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## SYMPOSIUM

# Developmental Stress, Condition, and Birdsong: A Case Study in Song Sparrows

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**Synopsis** Sexual-selection theory posits that ornaments and displays can reflect a signaler’s condition, which in turn is affected both by recent and developmental conditions. Moreover, developmental conditions can induce correlations between sexually selected and other traits if both types of traits exhibit developmental phenotypic plasticity in response to stressors. Thus, sexually selected traits may reflect recent and/or developmental characteristics of signalers. Here, we review data on the relationships between birdsong, a sexually selected trait, and developmental and current condition of birds from a long-term study of a population of song sparrows (*Melospiza melodia*). Field studies of free-living birds indicate that the complexity of a male’s songs, a permanent trait, reflects the size of a song-control region of his brain (HVC), and is correlated with body size and several parameters of immunity, specifically investment in protective proteins. However, the performance of a male’s songs, a dynamic trait, is not correlated to immune investment. Complexity of song is correlated with the glucocorticoid stress-response, and in some years response to stress predicts overwinter survival. Experimental manipulations have revealed that stressors in early life impair development of HVC, but that HVC recovers in size by adulthood. These manipulations result in impaired song-complexity and song-learning, but not song-performance. Experimental developmental stressors also affect growth, endocrine physiology, metabolism, and immune-function, often in a sex-specific manner. Combined, these studies suggest that song-complexity provides reliable information about early developmental experience, and about other traits that have critical developmental periods. Birdsong thus provides a multi-faceted sexually selected trait that may be an indicator both of developmental and recent conditions.

## Developmental stress and developmental phenotypic plasticity

### Developmental phenotypic plasticity

Phenotypic plasticity allows a given genotype to give rise to a variety of phenotypes as a result of interactions with the environment (Whitman and Agrawal 2009). One important form of this plasticity is developmental phenotypic plasticity, in which the environment can alter developmental trajectories that induce variation in adult phenotypes. Such changes often are long-lasting or permanent in nature. Development, then, is an epigenetic process whereby the environment induces differential gene-expression

and thus produces a differentiated phenotype (Waddington 1957).

Two environmental factors widely studied with respect to developmental phenotypic plasticity are diet and exposure to predators. For example, in the moth *Nemoria arizonaria* individual caterpillars develop one of two distinct phenotypes depending on the diet to which they are exposed. Broods that hatch in the summer feed on oak leaves and resemble oak twigs whereas broods in the spring feed on, and resemble, oak catkins (Green 1989). In addition to the effects of diet, cues from predators or other stressors can also induce developmental phenotypic plasticity

in a variety of species. For example, in response to chronic exposure to predators, amphibian tadpoles develop smaller trunks and larger tails, which enables enhanced burst-locomotion during escape (Benard 2004). Such phenotypic plasticity induced by exposure to predators may be mediated by glucocorticoid hormones (Middlemis Maher et al. 2013). Indeed, glucocorticoid hormones increase in response to many environmental stressors and have long-lasting effects on many aspects of the adult's phenotype (Welberg and Seckl 2001; Harris and Seckl 2011). Therefore, a wide range of environmental factors may influence development by affecting glucocorticoid hormones.

In some cases, phenotypic plasticity in response to developmental stressors may be a form of phenotypic programming that allows an individual to be well matched to a particular environment (environmental matching hypothesis; Monaghan 2008). This is illustrated by the example of *N. arizonaria* (described above) in which caterpillars exhibit phenotypic plasticity in response to variation in diet, which allows them to maximize their camouflage and avoid predation (Green 1989). On the other hand, changes induced by exposure to developmental stressors may simply represent an individual making the best of a bad situation. For example, in western spadefoot toads (*Scaphiopus hammondi*), experimentally simulating the drying of a pond causes tadpoles to accelerate maturation and complete metamorphosis at a younger age (Denver et al. 1998). This response may be advantageous in the short-term because it allows the young animal to escape the deteriorating conditions but appears to come at the cost of decreasing body size at metamorphosis (Denver et al. 1998), which may decrease fitness (Denver and Crespi 2006). In this case, the phenotypic response to stress decreases the adults' fitness but is necessary in order for the young animal to survive the harsh conditions experienced in the larval stage; thus, the animal is making the best of a bad situation. Hypotheses regarding how developmental stressors affect adults' traits must thus consider whether the developmental phenotypic plasticity represents adaptive environmental matching or compensation for a bad situation.

