



Review

The value of comparative approaches to our understanding of puberty as illustrated by investigations in birds and reptiles



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ABSTRACT

This article is part of a Special Issue "Puberty and Adolescence".

Studies of birds and reptiles have provided many basic insights into the neuroendocrine control of reproductive processes. This research has elucidated mechanisms regulating both early development, including sexual differentiation, and adult neuroendocrine function and behavior. However, phenomena associated with the transition into sexual maturation (puberty) have not been a focus of investigators working on species in these taxonomic classes. Research is complicated in birds and reptiles by a variety of factors, including what can be extended times to maturation, the need to reach particular body size regardless of age, and environmental conditions that can support or inhibit endocrine responses. However, careful selection of model systems, particularly those with available genetic tools, will lead to important comparative studies that can elucidate both generalizability and diversity of mechanisms regulating the onset of reproductive maturity.

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Introduction: sexual maturation in birds and reptiles

Puberty refers to the physiological processes by which an individual attains sexual maturity and is thus able to reproduce successfully as an adult. As such, it is a key component of the general process of development. Developmental biology has a long and distinguished history of employing a wide range of species in order to understand the complex sequence of events that underlies how a

zygote is transformed into an adult (Wolper and Tickle, 2010). Curiously, although all vertebrate species go through some process to attain sexual maturity, scientists who study non-mammalian vertebrates generally have not made puberty a major focus of their investigations. Thus, while substantial data are available from a comparative perspective on early brain development of, and adult plasticity in, brain and behavior, this critical phase of maturation is under-investigated.

A common pattern is that experiments completed on the physiology of puberty in non-traditional organisms have come from investigators interested in the control of reproductive cycles. As has been noted by several of these scholars (e.g., Foster et al., 1986; Nicholls et al., 1988), the study of seasonal breeding involving an individual cycling between

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states of being reproductively active or inactive is a potentially powerful model to gain insights into puberty. This developmental stage involves processes that control the transition from being a juvenile that is reproductively inactive to a reproductively functional adult, a process that is to a large degree recapitulated in seasonally breeding adults. Comparative studies of neuroendocrine control of seasonal reproduction have thus been one important source of insight into the physiology of puberty. However, it is not clear whether the mechanisms are fully parallel across these processes or whether the first instance of reproductive competence differs in terms of mechanism or function. In this review, we briefly summarize the current state of knowledge about puberty in birds and reptiles, and will offer some general ideas about the potential value of comparative studies on this topic.

The development of sexual maturity in birds

There are approximately 10,000 extant species of birds present on earth today (Clements, 2007). Nearly 50% of these are members of the order Passeriformes (songbirds; Clements, 2007). Passeriformes typically reach sexual maturity within one year. In temperate zone species this would mean that a nestling hatched in the late spring or summer would either migrate or overwinter and then be capable of breeding the following spring. This sort of pattern or some variant (depending on when breeding occurs and whether the species is highly seasonal) is the most common pattern of puberty among avian species (Follett, 1991). Some species, especially those that live in highly variable habitats such as the Australian outback will reach sexual maturity in less than one year. The zebra finch (*Taeniopygia guttata*) and other estrildid finches are an example of such a pattern of puberty. In larger long-lived species such as member of the Charadriiformes (i.e. gulls and terns), the Procellariiformes (fulmars, petrels and albatrosses), the Pelicaniformes (i.e., gannets, boobies and frigatebirds) or the Ciconiiformes (i.e., storks) the attainment of sexual maturity can be delayed two, three years or even more (Diomedea albatrosses usually require 7 years for example). These patterns encompass pubertal patterns exhibited by the vast majority of avian species. A consideration of what is known about the neuroendocrine basis of these patterns will form the focus of this section on puberty in birds.

What is the pattern of sexual development in birds that mature within their first year of life?

