of methyltransferase enzymes (Zhu et al., 2019). Dietary micronutrients and supplements may influence methylation and methyltransferase activity by altering the availability of methyl groups (SAM) needed for DNA methylation (Murdoch et al., 2016). Recent studies further indicate that exposure to maternal hormones is linked to changes in DNA methylation: high concentrations of *in ovo* corticosterone increase offspring GC receptor methylation and decrease receptor protein expression in the chicken hypothalamus (Ahmed et al., 2014). By contrast, in wild birds (great tit *Parus major*), physiologically relevant egg corticosterone levels were found to decrease GC receptor methylation (but only at specific sites), but not gene expression (likely related to small sample size; Hukkanen et al., 2023). To understand the reasons for inconsistent patterns, replication and more detailed analyses of gene target regions is needed, as patterns may differ across specific gene regions.

In addition to prenatal resource availability, early postnatal macro- and micronutrient availability has been found to influence DNA methylation patterns in chickens (reviewed by Dunislawaska et al., 2022; Murdoch et al., 2016): Similarly to prenatal nutrition, certain supplements could influence DNA methylation via the availability of SAM: for example, betaine supplementation increased methylation of the lipoprotein lipase gene (site specifically) with an associated decrease in expression (Xing et al., 2011). Interestingly, probiotic and antibiotic exposure has been shown to lead to changes in the microbiome and decreased overall methylation in the gut (Ognik et al., 2020), linking changes in the microbiome with epigenetic markers. In non-model species, effects of food quality have not yet been studied, but in zebra finches (*Taeniopygia guttata*) brood size (an index of food quantity) positively correlate with overall whole-blood methylation levels in nestlings (Sheldon et al., 2018), and enlargement of broods increases methylation of GC receptors even in 2- to 4-year-old adults (Jimeno et al., 2019). In another passerine, the great tit, brood size manipulation revealed differential methylation in genes related to development, growth, metabolism, behavior and cognition in nestlings (Sepers et al., 2021). Using correlative data from wild populations, Rubenstein et al. (2016) showed that unpredictability of resources during postnatal rearing reduces GC receptor gene methylation. Furthermore, in chickens, changes in food quantity (through direct food removal) can also lead to changes in the brain in methylation of genes of the stress axis (e.g. corticosterone release factor, CRF) and histone 3 (Xiao et al., 2020; Xu et al., 2012). Taken

together, although still limited, the data suggest that pre- and postnatal resources can induce epigenetic changes, for example, in stress axis (hypothalamus-pituitary-adrenal, HPA)-related genes, which could therefore influence HPA action later in life (see below).

Early-life resource availability and the microbiome

In chicken, prenatal availability of micronutrients, prebiotics or synbiotics (different fibers and dietary supplements influencing bacterial growth) have been shown to influence nestling and juvenile microbiome composition (reviewed by Siwek et al., 2018). These changes are linked to improved physiological (Zhang et al., 2020), immunological (Stefaniak et al., 2020), developmental, and performance traits (Bednarczyk et al., 2016). Yet, to date, studies on the effects of prenatal resources on the microbiome in non-model and wild species are lacking.

Similarly, the influence of postnatal nutrition on the microbiome has been well described in chickens: different fodder (different grains), micro- and macronutrient availability widely influences both diversity and composition of the microbiome in young chickens (reviewed by Shehata et al., 2022; Gabriel et al., 2006). In wild/non-model birds, to date only one study has directly analyzed the effects of food quantity, using a brood size manipulation in great tits, and no effects on the microbiome diversity were observed (Liukkonen et al., 2022). Studies from wild bird populations do show that early-life habitat (such as deciduous or coniferous forests) or location contributes to differences in both diversity and composition of the microbiome (greater flamingos, *Phoenicopterus roseus*, Gillingham et al., 2019; great tits, Goossens et al., 2022), yet it is unclear whether resource availability or quality are the drivers underlying such differences. Therefore, experimental studies on the influence of early-life diet quality on the microbiome and its long-lasting effects on the host are needed in wild populations.

