

ORIGINAL ARTICLE

Biased learning of sexual signals by female Bengalese Finches

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ORNITHOLOGICAL SCIENCE

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Abstract Peak shift in mate preference learning can be a driver of rapid repeated speciation. Therefore, clades that have undergone recent adaptive radiations are predicted to show biased learning of signals from the opposite sex. The estrildid finches are one such clade. In species including the Zebra Finch *Taeniopygia guttata* and the Bengalese Finch *Lonchura striata* var. *domestica*, females choose mates in part based on their songs. Consistent with theory, female Zebra Finches show peak shift in their learned response to male song characteristics. We used operant conditioning to train female Bengalese Finches to respond to songs with trills of one length and to ignore songs with trills of another length. Then, we exposed those females to songs with a range of trill lengths, and we observed their responses. We found that at least some Bengalese Finches also show behaviour consistent with peak shift in their response to male songs. Moreover, females evaluated songs relative to other songs they had recently heard. Our results suggest that females respond to male sexual signals with bias in multiple species in the rapidly speciating estrildid clade.

Key words Bird song, Estrildid finch, Mate choice, Operant conditioning, Peak shift

Many animals learn to respond to signals in their environments. For example, bees learn that some flower shapes or colours indicate high rewards, and preferentially visit those flowers (Pleasants 1981; Thomson 1988; Real & Rathcke 1991; Dreisig 1995; Cartar 2004). Predators learn to avoid prey with aposematic colouring that indicates prey defences (Gamberale-Stille 2001; Bonacci et al. 2008). Some animals learn signals associated with mate quality or receptivity, and so learn to prefer some mates or mate types over others (Dukas 2004; Verzijden et al. 2015). In sexual imprinting, individuals learn preferred mate phenotypes as juveniles and then exercise those preferences as adults. Often the learned prefer-

ence is for the phenotype of one of the individual's parents (Immelmann 1975; ten Cate & Vos 1999).

Learning can be biased. An animal that learns to respond to one signal (the S+ signal) and to avoid or ignore another (the S− signal) may later respond more frequently or more strongly to signals shifted away from S+ in the direction opposite S− (Hanson 1959; Ghirlanda & Enquist 2003). For example, Zebra Finch *Taeniopygia guttata* males that sexually imprint on their mothers' red beak colour (S+) and their fathers' yellow beak colour (S−) later display biased mate preferences for females with redder beaks than their mothers' (ten Cate et al. 2006). This phenomenon is called “peak shift” (Terrace 1966a). Alternatively, animals may respond most strongly to the S+ signal, but respond more strongly to signals on the side of S+ farther from S− than on the side closer to S−. This is called “area shift” (Terrace

(Received 15 November 2018; Accepted 23 May 2019)

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1966b; Thomas et al. 1973; Bushnell & Weiss 1980), because the area under the curve that describes the response frequency is shifted asymmetrically around S+. Thus, area shift differs from peak shift in the shape of the response curve. Throughout this paper, we will use the terms “peak shift” and “area shift” to indicate learned biases as described by Thomas et al. (1973), and we will use the generic term “shift” for biases in responses to signals that may or may not be due to learning.

In addition to the learned biases described in the previous paragraph, a biased response to signals can also arise when animals evaluate signals relative to others they have recently encountered, a phenomenon called context-dependence (Lawless et al. 1991; Bailey & Zuk 2009). For example, consider an animal that has learned S+ and S- signals, and is then confronted by a series of signals. The animal may be more likely to respond to a particular signal if it differs from the previous signal in the same way that S+ differs from S-. Like peak shift, context-dependence increases the rate at which animals respond to signals other than S+. However, while this occurs in peak shift because the animal's learning of S+ is biased, it occurs in context-dependence because the animal's perception of subsequent signals is biased.

A large body of work has studied how learning affects signal evolution and speciation (ten Cate & Rowe 2007; Verzijden et al. 2012), but less work has focused on the role of biased learning (Lynn et al. 2005; Grant & Grant 2010; Westerman et al. 2012). Recently, Gilman and Kozak (2015) reported a model in which biased sexual imprinting promotes rapid repeated speciation. This occurs because speciation by assortative mate choice requires that the phenotypes individuals use to assess mates (i.e., target traits) diverge (Ritchie 2007). Strong sexual selection reduces the variability in target traits, and so impedes divergence and speciation (Kirkpatrick & Nuismer 2004). However, if mate preferences are learned or applied with bias, then target trait variability can be maintained, and divergence and speciation can occur repeatedly within the same lineage and based on the same target trait (Gilman & Kozak 2015).

