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Megan Lagerquist April 12, 2022

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Neuroscience and Behavioral Biology

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Abstract

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Background: In Estrildid finches, juvenile males learn species-typical vocalizations through social interactions with an adult tutor, usually their father, during a sensitive period in development. It is not well-established how a juvenile's social behavior changes in the presence of a tutor during song learning. Here, I tested whether juvenile male Bengalese finches (*L. striata domestica*) would behave differently in a song learning context than in another social context.

Methods: At ~45 days post hatch, during the sensitive period for song memorization, juveniles raised by both parents were randomly assigned to one of two social conditions with either their father ("tutor") or an unrelated familiar female ("control") present. Behaviors of each juvenile were scored throughout a 45-minute stimulus period during which they could interact socially with the tutor or control animal in an adjacent cage.

Results: Juveniles presented with the tutor made more two-foot contacts with the walls nearest the stimulus cage, a high-effort approach behavior, than did juveniles presented with the control female. Juveniles also pecked more frequently in proximity to the stimulus animal in the tutor condition than in the control female condition. Finally, juveniles were more likely to approach the tutor when he was singing than expected by chance.

Conclusion: My results suggest that these juvenile songbirds were motivated to approach the tutor, were attracted to his song, and may have sought to elicit a response from the tutor through social bids. This study strengthens the applicability of the Bengalese finch model to socially-guided vocal learning (SGVL) in other species, including humans, and suggests future directions for investigating the role of specific behaviors in SGVL.

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Acknowledgements

I would like to thank all members of the Bird Brain Lab for welcoming me into the research space and sharing their expertise and passions with me. Specifically, I would like to thank Isabel Fraccaroli for her technical assistance and her willingness to teach me experimental procedures, and Aditya Bhise for volunteering his time to score behavior.

I am grateful to Dr. Nicole Baran for her expertise and encouragement from my first days in the lab through the final thesis defense. From her intentional mentorship, I have learned valuable skills and knowledge of experimental techniques and the field, and I have gained invaluable insights into scientific research and my place in it.

I am glad to have had Professors Malavika Murugan and Rick Thompson as members of my committee. I am thankful to them for reading my paper and for their thoughtful questions and advice. I am also grateful to Professor Leah Roesch for providing guidance and resources during this process.

I am thankful to my adviser, Professor Donna Maney, who has guided me and challenged me throughout this process. Her deep investment in me and my work have made this paper possible and helped me to grow as a writer and researcher.

Finally, I would like to thank my friends and family for their endless support and belief in me.

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Behavioral responses to social tutoring in male Bengalese finches

Adaptive behaviors, from tool use to social norms, can be transmitted from one generation to the next via social learning, in which a young learner imitates an adult model. A special case of social learning, socially-guided vocal learning (SGVL), or the ability to modify vocal output based on social guidance, is particularly reliant on early interactions with conspecifics. Speech learning in human infants is enhanced by social interactions with adults providing feedback on early vocalizations (Kuhl, 2007) and humans raised in social isolation learn only rudimentary speech (reviewed by Syal & Finlay, 2011). The effects of social context on vocal learning have been attributed to motivation to interact with caregivers, as these social exchanges constitute a rewarding experience for young children (Kuhl, 2007; Syal & Finlay, 2011). Rather than viewing SGVL as passive acceptance of social guidance provided by a teacher, emphasizing motivation leads to the view that the pupil is an active participant whose behavior also impacts their own learning outcomes. The impact of the learner’s motivation on SGVL is perhaps most noticeable in children with autism spectrum disorder (ASD). ASD is characterized by low social engagement (e.g. gaze following), which can contribute to delayed or atypical communication and social development (Mitchell et al., 2006). According to the social motivation hypothesis (SMH), children with ASD find social engagement less rewarding than do neurotypical children and therefore have lower motivation to engage in social activities (Dawson, 2008), such as initiating communication with caregivers (Su et al., 2021). The convergent evidence from language acquisition studies generally and from ASD research specifically suggests that bidirectional social interaction with caregivers is crucial for typical language development in large part because of the enhanced motivational state that is activated in a social setting. To better understand the role of social motivation and the learner's behavior in vocal development, there is a need for animal models that are both highly social and have vocal learning.

Songbirds are excellent models for studying SGVL, given that song learning relies not only on the acquisition of a motor skill but also on social interactions. Unlike most laboratory mammals (e.g., rats and mice), songbirds produce complex, learned vocalizations, and songbirds and humans share neuroanatomical pathways linking social motivation to vocal learning (Goodson & Kingsbury, 2011; Syal & Finlay, 2011). Vocal learning in both songbirds and humans occurs during sensitive periods of development during which song or speech are learned from adult caregivers (Baran, 2017). In Estrildid finches, a particularly popular model for song learning, juveniles learn only simplified, abnormally-structured songs when reared in isolation (Clayton, 1987; Eales, 1985), mirroring the rudimentary speech of isolated children (Syal & Finlay, 2011).

Songbirds learn species-typical song by copying the model song of one or more adult conspecifics, or 'tutors'. In Estrildid finches, such as zebra finches (*Taeniopygia guttata*) and Bengalese finches (*L. striata domestica*), only males sing, and song is used primarily for courtship purposes (Immelmann, 1969). Juvenile males first memorize a tutor's song during a phase called sensory learning (Fig. 1), then practice and refine their own song, which includes elements of tutor song and some improvisation, in a phase called sensorimotor learning (reviewed by Gobes et al., 2019). Birds reach crystallization, the final phase during which song obtains its mature, unchanging form, a few months after hatching (Immelmann, 1969; Okanoya, 2004). In Estrildid finches, the sensory and motor learning periods overlap temporally (reviewed by Gobes et al., 2019), providing an opportunity for the juveniles to practice and modify vocal behavior based on feedback from parents, peers, and potential mates (Carouso-Peck & Goldstein, 2019; Ljubičić et al., 2016).

It has long been hypothesized that the social relationship between tutor and juvenile impacts tutor choice (Beecher & Burt, 2004; García, 2019; Williams, 1990). Songbirds learn best, as measured by high similarity between the pupil's mature song and the tutor's song, through live interactions with a tutor (Chen et al., 2016; Eales, 1989; Payne, 1981). In comparison, juveniles do not learn as well from auditory-only interactions or passive song playback (Chen et al., 2016; Eales, 1989; Payne, 1981). These findings indicate that the social stimulus of a live singing tutor is necessary for developing a normal song, with typical phrase structure and song elements, in many passerine species, supporting the idea that vocal development is heavily gated by the social environment. In most natural contexts, juvenile songbirds have the opportunity to learn from several tutors and may incorporate elements from more than one model, but mainly copy only one or two tutors (Mann & Slater, 1995). Accordingly, juveniles' choice of one tutor over others may reflect their motivation to learn from that tutor. Juvenile preference for a tutor or his song, and ultimately tutor choice, is a popular paradigm for studying the influence of social bonding and interaction on song learning. Both male and female Bengalese finches recognize and prefer their father's song during sensory learning (Fujii et al., 2021) and juvenile male zebra finches in the sensory learning phase worked to hear their father's song in an operant conditioning task (Adret, 1993) over another familiar adult's song (Rodríguez-Saltos et al., 2021). Furthermore, the degree of a juvenile's peak preference for father's song was positively correlated with the degree of learning (Rodríguez-Saltos et al., 2021). Consistent with this finding, juveniles generally prefer to affiliate with their parents over other conspecifics, and the degree of affiliation is correlated with quality of song copying (Baran et al., 2017). Finally, although juvenile male songbirds prefer to copy the song of a conspecific tutor, zebra finches raised by a heterospecific pair will learn the song of their heterospecific foster father even if they are able to interact with a conspecific visually and vocally through a wire lattice (Eales, 1987); even if juvenile zebra finches are able to hear their father singing, they will still copy the foster father's song (Immelmann, 1969). Thus, juveniles form a preference for adult males playing a parental role, rather than being limited to their genetic father, and affiliative social experience with an individual tutor can enhance a juvenile's motivation to learn that tutor's song.