Early-life social environment and developmental plasticity in birds

Early social environment shapes both avian physiology (such as the stress axis) and behavior in adulthood (Brandl et al., 2019; Mariette et al., 2013; Müller et al., 2011; Grace and Anderson, 2018). For example, in songbirds, the early-life social environment has been found to influence song learning and plasticity (Rundstrom and Creanza, 2021). Here, given the scarcity of data for particular categories of social environment or interactions, social environment is defined broadly to include both intraspecific interactions (such as sibling competition) and interspecific interactions (such as predation).

Early-life social environment and epigenetics

Compared with resource (nutritional) availability, the mechanisms underlying social environment-related developmental plasticity are less understood in birds, and only a few studies have explored this question. In chickens, solitary rearing has been shown to influence DNA methylation patterns in genes related to cancer in juveniles (Pertille et al., 2017). In zebra finches, embryo exposure to heterospecific social cues or silence increased hypothalamic whole-genome DNA methylation compared with exposure to conspecific social cues (Antonson et al., 2021), but methylation of specific target genes was not analyzed. However, prenatal (light) cues from siblings, reflective of postnatal sibling competition, did not influence DNA methylation of GC receptors in yellow-legged gulls (*Larus michacellis*; Ruiz-Raya et al., 2023). Another key element of the social environment is predation risk. Interestingly, a recent study showed that prenatal exposure to (acoustic) predator cues decreases DNA methylation of the GC receptor shortly after hatching in yellow-legged gulls, but the strength of this effect varied between years

(Ruiz-Raya et al., 2023). More studies on different aspects of the social environment (such as competition, social hierarchy position, social instability, predation, and level of parental care other than nutritional resources), both pre- and postnatally are needed to draw conclusions about the role of epigenetics in mediating developmental plasticity to social environment (see Franklin et al., 2010; Weaver et al., 2004 for other taxa).

Early-life social environment and the microbiome

Surprisingly, to date the effects of the early social environment on the microbiome have not been thoroughly studied. An interesting case study compared microbial communities of juveniles incubated and raised by conspecific (zebra finch) and heterospecific (Bengalese finch, *Lonchura striata domestica*) foster parents, and found that the microbiome community composition resembled those of their foster family, emphasizing the importance of the social environment (Maraci et al., 2022). In later life, the early social environment continued to influence the microbiota, but genetic background (species) increased in importance (Maraci et al., 2022). However, in another system in which brood parasitic cuckoos (*Clamator glandarius*) were raised in magpie (*Pica pica*) nests, species-specific variation in the microbiome composition was reported, suggesting that genetic background rather than social environment can explain microbiome variation (Lee et al., 2020). Again, it remains to be studied how other social environmental cues, known to influence an organism's adult phenotype, affect microbiome diversity and composition.

Early-life thermal environment and developmental plasticity in birds

The majority of literature on avian developmental plasticity and its underlying molecular mechanisms concerns the effects of pre/postnatal temperature, including both developmental temperature stress studied in poultry (reviewed by Wada and Coutts, 2021), and temperature variation experienced by birds in wild populations (reviewed by Andreasson et al., 2020). Pre- and postnatal thermal challenges in poultry affect thermoregulatory capacities in adulthood, but the direction of this effect depends on the duration and severity of the thermal challenge: repeated cold/heat exposure generally leads to negative consequences to offsprings' (thermal) performance in adulthood, whereas a short-term exposure in a specific time-window leads to acclimation with associated changes in, for example, neurophysiology (Nord and Giroud, 2020; Wada and Coutts, 2021). Thermal challenges pre- or postnatally also influence post-hatching growth, metabolism, food-conversion efficiency, and morphology (DuRant et al., 2013; Loyau et al., 2015). Although in non-chicken models similar effects can be seen post hatching, long-term data on thermal traits, such as cold tolerance, in adulthood are limited (Nord and Giroud, 2020; Andreasson et al., 2020). However, early thermal conditions have been found to influence a plethora of related physiological traits, such as mitochondrial function (Pacheco-Fuentes et al., 2023; zebra finch), oxidative stress following exposure to heat stress in adulthood (Costantini et al., 2012; zebra finch), beak thermal physiology (Burness et al., 2013; Japanese quail), and even survival (Costantini et al., 2014; zebra finch).