According to Gilman and Kozak's (2015) theory, we should expect to see examples of biased sexual imprinting (i.e., peak shift or area shift) or the biased application of mate preferences (i.e., context-dependent mate choice) in clades that have undergone recent adaptive radiations. The estrildid finches, which comprise 158 species in 29 genera including

the waxbills (*Estrilda*, 16 species) and mannikins (*Lonchura*, 32 species) (Arnaiz-Villena et al. 2009; Clements et al. 2018), are one such clade. In the estrildid Zebra Finch, females imprint on the songs of their fathers (Adret 1993). Verzijden and colleagues (2007) found that adult female Zebra Finches trained to respond to S+ and S- songs that differed in their number of repeats exhibited peak shift towards exaggerated versions of S+. However, whether there is biased learning or context-dependent evaluation of signals under sexual selection in other estrildid species has not been studied.

Here, we used operant conditioning and behavioural assays to study how Bengalese Finches *Lonchura striata* var. *domestica* learn to respond to songs that contain trills (the repetition of the same note more than twice) of different lengths. Like Zebra Finches, female Bengalese Finches learn their song preferences by imprinting on the songs of their fathers (Kato et al. 2010). Although there is currently no evidence that trill length is under direct sexual selection, several papers have hypothesised sexual selection on song elements (Okanoya 2002; Okanoya 2004a). Moreover, in a sample of 16 Bengalese Finches and 20 Munias (the wild ancestor of the Bengalese Finch), we found that Bengalese Finches had more trills in their songs (average 1.69 and 0.85, $P=0.02$), and that the number of repetitions per trill was significantly higher ($P=0.03$) in Bengalese Finches (average 6.69) than in Munias (average 3.55), which suggests that trill number and length is actively evolving under sexual selection in captivity (Okanoya 2004a). We trained adult female Bengalese Finches to respond to songs with trills of one length and to ignore songs with trills of another length. Then, we exposed the same females to songs with trills having a range of lengths. We asked i) do birds respond more frequently to songs with trill lengths more extreme than the trained S+ signal in the direction opposite the S- signal, and ii) does the response rate to each song depend on the trill length of the song the bird had most recently heard. Our results provide evidence for both peak shift and context-dependent responses to a signal (i.e., trill length), which is thought to be under sexual selection in a rapidly speciating clade.

MATERIALS AND METHODS

1) Animals

We selected five female Bengalese Finches age

8–24 months and naïve to operant conditioning from the Okanoya laboratory colony at the University of Tokyo. Bengalese Finches reach sexual maturity at four months, so all birds in our study were sexually mature. Subjects were housed together in a cage (W31 x D27 x H39 cm) with a light:dark cycle of 14:10 hours. Temperature and humidity were maintained at 25°C and 60% respectively. Experiments were performed in accordance with regulations from the Office for Life Science Research Ethics and Safety at the University of Tokyo (UTokyo-OLSRES).

2) Apparatus

Auditory discrimination training and experiments were performed in an operant apparatus in a sound-attenuating chamber (internal dimensions: W40×D58×H38 cm). Each bird in turn was placed in a cage (W15×D30×H22 cm) with an opening facing the front wall of the apparatus. From its perch, the bird could reach a hole (diameter 10 mm) on its left with a green peck key, a hole on its right with a red peck key, and an opening 15 mm below the peck holes that gave access to a food cup on the front wall of the apparatus, at a distance of 50 mm from the peck holes. A monitor positioned behind the peck keys and connected to a desktop computer caused the keys to light up. The peck keys recorded information about pecks. The operant apparatus was illuminated by a light-emitting diode, and a feeder delivered food to the food cup as a reward for correct response to the S+ stimulus. A speaker (S36G04K-3, Toptone, Kasukabe, Japan) above the bird and connected to a digital stereo amplifier (RSDA302U, Rasteme Systems Co., LTD) presented song stimuli during training and experiments. A camera at the back of the cage allowed the experimenter to observe the bird from an external monitor.