### Developmental phenotypic plasticity and ornaments

Many physiological, morphological, and behavioral traits exhibit developmental phenotypic plasticity in response to environmental stressors and/or glucocorticoid hormones. Interestingly, this includes sexually

selected ornamental traits. For example, exposure to elevated levels of corticosterone (CORT) affects melanin-based coloration of plumage in barn owl nestlings (*Tyto alba*) (Roulin et al. 2008), which is a sexually selected trait in this species (Roulin 1999). In addition, fluctuating asymmetry (small deviations from symmetry in bilaterally symmetric traits) of visual or morphological traits may be involved in mate-choice in some species (Møller 1992; Thornhill and Gangestad 1994) and is affected by a variety of environmental stressors including restriction of nutrients, extreme temperatures, and exposure to parasites and pathogens (Parsons 1990). Since these sexually selected traits exhibit developmental phenotypic plasticity in response to environmental stressors, they may serve as reliable indicators of the quality of an individual's ontogeny (Spencer and MacDougall-Shackleton 2011).

Birdsong is another developmentally plastic sexually selected trait that may serve as a reliable indicator of the signaler's early ontogeny. Indeed, the developmental-stress hypothesis (originally called the nutritional stress hypothesis) proposes that exposure to developmental stressors maintains the honesty of birdsong as a signal (Nowicki et al. 1998, 2002). Most songbirds learn their songs early in life during the same time that the neural regions specialized for the learning and production of song (collectively referred to as the song-control system) are developing. During this time, altricial songbirds are likely to be exposed to a variety of environmental stressors, especially nutritional stress since they are entirely dependent on their parents for food. This hypothesis proposes that individuals who experience fewer stressors early in life, or are more resistant to stressors, will experience superior neural development and consequently produce the highest quality songs during adulthood (Nowicki et al. 2002). In this way, the learned features of song serve as reliable indicators of a male's early ontogeny and his subsequent phenotype as an adult. In support of this hypothesis, many studies have now shown that both the song-control system and the production of song are affected by exposure to developmental stress (reviewed by MacDougall-Shackleton and Spencer [2012]). For example, zebra finches exposed to food-restriction or treated with CORT early in life had smaller volumes of HVC in adulthood (Buchanan et al. 2004) and also produced songs that were shorter and contained fewer syllables (Spencer et al. 2003).

In addition to song, stress during early life has long-term effects on many other physiological and behavioral traits (Welberg and Seckl 2001; Harris and

Seckl 2011). For example, in songbirds, early-life stress may affect the immune system (Buchanan et al. 2003), regulation of the hypothalamus-pituitary-adrenal (HPA) axis (Pravosudov and Kitaysky 2006), and spatial learning (Farrell et al. 2011). In many species of songbirds, song correlates to measures of males' phenotypic quality and condition (Nowicki et al. 2002). In females, developmental stressors could affect song development in those species in which females sing, the ability of females to perceive song, and/or the preferences of females for different variants of song (Woodgate et al. 2010, 2011; Schmidt et al. 2013b). Thus, stress early in life can induce a variety of kinds of developmental plasticity related to birdsong and numerous other traits.

