Within-species differences in vocal production learning in a songbird are associated with differences in flexible rhythm pattern perception

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Media summary

When we recognize a song that's played faster or slower than usual, we are detecting timing *relations* between sounds, not just absolute durations. This ability relies on auditory-motor interactions in the brain. Auditory-motor processing is more developed in animals that learn their vocalizations so we hypothesize that such animals are advantaged in recognizing a rhythmic pattern at different rates. We test this idea in a songbird where only males learn to sing, and find males are slightly better than females at rhythmic pattern recognition. Thus, songbirds are ideal for investigating individual differences in rhythm pattern perception and underlying brain mechanisms.

Abstract

Humans readily recognize a familiar rhythmic pattern, such as isochrony (equal timing between events) across a wide range of rates. This ability reflects a facility with perceiving the relative timing of events, not just absolute interval durations. Several lines of evidence suggest that this ability is supported by precise temporal predictions that arise from forebrain auditory-motor interactions. We have shown previously that male zebra finches, which possess specialized auditory-motor networks and communicate with rhythmically patterned sequences, share our ability to recognize isochrony independent of rate. To test the hypothesis that flexible rhythm pattern perception is linked to vocal learning, we ask whether female zebra finches, which do not learn to sing, can also recognize global temporal patterns. We find that non-singing females can flexibly recognize isochrony but perform slightly worse than males on average. These findings are consistent with recent work showing that while females have reduced forebrain song regions, the overall network connectivity of vocal premotor regions is similar to that in males and supports predictions of upcoming events. Comparative studies of male and female songbirds thus offer an opportunity to study how individual differences in auditory-motor connectivity influence perception of relative timing, a hallmark of human music perception.

1. Introduction

The ability to recognize auditory rhythms is critical for many species [1–3], but the underlying neural mechanisms are only beginning to be understood. One area of progress is recognition of communication signals based on tempo. For example, female field crickets are attracted by male calling songs composed of trains of short sound pulses when the tempo is ~ 30 syllables/sec. This selectivity is hard-wired and mediated by a small network of interneurons that processes instantaneous pulse rate [4]. While this preference is genetically fixed, in other animals experience can sculpt neural responses to behaviorally salient call rates. For example, in the mouse auditory cortex, excitatory cells are innately sensitive to the most common pup distress call rate (~5 syllables/s), but their tuning can broaden to a wider range of rates following co-housing with pups producing calls across a range of rates [5].

Much less is known about how the brain recognizes rhythmic *patterns* independently of rate. While humans can encode and remember the rate of auditory sequences [6], we also readily recognize rhythmic patterns across a broad range of rates [7]. In music, one of the most widespread temporal patterns is isochrony, or equal timing between events [8], and the ability to detect and predict periodicity is central to the positive effects of music-based therapies on a variety of neurological disorders, including normalizing gait in Parkinson's disease [9,10]. The ability to recognize a rhythmic pattern whether it is played fast or slow is present in infants [11] and is based on recognition of the relative timing of events more than on their absolute durations. In humans, there is growing evidence that the neural mechanisms underlying detection of relative timing are distinct from those involved in encoding absolute timing [12–14]. In addition, neuroimaging studies have shown that both auditory *and* motor regions are active when people listen to rhythms, even in the absence of overt movement. Responses in several motor regions are greater when the stimulus has a strong, periodic pulse, or "beat" [15,16] and transient manipulation of auditory-motor connections using transcranial magnetic stimulation can disrupt beat perception without affecting single-interval timing [17].

Based on such findings, we and others have suggested that perception of temporal regularity (independent of rate) depends on the interaction of motor and auditory regions: the motor planning system uses information from the auditory system to make predictions about the timing of upcoming events and communicates these predictions back to auditory regions via reciprocal connections [18,19]. Such predictions could support detection of rhythmic patterns independent of tempo because the relative duration of adjacent intervals remains the same across different rates (e.g., 1:1 for an isochronous pattern).

Given that vocal learning species often communicate using rhythmically patterned sequences [20,21] and have evolved specialized motor planning regions that are reciprocally connected to auditory forebrain regions, we have hypothesized that vocal learners are advantaged in flexible auditory rhythm pattern perception [22]. Consistent with this hypothesis, zebra finches and starlings (vocal learning songbirds) trained to discriminate isochronous from arrhythmic sound sequences robustly generalize this ability to stimuli at novel tempi, including rates distant from the training tempi [22,23]. This ability, also seen in humans [7], demonstrates a facility with recognizing a rhythm based on global temporal patterns, since absolute durations of intervals differ markedly at distant tempi. These findings contrast with similar research conducted with vocal non-learning species. For example, pigeons can learn to discriminate sound sequences based on tempo but cannot learn to discriminate isochronous from arrhythmic sound patterns [24]. Rats can be trained to discriminate isochronous from arrhythmic sounds, but when tested at novel tempi, they show limited generalization, suggesting a strong reliance on absolute timing for rhythm perception [25].

Here, we further test the hypothesis that differences in vocal learning abilities correlate with differences in flexible rhythm pattern perception by taking advantage of the sexual dimorphism in zebra finches [26]. In this species only males learn to imitate song, and the neural circuitry subserving vocal learning is greatly reduced in females. Thus we predicted that male zebra finches would exhibit faster learning rates for discriminating isochronous versus arrhythmic stimuli and/or a greater degree of generalization in recognizing these categories at novel tempi. By using the same apparatus, stimuli, and methods, we can meaningfully compare the flexibility of male vs. female rhythmic pattern perception in this species.

Several prior findings, however, support the opposite prediction - either no sex differences in rhythmic pattern perception or better rhythmic pattern perception in female zebra finches. One reason females may not be disadvantaged in our task is that they analyze male song when choosing a mate, and a previous meta-analysis found that female zebra finches are faster than males at learning to discriminate spectro-temporally complex auditory stimuli [27]. In addition, while the volume of vocal motor regions that subserve song performance is substantially greater in male zebra finches, a recent anatomical study found male-typical patterns of connectivity in the vocal premotor region in females, including minimal sex differences in afferent auditory and other inputs [28]. Moreover, a recent study of antiphonal calling suggests that female zebra finches may perform better than males on rhythmic pattern processing [29]. In that study, both male and female zebra finches could predict the timing of calls of a rhythmically calling vocal partner, allowing them to adjust the timing of their own answers to avoid overlap. This ability to predictively adjust call timing was enhanced in females and was disrupted by lesions of vocal motor forebrain regions, suggesting that brain regions associated with singing in males may subserve auditory perception and/or timing abilities in female zebra finches.