3) Stimulus songs

Songs of Bengalese Finch males were chosen from the Okanoya laboratory song library and edited using sound analysis software (Avisoft SASLab-Pro ver 5.2.08, Avisoft Bioacoustic, Berlin, Germany). Auditory stimuli were obtained from a 15-note string (duration 1.78 s) containing one trill of eight note repetitions. From this song, we created six additional stimuli by adding or removing notes from the trill, so that trills ranged from five notes (song -3) to 11 notes (song +3) as shown in Fig. 1. Songs +2 and -2 were used as the S+ and S- training stimuli, and all songs were used for testing. When we analysed the song

motifs of 14 random Bengalese Finch males from the Okanoya aviary, we found an average occurrence of trilled notes of 1.69 (range 0–4) per song motif and an average of maximum repetition of 7.13 (range 3–31). Thus, the song stimuli used in this experiment had a natural range of trilled note repetitions. Birds were initially naïve to all song stimuli presented.

4) Experiment 1: Longer trills functioning as S+

Training took place every day from October 2014 to July 2015 between approximately 0900 and 1300. During this period, access to food was limited to four hours per day, and vitamin-enriched water was available *ad libitum*. Birds were fed at 1300 and deprived of food from 1700 until the following day. Food deprivation enhances motivation in operant tasks (Park et al. 1985). Bird weight was monitored each day before training and deprivation.

Birds were trained to respond to song +2 (S+) and ignore song -2 (S-). Each training session comprised 120 trials, with the two stimuli presented with equal frequency in random order. Lighting of the green peck key indicated the start of each trial. When the bird pecked the key, the green light turned off and a stimulus song was played. Then, the red peck key turned on and the bird's response to the key was recorded. Pecks in response to S+ ("hits") were rewarded by the delivery of egg-coated yellow millet, signalled by lighting the food cup for three seconds. Pecking in response to S- ("false alarm", FA) was corrected with a mild punishment consisting of a period of darkness ranging from 15 to 36 seconds, depending on the birds' performance. Missed responses ("misses"; when birds ignored S+) and correct rejections ("CRs"; when birds ignored S-) were not reinforced. If the bird did not respond to a stimulus within two seconds, the red light turned off and the next trial began after three seconds. Training ended after 120 trials or 50 minutes. The accuracy of each bird during each session was calculated as the sum of correct responses (hits and CRs) divided by the total number of trials (Seki et al. 2013). An accuracy $\geq 85\%$ and a FA rate $<25\%$ maintained for at least two consecutive sessions indicated that the bird had learned the task and was ready for testing.

Testing sessions studied birds' responses to the full range of song stimuli. Sessions comprised 24 test trials with songs +3, +1, -1, -3 (6 trials per stimulus) randomly interspersed with 96 trials with the training stimuli (48 trials each for S+ and S-). Correct and incorrect responses to the training stimuli were