Despite the evidence that social interactions during development are critical to vocal learning in songbirds, the exact behaviors that characterize those interactions and that may be important to SGVL are understudied (Beecher & Burt, 2004). What behaviors, if any, do tutors engage in when interacting with a juvenile that promote song learning? Strikingly, a tutor changes his song in the presence of a juvenile receiver compared to when he is singing alone. Much like how human adults change pitch, affect, and vowel emphasis when speaking to infants (Burnham et al., 2002), adult males increased the length of pauses, lowered mean syllable frequency, and increased goodness of pitch (a measure of how periodic song is) when singing to juveniles (Chen et al., 2016). Such targeted and consistent changes demonstrate that tutors modify singing behavior in a tutoring context and suggest that they may be actively invested in the vocal development of their offspring. No other examples of tutor responsiveness to juveniles during the sensory learning phase have been reported; although

Houx & ten Cate (1998) hypothesized that tutor behaviors, such as approaching the juvenile, may prime juveniles to pay attention, they found little evidence that tutor behaviors predicted juvenile learning.

Heretofore I have focused on the behavior of the sender; assuming that a tutor sings reasonably often, the behavior of the juvenile, who is the receiver, is perhaps more likely to be relevant to SGVL. It is possible that juvenile behaviors during interactions with a song tutor directly influence song learning or else reflect underlying changes in the juvenile's motivational or attentional state that promote learning. Several previous studies have indicated that juveniles respond behaviorally to a tutor and that their behavior in a song learning context predicts the quality of song learning, though none have established whether these behavioral responses are elicited by the song learning context specifically. In a study by Houx & ten Cate (1998), juveniles approached tutors at above-chance levels after the onset of tutor song, demonstrating attraction to a singing tutor. Subsequently, Houx et al. (2000) found that the quality of a juvenile's song learning was positively correlated with approaching a loudspeaker playing tutor song, indicating that attraction to tutor song may influence learning. In a more recent study, Chen et al. (2016) found that elevated attention to tutor song, as defined by being alert but still during song, improved song learning. For juveniles, being silent after playback and preening their feathers was also positively correlated with learning (Houx et al., 2000); juveniles may be more attentive to song when engaged in preening, a relatively quiet and still behavior, than when engaged in high-energy activity. These findings showed that active behaviors reflecting attraction and attention, like juvenile's approach to tutor, and quiet, attentive behaviors, like stillness and preening, could both be important to song learning.

Whereas many researchers have compared song learning outcomes in social and passive tutoring experiences, and some have measured juveniles' behaviors during tutoring sessions, few have explicitly tested which juvenile behaviors are elicited by the presence of a tutor. In the current study, I sought to elucidate how juvenile songbirds respond to the song learning context. To accomplish this goal, I chose to study the Bengalese finch, an Estrildid finch domesticated from the white-backed munia (*Lonchura striata*) ~250 years ago (Okanoya, 2004) Bengalese finches are highly gregarious, rarely aggressive, and learn from social guidance (Birke, 1974; Carouso-Peck & Goldstein, 2019; Honda & Okanoya, 1999). Additionally, Bengalese finches are thought to have been bred for high levels of parental care, making them an interesting model for juvenile-caregiver bonds (Honda & Okanoya, 1999). In this study I sought to develop the Bengalese finch as a model for SGVL from a behavioral perspective by testing whether juveniles behave differently in a song learning context than a control social context. To test this hypothesis, I compared behaviors between juveniles engaged with a tutor and juveniles presented with a familiar adult female. In both social conditions, the juvenile had the opportunity to interact with a familiar conspecific, but only the tutor was a caregiver and a potential song model. I predicted that juvenile male Bengalese finches would be more motivated to approach their father, who is a caregiver and song tutor, than to approach a familiar female. I also predicted that juveniles would approach the tutor in response to song, reflecting attraction to tutor song in particular. Finally, because preening behavior was previously associated with enhanced song learning, I predicted that juveniles would preen more in the tutor condition.

Methods

Animal Husbandry

All animal procedures were approved by the Emory Institutional Animal Care and Use Committee (protocol #201800016). Eight breeding pairs of Bengalese finches were housed in 14 x 14 x 18 in. cages in walk-in sound-attenuated booths, with two cages per booth. Birds were provided with *ad libitum* finch seed, water, cuttlebone, and a water bath, and their diet was supplemented with a blend of hard-boiled egg and vegetables twice a week. Nest-boxes and coconut coir as nesting material were provided during breeding and were removed after all juveniles fledged. Juveniles were reared with both parents and their siblings until the time of the experiment. The birds in each cage could see and hear the birds in the adjacent cage, meaning that juveniles were exposed to the songs of both their father and the neighboring adult male.

Sex Determination

Prior to fledging, 4-5 pin feathers were collected from each nestling for sex determination via PCR. DNA was extracted from pin feathers as described by Baran & Adkins-Regan (2014). Each 20µl PCR reaction contained 1 µl of DNA sample, 0.55 µM of each primer, 0.4µl Taq DNA Polymerase (New England Biolabs), 3.6µl 10x Standard Taq Reaction buffer, and 0.6µl deoxynucleotide (dNTP) solution mix (10µM each). The following PCR program was used: an initial hot-start 5 min denaturation step at 95ºC; followed by 30 cycles 94ºC for 30s, 48ºC for 30s, 72ºC for 1 min; and a final extension at 72ºC for 5 min. PCR product was then analyzed on a 1% agarose gel. This PCR assay produces one band for males (ZZ) and two bands for females

(ZW). The sex of all but one juvenile was determined using the PCR, and the juvenile of unknown sex was excluded, leaving 17 juveniles in the study.

Although females recognize and form preferences for song in Bengalese finches (as in zebra finches), only males sing (Immelmann, 1969). Because we were primarily interested in the contributions of tutor behavior to song learning, the current study included males only.

Behavioral Assay

Juvenile male Bengalese finches (n = 17) experienced a live tutoring session at 44-46 days old, a time of peak sensory learning (Fujii et al., 2021) during which juveniles memorize tutor's song (Fig. 1). On each day of the experiment, a different juvenile was placed with its mother in a test cage located in another sound-attenuated booth. Each test cage contained food, water, and three horizonal perches bisecting one lateral perch (Fig. 2). After a two-hour acclimatization period, the juvenile's father (tutor condition, n=9) or a familiar adult female (control condition, n=8) was placed in an adjacent cage allowing the birds to see and hear each other. The mother remained in the juvenile's cage. The control female was always the adult female of the breeding pair housed in the same home booth as the juvenile. Juveniles from the same family were tested with the same stimulus animal, except in one case when a breeding pair was moved between broods, resulting in a different control female being used. Cage orientation (left or right side of the booth) was randomly assigned. Each behavioral trial lasted for 90 minutes.

Video Analysis

The 90-minute stimulus period was recorded using a Canon Vixia HF R800 HD video camera with a RØDE VideoMic Camera-Mount Shotgun Microphone. Juvenile behaviors and tutor song were scored from recordings using BORIS (v7.10.5) for the first 45 minutes of the stimulus period. The author and an additional observer independently scored juvenile behaviors in all videos. Intercoder reliability was assessed with Pearson's correlations and is reported in the Results.

Behavioral Scoring

Behaviors were scored either as point events (one timestamp per event) or state events (with a timestamp for onset and conclusion of each event). The scored behaviors were beak wiping, preening, pecks to the stimulus animal, time spent on each wall, and time spent in the proximal zone, defined as the area within approximately five inches of the stimulus animal's cage (i.e., the third of the juvenile's cage closest to the stimulus animal) (Fig. 2). Operational definitions of all behaviors are given in Table 1.