Early-life thermal environment and epigenetics

Prenatal ambient temperature has been found to influence multiple epigenetic regulatory mechanisms (reviewed by Xu et al., 2022). Prenatal thermal challenge can influence histones, especially H3K4 methylation, associated with neurodevelopment in the hypothalamus of juvenile chickens (David et al., 2019), suggesting that neurogenesis could play a role in adaptation to heat stress later in

life. Whole-genome methylation data from juveniles with increased incubation temperature revealed a large number of differentially methylated CpGs that were associated with heart developmental processes, including cardiomyocyte proliferation and differentiation in chickens (Corbett et al., 2020) and methylation and expression of myogenesis-related genes in Peking ducks (*Anas platyrhynchos domestica*; Wang et al., 2019). In chicken, methylation of the heat shock protein (HSP) genes, essential for coping with heat challenges, was found to increase after prenatal heat stress exposure, and was associated with lower HSP expression (Vinoth et al., 2018).

Probably the most convincing evidence of epigenetic mechanisms mediating developmental plasticity in birds comes from studies on postnatal thermal adaptation in chickens. Exposure to high heat shortly after hatching led to multiple epigenetic changes 1 week later: methylation of the *CHR* gene was lower, and its expression higher in individuals exposed to high heat (heat vulnerable) compared with low heat (heat-resilient) or control individuals (Cramer et al., 2019). Some of these patterns can be explained by corresponding changes in enzyme activities: an increase in demethylation enzyme activities (TET) and decrease in methylation enzymes (methyltransferases) in the low-heat group compared with the high-heat group was observed (Cramer et al., 2019). Furthermore, histones were also influenced by postnatal heat: exposure to high heat increased histone H3K27 acetylation of the *CRH* intron, which increases chromatin accessibility, therefore increasing transcription of *CRH* (Cramer et al., 2019). Furthermore, in another study, changes in the methylation level of CpG sites and histone modifications in the *BDNF* gene (important, for example, in the sensory nervous system and memory) were observed during the acquisition of thermal tolerance on the third day after hatching (Yossifoff et al., 2008). Lastly, a role of small RNAs has also been discovered: miRNAs have been shown to decrease expression of the histone-modifying methyltransferase enzyme *EZH2*. *EZH2* further alters methylation of histone H3 at lysine 27 in the hypothalamus during the critical period of thermal control establishment, a phenomenon that seems necessary to regulate long-term thermal responses (Kisliouk et al., 2011). Note that all the studies described above only considered heat stress; cold stress/adaptation-related epigenetic mechanisms are not understood in birds despite cold exposures during embryogenesis being known to increase cold tolerance in chickens (Shinder et al., 2011; 2009). Furthermore, data from species other than poultry are lacking.

Early-life thermal environment and the microbiome

Although the potential effects of prenatal thermal conditions on the microbiome have not yet been studied, there is evidence that postnatal heat stress influences the microbiome, in terms of both the microbiome composition and diversity in chickens (e.g. *Firmicutes* increased, *Bacteroidetes* decreased; Shi et al., 2019; Cao et al., 2021). Heat stress in poultry can cause intestinal disorders, leading to imbalances between beneficial bacteria and pathogenic bacteria; heat stress also seriously affects the metabolism of probiotics in the intestine and reduces the synthesis of SCFAs (Chen et al., 2022; Cao et al., 2021). Cold stress in chickens has also been found to influence the microbiome composition when young (Yang et al., 2021). In wild birds, the effect of postnatal temperature has been recently studied: in eastern bluebirds (*Sialis sialis*) and tree swallows (*Tachycineta bicolor*), moderate warming during the nestling stage tended to lead to lower microbiome diversity (Ingala et al., 2021), whereas experimental cooling of the nesting environment for pied flycatchers (*Ficedula hypoleuca*) of two degrees did not influence nestling microbiome diversity or composition (S.R. et al., unpublished data). Therefore, more data

is needed for both pre- and postnatal cold and heat stress conditions to understand the potential role of the microbiome in mediating developmental plasticity to thermal variation.

