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Social cues are unlikely to be the single cause for early reproduction in urban European blackbirds (*Turdus merula*)

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HIGHLIGHTS

- Male European blackbirds were house in cages with or without a conspecific female.
- We recorded the time of gonadal growth and measured LH concentration.
- There was no effect of social condition on reproductive growth.
- Urban and rural birds responded equally to the experimental treatment.
- In comparison to light at night, social cues had a limited impact on gonadal growth.

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ABSTRACT

Despite urban ecology being an established field of research, there is still surprisingly little information about the relative contribution of specific environmental factors driving the observed changes in the behavior and physiology of city dwellers. One of the most reported effects of urbanization is the advanced phenology observed in birds. Many factors have been suggested to underline such effect, including warmer microclimate, anthropogenic food supply and light pollution. Since social stimuli are known to affect reproductive timing and breeding density is usually higher in urban populations compared to rural ones, we experimentally tested whether social interactions could advance the onset of reproduction in European blackbirds (*Turdus merula*). We housed male blackbirds of rural and urban origins with or without a conspecific female, and recorded their seasonal variation in gonadal size and production of luteinizing hormone (LH). Paired and unpaired males of both urban and rural origins did not significantly differ in their timing of gonadal growth. Moreover, rural and urban birds did not differ in their response to the social stimuli, rather they became reproductively active at the same time, a result that confirms previous studies that attributed the difference in reproductive timing observed in the field to phenotypic plasticity. We conclude that social stimuli do not contribute substantially to the observed early onset of reproductive physiology in urban bird species, rather other factors such as light pollution are likely to be stronger drivers of these physiological changes.

1. Introduction

The physiological control of reproductive timing is one of the most studied concepts in organismal biology, from plants [1] to insects [2], to vertebrates [3], including humans [4]. Reproduction, as other annual life-history events, has evolved under the natural rhythm of seasons [5], and different organisms have evolved different mechanisms to track seasons and effectively time annual events. In birds living at temperate latitudes, photoperiod is used as the most important proximate cue to initiate reproductive activities [6]. Indeed, because the reproductive system becomes quiescent after breeding in the majority of temperate bird species, a very precise and reliable cue is needed to re-activate the reproductive system in anticipation of breeding. While photoperiod is used as proximate cue, birds rely on other external information to fine-tune the ultimate breeding decisions, e.g., laying the first egg of their clutch. Among the most important of these supplementary cues, food availability, temperature and social cues are the most understood [7–9].

Social cues have been shown to affect the reproductive axis at various levels [10]. For example, the volume of song control nuclei in the

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brain was enlarged when male white-crowned sparrows (*Zonotrichia leucophrys gambelii*) were housed with a fertile female compared to when they were housed alone [11]. At the physiological levels, receptive male sparrows and cowbirds showed an increase in plasma concentrations of luteinizing hormone (LH) and testosterone when housed with a conspecific female [12,13], and social stimuli emitted by females prevented testicular regression of male starlings (*Sturnus vulgaris*) [14]. At the behavioral level, playback of male song was shown to influence the behavior of both male and female zebra finches (*Taeniopygia guttata*); males supplemented with playback song sang more frequently than non-supplemented males, and females who heard these extra-songs laid eggs earlier and increased clutch size compared to control females [15]. Ambient temperature and food availability have also been suggested to affect reproductive timing [16]. In the last 15 years the role of temperature for avian seasonal timing has become a major focus of ecology as attention has been drawn on the relationship between global warming and population decline of avian species [17], but the physiological mechanisms through which temperature affects timing of reproduction in birds are only partially understood [7,18]. Natural variation in food availability, as well as food supplementation experiments, has been long known to affect timing of breeding: in general, the more abundant the food availability is in a certain year, the earlier birds tend to lay their eggs [8,19–21].

In recent years, these concepts and findings on the relationship between timing of reproduction and environment in birds have been applied to an environmental change framework [22]. Besides climate change (see above), another important anthropogenic source of environmental change is urbanization. Bird species colonizing urban areas have been often reported to show an earlier onset of reproduction [23–26]. In European blackbirds (*Turdus merula*), urban males and females grow their gonads three weeks earlier during the reproductive season than their rural conspecifics. Phenotypic flexibility is the most probable mechanism underlying such differences in the timing of gonadal growth, although genetic differences cannot be completely ruled out [27,28]. These results may indicate that some urban-specific environmental factors should be the main driver of early breeding in urban areas. Indeed, the exposure to artificial light at night is able to explain great part of the variation in the timing of reproductive physiology in the field. European blackbirds exposed to only 0.3 lux at night showed a three- to four-week earlier onset of testicular growth and had lower levels of plasma melatonin compared to conspecifics exposed to dark nights [29–31]. Although these data suggest that artificial light at night may be the most important factor underlying early breeding in urban areas, more comparative data and experimental testing are needed to elucidate the relative role of other environmental cues. In particular, urban populations of many successful urban dwellers, including the European blackbird, often exhibit high population densities [32,33]. These high densities may cause higher social interactions between males and females and could thus result into an earlier onset of gonadal growth during spring via frequent stimulation of the opposite sex.