In seasonally breeding birds, the annual cycle of reproductive and non-reproductive activity can in many species be characterized by understanding how the birds' transition among three different physiological states that are defined based on their responsiveness to seasonal variation in photoperiod (Dawson et al., 2001; Nicholls et al., 1988). Lessons learned from the annual cycle of adults are then applied to thinking about the development of a mature reproductive state in juvenile birds. This strategy has been applied in most detail to European starlings (*Sturnus vulgaris*; Follett, 1991). Adult starlings in late fall and winter have regressed reproductive systems (i.e., small gonads and negligible concentration of sex steroid hormones in the blood). Long days in the late spring and early summer stimulate rapid reproductive growth and put the birds in a state of photostimulation. Other factors then act to fine tune the exact timing of breeding. Long days also set into motion another process that leads to the regression of the reproductive system so that the birds stop breeding before food supplies decline and there are no sufficient resources to feed their progeny. This state is known as 'photorefractoriness'. Starlings that are photorefractory will not respond with appropriate reproductive growth to any daylength including constant light. The photorefractory state is usually dissipated over the course of the fall by the experience of short days. Short days essentially re-set the hypothalamo-pituitary–

gonadal (HPG) axis so that the starlings are ready to respond to long days again. Birds that have experienced short days and are ready to respond to long days are said to be photosensitive.

Starlings hatch in the summer when daylengths are long but they do not respond to these with any sign of reproductive development (McNaughton et al., 1992; Williams et al., 1987). It was hypothesized that starlings were born in a photorefractory state and then experienced short days in the fall of their first year which rendered them photosensitive and allowed them to respond to long days and experience sexual maturity during the spring of their first year of life (Farner et al., 1983). A series of clever experiments involving raising young starlings (even while in the egg!) on short days showed that that is indeed the case (McNaughton et al., 1992; Williams et al., 1987). Starlings will start to respond to short days only after they reach adult size at 21 days after hatch. If placed on short days at this time they will start the process of breaking this juvenile photorefractoriness and exhibit changes in reproductive physiology characteristic of sexual maturity as early as day 21 or 28 after that (McNaughton et al., 1992). However, prior to day 21 of life they will not respond to short days. It is not because they are insensitive to daylength; studies of the timing of their pattern of molt indicate that these birds can indeed respond to daylength (McNaughton et al., 1992). The reproductive axis is unable to respond to daylength and therefore exhibit sexual maturity. What is the nature of this deficit? Is it a simple lack of development?

Studies relevant to this question have recently been completed in Japanese quail, a photoperiodic species that breeds after less than one year of life year. These studies investigated the ontogeny of neurons that migrate to the preoptic area (POA) a critical brain area for the control of the HPG axis and reproductive behavior. In contrast to starlings, the young in quail is precocial. The ontogeny of new neurons is largely complete in quail by embryonic day 16, but new neurons are incorporated in a second wave at the onset of sexual maturity in the first spring of life (Mouriec et al., 2011). This second wave that occurs in association with the onset of puberty and the activation of sexual behavior and may be one cellular marker of essential changes needed for puberty to commence.

What is the pattern of sexual development in birds that mature in substantially less time than one year?

Some avian species breed in an opportunistic manner (Hahn et al., 2008). Opportunistic in this case, refers to the fact that some critical resources are unpredictable and species that rely on them respond with a rapid increase in reproductive physiological activity when these resources become available. Species exhibiting this pattern for the organization of reproductive activity can be contrasted with seasonally breeding species that generally track highly predictable resources such as the availability of a food source that emerges every year in a reliable fashion (Wingfield, 2008). One of the most intensively studied opportunistic species is the zebra finch (Zann, 1996). This species lives in the harsh Australian outback and can only breed when the environment improves in response to increases in rainfall. Developing zebra finches can reach sexual maturity within 60 to 120 days depending on their sex and the specific environmental conditions when they are born and develop (Zann, 1996). The development of the HPG axis has not been investigated in detail but once these birds reach sexual maturity they tend to maintain the reproductive neuroendocrine system in a tonically elevated state (Hahn et al., 2008). One possibility is that puberty occurs once the birds develop sufficiently for their HPG axis to be functional. However, this hypothesis would need to be investigated carefully. A related issue is whether "opportunistic" species that occur in diverse taxa and diverse habitats all exhibit a similar pattern of sexual development (Hahn et al., 2008).