2. Methods and analysis

(a) Subjects

Subjects were 24 experimentally naïve female zebra finches from our breeding colony (mean age=72 ± 8 (SD) days post-hatch (dph) at the start of training; range=61-87 dph). Data from 14 age-matched male

zebra finches were collected previously [22]. All procedures were approved by the Tufts University Institutional Animal Care and Use Committee.

(b) Auditory Stimuli

All stimuli used in this experiment were the same as those described previously [22]. Briefly, we used sequences of natural sounds from unfamiliar male conspecifics: either an introductory element that is typically repeated at the start of a song, or a short harmonic stack [30] (see Fig. S1). For each sound, an isochronous sequence with equal time intervals between event onsets and an arrhythmic sequence with a unique temporal pattern (but the same mean inter-onset interval (IOI)) were generated at two base tempi: 120 ms and 180 ms IOI (Fig. 1A). These tempi were chosen based on the average syllable rate in zebra finch song (~7-9 syllables per second, or 111-142 ms IOI [30]). The number of elements, overall duration, amplitude, and spectral profile were matched across a pair of isochronous and arrhythmic stimuli. For the arrhythmic stimuli, the minimum gap between consecutive elements was 0 ms, and the maximum possible IOI was 1.5 times the base IOI. An additional pair of probe stimuli (one isochronous, one arrhythmic) was generated at a tempo (144 ms IOI) 20% faster/slower than the training stimuli.

For "rule training" (see below), an isochronous/arrhythmic pair was generated with a novel harmonic stack (sound D, see Fig. S1) at tempi ranging from 75 ms to 275 ms IOI in 1 ms steps (i.e., at 201 rates). Each arrhythmic stimulus had a temporally unique pattern. For a subset of these stimulus pairs (n=15/201), the amplitude was inadvertently higher as described previously [22].

(c) Auditory Operant Training Procedure

Training and probe testing used a go/interrupt paradigm as described previously [22]. Briefly, female birds were mildly water restricted and worked for water rewards, routinely performing ~520 trials/day. Pecking the trial switch triggered playback of a stimulus: either rewarded (S+, 50% chance) or unrewarded (S-, 50% chance). For rhythm discrimination experiments, the isochronous patterns were rewarded (S+ stimuli). Trial and response switches were activated 500 ms after stimulus onset, and pecking either switch stopped playback. "Hits" were correct pecks of the response switch during S+ trials. "False alarms" were pecks of the response switch on S- trials and resulted in lights out (up to 25s) [31]. If neither switch was pecked within 5 s of trial onset, the trial would end ('no response'). No response to the S- stimulus was counted as a "correct rejection", and no response to the S+ stimulus was considered a "miss". During this 5 s window, a bird could also peck the trial switch again to "interrupt" the current trial, which was counted as a 'miss' or 'correct rejection' depending on whether a S+ or S- stimulus had been presented. The use of the trial switch to interrupt trials varied widely among birds and was not analyzed further. Regardless of response, birds had to wait 100 ms after the stimulus stopped playing before a new trial could be initiated.

Shaping and Performance Criteria. To learn the go/interrupt procedure, birds were first trained to distinguish between two unfamiliar conspecific songs (~2.4 s long, "shaping" phase), one acting as the S+ (rewarded or 'go') stimulus, and the other as the S- (unrewarded or 'no-go') stimulus. Lights-out punishment was not implemented until a bird performed ≥ 100 trials. The criterion for advancing to the next phase was ≥ 60% hits, ≥ 60% correct rejections, and ≥ 75% overall correct for two of three consecutive days. Five females did not complete the shaping process within 30 days and were removed from the study.

Training: *rhythm discrimination*. Once a bird reached criterion performance on the shaping stimuli, she was trained to discriminate isochronous versus arrhythmic stimuli (n=13 birds). As described previously, each bird was trained using multiple sound types and multiple stimulus rates. In the first training phase, each bird learned to discriminate two isochronous stimuli (120 ms and 180 ms IOI) from two arrhythmic

stimuli (matched for each mean IOI). Once performance criterion was reached, the bird was presented with a new set of stimuli at the same tempi but with a novel sound element (and a novel irregular temporal pattern at each tempo; see Fig. 1B). One group of females ('ABC'; n=6) was trained to discriminate sound A stimuli, followed by sound B and then sound C; a second group ('CAB'; n=7) was trained with sound C, followed by sounds A and B. One female 'ABC' bird was presented with stimuli at 3 additional tempi, but those trials were not reinforced and were excluded from analysis.

Probe Testing/Generalization. To test whether birds could generalize the isochronous vs. arrhythmic classification at a novel tempo, females were tested with probe stimuli at 144 ms IOI, 20% slower/faster than the training stimuli (120 ms and 180 ms IOI). We previously showed that male zebra finches can discriminate between two stimuli that differ in tempo by 20% [22] and confirmed this in female zebra finches (see below and Fig. S2B). Probe sounds were introduced after a bird had successfully completed two phases of training (see Fig. 1B). Prior to probe testing, the reinforcement rate for training stimuli was reduced to 80% for at least two days. During probe testing, training stimuli (90% of trials) and probe stimuli (10% of trials) were interleaved randomly. Probe trials and 10% of the interleaved training stimuli were not reinforced or punished[22,32].

Rule Training. Following probe testing with all three sound types, subjects (n=5 birds) were presented with a new set of isochronous and arrhythmic stimuli using a novel sound (sound D). In this phase, stimulus tempi included every integer rate from 75 ms to 275 ms IOI (201 total rates). Arrhythmic stimuli were again generated independently so that each rate had a novel, random irregular pattern, with the same mean IOI as its corresponding isochronous pattern. Trials were randomly drawn with replacement from this set of 402 stimuli, and all responses were rewarded or punished as during training. This large stimulus set made it unlikely that subjects could memorize individual temporal patterns.