The aim of this study was to experimentally test the direct effect of social cues (e.g. presence of a female) on the timing of testicular growth of male European blackbirds. To this end, we placed individual male blackbirds in outdoor aviaries with or without a female, and recorded testicular size and plasma concentrations of luteinizing hormone (LH) from January to April. Elevated levels of circulating LH indicate that a bird approaches reproductive condition even before macroscopic changes in the appearance of the gonads can be detected. We predicted that the presence of a social female would lead to an earlier onset of reproduction. In addition, we aimed at further investigating whether urban and rural birds respond differently to social stimuli. As breeding densities is higher in cities, it is conceivable to hypothesize that urban birds are commonly used to a higher degree of social interactions than rural birds, and would therefore respond less strongly to the presence of a female than the forest counterparts.

2. Material and methods

2.1. Animals and experimental set-up

The birds of this study were collected in 1998 from 10 nests in Munich (48° 07′ N, 11°34′ E; 518 m asl) and from 10 nests in a forest (Raisting: 47°53′ N, 11°04′ E; 553 m asl). In total we collected 30 urban (17 males, 13 females) and 30 rural (16 males, 14 females) European blackbird nestlings (average age of 8 days). Previous data on the reproductive timing of free-living urban and rural European blackbirds were collected from the same sites [23]. All nestlings were hand-reared and then moved to one large experimental room. In this room, females of both origins were kept too, but birds could hear but not see each other, because all birds were housed in individual cages (width × height × length: 45 × 70 × 80 cm). Natural light was provided through large roof windows, thus birds were exposed to the local natural photoperiod at the Max-Planck-Institute for Ornithology, Seewiesen, Germany (47°97′ N, 11°23′ E). Ambient temperature ranged from +15 °C (minimum) in winter to +30 °C (maximum) in summer. Food (Granvit; Hungenberg) and drinking water were available ad libitum. Birds were kept under these conditions for two consecutive reproductive years (1999 and 2000) during which their reproductive development was monitored, before the experiment on social effects began (see below). During this time five males died of natural causes, thus the initial sample size of this study was N = 28 males.

2.2. Effects of social cues on timing of gonadal recrudescence

On October 20th, 2000, all male birds were randomly assigned to two treatment groups and moved into outdoor aviaries (width × height × length: 200 × 250 × 300 cm) in Seewiesen (47°97′ N, 11°23′ E) that were exposed to natural variation in photoperiod and temperature. The ceilings as well as the top and front parts of the aviaries were constituted of metal mesh, while the lateral sides were concrete walls. Thus, birds could again hear but not see each other, and food and drinking water were provided ad libitum. Each male was assigned to a single aviary but half of the males (N = 14; 7 urban and 7 rural males) were paired with a female blackbird of the same origin as the male (treatment group), whereas the other half (N = 14, 8 urban and 6 rural males) was housed alone. All the experimental procedures were carried out in accordance with the guidelines of the animal experimentation committee of the Regierung von Oberbayern, Germany.

2.3. Testicular size measurement, hormone analysis and assessment of body condition

We measured the size of testes by laparotomies [34] four times between January and March 2001 on Julian date 9 = Jan 9, Julian date 37 = Feb 2, Julian date 58 = Feb 27 and Julian date 79 = Mar 20). Incisions were made under Isoflurane anesthesia (CP-Pharma, Germany). The width of the left testis was measured to the nearest 0.1 mm. Incisions were treated with Actihaemyl gel (Meda Pharma GmbH, Germany) and sealed with Histoacryl (Braun, Germany). All birds recovered rapidly from the procedure. To compare the timing of gonadal growth, we used a threshold value of gonadal size of 5 mm, assuming that testes produce fertile sperm at half their maximum size (ca. 9 mm for the blackbirds) [35]. To compare reproductive timing between free-living and experimental blackbirds in different years, data for the timing of reproductive activity published in two previous manuscripts [23,28] were used.

We collected blood samples from every individual one week before each laparotomy session, between 9:00 and 12:00 am, by puncturing the brachial wing with a 23 mm needle. Blood was immediately centrifuged, plasma was separated from red blood cells and stored at −80 °C. Plasma samples were analyzed for luteinizing hormone concentration (LH) via a double-antibody radioimmunoassay [47]. Chicken LH was