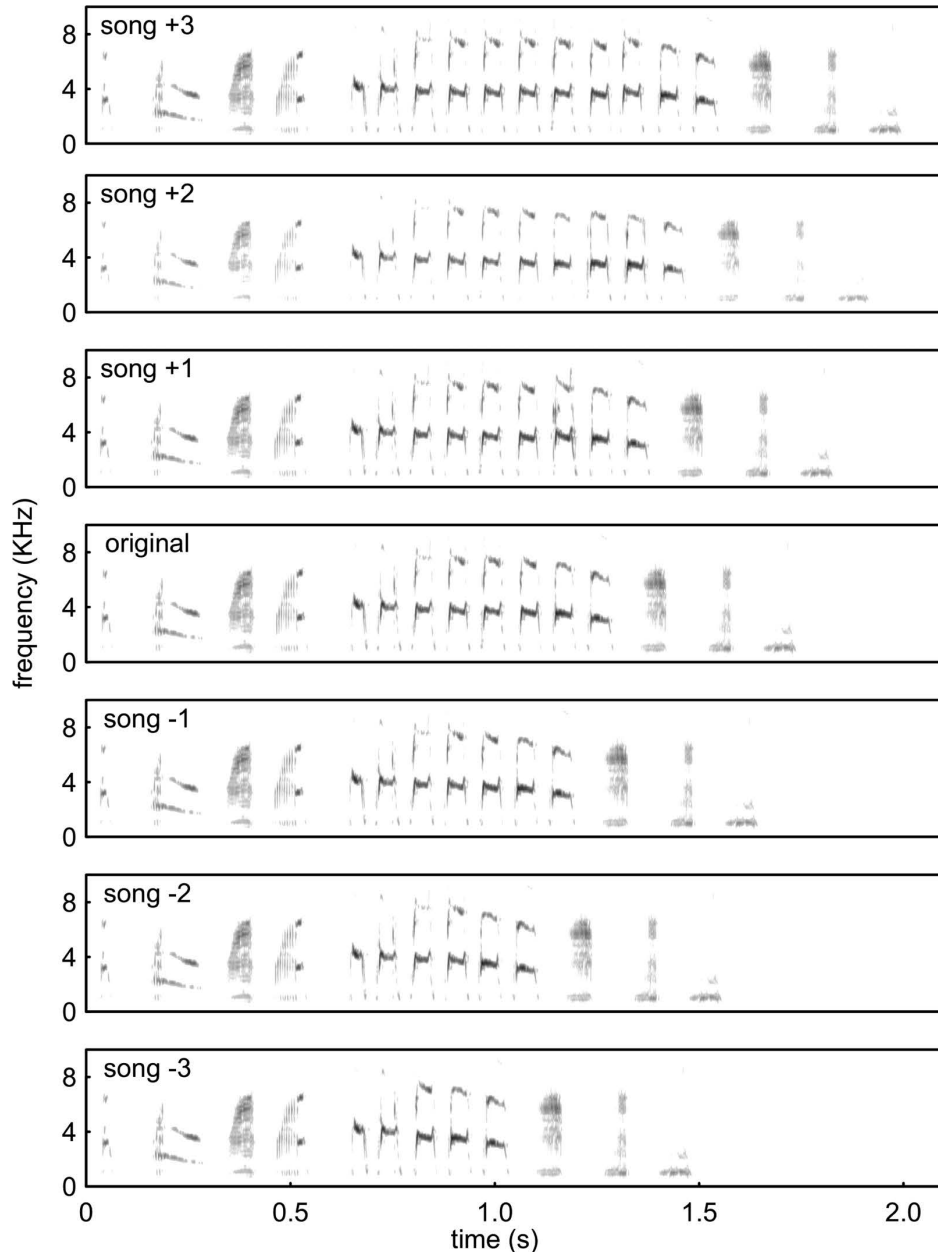


Fig. 1. Stimulus songs used for discrimination training and testing. Song 0 was selected from the Okanoya laboratory library of Bengalese Finch songs and includes a trill of eight notes. We created six new songs by adding (+1, +2, +3) or removing (-1, -2, -3) notes from the trill of song 0. Songs +1, +2, and +3 were used for discrimination training (*i.e.*, as S+ or S- stimuli), and all modified songs were used for response testing. Boxed notes in the figure indicate the trill component of each song.

rewarded or punished as in the training sessions, while responses to test stimuli were not rewarded or punished. Results were discarded if accuracy was <85% or if the FA rate was $\geq 25\%$ for the training stimuli. Testing sessions were separated by a minimum of two days of retraining.

We analysed the data from experiment 1 to answer

three questions. First, do birds show peak shift in their response to songs with different trill lengths? If birds show peak shift, they will respond more frequently to the +3 song than to the +2 song. Second, do birds show area shift in their response to songs with different trill lengths? If birds show area shift, they will not respond more frequently to the +3 than

to the +2 song, but they will respond more frequently to the +3 than to the +1 song. Finally, does the response to the current song depend on the song that the bird heard immediately previous to that song? We hypothesised that birds would respond more frequently to the current song if it differed from the previous song in the same direction that S+ differs from S-. If this were true, then birds in experiment 1 would respond more frequently to a given song, if the previous song had a shorter trill.

5) Experiment 2: Shorter trills functioning as S+

Apparent peak shift or area shift discovered in experiment 1 might indicate the biased learning of stimuli, or might indicate an innate tendency to respond more frequently to songs with longer trills. Therefore, we must account for possible innate biases before we can attribute our results to biased learning. We cannot test for innate biases in untrained birds, because untrained birds do not peck keys in response to signals. We cannot test effectively for innate biases in birds trained with only S+ songs, because birds trained with only S+ signals respond with high probability to all signals (Verzijden et al. 2007). Therefore, we opted for an approach that would demonstrate biased learning even if birds have innate biases. We retrained the same birds used in experiment 1, this time using song -2 as the S+ and song +2 as the S- stimulus. Then, we conducted testing sessions looking for peak shift and area shift toward shorter trills. If peak shift occurs in the same bird but in different directions in experiments 1 and 2, then an innate bias cannot explain peak shift in that bird.

