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Relative Importance of Male Song on Female Mate Selection in the Zebra Finch (Taeniopygia Guttata)

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Relative importance of male song on female mate selection in the zebra finch (*Taeniopygia guttata)*

A Thesis Presented
By
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Abstract

In the zebra finch (*Taeniopygia guttata*), song and its social context play an important role in female mate selection. The song of the zebra finch is unique in that it can only be produced by males, which makes the species ideal for analyzing the components of male song that influence female song preference and mate selection. There are three consistent features of zebra finch song that affect female mate preference: 1) the amount of time a male sings, 2) the size and complexity of his song repertoire, and 3) the structural conformation to species or population norms (reviewed in Nowicki et al. 2002). During courtship, male zebra finches often express 'static-visual' and 'dynamic-visual' elements in sync with song (Morris 1954), which would suggest that such behaviors also play a role in influencing female mate preference.

However, with courtship comes the competition between males for the attention of potential mates. These agonistic interactions between males act settle disputes over access to mates, and as such, are also likely to influence female mate selection. Thus we predicted that, at the initial onset of courtship, there would be a higher prevalence of male-to-female courting interactions and singing behavior in comparison to male-to-male agonistic tendencies if female mate selection is fundamentally determined by song preference. However if female mate selection is more complicated than simple song preference, we would expect to initially observe a higher number of agonistic interactions between males as they compete for dominance.

This hypothesis was tested by recording and analyzing the male-to-female courtship displays and male-to-male agonistic interactions in the context of song complexity. Our data comparing male song complexity to expression of courtship behaviors, indicates that dif-

ferent elements of male courtship are important in determining female mate preference. Our data comparing male-to-male interactions and male-to-female interactions within the first 30 minutes after introduction was statistically insignificant, suggesting that female mate selection is more complicated than simple song preference. This preliminary research can be used as a basis for future studies using quantitative movement tracking analysis, which would further strengthen these initial observations. By increasing our understanding of the influence the male song has on female mate selection, we can better expound upon the nature and function of the favored traits that male songbirds possess and the benefits that females and their progeny might gain from choosing a male with these particular attributes.

Introduction

Birdsong is a complex signal which plays a significant role in advertising and stimulating mate attraction. In the zebra finch, both of the sexes are highly social and vocal, however only the male can produce a mate advertising song (Figure 1). To more fully understand the function of this birdsong, consideration must be taken not only of the male's song but also of the female's preference. In many bird species, the female selects her mate based on her preferences for the differences between male song (Collins 2004). Song is but one important factor in mate choice selection, as mate advertising is usually a combination of song and courtship display (Morris 1954). Such is the case of zebra finch courtship. As vocalizations can facilitate communication, spanning long distances, song is thought to be more important in initial mate attraction (Bradbury and Vehrencamp 1998, reviewed in Riebel 2009), while visual displays play a larger role later in courtship. Though it is often the case that the vocal performance and courtship display act in concert to produce a sexual signal.

Figure 1. Male (top) and female (bottom) zebra finch. Distinguishing physical characteristics of the male include their orange cheeks and black-and-white banded breast, while the female appears light grey in color.

Characteristics of male song

In order to better understand the importance of male song on female mate selection in zebra finches, the elements of male song that make it attractive to females requires further study. Several studies have shown that characteristics of male song such as structure, performance, indicators of male condition, and song familiarity, play a significant role in attracting a receptive female.

Zebra finch songs are made up of elements or syllables, which are distinct from neighboring elements and are the most basic functional units of song production (Figure 2). The song is usually preceded by several identical syllables, called the introductory elements, that are sometimes incorporated into the song itself (Zann 1993). On average, a song will consist of seven different syllables sung in a specific order called a phrase or motif (Böhner 1990, in Zann 1993), however the number and complexity of these syllables will often differ between birds. The motif is then repeated several times to complete a song or a 'bout' (Price 1979, in Zann 1993). Despite having a basic pattern, each male zebra finch develops a unique song phrase used to stimulate courtship in females. Specific elements of the male song structure, such as motif and song duration, repertoire size, and phonetic syntax, have been shown to be more effective in eliciting a positive response to courtship.

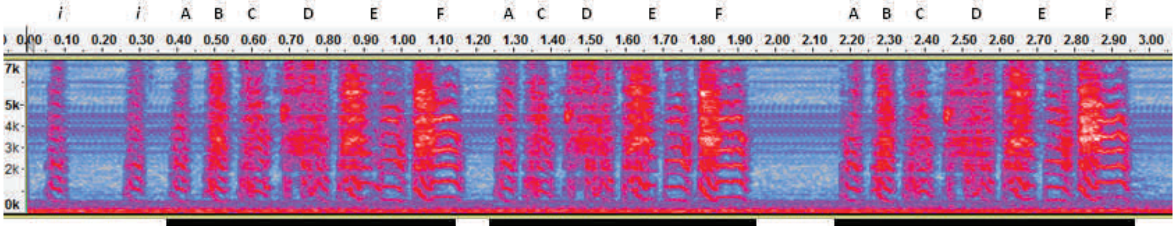

Figure 2. Sonogram of zebra finch (green164) song. X-axis measures time (seconds), Y-axis represents a logarithmic scale of the amount of energy in different frequency bands. Blue color indicates the least power, while red and white indicate highest power. Introductory syllables are labeled "*i*", while subsequent song syllables are labeled alphabetically. Within each syllable, increase in pitch is indicated by upward sloping line while decrease in pitch is indicated by downward sloping line. Black bars indicate motifs.