Approach Behaviors

Approach to a tutor or tutor's song has been interpreted as a sign of attraction and tutor preference (Houx et al., 2000; Houx & Cate, 1998; Fujii et al., 2021). Previous studies have defined approach behavior in songbirds as moving towards a social target or stimulus, such as a juvenile approaching a parent or song playback (Adret, 1993; Baran et al., 2017; Houx et al., 2000; Houx & Cate, 1998; Rodríguez-Saltos, 2017). In this study, approach was operationalized as the proportion of time spent in the proximal zone over the entire session (Table 1). Two-foot contacts on the walls of the proximal zone (two-foot contacts), were considered to indicate approach that is especially energy-costly, therefore potentially reflecting high motivation to work for proximity.

Pecks on the cage wall or a perch, in proximity to the stimulus animal, were also scored as a measure of social approach, as described by Pilgeram et al. (2021). Pecks to the stimulus animal were measured as a rate of pecks per minute in the proximal zone.

Preening

Preening is a grooming behavior that often occurs in long bouts, during which the bird is otherwise still and relaxed, and may co-occur with active listening as the bird is quiet but awake (Houx et al., 2000). Juvenile zebra finches that preened more often during tutoring sessions copied the song better (Houx et al., 2000). In this study, I counted each preening motion, as defined in Table 1, over the session.

Beak-Wiping

Beak-wiping behavior functions to clean a bird's beak and also is part of the ritualized mating display of zebra finches, which is similar to that of Bengalese finches (Morris, 1954). It is commonly observed before song and between characteristic side-to-side hops in the mating display of adult birds, though it is not necessary for mating and is secondary to singing behavior in importance (Morris, 1954). During the experimental session, the female mate of the tutor was present in the juvenile's cage and thus the tutor may have performed ritualized behaviors related to female-directed song, such as beak-wiping. Bengalese finches do not typically breed until three months of age (Eisner, 1960) and likely do not begin courtship behaviors until at least 60 dph (Kruijt et al., 1983). Thus, juveniles were not expected to perform courtship behaviors directed to the familiar female. However, as beak wipes are a ritualized behavior in adults, it is unknown whether they are elicited in juveniles by any social condition and were therefore a behavior of interest in this study. Beak wipes, as defined in Table 1, were counted over the entire session.

Vocal Behaviors Excluded

Juvenile subsong, an early form of sensorimotor learning (Pytte & Suthers, 2000), was observed in only 5/17 trials and therefore was not analyzed statistically. My experimental set-up did not include individual microphones to distinguish between calls from each bird, so no vocal cues other than song and subsong were measured.

Juvenile Approach in Response to Tutor Song

Approach in response to song was operationalized as the average latency, after onset of tutor song, to 1) move to the proximal zone and 2) make a two-foot contact with a wall in the proximal zone. To calculate the latency, I first measured the amount of time, rounded to the nearest half-second, between the onset of each tutor song and the onset of each of these two behaviors for each juvenile. Next, I averaged those latencies within each juvenile to arrive at an average latency for each behavior for each juvenile. Instances in which the tutor began song while the juvenile was already in the proximal zone were excluded. Latency was capped at 15 seconds after song onset, consistent with the behavioral scoring period used by Houx & ten Cate (1998). Finally, to determine whether the observed latency was meaningfully different from the latency expected by chance, I calculated a baseline approach latency. To estimate this baseline, I randomly generated timestamps as the starting times from which to measure latency, equal in number to the number of songs heard by that juvenile. For each juvenile, I calculated latencies from these timestamps to the two behaviors. I then averaged these latencies for each juvenile to arrive at an average latency, and repeated the process 1000 times, resulting in a distribution of average latencies for each juvenile. The mean of this distribution was used as the baseline for each juvenile, representing chance latency.

In addition to latency, juvenile approach behavior in response to song was operationalized as synchrony, or temporal overlap, between approach behaviors and tutor song. Whereas latency measures approach as a point behavior after song onset, synchrony is sensitive to the duration of juvenile approach behavior throughout a song event. Because synchrony reflects the total approach behavior during song, it is sensitive to whether and how quickly a juvenile approaches after song onset as well as whether and how quickly a juvenile stops the approach behavior during song. Synchrony is also sensitive to when tutors start singing and how long they sing.

Synchrony was calculated for time in the proximal zone and time in two-foot contacts in the proximal zone as the total temporal overlap between tutor song and each respective approach behavior by the juvenile. I first calculated the number of half-second windows in which juveniles were engaged in each of the two approach behaviors while the tutor was singing. The total duration of overlap between each behavior and tutor song was then summed across the 45-minute session for each juvenile. For comparison with baseline, chance overlap was estimated post-experiment for each juvenile using the following process: the timestamp indicating the onset of each song was shifted uniformly by a randomly generated number of seconds while maintaining song duration and duration of time between songs. Next, the total duration of overlap between song and each approach behavior was calculated. This circular permutation was repeated 1000 times, resulting in a distribution of total overlap that could be explained by chance. The mean of this distribution was used as the baseline for each juvenile, representing chance synchrony.

Statistical Analysis

The frequency (beak wipes, preening motions, pecks) or duration (proximity, two-foot contacts) of each behavior was compared between the two conditions using a general linear mixed model controlling for random effects of shared parentage. For latency and synchrony, observed and chance values were compared using GLMMs controlling for individual juvenile and shared parentage as random effects. The predetermined alpha level, 0.05, was corrected across all significant tests using the Benjamini–Hochberg procedure with an acceptable false discovery rate of 10% (Benjamini & Hochberg, 1995). Raw and corrected alpha levels are reported. Cohen's *d* was reported as an estimate of effect size. As an estimate of the effect size within individual, the normalized difference between observed and chance approach latency for each bird was calculated as $Obs_{avg} – Chance_{avg} / SD$, where SD is the standard deviation of the chance latency distribution. Normalized difference was similarly calculated and reported for synchrony.

Results

All results are reported in Table 2, and ranked results with corrected alpha values are reported in Table 3. The effects of type of stimulus animal (tutor or familiar female) on approach behaviors are shown in Fig. 3. Because the proximal zone encompassed approximately one third of the cage, juveniles with no location preference would be expected to spend approximately one third of their time there; in this study, juveniles in both social conditions spent significantly more than one third of the session in the proximal zone, defined as the zone nearest the stimulus animal (Tutor: t(8) = 6.41, $p < 0.001$, d_{av}= 4.46, Control: t(7) = 6.31, p 0.001, d_{av}= 4.85), despite the fact that food and water were placed in other zones. I did not find a difference between social conditions in the proportion of time spent in the proximal zone (Fig. 3A; $p = 0.71$). Each of the other two approach behaviors occurred more frequently in the tutor condition. Juveniles spent more time engaged in two-foot contacts on the walls of the proximal zone, a high-effort approach behavior, in the tutor condition compared with the control condition (Fig. 3B; $p < 0.001$). Pecks to the stimulus animal, defined as pecks on the walls or perches in the proximal zone, occurred at a higher rate in the tutor condition than the control condition (Fig. 3C; $p = 0.0016$).

The effects of social condition on the other juvenile behaviors are shown in Fig. 4. I found no significant effect of condition on the frequency of beak wipes, defined as a wiping motion during which a juvenile's beak contacts a perch (Fig. 4A; $p = 0.13$). I also observed no difference in the frequency of preening motions between the conditions (Fig. 4B; $p = 0.50$).