One hypothesis to explain how song comes to be correlated with these functionally independent traits in adulthood is that the period during which song is acquired may overlap with the development of these other traits, and that song and these other traits both may be affected by early-life stressors. That is, song and other aspects of phenotype may be developmentally correlated traits (Nowicki et al. 2002; Spencer and MacDougall-Shackleton 2011). In addition to the importance of developmental timing, the nature of developmental correlations will also depend on life-history strategies and how an organism may trade-off development of one system over another. Such developmental strategies will vary among species, and often between the sexes (Schmidt et al. 2012b). Developmental phenotypic plasticity in response to stressors may then help to explain how song serves as a reliable indicator of a male's early ontogeny and subsequent phenotype as an adult, as well as how song is correlated to other traits.

## Stress, development, and song in song sparrows

### Song in song sparrows

In this review, we highlight research from a long-term study of a population of song sparrows (*Melospiza melodia melodia*) breeding in eastern Ontario, and use these data to test predictions from the developmental-stress hypothesis and the framework outlined above. Our group has studied this population since 2003, both monitoring breeding biology in the field and conducting controlled experiments with captive birds. Birds in this subspecies are migratory, but return to breeding territories with high philopatry. We can thus follow birds from their first breeding season to death. Individual males in this population sing repertoires of 5–12 song-types (MacDougall-Shackleton et al. 2009a), composed of

about 20–48 distinct elements or syllables (Stewart and MacDougall-Shackleton 2008). In contrast to non-migratory western song sparrows (*M. m. morphna*) (e.g., Beecher et al. 2000), but similar to a migratory Pennsylvanian population of *M. m. melodia* (Hughes et al. 1998), males in our study population often share syllables with neighbors or other males at the site, but very rarely share complete song-types (Lapierre et al. 2011). Males with a greater number of locally common syllables in their songs have more locally typical neutral-locus genotypes, suggesting that the sharing of syllables reflects their population of origin; such males also have reduced indicators of physiological stress (Stewart and MacDougall-Shackleton 2008).

The different timescales along which different aspects of birdsong develop make song sparrows' song a compelling trait with which to examine predictions stemming from the developmental-stress hypothesis. Because song sparrows do not add to, or alter, the content of their repertoire after reaching adulthood (Nordby et al. 2002), repertoires of songs and syllables are established early in life and may thus provide a signature of early development. Although these two measures of song-complexity are strongly positively correlated, the size of their repertoires of songs and syllables may nonetheless convey distinct messages regarding the quality of the singer (MacDougall-Shackleton et al. 2009a). Other aspects of song, such as output, vocal consistency, and use of repertoire, are dynamic rather than permanent, presumably reflecting long- or short-term variation in the singer. For example, consistency or stereotypy of the song varies with the status of infection in white-crowned sparrows (*Zonotrichia leucophrys*), suggesting that this aspect of song may reflect current status of health of the singer (Gilman et al. 2007); characteristics of the species-typical song also vary seasonally in song sparrows and white-crowned sparrows (Smith et al. 1997a, 1997b). Song thus potentially can provide information regarding early development as well as about more recent experience.

In song sparrows, song appears to play a role both in the defense of territories (e.g., Beecher et al. 2000) and in attraction of mates (e.g., Reid et al. 2004), providing an example of a sexually selected trait. The developmental-stress hypothesis predicts that song in this species likely indicates early development and thus should be correlated with other developmentally plastic traits. Below we review field studies on our population of sparrows that examine potential correlations between song and other traits, as well as experimental studies that manipulate early

development in order to test the developmental-stress hypothesis.

### Field studies of developmentally correlated traits

As noted above, one prediction of the developmental-stress hypothesis is that multiple traits that are sensitive to early stressors should be correlated with each other. We have tested whether a variety of neural, physiological, and behavioral measures are correlated with song in our study population. In one study, we found that the size of song-repertoires was indeed correlated with the volume of a song-control region of the brain (HVC) (Pfaff et al. 2007). This corroborates previously observed correlations between HVC size and the complexity of songs within other species (e.g., Airey et al. 2000) and the correlation between complexity of songs and the size of HVC observed across species (e.g., Székely et al. 1996). In addition, the size of song-repertoires was correlated with an index of body condition (size-corrected mass), and two hematological measures (Pfaff et al. 2007). Birds with larger repertoires of songs were heavier for their size, and had relatively more lymphocytes and polychromasia than did birds with smaller song-repertoires. Thus, the size of the repertoire of songs was correlated with underlying structures of the brain, as well as with current physiological measures, indicating that song could provide receivers with information regarding a singer's phenotype.