Discrimination of other acoustic features. To determine whether the 20% difference between the training and probe stimuli could be detected by female zebra finches, we tested a separate cohort of females on tempo discrimination (n=6; 71 ± 9.1 (SD) dph at start of shaping). Following shaping, these birds were trained to discriminate isochronous sequences of sound A based on rate: 120 ms vs. 144 ms IOI. The rewarded (S+) stimulus was 120 ms IOI for four females and 144 ms IOI for two females. These females were also tested on their ability to discriminate spectral features using frequency-shifted isochronous sequences of sound A: one shifted 3 semitones up, and the other shifted 6 semitones up ("Change pitch" in Audacity v 2.1.2). Four females were tested with the 6-semitone shift as the S+ stimulus, and two females were tested with the 3-semitone shift as the S+ stimulus.

(d) Data Analysis

All statistical tests, except for the binomial tests for training and generalization, were performed in R (v. 3.6.2) within RStudio (v. 1.2.5033). Binomial logistic regressions were performed with lme4 (glmer) statistical package for R.

Training and generalization testing. To quantify performance the proportion of correct responses ((Hits + Correct Rejections) / Total number of trials) was computed for each stimulus pair (isochronous and arrhythmic patterns of a given sound at a particular tempo). For training phases, proportion correct was always computed based on the last 500 trials. For probe testing, performance was computed for the 80 probe trials. The proportion of correct responses was compared to chance performance (p=0.5) with a binomial test using α=0.05/2 in the training conditions (2 tempi), α=0.05/3 for probe trials (3 tempi). A linear least-squares regression was used to examine the correlation between each subject's average performance on interleaved training stimuli and probe stimuli.

Performance of females (n=7) during probe trials was compared to that of male zebra finches (n=7) collected in a prior study [22]. Performance across all probe trials (n=240 probe trials, 80 probe trials per song element x 3 song elements) was analyzed with a binomial logistic regression using a generalized

linear mixed model with sex, probe phase number, and sex-by-probe phase number interaction as fixed effects and subject as a random effect.

Rule training. As described previously [22], the first 1000 trials were analyzed for each bird (n=5) to minimize any potential effect of memorization. Trials were binned in 10 ms increments, and the number of correct responses was analyzed with a binomial logistic regression using a generalized linear mixed model (tempo bin as a fixed effect and subject as a random effect). Performance in each bin was compared to performance in the 75-85 ms IOI bin, where performance fell to chance since the degree of temporal variation in inter-element intervals was severely limited by the duration of the sound element. To identify possible sex differences, we performed an additional analysis using data from a range of tempi (95-215 ms) in which both males and females performed significantly above chance ($p<0.005$; binomial test). The number of correct and incorrect trials were calculated per sex and analyzed for significant group differences with a $\chi 2$ test.

Discrimination of Other Acoustic Features. Performance on tempo or frequency discrimination was analyzed in the same manner as for rhythm discrimination: the proportion of correct responses in the last 500 trials was compared to chance performance using a binomial test with $\alpha=0.05$.

Reaction Time. For the last 500 trials in a training phase, the time between trial initiation and response selection (when the bird pecked a switch) was computed and averaged. For the rhythm discrimination birds, only the first training phase (prior to probe testing) was used. Mean reaction time values were compared between 14 birds that completed all rhythmic training phases, 9 birds that failed to complete rhythm discrimination training, and 10 birds that learned the frequency discrimination (data from 6 females in this study and from 4 males collected as part of [22]) using a two-way ANOVA (sex, group, and sex-by-group interaction) followed by a Tukey multiple comparisons post-hoc test. Data met criteria for homoscedasticity and normal error distribution.

3. Results

(a) Rhythmic pattern training and generalization testing in female zebra finches

To test the ability of female songbirds to recognize a rhythmic pattern based on the relative timing of events, 13 female zebra finches were first trained to discriminate isochronous from arrhythmic sequences using a go/no-go paradigm with three training phases (Figs. 1A–B & S1; age: 61–87 dph on the first day of training). Seven out of 13 females learned to discriminate these sequences in all three phases, each of which used a different zebra finch song element. The number of trials for these females to reach criteria for rhythmic discrimination (see Materials and Methods) was comparable to that of male zebra finches tested previously (Fig. 1C; data from male birds in [22]). Fig. 2A shows the time course of learning for a representative female zebra finch (*y7o97*), which gradually learned to withhold her response to the arrhythmic stimulus. Across the seven successful females, rhythm discrimination performance was ~81% accurate at the end of the first training phase (Fig. 2B, left column), and a comparable accuracy level was attained in each training phase (median = 80% proportion correct; see Fig. S2A for learning curves for each bird on the rhythm discrimination task).

After successful completion of two phases of rhythm discrimination, females were then asked whether they could generalize the discrimination of isochronous versus arrhythmic stimuli at a novel tempo (Fig. 1B, 'probe testing'). Fig. 2B shows the performance on randomly interleaved training stimuli (*dark gray bars*; 90% of the trials*)* and 80 probe stimuli at a novel tempo (*light gray bars;* 10% of the trials) for the seven females that successfully completed all three phases of rhythm discrimination training. Performance on probe stimuli was significantly above chance for 17 of 21 probe tests (n=3 probe tests/bird x 7 birds, $p<0.0167$, binomial test with Bonferroni correction), indicating that female zebra

finches robustly generalized the discrimination of isochronous vs. arrhythmic stimuli to a novel tempo distant from the training tempi (Fig. 2B).

(b) Sex differences in rhythm pattern perception

To test the hypothesis that the ability to flexibly perceive rhythmic patterns is linked to vocal learning, we directly compared performance of female zebra finches, which do not learn to imitate song, with that of vocal learning males (Fig. 3). Across all generalization tests, discrimination of isochronous versus arrhythmic stimuli at a novel tempo (probe stimuli) was significantly lower for female zebra finches compared to males (n=7 males and 7 females; 240 probe trials per bird; mixed-effects logistic regression, $p = 0.033$). The observed sex difference in the ability to generalize rhythm discrimination did not reflect differences in motivation to perform the task. On average, female zebra finches performed ~520 trials/day, compared to ~530 trials/day for males in our previous work. Across all subjects (n=14), performance on interleaved training stimuli (learned discrimination) was positively correlated with performance on probe stimuli (measure of generalization; Fig. S3; $p = .0375$).