All but one juvenile in the tutor condition was exposed to the tutor's song at least once during the session. For those juveniles, I first measured the latency to engage in two of the approach behaviors (entering the proximal zone, moving to a two-foot contact in the proximal zone) after the onset of song. A lower latency value indicates faster approach. Juveniles approached the proximal zone more quickly after song onset than expected by chance (Fig. 5A; $p = 0.012$). In contrast, I found no evidence that latency to two-foot contacts in the proximal zone differed in response to tutor song compared with chance (Fig 5B; $p = 0.51$). Next, I measured the synchrony between juvenile approach behaviors and tutor song, defined as the total temporal overlap between the behaviors and song. A higher synchrony value indicates greater temporal overlap between behaviors and song. I did not find that time in the proximal zone was more likely to occur during song than by chance (Fig. 5C; *p* = 0.70). Two-foot contacts were also not more likely to occur during song than by chance (Fig. 5D; *p* = 0.16). The distributions of chance values for latency and synchrony are shown in Figs. S1 and S2, respectively.

Discussion

In Estrildid finches, juvenile males learn species-typical song through social interactions with an adult tutor, usually the father, during a sensitive period in development. The social bond with the tutor is thought to motivate the juvenile to seek out interactions that facilitate song learning (Eales, 1987; Mann & Slater, 1995; Rodríguez-Saltos, 2017; Rodríguez-Saltos et al., 2021; Williams, 1990). It is not well-established, however, precisely how a juvenile's social behavior changes in the presence of a tutor during song learning. In this study, I found that juvenile male Bengalese finches presented with a tutor, who was their father, engaged in more approach behaviors than did juveniles presented with an unrelated, familiar female. Furthermore, juveniles presented with the tutor were more likely to approach immediately after he started singing. These results suggest that juveniles are motivated to be near their song tutor and are attracted to tutor song. This study contributes to the development of the Bengalese finch as a model for SGVL by showing how juvenile behavior changes in a vocal learning context.

Social Bonds and Motivation

The results of this study suggest that the juvenile Bengalese finches were more motivated to approach an individual with whom they had a strong social attachment than to approach another familiar conspecific. In humans, the quality of parent-child attachment is associated with motivation and joint attention, which are thought to be foundational to SGVL (Kuhl, 2007). One of two main categories of behaviors that indicate mother-infant attachment is approach, which functions to bring child to mother (the second being signaling behavior, which brings mother to child) (Bowlby, 1969). Approach may similarly indicate attachment in non-human animal models of sociality. The tutors in this study were not only important caregivers throughout juvenile development, but also represented an attractive model for learning species-specific vocalizations. These two characteristics are interrelated, as the attractiveness of a song model, and hence a juvenile's desire to learn from an individual tutor, is correlated with the quality of previous social interactions and the juvenile's attachment with a caregiver (Eales, 1987; Payne, 1981; Williams, 1990). Motivation has previously been implicated in song learning through operant conditioning tasks demonstrating that juveniles will work to hear song and will work harder for the song of their preferred tutor (Adret, 1993; Fujii et al., 2021; Rodríguez-Saltos et al., 2021). Furthermore, juveniles' peak preference for father's song coincides, on average, with the sensory learning phase and predicts song learning (Rodríguez-Saltos, 2021). The tutor, by building a social bond with the juvenile, may shape the juvenile's motivation to learn the tutor's song and therefore the juvenile's mature song.

Juveniles in this study were attracted to both the tutor and a familiar female but appeared to work harder to be close to the tutor. Juveniles in the tutor condition spent significantly more time engaged in two-foot contacts with the walls of the zone closest to the tutor's cage (Fig. 3B). Two-foot contacts are a high-effort approach behavior and may reflect high motivation to be near and interact with the tutor. Beery et al (2021) argued that the effort an animal expends to access another individual more reliably implies motivation than do measures of social selectivity, or an animal's preference simply to associate with one individual over another. Social selectivity may indicate attraction without strong motivation, as male prairie voles strongly preferred to huddle with familiar conspecifics over strangers but worked to access only females, not males, regardless of familiarity (Beery et al., 2021). Similarly, in this study, juveniles may have been attracted to any familiar (or even unfamiliar) conspecific but be motivated to work to approach only song tutors. Juveniles in both social conditions did show attraction to both stimulus animals, as they spent most of the session in the proximal zone regardless of condition (Fig. 3A). Baran et al. (2017) showed that in a four-way preference test with other stimulus animals, juvenile zebra finches early in sensory learning spent the most time near their parents. Juveniles in the current study were not presented with a simultaneous choice between a tutor or a familiar female. The juveniles may have preferred to associate with their father if presented with both social options simultaneously. Finally, juveniles pecked the cage walls or the perch in proximity to the tutor more often than they did near the familiar female. Pecking behavior has previously been interpreted as a display of interest in a tutor (Pilgeram et al., 2022). Pilgeram et al. (2022) showed that when juveniles were treated with an oxytocin receptor antagonist, they performed fewer pecks to a tutor than in control sessions. As oxytocin is thought to be involved in social motivation and bonding (Syal & Finlay, 2011) including juveniles bonding with caregivers (Hammock, 2014), pecks to a tutor may reflect social motivation. In this study, the tutor's presence appears to have elicited greater social motivation in juveniles than the familiar female's presence.

Song-elicited Approach and Listening Behaviors

My results suggest that the juveniles were attracted to tutor song specifically. Juveniles moved into the proximal zone after song onset, as demonstrated by a shorter latency (or faster initiation) of approach than expected by chance. It would be interesting to test whether juveniles would approach equally quickly if the tutor's song were played from a recording, but only a familiar female was present, to determine whether the surrounding social context impacts juvenile's approach in response to song.

Juveniles did not make two-foot contacts more quickly after song, nor were their two-foot contacts more synchronous with song than expected (Figs. 5A-B, S1). Although social condition had a large effect on total time spent in two-foot contacts, the finding that they are no more likely to happen after song onset suggests that some juvenile behaviors may be elicited by the song learning context without being responsive to song specifically. As juvenile attention during tutor song, operationalized as being alert, quiet, and still, positively predicts quality of song learning (Chen et al., 2016), it may be disadvantageous to engage in high-energy or disruptive behaviors, such as two-foot contacts, while the tutor is singing. Such behaviors may interfere with listening attentively during song.

Preening

Preening, unlike two-foot contacts, is a restful behavior that has been hypothesized to facilitate listening (Pilgeram et al., 2022). Preening during tutoring has been found to be positively correlated with song learning (Houx et al., 2000). I hypothesized that, if preening is adaptive to song learning, the juvenile may be compelled to preen more often in a song learning context. Given this hypothesis, preening was expected to be greater in the tutor condition, but no effect of the social condition was detected (Fig. 4B). This finding suggests that preening is not elicited by the song learning environment. Instead, juveniles may simply learn better when they are already in a restful state.

Attention-Seeking and Social Bids

Along with approach, the second category of behavior classically thought to indicate mother-infant attachment in humans is signaling behavior, which brings the parent to the child (Bowlby, 1969). Beyond simply seeking a parent's proximity, children seek the attention of a caregiver, such as when they need assistance to complete new tasks (Gosselin & Forman, 2012). Attention-seeking behaviors may benefit social learning by promoting mutual responsiveness and joint attention between a young child and caregiver (Gosselin & Forman, 2012). Human infants and juvenile songbirds are dependent on caregivers and learn complex vocalizations from social feedback (reviewed by Baran, 2017; Kuhl, 2007; Varella & Ghazanfar, 2021), which may make attention-seeking behaviors especially important to these species (Varella & Ghazanfar, 2021). Studies investigating direct song contingency during the sensorimotor phase (e.g., tutors singing immediately after juvenile subsong) in zebra finches suggest that only a small portion of tutor songs are contingent on juvenile subsong (Chen et al., 2016) but that nonetheless, such contingency over the course of weeks may predict song learning (Carouso-Peck et al., 2020). In the sensory learning phase, juveniles may make social bids, such as two-foot contacts, beak-wipes, and pecks to a tutor, to elicit attention or other responsive behaviors from the tutor. This possibility raises a number of questions about the extent to which juvenile songbirds engage in social bids, whether a tutor might change singing behavior or otherwise react to these social bids, and whether that exchange enhances song learning. Houx & ten Cate (1998) found no evidence that juvenile zebra finches elicit song from tutors, for example by clumping (making physical contact with the tutor), begging (gaping beak up and calling), or approaching within one inch of the tutor. A recent study found that treating juveniles with an oxytocin antagonist before a social tutoring session decreased the number of times tutors sang, suggesting that tutors may be responsive to oxytocin-dependent behaviors of juveniles (Pilgeram et al., 2022). Because pharmacological manipulations were performed on the juveniles, not the tutors, these results indicate that the juvenile's behavior affected the tutor's behavior. However, the authors did not test whether song was contingent on, or correlated with, the juvenile behaviors that were affected by the treatment (such as pecks to a tutor).