### Song and immune-function

Since the conception of the Hamilton–Zuk hypothesis and immunocompetence-handicap hypothesis (Hamilton and Zuk 1982; Folstad and Karter 1992) there has been intense interest in the relationship between sexually selected ornaments, parasites, and immune-function. Many aspects of the immune system require extended and costly development early in life (Klasing 2004). As such, traits such as size of the song-repertoire might be predicted to correlate with allocation of resources to components of the immune system if they are developmentally correlated traits. On the other hand, some aspects of immune-function are costly at the time of an immune response, and these might be predicted to correlate with current physiological state, and perhaps more dynamic aspects of ornaments. We have recently characterized multiple components of the innate immunity in free-living song sparrows using eight immune assays; we compared variation in allocation of resources with different components of innate immunity with characteristics of the song. Cellular phagocytosis-based components of innate

immunity were negatively correlated with non-cellular components of innate immunity, such as natural antibodies and other soluble protective proteins (Kubli and MacDougall-Shackleton 2014). These results indicate, first, that birds may trade-off different components of innate immune-function, and second, that studies that use only one or two assays of immune-function may fail to detect important relationships between immune-function and sexually selected traits. Consistency in singing was not related to cellular versus non-cellular immune-function, suggesting that the dynamic aspects of song are not related to individual differences in allocation of resources to different components of the immune system (Kubli and MacDougall-Shackleton 2014). Size of the song-repertoire, however, was positively related to non-cellular, relative to cellular, components of the immune system, suggesting that birds that suffer from developmental stress may learn fewer songs and also invest less in developmentally costly aspects of the immune system, such as repertoires of natural antibodies. These individuals rely more on cellular components of innate immunity perhaps because they are unable to invest in more costly humoral components of immunity during early life (Kubli and MacDougall-Shackleton 2014). Overall, these results indicate that differential allocation to development of innate components of immunity may be developmentally correlated with song-complexity.

### Complexity of song and response to stress

One trait that is organized by stressors in early life is the stress response itself. In rats, brief maternal separation followed by maternal licking provides a stress-immunizing effect, such that in adulthood these animals have reduced response to a standardized stressor (Liu et al. 1997). In contrast, perinatal exposure to elevated glucocorticoids results in heightened response to stress and impaired negative feedback (Henry et al. 1994). Although there are fewer data available for birds, recent studies indicate that stress in early development has similar effects and increases stress-responses in the adult (Pravosudov and Kitaysky 2006; Spencer et al. 2009).

In free-living song sparrows, the size of the response to stress correlated negatively with song-complexity. That is, birds with the least complex songs increased CORT the most in response to stress imposed by a standardized restraint. This negative correlation was found in two separate studies on different cohorts of birds from our study population (MacDougall-Shackleton et al. 2009b; Schmidt et al. 2012a), suggesting that it is a stable relationship. These findings are also consistent with other studies

indicating a link between HPA function and sexually selected traits (Husak and Moore 2008). However, a recent study of a western subspecies of song sparrows (*M. m. montana*) found different results, with song-repertoire being positively correlated with the CORT stress-response (Grunst and Grunst 2014). Further work is needed to clarify why the complexity of song correlates negatively with stress-response in our population, but positively in another. However, research on rodents indicates that early experience can have quite opposite effects on adults' HPA function depending on the timing and nature of a developmental stressor (see above).