It is possible that the training in experiment 1 had a lingering effect on behaviour even after retraining in experiment 2. Therefore, if we fail to find evidence for peak shift in experiment 2, we cannot be confident that peak shift toward shorter trills does not occur. This might mean simply that our method failed to produce peak shift. However, if we do find peak shift toward shorter trills, then we can be confident that peak shift toward shorter trills does occur.

6) Analysis

We analysed the data from experiments 1 and 2 separately with mixed logistic regression models using the package lme4 in R (Bates et al. 2015). Each trial, in each session, provided one data point. The response variable was whether the bird pecked the red key after hearing the song in that trial. The potential predictors were trill length (categorical with

six values: -3, -2, -1, +1, +2, +3), the sign of the difference in trill length between the test song and the previous song (numeric with values -1, 0, or +1), and the position of the test song in the session (numeric, scaled from 0 to 1, where the first test song was zero and the last test song was 1). Random effects were session date nested within bird identity. These account for the fact that different birds may have had different baseline response rates, and that those rates may have varied independently for each bird on each day of the study. We determined whether each random effect belonged in the best model using likelihood ratio tests (Fox 2002). We selected the model with all or a subset of the fixed effects and the lowest Akaike information criterion (AICc) value as the best model for the data (Burnham & Anderson 2004).

The data from experiment 1 shows complete separation: birds always responded to the +3 song. Thus, the maximum likelihood effect size of the +3 song is not meaningful, and we used a Monte Carlo approach (Metha et al. 2000) to ask whether responses to the +3 song differed from responses to the +2 and/or +1 songs. To do this, we fitted a mixed logistic regression model to the data assuming that there was no difference in the probability of response to the song types of interest. We used the fitted model to predict the probability that birds would respond to each song in the test session. Then, we permuted the observed responses among the song types of interest, and we calculated the exact probability of obtaining each permuted set of responses. We did this for 10^8 permutations. We calculated the summed probability of all permutations in which birds always responded to the +3 song, and we divided this by the summed probability of all 10^8 permutations. This estimates the probability that birds in the experiment would have responded to every instance of the +3 song if there were no difference in response to the song types of interest.

RESULTS

1) Experiment 1

Three of five birds achieved 85% accuracy in training and could be used in experiment 1. These three birds successfully completed 10, 10 and eight testing sessions, providing a total of 3,360 responses that we could analyse. Another one, four and five testing sessions were excluded from analysis because the birds did not achieve the predefined accuracy threshold on the trained S+ and S- signals during those sessions. The best model for the responses of birds to

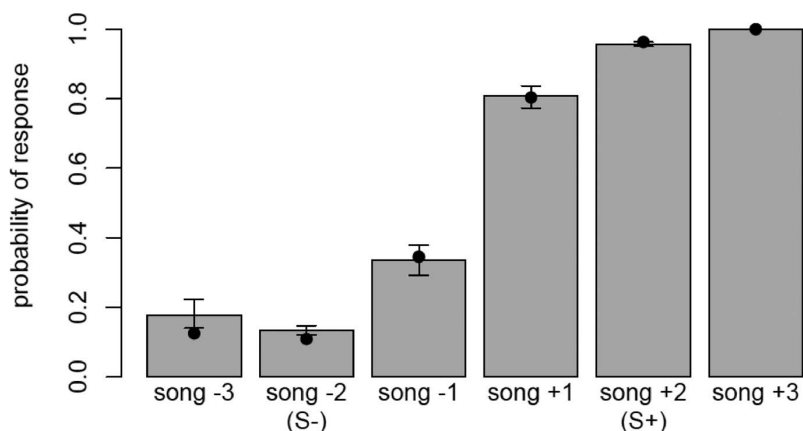


Fig. 2. Probabilities of response to songs with different trill lengths in experiment 1. Birds were trained to respond to song +2 (10-note trill) and to ignore song -2 (6-note trill). Birds were more likely to respond to song +3 than to song +1 (area shift, $p < 0.0001$) and more likely to respond to song +3 than song +2 (peak shift, $P = 0.0080$). Bars show predicted values from the best model for a song that i) follows a song with the same trill length and ii) occupies position 60 in the test session. Error bars show standard error of the predictions. Filled circles show observed mean response rates. In test sessions, songs with very short (long) trills usually followed songs with longer (shorter) trills, and birds evaluated each song in part relative to the immediately preceding song. Therefore, the observed mean response rates to songs with very short (long) trills were lower (higher) than the predictions shown in the figure for songs that followed songs with the same trill length.