Like pecks and other behaviors directed towards the tutor, beak-wiping may be an attention-seeking behavior. In adults, beak-wipes serve a dual purpose of grooming and courtship display; male zebra finches beak-wipe more frequently when a female is unresponsive (Morris, 1954), suggesting that this behavior could be a social bid for the female's attention. As the juveniles in this study were not old enough to mate and beak-wiping has not previously been ascribed significance in juveniles beyond grooming, this behavior was not expected to differ between the social conditions. Although it was not statistically significant, there was a medium-sized effect of social condition on beak wipes such that the behavior occurred more frequently in the tutor condition (Fig. 4A). It may be that beak-wipes are performed flexibly, in more contexts than exclusively during courtship displays and grooming, and that in juveniles they may serve a purpose related to communication.

Limitations

My interpretations of my results are subject to a number of limitations, the first being sample size. The sample size in this study was sufficient to detect only large effects of social condition and the tests of responsiveness to song were limited to the tutored group only, further limiting power. Both latency after song and synchrony with song may have been especially sensitive to outliers given the small sample size (n = 8) of juveniles in the tutor condition that heard at least one song. Furthermore, the study design itself presented some limitations. First, the between-subjects design has less statistical power than a within-subject design. Second, it is impossible to rule out the influence of the juvenile’s mother, who was in the same cage as the juvenile throughout the session. Because one aim of the present study was to measure juvenile behavioral responses to song and because adult males frequently sing after being reunited with their mate, the mother was included to increase the likelihood that tutors would sing. Furthermore, the mother’s presence contributed to the ecological validity of the design, as the learning environment would typically consist of overhearing males singing to females and observing contingent social interactions, including female responses to song. However, the mother’s behavior is a potential confound; although the mother was present in both conditions, she would be expected to behave differently in response to her mate than in response to a familiar female. Female zebra finches respond to song through social and sexual displays such as hops on perches, tail-quivering, fluffing, beak-wipes and calling (reviewed by Hauber et al., 2010). Like juveniles, females also approach song and work to hear song (reviewed by Hauber et al., 2010). Female behavior is known to impact song learning, as juvenile males raised with hearing females develop more normal songs than those raised with deaf females (Williams, 2004). Given previous research showing a closer bond with the father than the mother at a similar stage in development (Houx & ten Cate, 1998; Mann & Slater, 1995), it is likely that the juvenile's behavior was not driven entirely by the mother's behavior.

Conclusion

As researchers seek to understand the role of motivation and social interaction in SGVL in humans through songbird models, it is important to work with a toolkit of relevant behavioral paradigms. Part of developing the Bengalese finch model of vocal learning, and the songbird model generally, is understanding how their behavior depends on social and learning contexts. The current study builds on previous work by demonstrating that juveniles behave differently toward a caregiver and song model compared with another familiar conspecific. My results suggest that juveniles were motivated to approach the tutor, especially when he was singing. I propose that building and maintaining a social bond between tutor and juvenile critically enhances juvenile's motivation to learn and that juveniles engage in social bids to elicit a response from the tutor. Further work is needed to determine whether tutors are indeed responsive to juvenile behaviors such as pecking, two-foot contacts, or beak-wiping.

Human adults and children engage in rich behavioral interactions that coordinate joint attention and exchange social feedback, both of which are thought to be important to the acquisition of mature vocal skills (Kuhl, 2007). Furthermore, although SGVL is a rare skill among primates, it is a critical one for humans (Varella & Ghazanfar, 2021). The Bengalese finch is a suitable laboratory model for investigating the role of social behaviors and child-parent attachment in SGVL because it is highly gregarious, has been artificially selected for parental behaviors during domestication, and, due to overlapping phases of song memorization and practicing (Birke, 1974; Honda & Okanoya, 1999), juveniles have the opportunity to learn from social feedback. By demonstrating results in the Bengalese finch that are consistent with existing research that zebra finches approach tutors and tutor song (Houx & ten Cate, 1998; Houx et al., 2000), this study strengthens the applicability of the Bengalese finch model to SGVL in other species, including humans. Future experiments should investigate whether the social bond with a tutor makes a juvenile more receptive to learning his song, and the role of specific behaviors in both social bonding and SGVL.

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Table 1.

Behaviors and scoring criteria

Behavior	Event Type	Category	Definition
Proximal zone (s)	State	Approach	Time spent with majority of body within 5 in. of the side of cage closest to stimulus animal
Two-foot contacts (s)	State	Approach	Time spent hanging with two feet contacting the proximal wall or a side wall in the proximal zone
Pecks to stimulus animal (per min.)	Point	Approach	Pecks to wire of proximal wall or pecks to perch in proximal zone
Preening (count of motions)	Point	Grooming, restful	Self-cleaning of feathers with beak. Each new motion is a distinct event
Beak-wipes (count)	Point	Grooming, mating display	Wiping motion during which beak contacts a perch
Song (tutor only)	State	Vocalization	Loud, organized sequence of notes (Marler, 1970) of any length

Note. State events were coded as a duration (s) whereas point events were treated as an instant in time.

Table 2.

Results for each variable

Variable	Reliability [a]	Mean ± SD (Tutor)	Mean ± SD (Control)	$X^2(1)$	*p*	d_{av} [b]
Proximal zone (% time)	0.97	64.0 ± 14.3	61.7 ± 12.7	0.14	.71	0.17
TFC (% time)	0.99	23 ± 10	79 ± 5	11.86	**.00057**	1.89
Pecks/min	0.84	0.91 ± 0.35	0.44 ± 0.37	9.9	**.0016**	1.67
Beak wipes	0.59	12.78 ± 12.46	5.63 ± 5.63	9.64	.13	0.31
Preens	0.85	16.33 ± 16.80	23.13 ± 27.13	0.44	.50	1.15
		Mean ± SD (Observed)	Mean ± SD (Chance)			
Latency (P)		6.82 ± 3.95	10.21 ± 1.62	6.3	**.016**	1.15
Latency (TFC)		10.82 ± 3.32	11.28 ± 1.59	0.44	.51	0.20
Synchrony (P)		57.13 ± 35.91	60.14 ± 45.36	0.15	.70	0.08
Synchrony (TFC)		12.31 ± 9.74	23.40 ± 26.78	1.95	.16	0.55

Note. General linear mixed models were used to compare between conditions (top) or between observed and chance values (bottom) (See Methods). TFC = two-foot contacts, P = proximal zone, % time = percent of total time

[a] Intercoder reliability was calculated using a Pearson's correlation and the correlation coefficient is reported. [b] Effect size (d_{av}) was calculated using Cohen's *d* (Cumming, 2012).

*Bolded *p*-values are significant at corrected alpha levels (Table 3).

Table 3.