Because song-learning is closed-ended in song sparrows, repertoires of songs and syllables become permanently established during the first year of life. It is thus unlikely that reactivity to stress in adulthood directly affects our measures of the complexity of song. Rather, it is more likely that reactivity to stress and the complexity of songs are developmentally correlated, because both are susceptible to stressors during early life. It is also notable that this correlation was observed, given that reactivity to stress is a highly plastic trait influenced by many factors. It thus appears that some component of the stress response is repeatable for individuals.

The stress-response allows an organism to cope with stressors, but is also shaped by experiences in early life. Thus, we have also assessed whether reactivity to stress (as measured by HPA function) is correlated to other aspects of adults' phenotypes. Birds that had a lower response to restraint not only had more complex songs, but also had more heterophils and higher heterophil to lymphocyte (H:L) ratios. We also assessed negative feedback of the HPA axis using a dexamethasone-suppression test (Rich and Romero 2005). Birds that suppressed CORT to a greater degree following injection of dexamethasone were larger and had fewer heterophils and lower H:L ratios. Thus, song, circulating immune cells, and reactivity of the HPA all appear intercorrelated in song sparrows, supporting the hypothesis that these are developmentally correlated traits.

We have also assessed the relationship between reactivity of the HPA and measures of fitness in our song sparrow population (MacDougall-Shackleton et al. 2013). There has been tremendous interest in potential relationships between CORT and fitness (Breuner et al. 2008; Bonier et al. 2009a). However, the relationship between HPA function and measures of fitness is likely to vary with life-history stage (Bonier et al. 2009b) and as the environment of selection varies (Schoech et al. 2009).

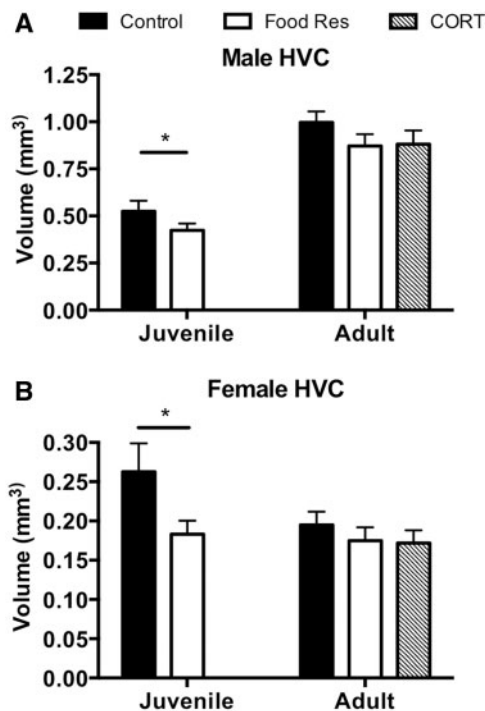
For example, having greater reactivity to stress may increase survival in some environments, but may be overly costly in others. Our results support the conclusion that relationships between CORT and fitness will vary in space and time. In 1 year, we found that song sparrows with higher reactivity to stress were less likely to survive over winter (MacDougall-Shackleton et al. 2009b), but this relationship was absent when assessed again 3 years later (MacDougall-Shackleton et al. 2013). HPA reactivity of males was also correlated with the date their females laid eggs. Females mated to males who had a greater increase in CORT in response to restraint stress (measured prior to nesting) tended to lay their eggs later in the season (MacDougall-Shackleton et al. 2013). This suggests that responsiveness to stress may be related to the onset of breeding and/or to the acquisition of a territory in this species. Thus, HPA function appears to be correlated with other traits such as song, and is potentially linked to fitness consequences, in at least some years.

### Experimental manipulation of developmental stress

In general, experimental tests of the developmental-stress hypothesis have involved manipulating early development and then measuring the songs and/or the brains of adult songbirds (e.g., Nowicki et al. 2002; Spencer et al. 2003; Buchanan et al. 2004). In addition, some studies have assessed the impact of early development on traits other than song. For example, in European starlings (*Sturnus vulgaris*) manipulation of food early in life affected both song and responses of the immune system in one study (Buchanan et al. 2003) and song and spatial cognition in another (Farrell et al. 2011). In song sparrows, we have manipulated stress during development through early restriction and predictability of food, as well as through directly administering CORT to birds. Following this we measured the effects on the brains of juveniles and adults, as well as on a suite of physiological and behavioral traits.