Early-life anthropogenic stressors and developmental plasticity

Anthropogenic stressors, such as pollutants, noise and artificial light have long-lasting effects on offspring physiology and survival (e.g. Szulkin et al., 2020). Organisms can be exposed to various anthropogenic pollutants prenatally via pollutants transferred to eggs or postnatally via diet (e.g. Ruuskanen et al., 2014; 2020b). The mechanisms underlying these non-adaptive changes have only recently started to be discovered (Head, 2014).

Early-life anthropogenic stressors and epigenetics

In contrast to other early-life environmental factors, literature on the effects pollution/toxic substances and other human-derived stressors have on mechanisms of developmental plasticity is rather broad in wild species. A recent study on house wrens (*Troglodytes aedon*) reported differences in methylation patterns of genes related to mechanosensory behavior between hatchlings of urban and rural populations (von Holdt et al., 2023), which suggests that exposures at the prenatal stage (via egg resources or incubation behavior) or genetic background contributes to these differences. Furthermore, an urban rearing environment strongly affected methylation of genes related to metabolism, e.g. respiratory electron transport, electron transport chain, mitochondrial respirasome, oxidative phosphorylation and cellular respiration (von Holdt et al., 2023). Similarly, data from a great tit population exposed to long-term pollution because they were situated close to a copper smelter showed that genes related to nervous development and the stress axis were differentially methylated compared with nestlings in an unpolluted population (Mäkinen et al., 2022). However, correlative data from polluted/urban populations cannot pinpoint whether the effect on methylation patterns is related to the chemical pollutants directly, or if it is due to indirect changes in diet or other habitat characteristics in wild populations. Two experimental studies in great tits manipulated environmentally relevant early-life exposure to pollution, namely lead (Mäkinen et al., 2022) and arsenic (Laine et al., 2021), testing the direct effect of pollutants on methylation. Both lead and arsenic exposure led to altered methylation patterns in genes related to development (Mäkinen et al., 2022; Laine et al., 2021), yet, importantly, the number of differentially methylated genes was much lower in birds with direct metal exposure than in birds inhabiting polluted habitats, suggesting that the combination of both direct and indirect pollution effects can have larger influence on DNA methylation patterns. Similarly, effects seen at the phenotypic level (development, oxidative stress, survival) have been shown to be weaker after direct exposure to metals compared with the effects of indirect environmental changes (Eeva et al., 2014). In addition to metal pollution, a recent study using the pesticide permethrin (applied to nest material) reported that the methylation of multiple genes related to cellular signaling and endocrine function was affected by pesticide exposure in zebra finch nestlings (McNew et al., 2021). Differences in methylation patterns between urban and rural populations of great tits have also been reported in adult birds (Watson et al., 2021; Caizergues et al., 2022; Riyahi et al., 2015), yet whether these differences are of developmental origin or are reversible is not fully understood.

Early-life anthropogenic factors and the microbiome

Anthropogenic stressors and pollutants can influence and disrupt microbiome diversity and composition, in both poultry and wild

bird populations. Some pollutants, such as the most widely used herbicide glyphosate, have direct antimicrobial properties (Ruuskanen et al., 2023). Early postnatal exposure to glyphosate in Japanese quails has recently been found to influence microbiome composition, specifically taxa such as *Lactobacillus*, along with causing changes in growth, hormone levels, and antioxidant defenses (Ruuskanen et al., 2020a,b). Postnatal exposure to the pesticide thiram, microplastics, and the heavy metals mercury, chromium and copper all led to declined diversity and altered microbiome composition in chickens, with associated changes in growth and the gut metabolism (Wu and Su, 2022; Li et al., 2023; Zhou et al., 2020; Li et al., 2021; Huang et al., 2021). Yet one limitation in all of these exposure studies is that the microbiome was characterized after continuous exposure; therefore, it is not understood whether these changes in the microbiome are permanent, reversible, or partly reversible (founder effects). However, some of the studies reported changes in gut histology (Zhou et al., 2020) that are likely to be long lasting, and therefore potentially permanently influence the adult microbiome. Furthermore, extrapolating these kind of exposure studies to ecologically relevant effects in wild populations should be done carefully, as the doses used are often larger than actual exposures in wild populations.