What is the pattern of sexual development in birds that mature after their first year of life?

In birds that mature after their first year of life the most obvious hypothesis is that they have evolved a life history strategy of delayed maturity. If this is the case they would not breed in their first year because they are just not mature enough. However, several lines of evidence indicate that this is not the case. For example, seabirds such as Albatrosses do take a long time to mature but they still reach their full body size well before they reach sexual maturity. Also, studies of endocrine changes in species of this sort with this delayed pattern of sexual maturity indicate that the HPG axis is not entirely quiescent prior to the year that these individuals breed. Instead, some species with delayed reproduction start exhibiting reproductive cycles in their first year which are not sufficiently pronounced for the birds to reach full breeding status (Follett, 1991). Thus, the birds are mature in the first year and the HPG axis is active in their first year. Other factors must be in play that result in a delay of full reproduction until the third year or later depending on the species. An interesting example of sexual maturity delayed beyond one year is observed for the “helpers” in cooperatively breeding species (Brown, 1987). In this case, individuals will help with rearing the young sired by another pair (sometimes their parents) rather than going off and breeding on their own (Brown, 1987). The adaptive significance of this behavior is relatively clear as there is often habitat saturation and the individuals cannot increase their fitness but setting up their own territory so they help with another either to promote their indirect fitness by raising more brothers or sisters or they help in order to position themselves to inherit the territory (Brown, 1987). Since individuals in these species very regularly can breed within one year, the question arises as to the physiological basis of this delay in breeding for a year or more. One hypothesis is that the helpers are suppressed by the dominant breeding individuals (Brouwer et al., 2009). This was tested by removing the primary males in cooperatively breeding Seychelles warblers (*Acrocephalus sechellensis*). It was found that the subordinate males did exhibit an enhancement in reproductive activity, at least as measured by changes in the concentrations of testosterone in the blood (Brouwer et al., 2009). These increases though did not reach the concentrations of the male who was previously dominant suggesting that the recovery from this suppression can take a substantial amount of time.

The development of sexual maturity in reptiles

Like birds, almost 10,000 species of reptiles exist; the vast majority of which are squamates (lizards and snakes). However, the literature on puberty in reptiles is extremely thin. A PubMed search of “reptile and puberty” in November 2012 produced nine articles, three that are actually on human pediatrics. Only six relate to endocrine development in non-mammalian vertebrates (two of these are not in English and therefore may be of limited accessibility). *Web of Science* provided four references, three of them overlapping with the non-human articles in PubMed. In contrast, PubMed searches for “human and puberty” and “rodent and puberty” produced almost 24,000 and 2500 references, respectively.

As these numbers suggest, almost nothing is known about factors regulating puberty in reptiles. Work conducted specifically on this developmental stage has mainly been in alligators. These data provide general information on an extended period of puberty in which gonads gradually increase their synthesis of steroid hormones. Animals undergoing this process are capable of responding to seasonal environmental cues that regulate reproductive timing in adults, both increasing gonadotropin release and enhancing gonadal responsiveness (Edwards et al., 2002). Advances in the study of molecular mechanisms in reptiles have recently been facilitated by genomic sequencing. Data are readily available for one species, the green anole lizard (*Anolis carolinensis*; Alfoldi et

al., 2011). Sequence analysis from this species indicates one gene for kisspeptin (*kiss2*) and one for a cognate receptor (*gpr54-a*), suggesting the possibility that these molecules could be important for timing of puberty and control of reproductive function in reptiles as in other vertebrates groups, although perhaps not birds (Tena-Sempere et al., 2012). At this point, though, no detailed studies are available on mechanisms associated with the initiation of puberty in reptiles. In fact, basic knowledge of the HPG axis is lacking; for many reptilian taxa it is not even clear whether one or two gonadotropins exist (Jones, 2011).