#### Effects on the song-control system

An important prediction of the developmental-stress hypothesis is that exposure to stressors early in life should affect development of the song-control system and ultimately the quality of an adult's song. We have tested this prediction in song sparrows. In the first study, nestling song sparrows had their food restricted or were fed *ad libitum* from 3 days of age onward (MacDonald et al. 2006). Birds were euthanized immediately after the experiment when they were approximately 23 days of age. The size of HVC,



**Fig. 1** The volume of the song-control nucleus HVC in (A) male and (B) female song sparrows exposed to control conditions (*ad libitum* food), restriction of food (food res), or treatment with CORT. Juvenile birds were euthanized at approximately 23 days of age, immediately after the experimental treatments. Adult males were euthanized at approximately 1 year and females at approximately 9 months, several months after the end of the experimental manipulation. In juvenile birds, the volume of HVC was significantly smaller, both in food-restricted males and females, compared with control birds. However, in adult birds, there was no significant difference in HVC volumes between treatment groups, suggesting that HVC recovers from the effects of developmental stress once the treatment subsides. Asterisks indicate a significant main effect ( $P < 0.05$ ) of treatment in juvenile birds. Data are from MacDonald et al. (2006) and Schmidt et al. (2013a).

but not the robust nucleus of the arcopallium (RA) or area X, was smaller in male and female food-restricted birds, compared with control birds, suggesting that stress in early life does impair development of the song-control system (Fig. 1; MacDonald et al. 2006). In a second study, song sparrows exposed to stress early in life (either by restricting their food or by treating them with CORT) were euthanized in adulthood, many months after the end of the imposed stress. In contrast to the earlier study, there was no long-term effect of early-life stress on adult HVC in males (Schmidt et al. 2013a) or females (Schmidt et al. 2013b) (Fig. 1). This suggests that the effects of early-life stress on HVC in song sparrows subside once the stressor is terminated, possibly because

HVC exhibits a large amount of neural plasticity in adult song sparrows (Smith et al. 1997a, 1997b). In addition, in this second study, males whose food had been restricted had smaller volumes of RA than did control or CORT-treated males (Schmidt et al. 2013a). This is in contrast to our first study in which restriction of food had no effect on the volume of RA when birds were euthanized immediately after the period of treatment (MacDonald et al. 2006). Therefore, in song sparrows, RA may be affected by later restriction of food (in the fledgling period), rather than earlier (in the nestling period).

Interestingly, stress may not only affect the song-control system during development, but also appears to have effects in adulthood. For example, implanting adult male song sparrows with CORT decreased the volume and number of neurons in HVC compared with males receiving control implants (Newman et al. 2010). This raises the intriguing possibility that the dynamic features of song (e.g., consistency of songs) that are influenced by seasonal changes in HVC may be affected by exposure to stress in adulthood and may therefore serve as reliable indicators of a male's current, or recent, condition/quality.

#### Effects on production of song by adults

We also investigated the effects of early-life stress on the production of song by adult male song sparrows. As predicted by the developmental-stress hypothesis, males exposed to restriction of food or treatment with CORT during development sang repertoires that contained fewer unique song-types and fewer unique syllables, showing that stress in early life decreases the complexity of the song in this species (Schmidt et al. 2012a). Males whose food had been restricted, but not males who had been treated with CORT, also sang less accurate copies of the tutor's song in adulthood (Schmidt et al. 2013a). Neither treatment affected consistency of the song or its trill-deviation (the speed of frequency modulation within a trill, thought to be constrained by a trade-off between the rate of trill and the frequency bandwidth) (Podos 1997), suggesting that early-life stress does not have long-term effects on vocal performance in this species (Schmidt et al. 2013a). As mentioned above, song sparrows do not alter the content of their song repertoire in adulthood and therefore cannot compensate for a stressful rearing through improving the complexity of their song repertoires or learning song more accurately in adulthood. In contrast, measures of vocal performance, such as consistency and trill-deviation, are dynamic traits that maintain plasticity in adulthood.