As a second test of the ability to recognize temporal regularity independent of tempo, five females that completed rhythm discrimination training were tested with sequences of a new sound element (sound D, see Figs. S1-S2) across a wide range of tempi (75-275 ms IOI; "rule training"). For each trial, one of 402 stimuli was played (with equal odds of an isochronous or arrhythmic sequence), reducing the likelihood that correct discrimination was based on memorization. Fig. 4 shows the average performance of all birds over the first 1000 trials, broken into 10 ms bins (~50 trials per bin). For female birds, performance was best between 95 and 215 ms IOI (tempi ~20% slower to ~25% faster than the original training range: darker bars with >=2 black asterisks, $p<0.005$; mixed effect logistic regression). Performance fell to chance at faster tempi (75-85 ms IOI), when temporal variability in IOIs was limited by the length of the sound element, and at slower tempi (225-275 ms IOI). To directly compare performance to that of male zebra finches, the number of correct and incorrect trials within the 95-215 ms IOI tempo range (where both sexes performed well above chance) were grouped by sex. In this range, female performance was significantly worse than male performance ($\chi^2(1) = 13.919$, $p<0.001$).

(c) Reaction times for discrimination of acoustic features.

Across female birds who succeeded in rhythm discrimination training (n=7), the average reaction time at the end of the first training phase was ~1.70 s after stimulus onset, or ~75% of the duration of the stimulus train (Fig. 5). Data from previously tested males that succeeded in rhythm discrimination training (n=7) shows a similar average reaction time (1.90 s), indicating that both males and females heard 10-15 intervals on average before responding (overall average reaction time of ~1.80 s). In contrast, the average reaction time for the females that did not reach the criterion for rhythm discrimination during training (n=6) was ~1.30 s, ~50% of the duration of each stimulus, similar to the average failed male (n=3) reaction time of ~1.18 s.

Shorter reaction times, however, do not necessarily indicate poor perceptual discrimination. In a separate cohort of birds that learned to discriminate isochronous sequences that differed in frequency by 3 semitones (i.e., a quarter of an octave; n = 6 females; average proportion correct responses in last 500 trials = 91%; see Fig. S2B and n = 4 males from [22]), the average reaction times for successful discrimination was ~1.24 s. This suggests that unlike rhythm discrimination, discrimination based on spectral features does not require hearing long stretches of the sequence.

Comparison of reaction times between successful rhythm discrimination birds, unsuccessful rhythm discrimination birds, and successful frequency discrimination birds showed a significant effect of group (Fig. 5; two-away ANOVA, $F(2,27)$, $p = 0.001$). Tukey post-hoc tests showed that the reaction times of the successful rhythm birds were significantly higher (slower) than both the unsuccessful rhythm birds and the successful frequency birds. There was no effect of sex or a sex-by-group interaction. Taken together,

these results suggest that success at rhythm discrimination may be related to how long birds listened to the stimuli before responding. Indeed, even among the birds that successfully generalized the discrimination of temporal regularity across a wide range of tempi, reaction time was longer for the tempi where the discrimination was more difficult (i.e., when the proportion of correct responses was closer to chance performance: 75-85 ms and >235 ms; Fig. S4).

4. Discussion

To test the hypothesis that differences in the capacity for flexible rhythm pattern perception correlate with differences in vocal learning, we investigated the ability of a sexually dimorphic songbird to recognize a fundamental rhythmic pattern common in music and vocal communication – isochrony [8,20,21]. In zebra finches and many other songbirds, only the males learn to sing and possess pronounced forebrain motor regions for vocal production learning [26]. Previously we showed that male zebra finches can readily discriminate isochronous versus arrhythmic patterns independent of absolute time intervals [22], and we predicted that female zebra finches would perform less well when tested with the same rhythm discrimination and generalization tasks. Using a sequential training paradigm with multiple sound types and tempi, we found that about half of females tested (n=7/13) could learn to differentiate isochronous from arrhythmic patterns, and the learning rates for these females were similar to those of males (Fig. 1C). Females that completed rhythm discrimination training readily generalized the discrimination to stimuli at novel tempi (Fig. 2B & 4). Correct discrimination of isochronous vs. arrhythmic stimuli was significantly above chance at tempi ranging from 20% slower to ~25% faster than the original training range, indicating that female zebra finches, like males, can recognize a rhythmic pattern based on the relative timing of events rather than on absolute durations. Notably, in both sexes, birds that succeeded in learning to recognize isochrony listened for longer before responding compared to birds who failed (Fig. 5, left two bars), consistent with attention to global temporal patterns.

While female zebra finches can learn to recognize isochrony independent of tempo, we found small but significant differences in performance between males and females. First, across all three tests of generalization at a novel tempo, males outperformed females (Fig. 3; average proportion of correct responses during probe trials: 75% vs. 69%). Second, males consistently slightly outperformed females when tested with stimuli across a broad range of tempi at which both sexes performed well above chance (Fig. 4; 95-215 ms IOI; average proportion correct: 76% vs 72%). These differences did not reflect differences in motivation to perform the task; on average, males and females performed comparable numbers of trials/day. Finally, the proportion of females that successfully recognized isochrony based on global temporal patterns (n=7/13) was lower than that of males tested previously (n=7/10), although more data would be needed to determine if this difference is reliable.

It is important to note that the sex difference we observed, while consistent with our hypothesis, is an *average* difference. Modest, but consistent, sex differences are well known in biology (e.g., on average, men are taller than women, due, in part, to sex-biased gene expression [33]), but these differences typically pertain to anatomy, not cognition [34]. Prior work on sex differences in the auditory domain has focused largely on neural mechanisms in the periphery [35–38], although more recent work has demonstrated hormone-mediated differences in forebrain auditory responses to conspecific songs in birds [39,40]. Here, we show a small, but consistent sex difference in an auditory cognitive task – flexible rhythm pattern recognition – in a sexually dimorphic bird.