Table 1. Fixed effects in the best model for 3,360 responses in experiment 1 (S+: song +2, S-: song -2). Relative effect sizes for songs with different trill lengths are shown in Fig. 2 and table S2.

Predictor	Effect size	Standard error	P-value
trill length	see Fig. 2		<0.0001
difference in trill length from previous song	-0.48	0.0914	<0.0001
position of trial in session	0.61	0.2284	0.0080

stimuli in experiment 1 includes random effects of bird identity ($\Delta \log \mathcal{L} = 5.22$, $P = 0.0054$) and session date ($\Delta \log \mathcal{L} = 2.64$, $P = 0.0715$). The effect of session date was marginally significant, but omitting session date from the model does not change our qualitative results. The best model includes fixed effects of trill length, the difference in trill length from the previous song, and the position of the song in the session (table 1; supplementary tables S1, S2). Birds were more likely to respond to song +3 than to song +2 ($P = 0.0080$, Fig. 2; see Supplementary Fig. S1 for response rates of each bird individually), showing a shift in stimulus response. Birds were more likely to respond to songs if the previous song had shorter trills ($p < 0.0001$, table 1).

2) Experiment 2

One bird was successfully retrained to respond to signal -2 (S+) and ignore signal +2 (S-), and this bird was used in experiment 2. The bird successfully completed nine testing sessions providing a total of 1,080 responses that we could analyse. Another three testing sessions were excluded from analysis because the bird did not achieve the predefined accuracy threshold on the trained S+ and S- signals during those sessions. We found no evidence for a random effect of session date on response probability in experiment 2 ($\Delta \log \mathcal{L} < 0.01$, $p > 0.99$). The best model for the response probability in experiment 2 includes fixed effects of trill length and the difference in trill length from the previous song (table 2; supplementary tables S3, S4). We found no difference in the response probability to songs -3 and -2 ($P = 0.6881$, Fig. 3), and thus no evidence for peak shift. We found no significant difference in the response probability to songs -3 and -1, but there was a non-significant trend in this direction (i.e., toward area shift, $P = 0.1175$, Fig. 3).

The shift in response probability in experiment 1 might be due to an innate tendency to respond more frequently to songs with longer trills. However, if birds had an innate tendency to respond to songs with longer trills, then we would expect the bird in

Biased learning by female birds

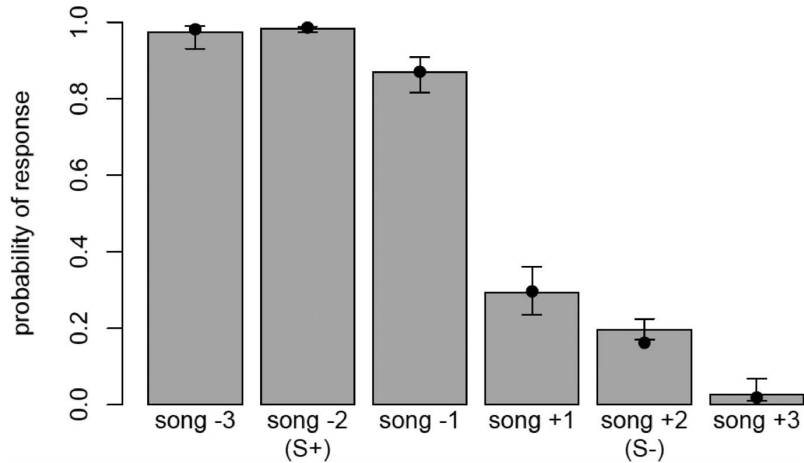


Fig. 3. Probabilities of response to songs with different trill lengths in experiment 2. The bird was trained to respond to song -2 (six-note trill) and to ignore song +2 (10-note trill). Probabilities are predictions from the best model for a song that follows a song with the same trill length. Error bars show standard error of the predictions. Filled circles show observed means. Filled circles show observed mean response rates. In test sessions, songs with very short (long) trills usually followed songs with longer (shorter) trills, and birds evaluated each song in part relative to the immediately preceding song. Therefore, the observed mean response rates to songs with very short (long) trills were higher (lower) than the predictions shown in the figure for songs that followed songs with the same trill length.

Table 2. Fixed effects in the best model for 1,080 responses in experiment 2 (Go stimulus: song -2, NoGo stimulus: song +2). Relative effect sizes for songs with different trill lengths are shown in Fig. 3 and table S4.