Therefore, it is possible that early developmental stress impaired song-learning, but that birds were able to develop normal vocal performance during the months following the end of the imposed stress. Thus, song-complexity and the accuracy with which it is learned may be more indicative of a male's early developmental conditions whereas the consistency of song and its trill-deviation may be more indicative of current or recent conditions. If so, different aspects of song may convey multiple messages with different features of song being indicative of the quality of their environment during different stages of their life history (Møller and Pomiankowski 1993).

In the study described above (Schmidt et al. 2013a), we also determined whether the quality of a father's song was related to the quality of an offspring's song (when adult) following exposure to developmental stress. This was achieved by recording the song-repertoires of the fathers in the field prior to experimentally manipulating their young. Interestingly, paternal song-complexity was significantly related to the offsprings' trill-deviation. Specifically, males with more song-types in their repertoires had sons that produced higher quality trills (Schmidt et al. 2013a). Because nestlings were raised in captivity from a very young age, they had extremely limited exposure to their fathers' songs. This finding, combined with the fact that there was no effect of developmental stress on trill-deviation, suggests that this measure of vocal performance may be more strongly influenced by hereditary factors than by environmental factors. This raises the possibility that males that sing songs of greater complexity, and that presumably may be of higher phenotypic quality, may pass on alleles to their offspring that allow them to produce certain features of song more competently, even in the face of a stressful upbringing.

#### Effects on physiological traits

In addition to song production and the song-control system, we determined the long-term effects of developmental stress on several physiological traits in order to determine whether the same stressors that affect song-learning also affect overall phenotypic quality and condition in adulthood. Indeed, early-life stress seems to have widespread effects on many traits and, interestingly, these effects are often sex-specific. For example, female song sparrows with restricted food during development weighed less than control females during the nestling period; however, food-restricted males weighed the same as controls (Schmidt et al. 2012b). In addition, females

treated with CORT also weighed less than controls, but CORT-treated males actually weighed more than control males (Schmidt et al. 2012b). This suggests that when faced with a low-quality environment during rearing, males preferentially allocate more resources to growth, possibly as a strategy that increases their chance of short-term survival. In this study, there were no long-term effects, either from restriction of food or from treatment with CORT, on adult body size, suggesting that females exhibited catch-up growth. This may come at a cost, however, as both food-restricted and CORT-treated females had elevated standard metabolic rates, which has been linked to catch-up growth (Crisuolo et al. 2008). Stress in early life did not affect metabolic rates in males (Schmidt et al. 2012b).

We have also found long-term effects of stress in early life on endocrine regulation. For example, CORT-treated males and females both exhibited exaggerated increases in CORT in response to a standardized dose of adrenocorticotrophic hormone (ACTH) in adulthood (Schmidt et al. 2014). Exposure to restricted food early in life had no effect on the CORT response to ACTH and neither restriction of food nor treatment with CORT affected the response of CORT to stress imposed by restraint or to a challenge by dexamethasone (Schmidt et al. 2014). Thus, stressors that increase exposure to CORT during development may have long-term effects on the maximum output of adrenal CORT in song sparrows, but do not seem to affect the response to restraint or the efficacy of negative feedback. These observations are consistent with the hypothesis that stressors early in life program the HPA axis to respond to stressors in adulthood. In the same study, developmental stress also affected the hypothalamic-pituitary-gonadal (HPG) axis. Specifically, males treated with CORT during development had higher baseline levels of testosterone in plasma during adulthood (Schmidt et al. 2014). Neither restriction of food nor treatment with CORT affected the response of plasma testosterone to a challenge by gonadotropin-releasing hormone. Interestingly food-restricted and CORT-treated females had lower levels of plasma estradiol in adulthood than did control females (Schmidt et al. 2014). Therefore, early-life stress appears to have opposite effects on levels of plasma sex steroids in males than in females. It is possible that stressors early in life program males to have higher testosterone responses to social challenges, thereby supporting greater aggressive behavior. Thus, both the HPA and HPG axes exhibit evidence for phenotypic programming by stressors experienced early in life.