Why the paucity of information about reproductive maturation in reptiles? A variety of factors probably contribute, not the least of which is that natural populations are usually investigated, which makes it difficult to get carefully controlled (or timed) data. Depending on the species, the transition from juvenile to adult can take years or even decades, particularly in large reptiles. While the parallel to humans might be useful from a biomedical perspective, interpretations can be challenging due to the difficulty of collecting data through this period. Even within a species, a large amount of variability exists in the timing of puberty; body mass or length is often a better predictor of reproductive maturity than age. For example, female iguanas can experience estradiol surges earlier, but do not ovulate until they reach a threshold size (Pratt et al., 1994). Growth, of course, is affected by numerous factors related to food availability and other features of the local environment, including the history of exposure to optimal temperatures.

While temperate zone reptiles generally reproduce on a seasonal basis, relationships between environmental cues and reproductive physiology are highly variable. Temperature, moisture and photoperiod can all play roles in stimulating gonadal function (Kumar et al., 2011). On a broader scale, the relationship between gonadal function and the display of reproductive behaviors is not necessarily linked. Among the species investigated to date, some reptiles display an ‘associated’ (or pre-nuptial) pattern typical of mammalian species, in which gonadal hormone synthesis and gametogenesis occur just prior to or in concert with mating behavior. However, in others the pattern is dissociated, such that post-nuptial gonadal function is the norm. In extreme cases, gonadal activity increases following the seasonal display of sexual behavior and gametes are stored for the following year for exchange at a time of basal circulating steroids. The dichotomy is not so simple, however, as the two sexes within a species do not necessarily match (one can show an associated pattern and the other dissociated; Crews, 1984). In some snakes, a mixed pattern within males can also occur, in which spermatogenesis begins in the spring but is not completed until a year later; mating can either occur at that time or in the following fall (Taylor and DeNardo, 2011).

The diversity across reptiles thus presents a great challenge and terrific opportunity for the study of mechanisms regulating reproductive physiology.

The difficulty is magnified if one wants to determine factors controlling the first reproductive event, for both practical and theoretical reasons. That is, not only can the conditions of study be complex, but also it is not completely clear how one would even define pubertal onset in some species.

Taking on these challenges, however, could provide substantial benefit in the understanding of neuroendocrine function, from both basic and biomedical perspectives. In addition to the types of diversity described above, within the class of reptiles, diverse modes of sexual differentiation exist. These include those based on XX/XY (parallel to the mammalian system with homogametic females) and ZZ/ZW (as in birds, homogametic males) sex chromosome systems and animals in which gonadal sex is determined by egg incubation temperatures. Similarly, examples of oviparity, viviparity, and ovoviviparity all occur within this vertebrate class. The enormous diversity of characteristics and functions among relatively closely related organisms makes reptiles ideally suited to a wide range of natural experiments for uncovering mechanisms.

Discussion and conclusions

Birds and reptiles represent two diverse vertebrate taxa that exhibit a range of adaptations in their strategies of reproduction. This diversity has informed the field of reproductive neuroendocrinology about many fascinating questions, including those associated with sexual differentiation and the control of seasonal breeding cycles. However, puberty has received much less attention in these taxa than in mammalian vertebrates. In birds, model species such as starlings have been developed that provide a foundation for additional studies on the mechanisms of puberty. In reptiles, the research is even further behind. The challenge, of course, is setting up a preparation that is easily accessible to investigation. One should not study puberty in diverse species just to describe a process comprehensively in all living taxa. However, studies of puberty in a set of diverse and carefully selected species should be pursued to the degree that they will provide novel basic insights and/or allow one to investigate the generalizability of a concept.

What value can birds and reptiles bring to such studies? The variation described above within and across taxa provides excellent opportunities for comparative investigations. This work can now be taken to the molecular level in a range of avian and reptilian species. The recent implication of epigenetic mechanisms acting via *Kiss1* provides an exciting general hypothesis that should be tested widely (Lomniczi et al., 2013). Discoveries from this type of comparative work could provide a stronger understanding of the evolution of processes related to puberty and neuroendocrine regulation of reproduction at other life stages, as well as the potential to reveal novel mechanisms that could inform treatments for related human disorders.

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