Do early-life changes in molecular markers associate with later-life phenotype?

The reviewed literature suggests that epigenetic markers and the microbiome are influenced by various early-life environmental attributes. The key challenge is to link these changes to actual phenotypic changes in adult traits to demonstrate causal effects. However, for methylation changes, many studies currently use methodologies that can only detect changes in overall methylation, which are difficult to link to specific changes in the adult phenotype. Also, many studies using genome-wide analyses, which have discovered epigenetic changes in certain genes, have not yet been followed up with expression studies on the putative target genes. Simultaneous analysis of both methylation and expression of genes found to be differentially methylated, as well as including adult phenotype, is an obvious first step in trying to link changes in epigenetic markers to developmental plasticity. Furthermore, poultry studies provide some interesting methods (e.g. Kislouk et al., 2017), for example *in vitro* methylation analyses in which the target sequence can be ligated into pGL-basic plasmids, methylated *in vitro* (CpG methyltransferase and its substrate) and then reared under the environmental stress. Currently, such approaches require specific expertise and funding, and are not feasible for many biotic stressors that cannot be applied to cells.

Similarly, for the microbiome, simple metrics of change in microbiome diversity are difficult to link to adult phenotypic traits. For associating changes in the microbiome to phenotype, identification of key groups, species, and strains of bacteria, and, most importantly, their functions and microbial metabolites is needed. To study this, metagenomic and metatranscriptomic methods can be applied to produce microbial functional analyses, combined with metabolomic assays. Once identified, metabolites such as SCFAs in early life can be directly manipulated to reveal links between microbiome function and adult phenotype.

A putative pathway through which many early-life environmental effects could permanently influence adult phenotype, and in which epigenetic and microbiome changes could play a role, is via permanent changes in a key regulatory axis, the HPA axis. Across taxa, it is well established that various early-life environmental stressors exert organizational effects on the HPA axis and

glucocorticoid production (Lupien et al., 2009; Mcmillen and Robinson, 2005; Schoech et al., 2011). In birds, stress-induced corticosterone levels (but not baseline) are often elevated by early-life stress (Wada and Coutts, 2021), although the effect is not universal (e.g. Zimmer et al., 2013; Schmidt et al., 2014; Goerlich et al., 2012; Grace et al., 2020). Prenatal stress has also been found to alter GC receptor expression, yet the direction of this change has been found inconsistent across studies (Zimmer and Spencer, 2014; Ruiz-Raya et al., 2023). The literature reviewed here suggests that changes in both epigenetic markers and microbiome composition following different early-life environmental stressors could be linked to permanent changes in HPA-axis function, which in turn could mediate many downstream effects on phenotype and fitness. First, several of the previous examples of early-life environmental effects (biotic and abiotic) on epigenetic markers show altered methylation patterns of histones, GC receptors, CRH or other components of the HPA axis (Table 1). Second, prior literature has demonstrated a bidirectional connection between the gut and the HPA axis: research on rodent models has shown how GCs can induce changes in intestinal motility and permeability, and cause intestinal inflammation, all of which can have lasting effects on gut bacterial communities (Dinan and Cryan, 2012); changes in the gut microbiome can further influence the HPA axis, for example via the vagus nerve (Dinan and Cryan, 2012). Recently, an association between GC levels and the gut microbiome has also been observed in birds; for example, Japanese quails differing in stress response exhibited differential microbiome (Lyte et al., 2021). It remains to be studied how general the phenomenon of HPA axis-driven developmental plasticity is across stressors and species.

Challenges and future directions

This literature review on the putative mechanisms underlying developmental plasticity in birds has raised several questions and challenges in the field, which will be discussed briefly below.

Are there permanent effects of early-life environmentally induced changes on epigenetic markers and the microbiome?