Song characteristics such as motif and song duration have been studied in the context of song attractiveness, although there are conflicting results in the literature concerning the significance of these elements on female song preference and mate selection. Motif describes the order or narrative of the syllables in a song, and a zebra finch song will normally contain several of these motifs strung together. There is also usually a positive association between motif and song duration in zebra finches, so the two elements have often been studied in relation to one another. Some studies have shown that females prefer longer songs over shorter ones (Clayton and Prove 1989, reviewed in Riebel 2009), while others did not find motif duration to be a significant factor in determining female song preference (Leadbeater et al. 2005). This variation in results may be due to the fact that both studies that did find a preference for longer motifs used extreme differences in stimulus duration, and as such, these findings cannot be attributed to more than natural variation. Studies that used more natural differences between song stimuli found no female preference for long over short single song motifs (Leadbeater et al. 2005).However, a study by Collins et al. (1994), found that female zebra finches preferred males with both higher song rates and complex song phrases.

Others studies indicate female preference for increased phonetic syntax in male zebra finch song. Phonetic syntax is the different arrangements of syllables within the motifs and motifs within the songs. There are no fixed positions for a particular category, however the sequences of the elements tend to follow some rules. For example, notes used in the call repertoire are found in the beginning and end of a song, while the loudest notes usually occur in the middle of a song (Zann, 1993). Each motif rendition is not always sung in the same syllable sequence, likely due to changes in preference of note order, note sequencing, deletions, insertions, and repetitions (Helekar et al. 2000, reviewed in Riebel 2009), that occur with male zebra finch development. Females have been shown to prefer songs that had the syllables of the motif rearranged in four different sequential sequences compared to songs that had four sequentially identical repetitions (Collins 1999). However, females also showed similar preference for songs made up of four motifs from four related males, which would indicate that this preference for variability in motif order could also be attributed to the a larger repertoire size (Collins 1999).

There is strong evidence for increased female receptiveness to complex songs with larger syllable or repertoire size than longer motif length (Vyas et al. 2009, reviewed in Riebel 2009). Difference in repertoire size are often measured by the number and variety of syllables present in a males' motif. In a preference test experiment, song duration was also found to be positively correlated with repertoire size of the male song (Clayton and Prove 1989, reviewed in Riebel 2009). The significant correlation between the repertoire size of male song and female song preference could also be related to the difference between tutored and untutored song, as it has been observed that untutored males have a reduced repertoire size (Immelmann 1969, reviewed in Riebel 2009), and thus their song is perceived as less attractive to females.

When considering song characteristics in zebra finch song that might play a role in attracting females, it is also important to consider the effect of the “tutor”. Young birds will often hear the male parent singing while been cared for, and later on during development, will be able to memorize the songs heard from male relatives (Collins 2004). Several studies have shown that the difference between learned and untutored song can influence female zebra finch mate selection. The male song can develop with or without exposure to an adult male tutor, however, studies show that untutored males will have song motifs with an uncommon note structure, reduced syllable variation, less complex and longer syllables, and a slower song rate, compared to their tutored counterparts (Immelmann 1969, reviewed in Riebel 2009). As female zebra finches have been shown to prefer males with more complex song structure (Vyas et al. 2009, reviewed in Riebel 2009), a lack of tutoring would consequently reduce a male's success in attracting a female mate. This difference in female preference was also confirmed in a study performed in the absence of behavioral cues (Lauay et al. 2004). The results of this experiment demonstrated that female zebra finches prefer tutor song over untutored song (Lauay et al. 2004). However, in the same experiment, female zebra finches, that had not been exposed to male song before, were shown to be equally attracted to the tutored and untutored song (Lauay et al. 2004), indicating that preference also depends on the female's early song exposure.

As discussed above, many of the features of zebra finch song have been shown to influence female mate preferences. However, three features of song are consistently shown to affect female mate preference (Searcy and Nowicki 2000, in Nowicki et al. 2002): 1) the amount of time a male sings (Kempenaers et al. 1997, in Nowicki et al. 2002), 2) the size and complexity of his song repertoire (Hasselquist 1998, in Nowicki et al. 2002), 3) the structural conformation to species or population norms (Searcy et al. 2002, in Nowicki et al. 2002). For the purposes of this project, three related features of song will be analyzed: 1) motif length, 2) the number of syllables per motif, and 3) the number of original syllables per motif. The motif length will be used to infer the amount of time a male sings, the number of syllables per motif will indicate the size of his repertoire, and the number of original syllables per motif will determine the complexity of his song repertoire. This experiment will not directly look at the conformation of song structure to species norms; however studies by Catchpole (1996), in Nowicki et al. 2002, show that repertoire complexity depends on normal song development, and thus can be used as an indicator of the conformation to species norms. Such analysis of specific song features will allow for the prediction of which male the female is likely to pair with, based on song alone without behavioral cues.