Yet just as some women are taller than some men, we find that some individual female zebra finches can outperform individual males in our tasks (e.g., probe tests in Fig. 3). How can this be reconciled with our hypothesis of a link between the neural circuitry for vocal learning and flexible rhythm pattern perception? Recent neuroanatomical work found that although female zebra finches possess smaller vocal motor regions, the overall network connectivity of vocal premotor regions is similar in male and female zebra finches [28]. Similarities in ascending auditory inputs to premotor regions, in particular, suggest that

auditory processing mechanisms may be conserved across sex. This raises the question of whether individual variation in auditory-motor circuitry correlates with individual differences in flexibility of rhythmic pattern perception, irrespective of sex. Indeed, in humans, the strength of cortical auditory-motor connections predicts individual differences in rhythmic abilities [41,42].

Our finding that female zebra finches perform slightly worse than males in recognizing isochrony contrasts with a prior finding that female zebra finches outperform males in a task involving temporal processing of rhythmic signals [29]. In that study, birds (6 males and 6 females) called antiphonally with a robotic partner that emitted calls at a rate of 1 call/second. Birds quickly learned to adjust the timing of their calls in order to avoid a jamming signal introduced at a fixed latency after the robot call, with females showing more pronounced adjustments of call timing than males. While both studies highlight the importance of prediction for rhythm perception, the underlying mechanisms for predicting upcoming events may differ between the two studies. In principle, avoidance of the jamming signal could have resulted from learning a single temporal interval – the time between the robot call and the jamming signal. In contrast, in our task birds had to learn to recognize the relative timing of successive events and to respond to the same pattern, even when absolute interval durations changed. Prior work suggests distinct mechanisms for single-interval versus relative timing [12–14], so a male advantage on a task the depends on relative timing is not necessarily inconsistent with a female advantage in single interval timing.

Several recent studies have begun to elucidate the neural mechanisms for rhythm perception in nonhuman animals. For example, Asokan et al. (2021) found that the responses of neurons in the primary auditory cortex of mice are modulated by the rhythmic structure of a sound sequence. While neurons in the midbrain and thalamus encode local temporal intervals with a short latency, cortical neurons integrate inputs over longer a timescale (~1 s), and the timing of cortical spikes differs depending on whether consecutive sounds are arranged in a repeating rhythmic pattern or are randomly timed. In gerbils, midbrain neurons have also been shown to exhibit context-dependent responses: on average, neural responses were greater for noise bursts that occurred on the beat of a complex rhythm compared to when the same noise bursts occurred off-beat [44]. Similarly, in rhesus monkeys, occasional deviant sounds in auditory sequences elicit a larger auditory mismatch negativity in electroencephalogram recordings when those sequences had isochronous vs. arrhythmic event timing [45]. While these studies found context-dependent modulation of auditory responses, they did not test the ability of the animals to recognize a learned rhythm independently of tempo. Demonstrating this ability requires behavioral methods, and an important lesson from prior research is that training methods can strongly influence to what extent such abilities are revealed (e.g., compare [46] with [22] and [47] with [48]; see also [49]). More generally, the ability to relate neural activity to perception and behavior is critical for understanding the contributions of motor regions to detecting temporal periodicity and predicting the timing of upcoming events, two hallmarks of human rhythm processing that are central to music's positive effect on a variety of neurological disorders.

Data accessibility. Concatenated trial data for female zebra finches, summarized data files, and female analysis code are available through Mendeley Data: https://dx.doi.org/10.17632/2r29x6gr7w.1

Published data for male zebra finches as well as stimulus files, circuit diagrams, male analysis code, and operant chamber setup diagram are available through Mendeley Data: https://dx.doi.org/10.17632/fw5f2vrf4k.2

The modified Pyoperant code for this experiment is available on GitHub: https://github.com/arouse01/pyoperant.

