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The Importance of Development: What Songbirds Can Teach Us

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Birdsong is a sexually selected signal that is learned early in life. Song learning (imitative vocal learning) by male songbirds has been extensively studied, but other aspects of development are important in birdsong as well. Female experience with song can affect song preferences in some species but not in others. The neural responses to song in females, as assessed by immediate-early gene expression, likewise appear related to early learning in some studies but not others. The development of song preferences by females requires further study to determine how genes interact through experience to produce adult preferences. Male song learning appears to be affected by exposure to environmental stressors during development. Developmental stressors impair both neural development and song learning. Thusly female song preferences may guide females to mate with males who have had benign developmental conditions and/or high developmental stability. These lines of research highlight the importance of considering development whether examining the function or the mechanisms of behaviour.

Keywords: birdsong, acoustic communication, song control system, HVC

Songbirds are one of the most extensively studied taxa in the field of animal behaviour. Several reviews have highlighted the importance of bird studies to the behavioural and life sciences (e.g., Konishi, Emlen, Ricklefs & Wingfield, 1989). In fields such as orientation and navigation, spatial cognition, learning and memory, and adult neural plasticity, studies of songbirds have yielded fundamental insights. The study of birdsong, in particular has proven an important behavioural system in fields ranging from behavioural ecology to cellular and molecular neuroscience.

In his seminal review, Tinbergen (1963) outlined a framework for the study of behaviour that has persisted to this day. He noted that there are four types of questions that can be asked about behaviour: the ultimate questions regarding evolutionary history and current adaptive value, and the proximate questions of physiological mechanisms and development. Tinbergen thusly highlighted development as fundamentally important to a complete understanding of behaviour. The study of birdsong has been so successful in part because it is well studied from all four of the above perspectives (Catchpole & Slater, 2008).

In this review, I highlight research from my lab and others that supports the importance of considering development in our understanding of behaviour. First, I review studies on the importance of early experience on the development of female song preferences.

Then I review the importance of stress during development for the imitative vocal learning of song and other traits. Collectively, these bodies of research demonstrate the importance of early development in a broad suite of learned and nonlearned behavioural traits.

Songbirds and Birdsong

Songbirds (suborder Passeri) are a diverse taxonomic group that comprise almost half of the living species of birds. The songbirds include species such as chickadees, finches, sparrows, robins, and crows. Features of this group include complex musculature of the sound producing organ, the syrinx, and the imitative vocal learning of their songs. Vocal learning appears to have evolved independently in two other avian groups parrots (Psittaciformes) and hummingbirds (Trochiliformes), but most other birds do not appear to learn their vocalizations (Jarvis, 2007). Thusly with the exception of humans, and perhaps a few other groups of mammals, birds demonstrate the most extensive evolution of vocal learning.

Vocal learning was discovered in birds because of the discovery that song often varies geographically within a species (Marler & Tamura, 1964; Thorpe, 1958). Such variation suggested the possibility that these geographic variants (termed song dialects) arise from vocal learning in an analogous way to human dialects. This has been confirmed experimentally in many species of songbirds—birds raised in isolation are unable to learn to sing normal songs, and birds raised with an appropriate tutor learn to imitate it. Although there is variation amongst species in several aspects of song learning (Brenowitz & Beecher, 2005), a general model of birdsong learning has emerged in which young birds must hear conspecific song during an early sensory phase to form an auditory memory of song, then later practise singing and eventually match their vocal output to the stored auditory memory during a sensorimotor phase. Underlying this imitative vocal learning ability is a specialised neural circuit, called the song-control system, that is
absent in species of birds that lack vocal learning (Nottebohm, 2005). The song-control system consists of a caudal motor path, critical for song production, and an anterior forebrain path, important for learning and control by auditory feedback. The brain region HVC (sometimes called the high-vocal centre) is part of both of these paths and is critical for both song learning and production in adulthood (Brenowitz, Margoliash & Nordeen, 1997).

