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A potential mate influences reproductive development in female, but not male, pine siskins

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ABSTRACT

The role of photoperiod in avian reproductive timing has been well studied, and we are increasingly recognizing the roles of other environmental cues such as social cues. However, few studies have evaluated the extent to which males and females of the same species respond similarly to the same type of cue. Moreover, previous studies have rarely examined how variation in the quality or nature of a given social cue might modulate its effect. Here, we examine the sensitivity of male and female pine siskins (*Spinus pinus*) to a potential mate as a stimulatory cue for gonadal recrudescence, and we investigate whether variation in the relationship between a bird and its potential mate modulates the effect of that potential mate. Birds were initially housed without opposite sex birds on a 12L:12D photoperiod with ad libitum food. After gonadal recrudescence had begun males and females were randomly paired with an opposite sex bird or housed alone. An additional group of males was paired with estradiol-implanted females. In males, these social treatments had no effect on testis length, cloacal protuberance length, luteinizing hormone (LH) levels, or testosterone levels. In females, presence of a potential mate had a significant and positive effect on ovary score, defeathering of the brood patch, and LH levels. Among paired birds, the degree of affiliation within a pair corresponded to the extent of reproductive development in females, but not males. Thus, reproductive timing in females appears to be sensitive to both the presence of a potential mate and her relationship with him.

Introduction

Understanding how environmental factors affect physiology and behavior in order to time important life history events, such as reproduction and migration, has been an area of long-standing interest in biology. Moreover, it is an area of growing interest as we seek to anticipate and potentially mitigate the consequences of rapidly occurring global environmental change (Bronson, 2009; Visser et al., 2004; Wingfield, 2008). For example, the ability to time reproduction such that it coincides with favorable environmental conditions is critical to an organism's reproductive success (reviewed in MacDougall-Shackleton et al., 2015). Temperate-zone seasonally breeding species, especially birds, have been the focus of much research aimed at understanding reproductive timing mechanisms. From this work, we know that in seasonally breeding birds increasing photoperiods typically stimulate the hypothalamic–pituitary–gonadal (HPG) axis, bringing about changes in physiology and behavior in preparation for breeding. However, additional environmental cues, such as food availability, temperature, or social information and interactions may also be important in fine-tuning the timing of breeding (Wingfield, 1983). Although these non-photic cues have received less attention, we are increasingly realizing their importance in determining reproductive timing in temperate-zone and seasonally breeding species (Schaper et al., 2012; Wingfield et al., 2003), as well as in tropical species (Hau, 2001) and more flexibly or opportunistically breeding species (Hahn, 1995; Ligon, 1974; Perfito et al., 2008).

The role of social cues in reproductive timing has been a topic of interest across taxonomic groups, and broadly, both inter- and intrasexual cues have been found to up- and down-regulate reproductive functions (Bronson, 1989; Crews, 1980; Helm et al., 2006; Wingfield et al., 1994). Previous studies of birds have found that cues from opposite sex individuals can stimulate gonadal recrudescence and advance the onset of breeding in both males and females (Brockway, 1965; Hinde and Steel, 1978; Lehrman, 1965; Morton et al., 1985; Perfito et al., 2015; and references below). Most studies of females have examined the effects of male displays, particularly vocal displays (e.g., Bentley et al., 2000; Friedman, 1977; Kroodsma, 1976; Waas et al., 2005), whereas most studies of males have examined the effect of the presence of a female (e.g., Burger, 1953; Haase et al., 1976). Rarely, have studies examined the effect of a particular type of social cue on both males and females. Also, studies of males and females have tended to use different systems. Studies of females have focused mostly on domesticated

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and captive-bred animals belonging to a few species such as the ring dove, *Streptopelia risoria*, and the canary, *Serinus canaria* (e.g., Bentley et al., 2000; Friedman, 1977; Kroodsma, 1976). On the other hand, studies of males have more often focused on wild-caught birds representing a different set of species (e.g., Burger, 1953; Dufty and Wingfield, 1986; Hahn et al., 1995). Consequently, our understanding of sex differences in responses to social cues (or other environmental cues for that matter) is extremely limited (Ball and Ketterson, 2008).

Additionally, studies examining the effect of social cues on reproductive timing have often ignored potential qualitative differences in the nature of a given cue. In most cases, a generalized form of a particular social cue (e.g. a vocalization) is considered sufficient to induce a change in the HPG axis, or experiments are designed specifically to minimize variation in the quality of the cue. Yet, with respect to the effect of intersexual signals on females, there is a large literature documenting the effects of male phenotype (e.g., song quality, ornamentation) on various aspects of female reproductive behavior (e.g., mate choice, frequency of extra-pair copulations, expression of proceptive behaviors) and parental investment, as well as acute effects on circulating hormone levels (Baker et al., 1986; Gil et al., 2004; Hasselquist et al., 1996; Kingma et al., 2009; Marshall et al., 2005; Safran et al., 2005; Vallet and Kreutzer, 1995; Zuk et al., 1992). Thus, it seems likely that variation in cues from potential mates might also have differing effects on reproductive development. Indeed, two studies provide important evidence that not all forms of a given cue are equally stimulatory with respect to reproductive physiology. First, Kroodsma (1976) found that the size of song repertoire influenced the degree to which male song served as a stimulatory cue for female canaries, with larger song repertoires being a more potent stimulus. Second, Bluhm (1985) found that female canvasback ducks (*Aythya valisineria*) only advanced to egg-laying with their chosen mate, not a mate who was force-paired, even though males exhibited intense courtship behavior in both circumstances. Thus, variation in the 'quality' of any given social cue may influence its potency.