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Figures

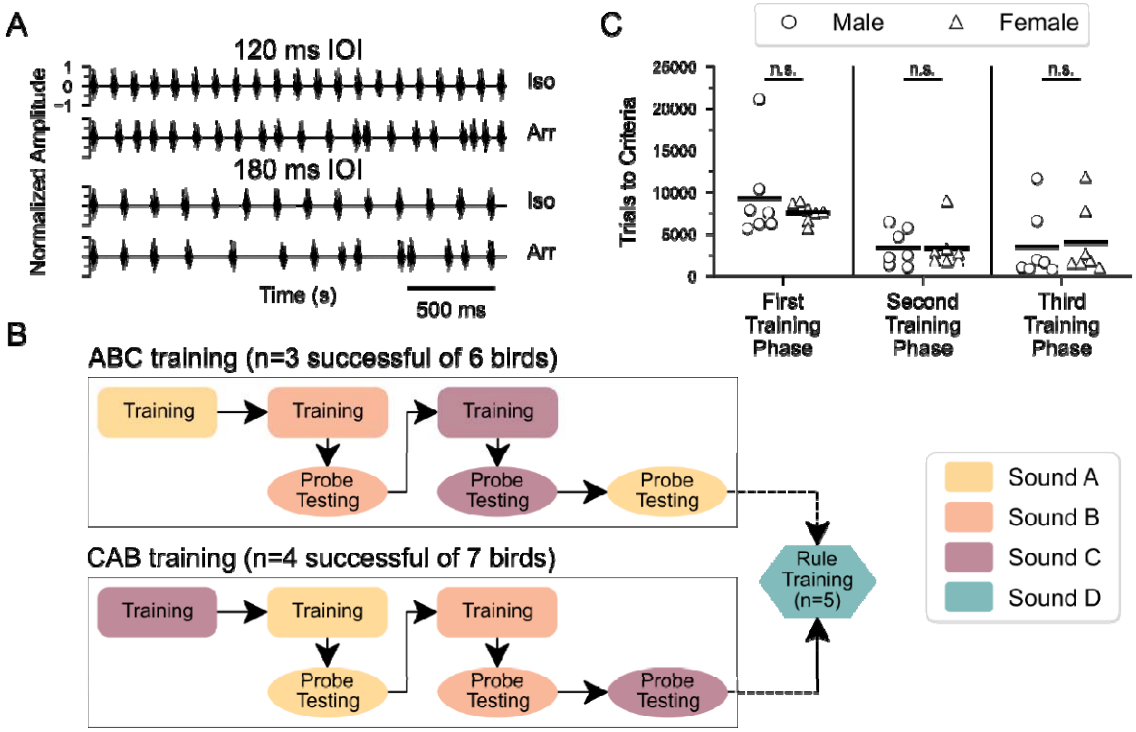

Figure 1. Experiment design for testing the ability to flexibly perceive rhythmic patterns. *(A)* Amplitude waveforms of isochronous (*Iso*) and arrhythmic (*Arr*) sequences of a repeated song element (sound B; see Fig. S1) with 120 ms (*top*) and 180 ms (*bottom*) mean inter-onset interval (IOI). *(B)* Schematic of the protocol. After a pre-training procedure (not shown), birds learned to discriminate between isochronous and arrhythmic sound sequences at 120 and 180 ms IOI, for sounds A, B, and C ('ABC training', n=6 females) or starting with sound C followed by A and B ('CAB training', n=7 females) (color indicates sound type; see Fig. S1). To test for the ability to generalize the discrimination to new tempi, probe stimuli (144 ms IOI) were introduced after birds had successfully completed two training phases. A subset of birds was then tested with a broader stimulus set using a novel sound element (sound D) and every integer rate between 75 and 275 ms IOI ("Rule Training"; see *Materials and Methods* for details). Color conventions indicating sound type are used in all subsequent figures. *(C)* Comparison of training time, plotted as Trials to Criteria, for male and female birds that completed rhythm discrimination training for all three phases. Symbols denote sex. For this and subsequent figures, data from male birds were collected previously [22], and are replotted here for comparison. A Mann-Whitney test for each training phase showed no sex-based differences in training time for any phase (p>0.05).

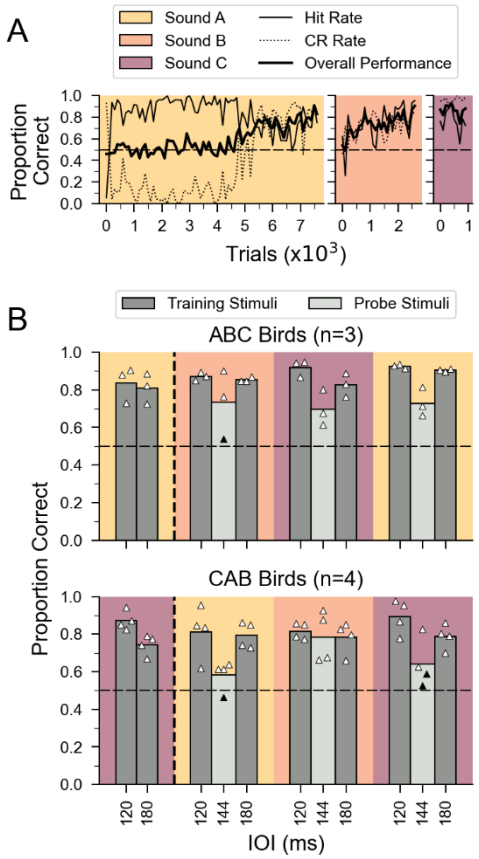

Figure 2. Learning of rhythmic pattern discrimination and generalization to new tempi for female birds. (*A)* Learning curves for rhythm discrimination for a representative female bird (*y7o97*) across three training phases (ABC training) in 100-trial bins. Overall proportion of correct responses is shown by *thick black* line. Proportion correct for isochronous stimuli (S+, "hit rate") is indicated by *thin black* line; proportion correct for arrhythmic stimuli (S-, "correct rejection") is indicated by *dotted line*. Chance performance is indicated by dashed horizontal line. Data are plotted until criterion performance was reached. (*B*) Results for rhythm discrimination training and probe testing for successful female birds (n=7). Data left of the vertical dashed line show performance in the final 500 trials of the first rhythm discrimination training phase (no probe testing, see Fig. 1B). Data right of the vertical dashed line show performance during probe testing with stimuli at an untrained tempo of 144 ms IOI (*light gray*) and for interleaved training stimuli (*dark gray*). Triangles denote performance for each female (n = 3 probe tests/bird x 7 birds). All seven females were able to generalize the isochronous versus arrhythmic discrimination to new tempi (20% different from the training tempi) for at least two sounds. Performance on probe stimuli was significantly above chance for 17 out of 21 probe tests (*white* triangles; $p<.0.0167$, binomial test with Bonferroni correction; black triangles denote performance not significantly different from chance). Average performance across birds in each group is indicated by bars.

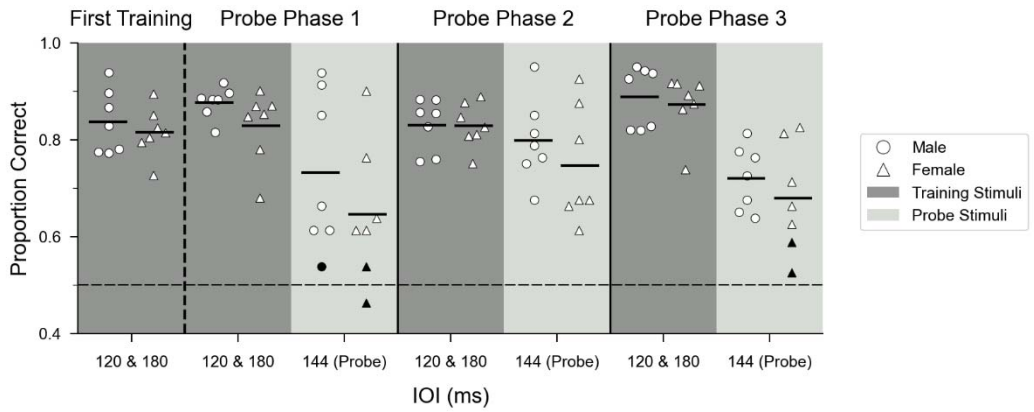

Figure 3. Comparison of generalization of rhythm discrimination by sex. Mean performance of all successful males (*circles;* n=7) and females (*triangles*, n=7) during the last 500 trials of the first training phase (first column) and for probe (*light gray*) and interleaved training stimuli (*dark gray)* in subsequent phases. Data are collapsed across training order, and interleaved training trials are combined across tempo. Dashed horizontal line indicates chance performance. Filled black symbols are not significantly different from chance. Across probe tests, average performance was significantly lower for females than for males by ~9% (p<0.05, mixed-effects logistic regression).