Experience and Female Song Preferences

Male songbirds typically sing much more than females and use song to defend breeding territories and/or attract mates. Although song learning has been extensively studied in males, the role of learning in females has been relatively understudied in regard to song learning in species in which females do sing and in regard to females learning to recognise and prefer certain songs to others (Riebel, 2003).

Just as the discovery of song learning by males stemmed from the discovery of song dialects, the first evidence for female songbirds learning to prefer certain songs to others also stemmed from the study of song dialects. Baker and colleagues (Baker, Spitler-Nabors & Bradley, 1981; Casey & Baker, 1982) conducted a series of experiments demonstrating that female white-crowned sparrows (Zonotrichia leucophrys) prefer their local dialect song over songs of other dialects. This result has been replicated in several other species of songbirds (e.g., O’Loghlin & Rothstein, 1995; Searcy, Nowicki & Hughes, 1997), and in other populations of white-crowned sparrows. For example, we captured juvenile female white-crowned sparrows from Sonora Pass in the Sierra Nevada of California and kept them in an acoustically controlled environment during their first year of life. These females performed more sexual displays in response to playback of song from their natal dialect than to playback of song from the Rocky Mountains or playback of another species’ song (MacDougall-Shackleton, MacDougall-Shackleton, & Hahn, 2001). These results, and other similar studies, are consistent with the idea that young female songbirds form auditory memories of songs early life and use these memories to guide mate choice decisions in adulthood.

There is an important shortcoming of the studies reviewed above, however. None of them fully controlled for potential inherited factors. That is, females from one location may inherit preferences for the local song rather than learn them. Although inherited song preferences may not seem plausible at first, we found exactly that in a different species of songbirds, house finches (Carpodacus mexicanus). Female house finches were captured from the nest in Mississauga, Ontario, and hand raised in the lab. Some females were tutored with either the local song dialect, some with songs recorded in Arizona, and a third group (isolates) were not exposed to song. When the birds were sexually mature we assessed their song preferences. Regardless of early experience, females in all groups preferred the local dialect song (Hernandez & MacDougall-Shackleton, 2004). This suggests that inherited factors, not early experience, guide the development of song preferences in this species. Similar reports of experience-independent song preferences have been reported for other songbird species (e.g., Vallet, Beme & Kreutzer, 1998).

How can we reconcile the evidence for early experience guiding song preferences on one hand, with evidence for inherited effects on song preference on the other? One solution may be that the importance of learning may depend on geographic scale. In the house finch study, the songs used were recorded from opposite sides of North America, a distance far greater than birds would ever disperse. In this case, inherited factors may guide the development of song preferences because the foreign songs were from so far away that they were no longer recognizable as a conspecific signal. At smaller geographic scales, experience early in life may shape song preferences amongst more similar song variants. A similar mechanism occurs in male imitative vocal learning—tutor songs must be close to the young bird’s own species song to be suitable models (e.g., Marler & Tamura, 1964). Inherited factors guide which songs are an appropriate model, then experience guides the development of specific songs.

To directly compare the inherited and experiential factors that guide female song preferences, Hernandez, Pfaff, MacDougall-Shackleton, and MacDougall-Shackleton (in press) recently conducted a study on song sparrows (Melospiza melodia). Young females were removed from the nest from two Ontario locations, about 450 km apart, and were tutored with song from one of the two locations or with no song (isolates). Prior research on this species has shown that females prefer local songs at this geographic scale (Searcy et al., 1997). Because Hernandez et al. used a fully counterbalanced design they could test the relative importance of inherited factors versus female experience in shaping song preferences at this geographic scale. Regardless of geographic origin, females in this study preferred songs that they were tutored with early in life (Hernandez et al., in press). Isolate females, conversely, had no consistent preferences. This finding demonstrates that songs from either location were not inherently more attractive than the other, and that experience early in life guides preferences for local song in this species and at this geographic scale.