The concept of developmental plasticity assumes that permanent changes in phenotype are driven by permanent changes in the underlying mechanisms, such as epigenetic marks. The relative stability of early-life changes in both methylation and the microbiome is poorly understood, especially in non-model bird species, as most studies measuring epigenetic markers or the microbiome are limited to the developmental or juvenile stage (see Tables 1, 2). Of the reviewed studies, only four measured the target traits after more than 3 months of age. In poultry models, very few studies measure the responses after 40–50 days of age, owing to the short rearing protocols in poultry. Most data from wild bird species concerns the nestling stage, and long-term studies in wild populations are often hindered by the ability to recapture birds as adults. An alternative to studying long-lasting effects in non-model species is through captive rearing; this is feasible for some species, e.g. passerines. However, it is known that not all epigenetic patterns are temporally stable, as an age-related decrease in global methylation has been reported in chicken (Gryzinska et al., 2013), and short-term temporal changes in methylation patterns occur in adult birds during breeding (Viitaniemi et al., 2019; Lindner et al., 2021; great tits). Yet all genes may not follow the global methylation pattern and could even exhibit opposite trends (De Paoli-Iseppi et al., 2019); therefore, characterization of longitudinal patterns of particular markers is needed. Concerning the microbiome, emerging studies in wild birds show that there is

some stability of the early community, but other factors (e.g. genetics) increase in importance with age (Maraci et al., 2022). In barn swallows and zebra finches, temporal stability was found to be relatively low, but consistent, especially for some key taxa (Kreisinger et al., 2017; Benskin et al., 2010). Importantly, many bird species, especially migratory species, encounter very different environments during their lifetime; therefore, if horizontal transfer of microbes from the environment contributes to the microbiome, stability may be low although the contribution of horizontal transfer in adulthood is not yet well understood. All in all, more work on the temporal stability of epigenetic markers and the microbiome is needed to understand in which circumstances early-life effects can be long-lasting.

Differences in mediators of developmental plasticity depending on the ontogenetic stage

Both the pre- and postnatal environment can induce changes in both epigenetic marks and the microbiome, yet a comprehensive analysis comparing different stages in ontogeny is missing, and therefore the role of the timing of exposure is difficult to judge. Importantly, altricial and precocial species largely differ in their pace of development: for altricial birds, thermoregulation, motor skills and cognition only develop long after hatching compared with precocial species. This means that the sensitive windows for early-life environmentally induced changes may also differ. Chicken studies have revealed sensitive windows for thermal adaptations, both during embryonic development and shortly post-hatching (e.g. Yossifoff et al., 2008), whereas for altricial species data are lacking. Also, the effects of early-life stressors can vary depending on the ontogenetic stage at which they are applied (e.g. Marasco et al., 2012; Japanese quail). In the future, such sensitive periods for other environmental influences should be characterized, taking into account the species ecology and evolutionary history.

Broadening the range of molecular pathways

A significant gap exists in our understanding of which molecular mechanisms underlie developmental plasticity. For epigenetic markers, besides a few poultry studies, most data relate to DNA methylation, and data on the importance of small RNAs and histone modifications are limited. Small RNAs and histone modifications are widely known drivers of cellular and whole-organism processes and traits, as shown in other model systems (Jaenisch and Bird, 2003) and, therefore, in the future such mechanisms should be addressed for a more complete understanding of the underlying mechanisms. Concerning the microbiome, studies have focused on only a part of the existing variation; most studies to date in avian research, especially in early-life research, concern data on the presence/absence of certain groups or species of microbes. Owing to practical limitations (most studies use 16 s RNA amplicon sequencing), species level data on microbes are also missing, thereby ignoring potential changes in the genetic background and functions of the very same microbes and communities. This clearly calls for more studies on functional aspects of the microbiome. Furthermore, current microbiome studies are almost solely focused on bacteria, but it is increasingly understood that the mycobiome, i.e. fungal microbes, can also contribute to host physiology (Davies et al., 2022).