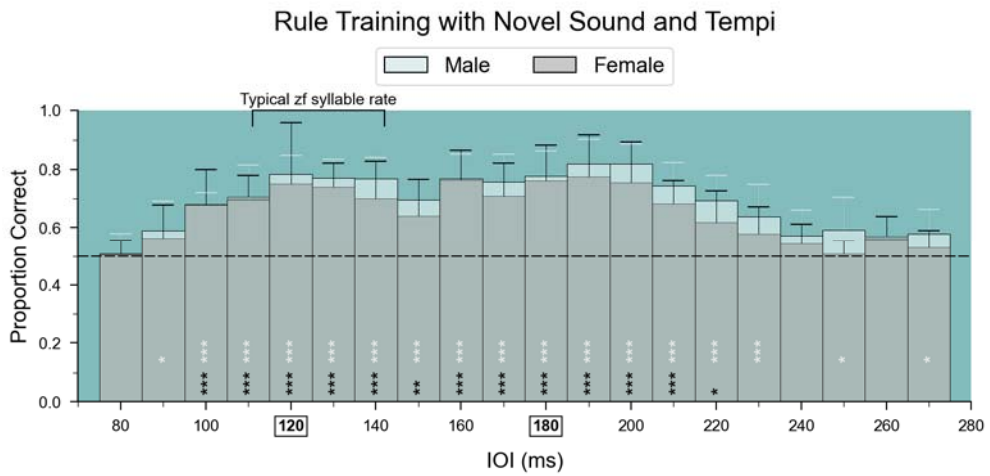

Figure 4. Sex differences in generalization of the learned discrimination across a wide range of tempi. Average performance of 5 female birds (dark bars) during the initial 1000 trials of "Rule training". Mean performance ± SD is plotted for 10 ms IOI bins. Chance performance is indicated by the horizontal dashed line. Female performance for binned IOIs from 95-225 ms is significantly above performance in the 75-85 ms IOI bin, where performance was at chance (***p<0.001; **p<0.005; *p<0.05, mixed-effect logistic regression, black asterisks). Data from 7 males from [22] are plotted for comparison (light green bars and white asterisks). IOIs used in rhythm discrimination training phases before rule training are shown in bold and boxes on the x-axis.

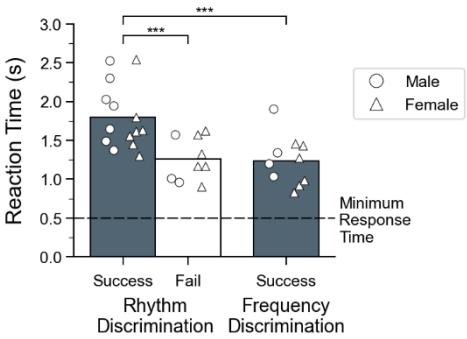

Figure 5. Reaction times during rhythmic pattern discrimination and frequency discrimination tasks. Mean reaction time for the final 500 responses in the first training phase are shown for three groups of birds. For rhythm discrimination, data are shown for birds which completed all three training phases (first bar, n=7 males and 7 females) and birds that did not complete rhythm discrimination training (middle bar, n=3 males and 6 females). For frequency discrimination, data are shown for a separate cohort of birds (third bar, n=4 males and 6 females). The 500ms period between trial start and activation of the response switch is indicated by the horizontal dashed line. Symbols indicate the average reaction time for each bird. A 2-way ANOVA of sex, group, and sex-by-group interaction showed only a significant effect of group ($F(2,27)$, $p = 0.001$). Successful rhythm discrimination birds responded significantly slower than the other two groups ($p<0.01$ compared to the failed birds, $p<0.005$ compared to the frequency discrimination birds; Tukey post-hoc tests).

Supplemental Figures

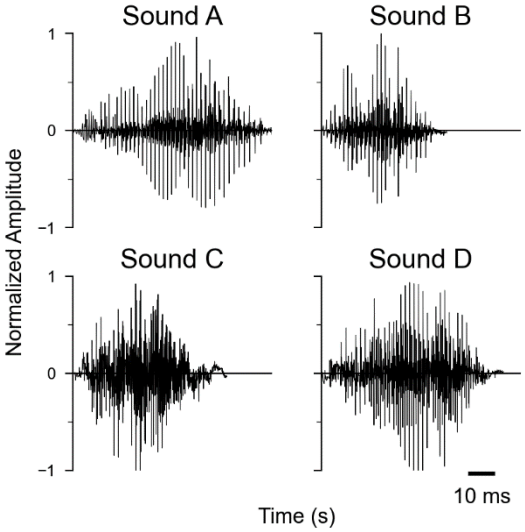

Figure S1. Amplitude waveforms of the four sound elements used in the stimuli. Sound files are available at https://dx.doi.org/10.17632/fw5f2vrf4k.2