Ranked results with corrected alpha levels

Variable	*p*	Rank (i)	Corrected α	d_{av}
Proximal (T)	**.00021**	1	.0091	4.46
Proximal (C)	**.00040**	2	.018	4.86
TFC	**.00057**	3	.027	1.89
Pecks/min	**.0016**	4	.036	1.67
Latency (P)	**.016**	5	.045	1.15
Beak wipes	.13	6	.055	0.72
Synchrony (TFC)	.16	7	.064	0.55
Preens	.50	8	.073	0.31
Latency (TFC)	.51	9	.082	0.20
Synchrony (P)	.70	10	.091	0.08
Proximal zone (% time)	.71	11	.100	0.17

Note. *p*-values were ranked from smallest to largest. Alpha levels (α) were corrected using the Benjamini–Hochberg procedure with an acceptable false discovery rate (Q) of 10%. α = i/m * Q where i = rank, m = total variables tested (11), and Q = 0.1. All effects that were significant at α = 0.05 remained significant given the corrected α. TFC = two-foot contacts, P = proximal zone, % time = percent of total time.

[a] Effect size (d_{av}) was calculated using Cohen's d (Cumming, 2012).

* Bolded *p*-values are significant at corrected alpha levels.

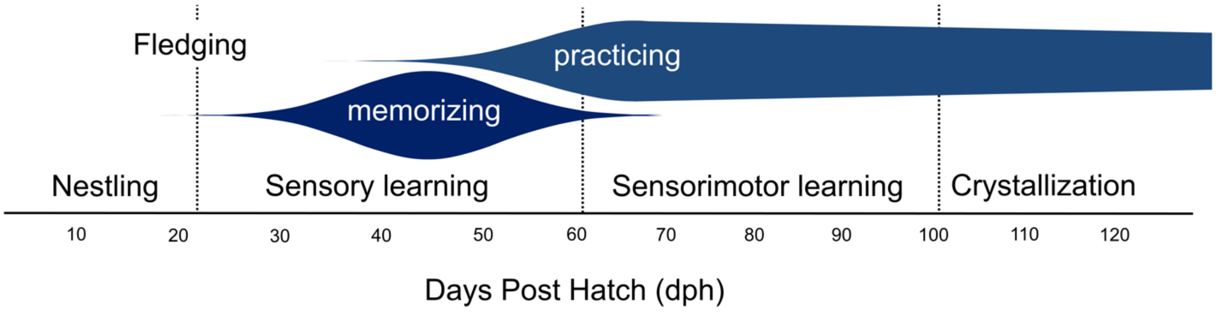


Figure 1. Sensitive period for song learning in Bengalese finches. Bengalese finches learn songs during overlapping sensitive periods for memorization and practicing. Full crystallization is attained by 120 dph (Okanoya, 2004). Figure adapted from Davis et al. (2022).

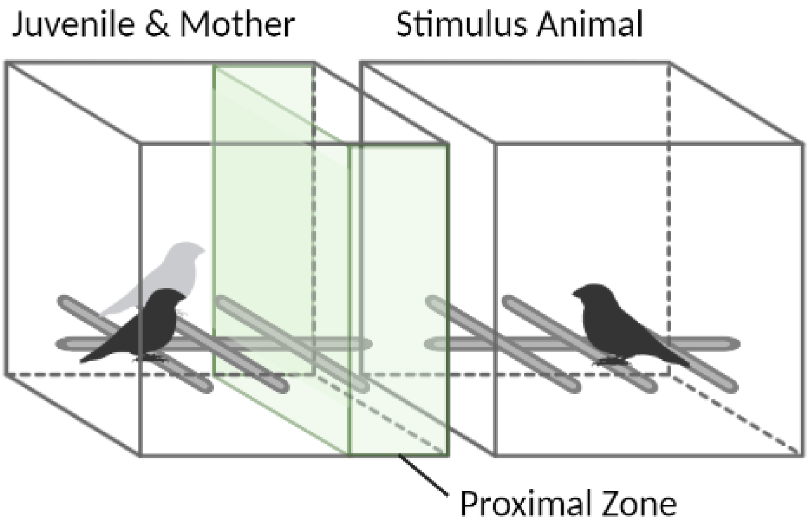


Figure 2. Experimental setup for behavioral assays. The juvenile was placed in a test cage where he could see and hear the stimulus animal. The proximal zone, the area within 5 inches of the stimulus animal's cage, is marked in green.

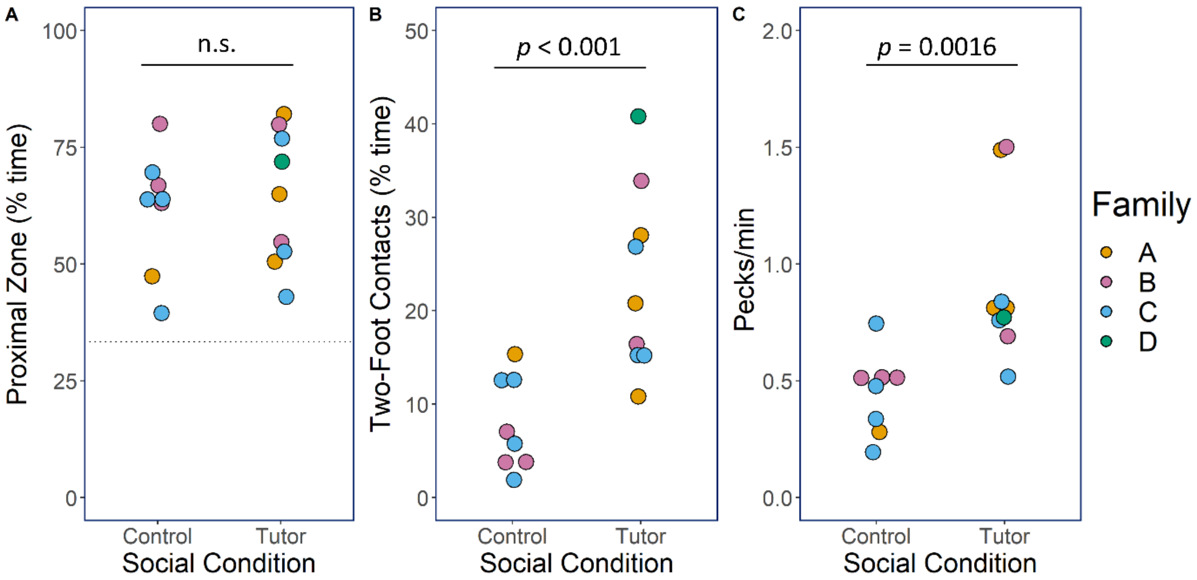


Figure 3. Effect of social condition on juvenile approach behaviors. Individual scores are plotted for juvenile approach behaviors in the control (n = 8) and tutor (n = 9) social conditions. The control stimulus animal was a familiar female. The *p*-values from general linear mixed models (see Methods) are shown, with asterisks indicating significance after corrected alpha (n.s., not significant). Proportion of time engaged in two-foot contacts in the proximal zone (B) and pecks to the stimulus animal in the proximal zone (C) were significantly higher in the tutor condition. Time in the proximal zone (A) was above chance (horizontal dotted line) in both conditions but did not significantly differ between conditions. Family (see legend) indicates shared parentage and housing and therefore juveniles that also had the same stimulus animal (with one exception in the control condition; see Methods).