Experience and Neural Responses to Song

Auditory processing of song by songbirds appears to take place in forebrain regions including the caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM). Lesions to CMM disrupted normal song discrimination in zebra finches (Taeniopygia guttata; MacDougall-Shackleton, Hulse, & Ball, 1998). Neural activity in CMM and NCM is higher following presentation of conspecific song as compared to other stimuli, as assessed through electrophysiology (e.g., Chew, Mello, Nottebohm, Jarvis, & Vicario, 1995) or measuring the expression of the immediate-early gene Zenk (also known as egr-1). For example, in zebra finches Zenk expression was highest following playback of conspecific song, lower following playback of heterospecific song, and lower still in response to tone bursts or silence (Mello, Vicario, & Clayton, 1992).

Zenk expression in NCM and CMM also appears to correlate with song preferences across a range of species. For example, in female budgerigars (Melopsittacus undulatus, a nonsongbird) playback of more complex songs (which are preferred over simpler songs) results in higher Zenk expression in NCM as compared to playback of less complex songs (Eda-Fujiwara, Satoh, Bolhuis, & Kimura, 2003). In European starlings (Sturnus vulgaris), females prefer longer songs to shorter songs, and this is reflected in levels of Zenk expression in the auditory regions of the forebrain (Gentner, Hulse, Duffy, & Ball, 2001). In female white-crowned sparrows Zenk expression is higher following playback of natal
dialect song than following playback of foreign dialects (Maney, MacDougall-Shackleton, MacDougall-Shackleton, Ball, & Hahn, 2003). Moreover, across birds exposed to natal dialect song the level of Zenk expression is correlated to the strength of song preference as assessed by sexual displays (Maney et al., 2003). Activity in NCM and CMM, then, appears to reflect song preferences, or perhaps attention to or the biological salience of song to a female.

If Zenk expression is correlated to song preferences, how is this response modified by early development? Do levels of Zenk expression in CMM and NCM reflect early experience with songs? Results are mixed (see the review in Hernandez, Phillmore, & MacDougall-Shackleton, 2008). In zebra finches, Zenk response is higher in response to playback of songs heard early in life (Terpstra, Bolhuis, Riebel, van der Burg & den Boer-Visser, 2006) and this has been proposed to result from NCM and CMM as the locus of the auditory memory of songs (Gobes & Bolhuis, 2007). However, such results have not been found in house finches or song sparrows. In these species Zenk responses did not differ following playback of tutor versus other songs, and in both cases Zenk responses were actually highest in birds that had been isolate reared (Hernandez & MacDougall-Shackleton, 2004; Hernandez et al., 2008).

Further work is required to clarify the reasons for differing results across species and laboratories. It is possible that Zenk responses reflect auditory memories in some species but not others. It is also possible that Zenk responses reflect the salience of a signal. The Zenk response habituates following repeated presentation of the same song (Mello, Nottebohm, & Clayton, 1995) and dishabituates following presentation of a novel song or even changing the location of the song playback (Kruse, Stripling, & Clayton, 2004). If the levels of Zenk response to reflect attention or salience, this may be related to auditory memories in some instances but not others. Further experiments are required to test this possibility.

Stress, Development, and Song Learning in Males

My research, and that of other research groups, has highlighted the important role of early experience in the development of female song preferences. Recently, early experience also has been shown to be critical for the development of song quality in males. Amongst species, females exhibit preferences for three main aspects of male song: song complexity, song dialect, and song performance (Nowicki & Searcy, 2005). The relative importance of these song features varies amongst species, but all three reflect, to a degree, how well a male was able to learn to sing.

Why females should attend to the quality of song learning when selecting a mate has long been a puzzle. Typically, for a signal to accurately indicate something about the signaller there must be a cost that prevents low-quality individuals falsely advertising superior status (Searcy & Nowicki, 2005). For song, however, the costs of producing a well-learned song have remained unclear. In the developmental stress hypothesis Nowicki and colleagues (Nowicki, Peters, & Podos, 1998) posited that the costs maintaining the honesty of birdsong are not incurred at the time of singing but through the early development of song.