Generalization of results on early-life effects of epigenetics and the microbiome across taxa

It is evident that current data on avian early-life effects originate from two separate research lines: precocial poultry and altricial wild

species. Also, within the studied non-model species, most examples concern passerines and a limited number of species have been investigated. Although poultry studies are excellent for mechanistic understanding, we do need to consider that domestication can lead to strong artificial selection, which can influence responses and their interpretation. Furthermore, the simplistic captive environment heavily affects some mediators, especially the microbiome (e.g. San Juan et al., 2021). To understand the effects of artificial selection, the patterns described in domestic poultry could be investigated in non-domesticated precocial species, such as the red junglefowl. In wild systems, complexity of the environment and selective pressures exerted can be studied, yet high environmental variation will also increase overall variation in the data, and therefore overshadow some associations. Birds express diverse life histories and adaptations (large differences in growth rate, physiology, dietary niche, social environment, lifespan, migratory/non-migratory lifestyle, to mention a few); therefore, a broader taxonomical coverage is needed to understand and make generalizations about the mechanisms underlying avian developmental plasticity. Luckily, current technological advances, such as new sequencing methods that do not rely on already sequenced genomes (Laine et al., 2022), can facilitate such approaches in non-model species.

To date, studies on developmental plasticity, and especially its mediators, in birds concern single stressors. In natural environments, individuals are, however, constantly exposed to multiple stressors. To understand the capacity of organisms to cope with these changes, such scenarios and their physiological responses should also be modeled. A rare example of a multi-stress experimental design looking into the underlying epigenetic changes was conducted by Chanthavixay et al. (2020), where simultaneous effects of heat stress and pathogen exposure were studied, and changes in histones H3K27 acetylation and H3K4 methylation were reported.

The early-life environmental microbiome as a key environmental factor

Our understanding of the key environmental factors influencing developmental plasticity may also need to be revised. One early-life biotic factor that may have been overlooked is the environmental microbiome (any microbiome external to the organism). The environmental microbiome provides a source for the microbiome, but could also lead to complex selection pressures (e.g. pathogen pressure). For example, before hatching the eggshell microbiome may contribute to the development of the embryos (Nyholm, 2020): in hoopoes (*Upupa epops*), bacteria from the uropygial gland are transferred in a secretion to specialized crypts on the surfaces of eggs during oviposition, where they are hypothesized to protect embryos from infection (Martín-Vivaldi et al., 2014). In many eggs, embryos have a membrane that may prevent microbes from directly interacting with host cells, and currently the data on the embryo gut microbiome is contrasting (Trevelline et al., 2018; van Veelen et al., 2018; Grizard et al., 2015). However, small microbial metabolites and viruses may still be able to reach the embryo (Nyholm, 2020), yet their influence has not been studied in birds. Even more important is the postnatal environmental microbiome, as this can contribute to the development of the offsprings' microbiome via horizontal transfer, which may have long-lasting consequences. The environmental microbiome includes the nest microbiome (in nest material), which is influenced by the parental microbiome and the nest material itself (see 'nidobiome' concept; Campos-Cerda and Bohannan, 2020) and the microbiome in food items (Kreisinger et al., 2017; Chen et al., 2020). In precocial species, parents provide no direct mouth-to-mouth feeding to offspring but can indirectly

influence the nesting microbiome, for example via exposure to parental fecal matter when pecking for food, or via brooding. Future studies should explore the importance of the environmental microbiome for developmental plasticity.

Conclusions

This overview on early environment-induced changes in putative mediators of developmental plasticity, epigenetic markers and the microbiome shows that both abiotic and biotic environmental factors can induce changes in these mechanisms. Yet whether these changes are long-lasting and therefore responsible for developmental plasticity is still poorly understood. The causal links between detected molecular changes and the corresponding phenotypic traits needs to be verified in many cases, and the generality of these patterns across bird taxa, especially in wild populations and species, further studied.

Acknowledgements

S.R. thanks Charli Davies and all other colleagues for inspiring discussions.

Competing interests

The author declares no competing or financial interests.

Funding

This work was funded by the Research Council of Finland (project 342907) and Emil Aaltosen Säätiö.

Special Issue

This article is part of the Special Issue 'Developmental plasticity: from mechanisms to evolutionary processes', guest edited by Patricia A. Wright and Kathleen M. Gilmour. See related articles at https://journals.biologists.com/jeb/issue/227/Suppl_1.

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