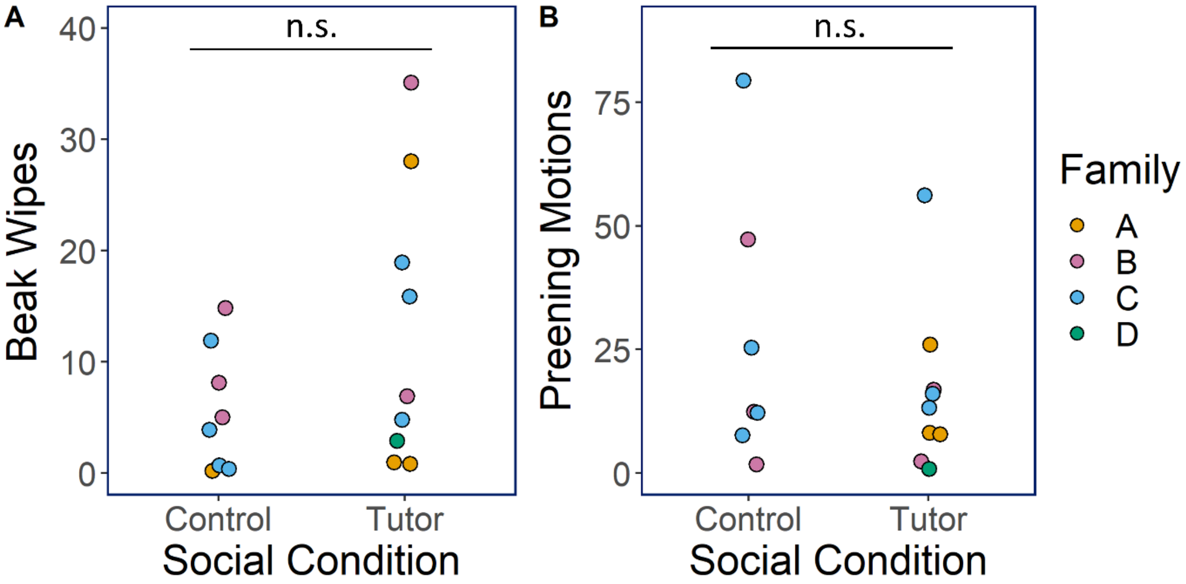


Figure 4. Effect of social condition on juvenile grooming behaviors. Individual scores are plotted for juvenile grooming behaviors (see Methods) in the tutor (n = 9) and control (n = 8) conditions. Neither beak wipes nor preening were significantly different between the two social conditions. n.s., not significant.

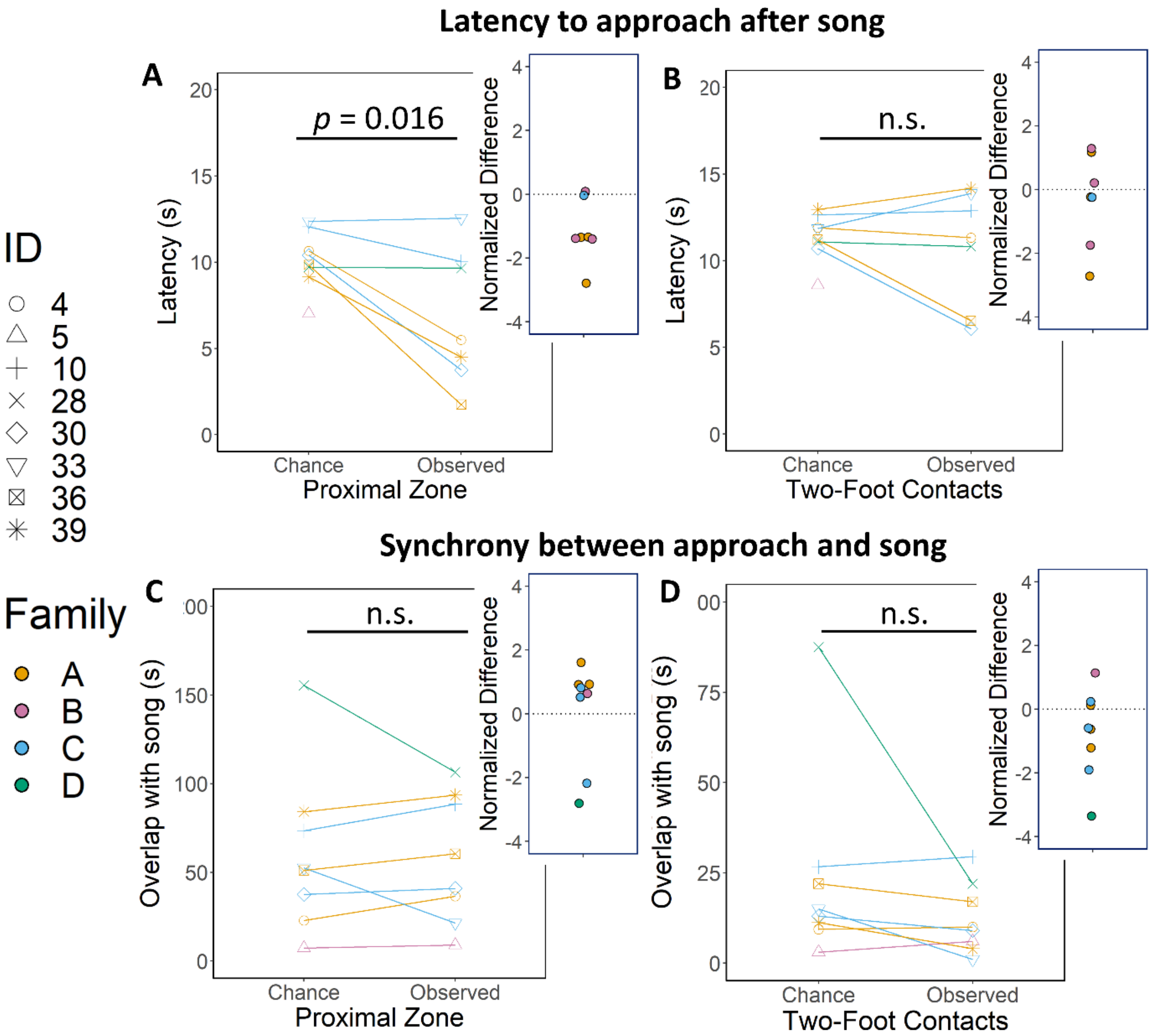


Figure 5. Juvenile approach in response to tutor song. Latency to begin approach behaviors after the onset of song (A, B) and synchrony between approach behaviors and song (C, D) are plotted (see Methods for definitions). Each line on each plot represents one of the juveniles in the tutor condition (n=8). Color-coded points indicate the mean of the observed or chance values for each juvenile (see Figs. S1 and S2). The values for both latency and synchrony are shown in seconds. One bird (ID=5) heard only one song and was already engaged in two-foot contact with a wall in the proximal zone, so no observed latency could be calculated. The *p*-values from general linear mixed models (see Methods) are shown (n.s., not significant). Insets depict the normalized difference (see Methods) between chance and observed latency (A, B) and chance and observed synchrony (C, D) as estimates of the individual effect size. Horizontal dotted lines represent the normalized difference when observed latency or synchrony is equal to chance.