Young songbirds undergo rapid growth during the nestling stage, and then face nutritional and other stressors when they fledge from the nest and begin feeding independently. It is during this time that the song-control brain regions are developing and the sensory phase of song learning takes place (see Figure 1). Thusly, adult song could act as a reliable indicator of conditions experienced early in development (Nowicki et al., 1998). That is, by attending to the quality of learned song, females may assess the degree to which a male experienced stressors during development, and/or how well that male coped with such stressors.

In the last decade much data supporting the development stress hypothesis has accrued. Experimental manipulations of early development have demonstrated effects on the development of song and the song-control regions of the brain in several species. For example, food restriction or corticosterone administration to young zebra finches results in the development of smaller HVC size and less attractive songs (Buchanan, Leitner, Spencer, Goldsmith, & Catchpole, 2004; Spencer, Wimpenny, et al., 2005). Making food unpredictable to young European starlings similarly reduces HVC size and song-bout length (Buchanan, Spencer, Goldsmith, & Catchpole, 2004; Spencer, Wimpenny, et al., 2005). In domestic canaries, parasitic infection during the fledging stage reduces HVC size and song complexity (Spencer, Buchanan, Goldsmith, & Catchpole, 2003; Spencer, Buchanan, Goldsmith, & Catchpole, 2004). In domestic canaries, parasitic infection during the fledging stage reduces HVC size and song complexity (Spencer, Buchanan, Goldsmith, & Catchpole, 2005). In swamp sparrows, food restriction during the nestling stage reduces the accuracy of song learning (Nowicki, Searcy, & Peters, 2002). In

**Figure 1.** Song learning and developmental stress in song sparrows. The song-control system develops shortly after fledging and as a bird is foraging independently. During the first summer of life the bird undergoes the sensory phase of song learning in which the auditory memories are formed that will guide song production for the rest of the individual's life (d = days). Song learning timelines based on data from Marler and Peters (1987).
sum, there is now substantial evidence that a variety of developmental stressors can impair development of the song-control regions of the brain and song learning.

Research in my lab has been testing the developmental stress hypothesis in song sparrows. Song sparrows are close-ended song learners, with all song learning typically taking place in the first year of life. Male song sparrows in our study population sing a repertoire of 5 to 13 different song types made up of about 25 to 56 different song syllables. Prior work has shown that males with more complex songs (larger repertoire size) have higher reproductive success (Reid, et al., 2004) and females also prefer songs from a local geographic area (Searcy, Nowicki, Hughes, & Peters, 2002). Thusly, song sparrows provide an excellent species to test the developmental stress hypothesis.

In Pfaff, Zanette, MacDougall-Shackleton, and MacDougall-Shackleton’s (2007) study of a population of song sparrows in Eastern Ontario, song repertoire size was correlated to HVC size, consistent with results in other species. This suggests that singing complex songs requires the development of a large HVC. Song repertoire size is also correlated to other traits such as body condition, aspects of immune function (Pfaff et al., 2007), and parental effort (Potvin & MacDougall-Shackleton, 2009). Males with larger repertoires also sing more often (MacDougall-Shackleton, Stewart, Potvin, & Tennenhouse, in press) suggesting they have more time or energy to devote to displaying than males with smaller repertoires. Hand-reared song sparrows fed ad libitum developed a larger HVC than those with a restricted diet (MacDonald, Kempster, Zanette, & MacDougall-Shackleton, 2006). It is thusly plausible song complexity in song sparrows accurately predicts other aspects of a male’s phenotype because song and these other traits are influenced by stressors during development.