In light of these gaps in our understanding of the role of social cues in reproductive timing, here we examine the sensitivity of both males and females to a potential mate as a stimulatory cue for gonadal recrudescence in a single species, the pine siskin (*Spinus pinus*; Experiment 1). Furthermore, we investigate whether variation in the relationship between a bird and its potential mate modulates the effect of that potential mate (Experiment 2). Pine siskins are temperate-zone songbirds with flexible breeding schedules; egg laying can occur from March to August–September and its timing varies inter-annually (Dawson, 1997; Hahn et al., 2004). Although pine siskins are sensitive to photoperiodic cues (Hahn et al., 2004; MacDougall-Shackleton et al., 2006), they are also likely to rely heavily on non-photic cues to time reproduction. We have previously found that both male and female pine siskins are sensitive to food availability for the initiation of reproductive development (Watts and Hahn, 2012). In this study, we focus on the effect of a potential mate during the later period of reproductive development, as an individual approaches full mature capabilities, rather than the period in which development is initiated. It is during this later period that females are expected to be most sensitive to social cues (reviewed in Ball and Ketterson, 2008; Perfito et al., 2015). And, we have previously found no effect of the presence of a female on the earlier period of initiation of reproductive development in male pine siskins (Watts and Hahn, 2012).

Methods

Ethics

Experimental procedures were approved by the Institutional Animal Care and Use Committees at University of California Davis (Experiment 1) and Loyola Marymount University (Experiment 2) and were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Birds were captured under permits from the U.S. Fish and Wildlife Service, Oregon Department of Fish and Wildlife, and Wyoming Game and Fish Department.

Experiment 1: Effects of a potential mate on reproductive development

Animals

Birds were captured in Jackson WY (43° 28′N, 110° 48′W) in August and September of 2009 and in Mt Ashland OR (42° 4′N 122° 43′W) in December 2009; two additional females were captured in Jackson WY in October 2008. Birds were transported to facilities at the University of California, Davis where they were housed in large indoor flight cages on photoperiods that simulated natural changes in photoperiod at a latitude of 38°N until winter solstice (21 December 2009), after which time they were held on winter solstice day length (9.7L:14.3D) until 13 January 2010 when they were put on a 12L:12D photoperiod. This 12L:12D photoperiod is permissive for breeding, but not highly stimulatory. Throughout the study birds were fed a diet of Roudybush Small Bird Maintenance Diet (Woodland, CA) and a mixture of seeds (black oil sunflower seeds, sunflower hearts and thistle seeds). Water and fine grit were also provided ad libitum. Birds were sexed either by laparotomy or genetic sex determination (Zoogen, Inc., Davis, CA).

Experimental design

The experiment included 3 treatment groups for males and 2 treatment groups for females. Both males and females were housed either alone (control, $n = 8$ males, $n = 7$ females) or with an opposite sex partner (a potential mate, $n = 9$ males and females, female subjects served as stimulus females for male subjects and vice versa). Additionally, a third group of males was paired with females that received estradiol implants ($n = 9$ males). These estradiol-implanted females were expected to behave similarly to reproductively mature females and thus be maximally stimulatory to males. Birds were randomly assigned to treatment groups, with birds captured at different locations balanced across groups. Male–female pairings were randomly assigned except that no bird was paired with an individual with which they were captured. One pair each from the unmanipulated female group and the estradiol-implanted group were removed partway through the study because one member of the pair died unexpectedly. Data from these pairs was included for those sampling points for which it was available.

Prior to winter solstice, males and females were separated into different cages and were visually isolated from opposite-sex birds. For the experiment, birds were housed in 'individual' cages that contained either a single bird or pair of birds. Cages were placed in 12 acoustic isolation chambers (Industrial Acoustics Company, Bronx, NY), arranged on multiple shelves such that each cage was visually isolated from the other cages in the chamber, but birds could hear other birds belonging to the same treatment group. Birds assigned to each treatment group were divided among 2–4 chambers. Chambers were arranged within a single room, in alternating order. The positions of cages on shelves within a chamber were rotated weekly throughout the experiment.

The experiment began on 20 February 2010 (Day 0), when birds were moved into acoustic chambers either alone in a cage or in a cage with a randomly assigned partner of the opposite sex. At this point, birds had been on a permissive photoperiod for 38 days and had begun gonadal recrudescence (Figs. 1 & 2).

Estradiol implants

Females receiving estradiol implants were implanted two days prior to the start of the experimental manipulation (18 February 2010, Day −2). Estradiol implants were created using silastic tubing (1.96 mm outer diameter) filled with crystalline 17β-estradiol (Sigma E-8875). Implants were 7 mm in length and sealed at both ends with silicon adhesive to create an implant with 5 mm of packed hormone. Implants were soaked in sterile saline overnight before implanting. Following application of a topical anesthetic, implants were inserted subcutaneously