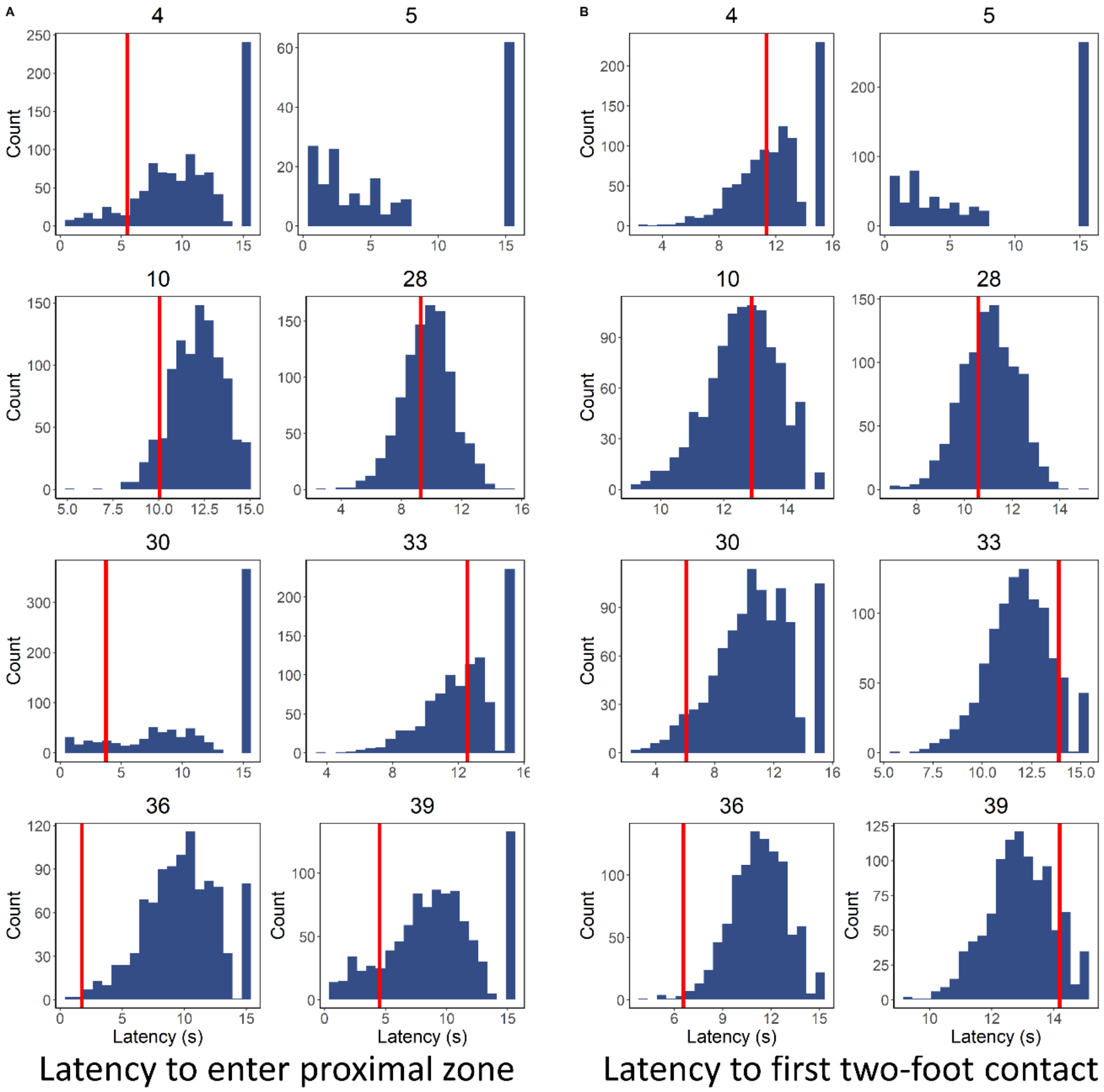
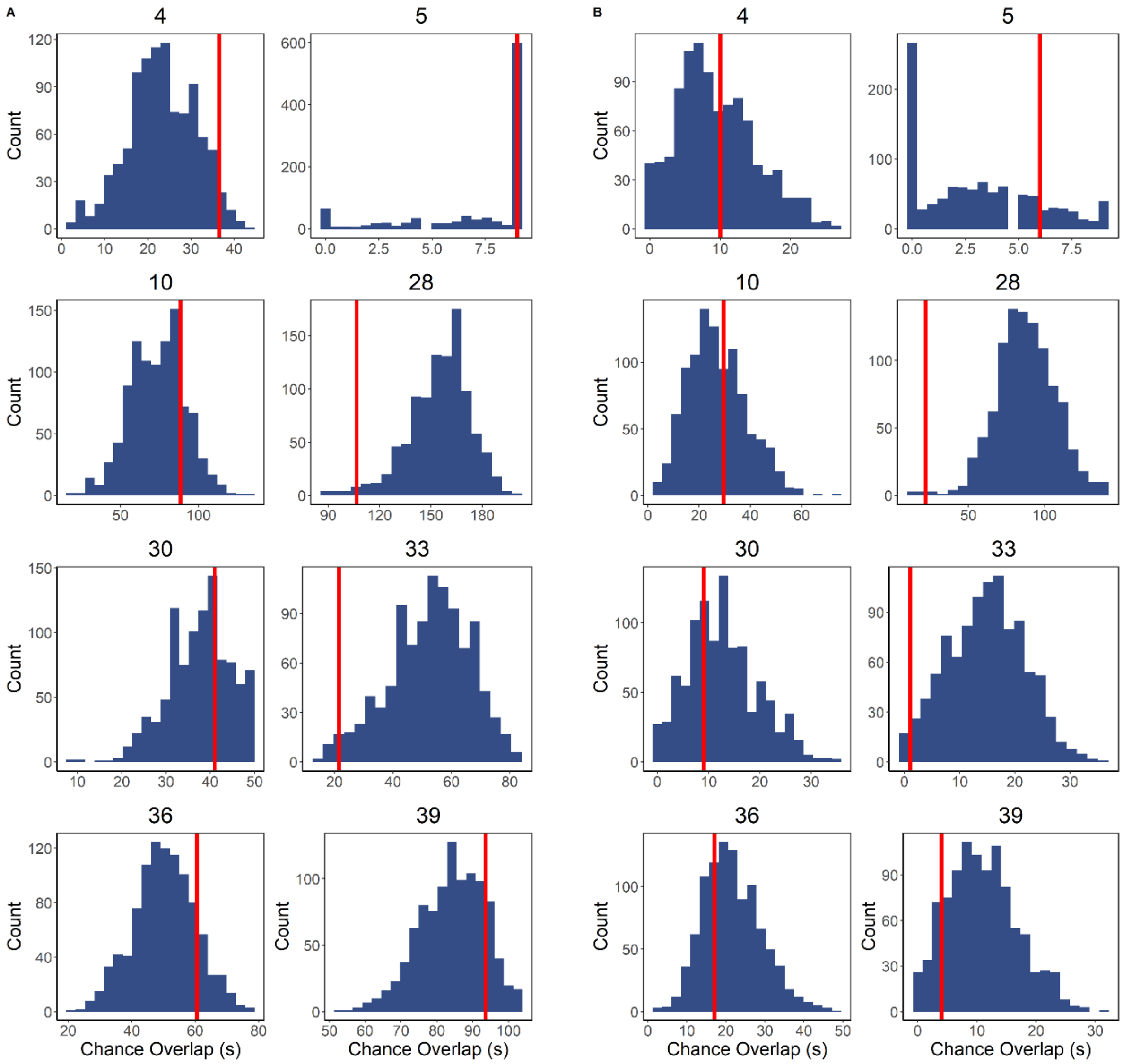


Figure S1. **Latency to begin approach behaviors after song onset for each juvenile.** Individual mean latency to enter the proximal zone (A) or to engage in two-foot contacts (B) is plotted over the distribution of mean latencies relative to randomly generated timestamps (see Methods). Vertical red lines indicate observed mean latency for that juvenile. Individual numerical ID is indicated above each graph. One bird (ID=5) heard only one song and was already engaged in a two-foot contact in the proximal zone, so no observed latency could be calculated.



Overlap between song and proximal zone

Overlap between song and two-foot contacts

Figure S2. Synchrony between approach behaviors and song for each juvenile. Overlap between tutor song and time in the proximal zone for each juvenile (A) and overlap between tutor song and two-foot contacts for each juvenile (B) are plotted over the distribution of chance overlap estimated from circularly permuted data (see Methods). Vertical red lines indicate the observed overlap. Individual numerical ID is indicated above each graph.