The experimental data reviewed above indicate that early stress can impair neural development and song learning. A remaining question, however, is whether this mechanism explains the variation we see in song amongst males in the wild. Just because a mechanism works in the lab does not mean that process is actually important in nature. If the developmental stress hypothesis does explain natural variation in song, we would predict that song should be correlated to other indicators of developmental stress. In great reed warblers (Acrocephalus arundinaceus), song repertoire size is correlated with feather quality, consistent with the idea that both were affected by stress during development (Nowicki, Hasselquist, Bensch, & Peters, 2000). Another trait that may be affected by early stress is the functioning of the stress response itself. Chronic stress during development can result in hyper-reactivity of the stress response in adulthood in mammals and birds (e.g., Hayward & Wingfield, 2004; Macri & Wurbel, 2006; Pravosudov & Kitaysky, 2006). MacDougall-Shackleton et al. (MacDougall-Shackleton, Dindia, Newman, Potvin, Stewart, & MacDougall-Shackleton, 2009) found that song syllable repertoire is correlated with stress reactivity in their population of song sparrows. Males with smaller repertoires show the greatest endocrine responses to a standardised stressor, consistent with the idea that both traits were affected by developmental conditions. More interesting, stress reactivity is also highly predictive of subsequent survival; males with the greatest stress reactivity are least likely to survive to breed the following year. Thusly, variation in song complexity in song sparrows may reflect differential stress reactivity and future survival.

There is now ample evidence in support of the developmental stress hypothesis. This hypothesis has the potential to explain variation in male song as well as variation in female song preferences. There still remain many outstanding questions, however. First, it remains to be determined whether song quality reflects a male’s developmental conditions, his developmental stability in the face of those conditions, or an interaction of the two. This is an important distinction in clarifying whether female mate choice for song results in direct benefits such as parental feeding, or indirect benefits such as genes for high developmental stability. Second, songbirds exhibit seasonal neural plasticity of the song-control system. It is an open question whether stress occurring in adulthood could have an impact on HVC and song. Finally, stress during development affects the brain development of female songbirds as well as males. How developmental stress affects a female’s ability to perceive and respond to song remains unknown. All of these questions are topics of ongoing research in my laboratory.

Summary

Birdsong is extensively studied from both ecological and mechanistic perspectives. Early experience affects both a male’s ability to produce song and female preferences for those songs. Female song preferences appear to be guided by both early experience and inherited predispositions. Future work is required to determine under which conditions early exposure to song can modify song preferences. Male song involves early learning and stressful conditions during this process can impair neural development and song learning. Thusly song can become correlated other traits that are affected by stress during development. Preferences for well-learned songs, therefore, may benefit females by leading them to choose mates who had benign development. These lines of research highlight the importance of development in a complete understanding of both the function and mechanisms of animal behaviour.

Résumé

Le chant des oiseaux est un signal déterminé sexuellement dont l’apprentissage survient tôt dans la vie. L’apprentissage du chant (apprentissage vocal par imitation) par les oiseaux mâles a été étudié amplement, mais d’autres aspects du développement sont également importants en ce qui a trait au chant des oiseaux. L’expérience des femelles avec le chant peut influencer la préférence de chant chez certaines espèces, mais pas chez d’autres. La réponse neuronale au chant chez les femelles, telle que mesurée par l’expression des gènes précoce et immédiat, semble aussi liée à l’apprentissage précoce selon certaines études, mais pas selon d’autres. Le développement de la préférence du chant chez les femelles requiert plus d’études afin de déterminer comment les gènes interagissent au fil de l’expérience pour produire la préférence à l’âge adulte. L’apprentissage du chant chez le mâle semble influencé par l’exposition à des facteurs de stress durant le développement. Les facteurs de stress développementaux nuisent autant au développement neuronal qu’à l’apprentissage du chant. Ainsi, les préférences de chant chez les femelles pourraient guider celles-ci à côtoyer des mâles ayant eu des problèmes de développement bénins et/ou une stabilité développementale. Ces questions de recherche soulignent l’importance de considérer le développement lorsqu’on étudie la fonction ou les mécanismes du comportement.

Mots-clés : chant d’oiseau, communication acoustique, système de contrôle du chant, HVC
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