Finally, stress in early life also has sex-specific effects on functioning of the immune system in song sparrows. For example, males on restricted food or treated with CORT during development exhibited less swelling of the wing-web in response to an injection of phytohemagglutinin (PHA) in adulthood, compared with control males. However, there was no effect of early-life stress on the swelling response to PHA in females (Schmidt et al. unpublished data). Treatment with CORT early in life also affected constitutive innate immunity in males, but not in females. Specifically, CORT-treated males had lower antimicrobial capacity toward a strain of the bacterium *Escherichia coli* but higher antimicrobial activity toward a strain of the fungus *Candida albicans*, compared with food-restricted or control males (Schmidt et al. in review). Combined, these studies suggest that stress in early life has long-term effects on many physiological traits, including growth, metabolism, immune-function, and regulation of hormones. Therefore, the same stressors that affect males' production of song also affect overall quality and condition of adults' phenotypes. Thus, correlations between features of song production and physiological traits in adulthood could arise because both song and these other traits are susceptible to the effects of developmental stress.

## Conclusions

Combined, the studies above provide support for the developmental-stress hypothesis as a framework for understanding song as an indicator signal in song sparrows. Experimental manipulations have demonstrated that early developmental stressors can impair neural development and song-learning, resulting in life-long alteration of a bird's song. In addition, these early manipulations also affect the development of a variety of other traits including metabolism, regulation of hormones, and immune-function. Thus, developmental stress can both affect development of birdsong and induce developmental correlations among traits. In addition, field studies demonstrate that these correlations also exist in the field, providing external validity for our laboratory studies. Importantly, many of the effects of developmental stressors were sex-specific. Thus, males and females appear to differ in how they trade-off allocation of resources in the face of environmental stressors. Traits also varied in how susceptible they were to developmental stress, and some effects appear permanent (e.g., number of song-types) whereas others appear transient (e.g., size of HVC). On the whole, this research tells us that song

sparrows' songs are a reliable indicator of other traits; however, determining exactly how these traits are traded off during development and which traits are most susceptible to developmental stress requires further research.

It will be important to conduct comprehensive field and laboratory studies like those described above on other species. In particular, many songbirds continue to learn songs throughout adulthood, which opens the opportunity that early developmental stress may become decoupled from adults' songs if birds can make up for early impairments in song-learning a song. Species may thus vary in whether song indicates developmental conditions, or more recent and dynamic conditions.

Birdsong is a sexually-selected trait, sometimes referred to as an acoustic ornament, which clearly exhibits developmental phenotypic plasticity. A young songbird's social experiences and exposure to stressors interact in ways that direct the song-learning process, which can result in permanent effects on a trait that is important for lifetime reproductive success. Similarly, many other ornamental traits exhibit developmental phenotypic plasticity, and stressors experienced early in life can affect developmental plasticity in ornaments and other traits (Husak and Moore 2008). Birdsong and the developmental-stress hypothesis thus provide a well-studied example of a more general process. The mechanisms by which stress affects development can be understood within the broad framework of developmental phenotypic plasticity. The effects of stress on ornaments and other sexually-selected traits can be understood as one category of traits that are affected by developmental stress. Future research should expand this approach to clarify how well other ornaments can be understood in the framework of developmental phenotypic plasticity. Such an approach could provide a useful interface between sexual-selection and theories about phenotypic plasticity.

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