Predictor	Effect size	Standard error	P-value
trill length	see Fig. 3		<0.0001
difference in trill length from previous song	0.34	0.1693	0.0419

experiment 2 to have responded more frequently to song +3 than to song +2, both because song +3 had a longer trill and because song +2 was the conditioned S- stimulus. In fact, we found the opposite. The bird was significantly less likely to respond to song +3 than to song +2 ($P=0.0299$, Fig. 3). Thus, an innate preference for longer trills cannot explain the patterns we observed.

The bird was more likely to respond to songs if the previous songs had longer trills ($P=0.0419$, table 2). Thus, in both experiments 1 and 2, birds responded more frequently if a song differed from the previous song in the same direction that S+ differed from S-.

DISCUSSION

It has been theorised that biased sexual imprint-

ing can promote rapid repeated speciation (Gilman & Kozak 2015). If this mechanism is important in nature, then we should expect to see biased learning of preferences for sexual signals in some clades that have undergone recent adaptive radiations. Male song is a target of sexual imprinting in estrildid finches including the Zebra Finch (Adret 1993) and the Bengalese Finch (Okanoya 2004a). Verzijden and colleagues (2007) found that female Zebra Finches learned to respond to male song elements with bias. Our results suggest that at least some female Bengalese Finches also show behaviour consistent with peak shift in their responses to male song characteristics. Moreover, whether a female Bengalese Finch responds to a particular song depends on the other songs she has recently heard. To our knowledge, this is the first evidence of a context-dependent response to sexual signals in estrildids.

Context-dependent mate choice may facilitate speciation. To understand why this is true, let us assume that the song of a given bird consisted of trills of fixed note number (although in reality, trill length varies within individuals). Recall that speciation by assortative mate choice requires the divergence of traits under sexual selection (Ritchie 2007), and that sexual selection itself tends to stabilise trait values and prevent divergence (Kirkpatrick & Nuismer 2004). Now, consider two females that each imprint on a song

with an eight-note trill (perhaps their father's song) and learn to ignore a song with a six-note trill. If one of these females encounters a male with a nine-note trill immediately before she evaluates a potential mate, she may be likely to ignore a potential mate with an eight- or nine-note trill and look instead for a mate with a 10-note trill. This would cause a peak shift similar to that caused by biased sexual imprinting. If the other female encounters a male with a five-note trill immediately before she evaluates a potential mate, she may be likely to accept the potential mate even if his trill has only seven notes. Across all females imprinted on eight-note trills, the net effect would be to reduce the probability of choosing mates with eight-note trills and increase the probability of choosing mates with different trill lengths. This would weaken stabilising sexual selection on male trill length, and potentially allow for the divergence of trill length in the population. This argument is consistent with the mechanisms proposed by Gilman and Kozak (2015) to promote speciation, but to our knowledge it has not been tested in formal models.

We observed shift around the S+ signal in experiment 1, when S+ contained longer trills than S-, but not in experiment 2, when S+ contained shorter trills than S-. Similarly, Verzijden and colleagues (2007) observed peak shift around their S+ signal when it contained more notes than S-, but not when it contained fewer notes. The bird in experiment 2 had previously been used in experiment 1, and its earlier training may have affected its behaviour in the later experiment. However, Verzijden and colleagues (2007) used different birds in each of their experiments, so prior training cannot explain why birds in their study did not show peak shift around the shorter S+ signal. Interestingly, when we trained birds to respond to shorter trills and to ignore longer trills, we observed a shift in responses around the long-trilled S- signal. Birds were more likely to ignore the +3 song than the +2 song they had been trained to ignore. Such shift around an S- has been called negative peak shift (Guttman 1965; Purtle 1973; Ghirlanda & Enquist 2003). We found no evidence for negative peak shift around the S- signal when the S- signal contained a shorter trill. Thus, in each case where we identified a shift, it occurred around a signal with a long trill. This suggests a *post hoc* hypothesis: when trills are long, it may be more difficult for birds to assess small differences in trill length (Weber's law, Rowe & Skelhorn 2004), and difficulty in discriminating trill lengths may result in

more pronounced shift.