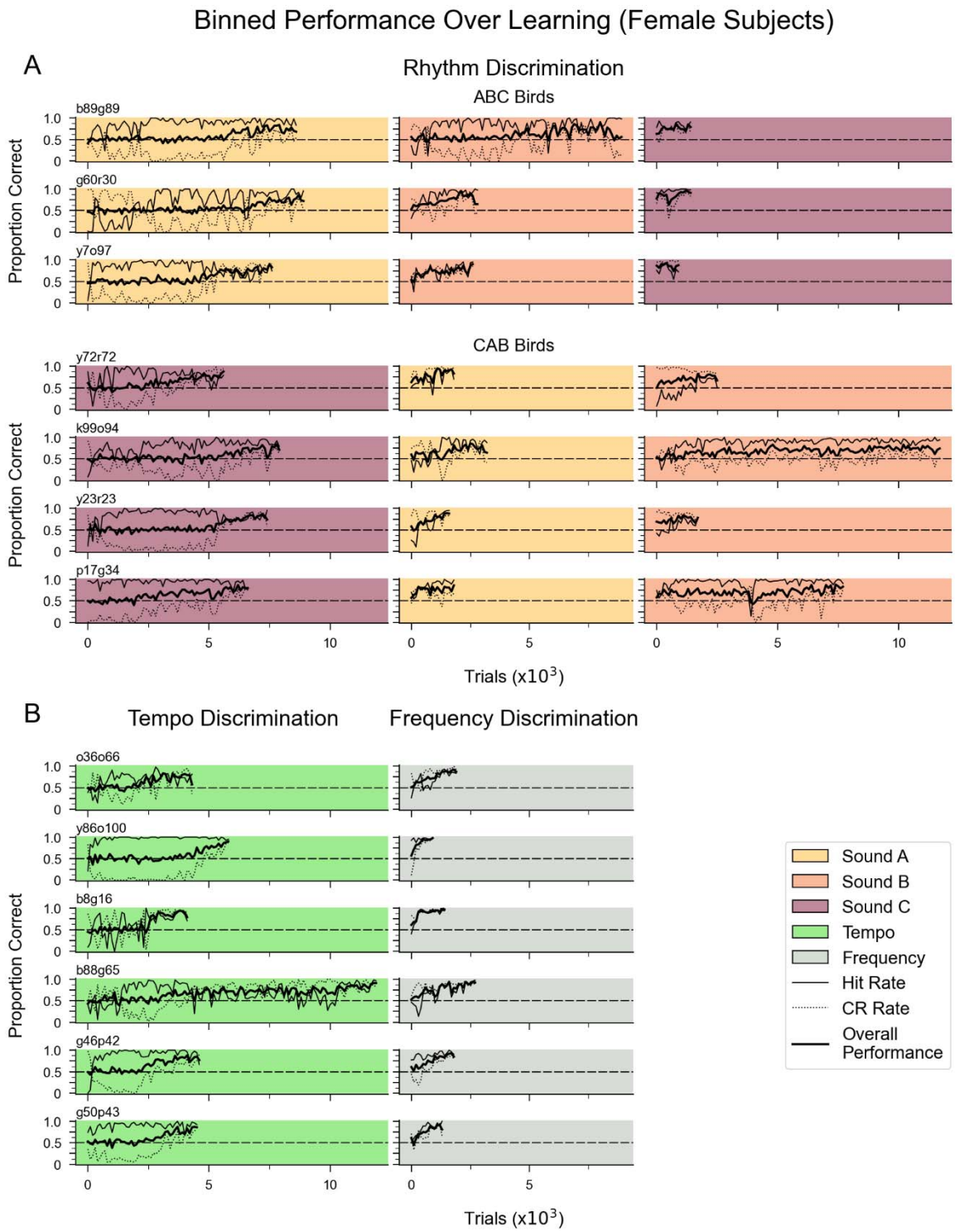

Figure S2. Learning curves for discrimination of stimuli based on rhythmic pattern, tempo, or frequency. (*A*) Learning curves for 7 female birds that successfully discriminated isochronous vs. arrhythmic stimuli within 30 days for each sound type. (*B*) Learning curves for a separate cohort of females (n=6) trained to discriminate isochronous sequences of sound A that differed in tempo (120 ms IOI vs. 144 ms IOI) or frequency (shifted up 3 semitones vs. 6 semitones). All females tested met the criterion for discriminating stimuli based on tempo or frequency. Conventions as in Fig. 2A**.**

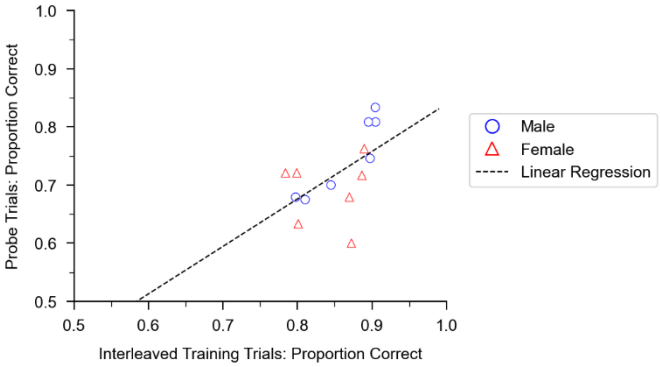

Figure S3. Relationship between performance on probe trials and interleaved training trials. Across birds that successfully completed all three training phases (n=14), there was a significant positive correlation between the accuracy on interleaved training stimuli (learned discrimination) and probe stimuli (generalization); least squares regression fit; R^2 =0.31; p = 0.0375). For each bird, data were combined across all three sound elements; each data point is the average performance across 240 probe stimuli and ~2000 interleaved training stimuli.

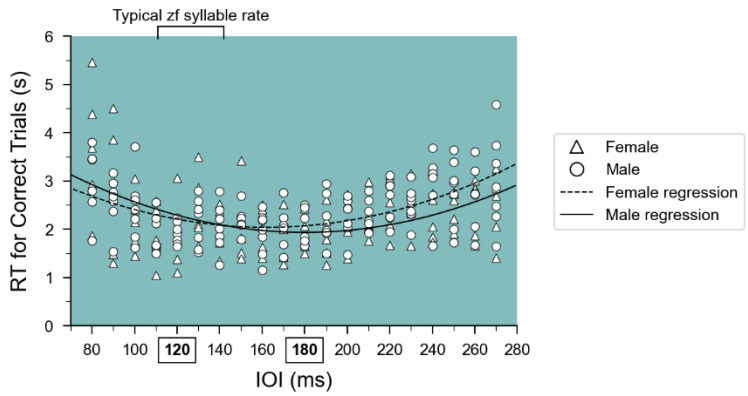

Figure S4. Reaction time (RT) across a broad range of tempi. Mean reaction time for correct trials versus tempo during Rule Training for male (*circles*) and female (*triangles*) birds. RT tended to be longer at the end of the tempo range (<100 ms and >220 ms), when discrimination of isochronous vs. arrhythmic stimuli fell to chance. Fitted quadratic curves are plotted to help illustrate the observed relationship.