Our analysis separates the effect of biased learning from that of context dependence. In practice however, both mechanisms affect a bird's responses simultaneously, and context dependence augments peak shift. For example, consider a case in which S+ includes a longer trill than S-. If a bird evaluates a test song with a trill longer than S+, peak shift favours a response to that song. Moreover, the longer the test song is, the more likely it is that the previous song was shorter than the test song. So, context dependence favours responses to songs longer than S+ and favours ignoring songs shorter than S-. This pattern is apparent in Figs. 2 and 3, where the observed response rates (represented by filled circles) to extreme songs are more extreme than the predicted rates (represented by the bar heights). This occurs because the predictions are for test songs that have the same trill length as the previous song, but in our experiment this was seldom true. We expect that context dependence will augment peak shift in the same way in nature.

The Bengalese Finch is a domesticated variant of the White-rumped Munia (Washio 1996). Since domestication, the song of the male Bengalese Finch has evolved a greater syntactical complexity (including longer trills) than that of its wild ancestor (Honda & Okanoya 1999). There is no evidence that humans have selected for song features in Bengalese Finches (Washio 1996), and song evolution in the Bengalese Finch is unexplained. Female Bengalese Finches learn to prefer mates with songs similar to those of their fathers (Kato et al. 2010). Our results suggest that female Bengalese Finches learn to respond to male songs with bias, and the response is consistent with Weber's law. Thus, if female response to song complexity is learned in the same way as female response to trill length, then sexual selection driven by (i) females learning their preferred trill length with peak shift, and (ii) a Weber effect causing peak shift to be greater when trills are longer, might explain song evolution towards longer trills in Bengalese Finches.

Because the number of birds in our study was small, we cannot confidently extrapolate our results to all Bengalese Finches. However, because the number of trials per bird was large, we can be very confident that the birds in our study showed biased learning of trill lengths. Thus, our work falls into the common class of studies that demonstrate the existence of a behaviour without attempting to assess how common that behaviour is in nature (e.g. Weir et

al. 2002; Plotnik et al. 2006; Krupenye et al. 2016). Studies that have observed biased learning in other species have found that it is remarkably consistent across individuals (Thomas et al. 1991; Verzijden et al. 2007; Martinez-Harms et al. 2014), and therefore we conjecture that biased learning is unlikely to be rare in Bengalese Finches.

Birds in this study showed biased learning of a signal that is known to be under sexual selection (Okanoya 2002; Okanoya 2004b). In our experiment, birds learned the S+ and S− signals by operant conditioning. Biased learning is thought to arise from an interaction between signal qualities and the sensory or neurological apparatus used to process that signal (Thomas et al. 1991). Thus, we conjecture that a signal learned with bias during operant conditioning would also be earned with bias during sexual imprinting. Other authors have made similar suggestions (Verzijden et al. 2007), but to our knowledge this has not been formally tested.

Adaptive radiations have fascinated biologists since Darwin (Schluter, 2000b), and have generated some of Earth’s most spectacular examples of biodiversity (Schluter 2000a; Allender et al. 2003; Grant & Grant 2014). Recently, increasing attention has turned to the potential role of learning in facilitating adaptive radiation (Verzijden et al. 2012; Gilman and Kozak 2015). Theory suggests that learned mate preferences can promote speciation (Kozak & Boughman 2009; Verzijden et al. 2005), and that biased mate preference learning can promote rapid and repeated speciation (Gilman & Kozak 2015). In many rapidly speciating clades mate preferences are at least partly learned (Verzijden et al. 2008; Kozak et al. 2011; Grant & Grant 2014), but whether that learning is biased has rarely been studied. Coupled with the results of Verzijden and colleagues (2007), our results provide the first evidence that the response to sexual signals may be learned with bias in at least two species in the same rapidly speciating clade. Many studies of species that are, and species that are not, products of recent adaptive radiations, will need to be conducted before a link between biased learning and adaptive radiation can be firmly established or refuted. Our work is a first step in that direction.

ACKNOWLEDGMENTS

We would like to thank G. M. Kozak for her comments on the manuscript. This work was supported by NERC [grant NE/K500859/1] to RTG and JSPS

[grant #26240019] to KO.

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Supplementary Fig. S1. Probabilities of response to songs with different trill lengths in experiment 1 for each bird individually. Birds were trained to respond to song +2 (10-note trill) and to ignore song -2 (six-note trill). Bars show predicted response probabilities from the best model for a song that i) follows a song with the same trill length and ii) occupies position 60 in the test session. Error bars show standard error of the predictions. Filled circles show observed mean response rates. The bird in panel (A) was also used in experiment 2.