Male song performance

In addition to song structure, elements of male song performance such as song rate, temporal performance, amplitude, and mode of singing can influence female mate selection.

The effect of male song rate on the perceived attractiveness of his song not clearly defined, but it has been correlated to female preference. In different studies on zebra finches, measurements of song rate have been defined in a variety of different ways such as the amount or duration of song motifs per unit of time. In Houtman's (1992) study, female zebra finches were shown to solicit extra-pair copulations with males that had higher song rates, with song rate measured as the number of song phrases in relation to the length of the song phrase. However, this positive association between song rate and female song preference, may not be a solid foundation to predict female mate selection. Increased song rate could be as much a cause as a consequence of female mate selection, as an increased male song rate might be elicited encouraging female behavior (Collins 1994).

It is also possible that the temporal performance, meaning the amount of sound in proportion to silence within a song, could influence female song preferences. A test performed by Leadbeater et al. (2005), looked at how the presence of inspiratory syllables--syllables sung during inspiration--alone could alter the attractiveness of a motif to females. The results were negative, but that doesn't mean that the effects of temporal performance is non-significant, because this trait is also associated with longer, more complex songs, it could be a combination of interdependent factors that influence female preference. Therefore, the next question that should be asked is whether motifs with these inspiratory syllables are more attractive than the syllables alone (Leadbeater et al. 2005).

In zebra finches, the amplitude of a male's song significantly increases in the presence of a female. The rate of amplitude increase has also been shown to vary with distance to the female (Brumm and Slater 2006, reviewed in Riebel 2009). Although this study does not answer whether amplitude effects female song preference, an increase in amplitude would be assumed to draw the attention of a female and thus increase the male's chance of being selected as a mate.

Zebra finch males have two modes of singing: directed song, which is used in courtship, and undirected song, which is sung in solitude (Zann 2004). These singing modes are triggered by different motivational states in view of a female, and thus serve different purposes (Zann 2004). In both types of song, the same motif is sung, but directed song has overall greater song parameters including, more introductory note repetitions, increased motif repetition, and faster rate, than in undirected song (Riebel 2009), which are detected and relevant to female mate selection.

Song as an indicator of male condition

In zebra finches, some song features may inform females on aspects of male fitness. This idea that song was an indicator of male condition, garnered support after the publication of a "nutritional stress hypothesis", which proposed that learned song attributes were influenced by the presence or absence of environmental stressors, such as nutrition (Nowicki and Searcy 2004). Young male zebra finches require a lot of energy for developing brain structures that can process and produce complex song structures, therefore those males that got more nutrition while growing, were more fit and could thus focus on producing better song (Nowicki and Searcy 2004). Following this hypothesis, many different tests have been performed to stress juvenile condition and see the different aspects of song that are consequently affected. This method of approach yielded surprising results, namely that traditional features of song that are associated with female song preference, such as song rate and number of different syllables, were largely unaffected (Riebel 2009). Instead, parameters like temporal patterning and the syntax copying accuracy seemed to be more significantly affected by environmental stress conditions (Riebel 2009). Therefore, such song elements could serve as indications of male fitness and inform females in their mate selection.

Song familiarity

When taking into consideration female preference for male song, it is also important to understand female learned preference for song variations. The zebra finch was one of the first species in which female preference for familiar songs was reported (Collins 2004). In several studies, females were shown to prefer songs similar to those of their father's (Clayton 1990, reviewed in Collins 2004) and those they were exposed to when younger (Riebel 2000, reviewed in Collins 2004). This preference for familiar songs--be it a father's or unrelated adult male's song--over unfamiliar songs, could also function in mate recognition (Collins 2004), and can clearly influence female preference of a mate.

Agonistic behavior

It is important to note that male song is often performed in concert with courtship displays. With courtship, comes competition, and when males compete for mates or other reasons, they tend to exhibit agonistic tendencies. The agonistic and courtship behavior of zebra finches has been well documented by Desmond Morris (1954). Morris defines “agonistic behavior” as all of the actions associated intraspecific fighting, such as attacking, threatening, submitting, and fleeing (1954). This provides a more comprehensive and well-rounded understanding of the behavioral interactions between competing mates, as a focus on the antagonistic or aggressive behaviors, neglects the pacifying behaviors of the submissive individual. An interaction of these behavioral responses results in threatening postures or movements used to convey intention to the receiver.

Most species use a form of "threat code" to settle potential disputes without engaging in physical violence. These threatening postures and movements are advantageous as they prevent the bodily harm and increased energy consumption associated with physical fighting, while allowing the individual to express them self. In zebra finches, most disputes are settled by chasing and pecking. Although, supplanting attacks will arise in situations where there is a distinct difference in dominance between birds. In such cases, the subordinate male will usually flee while the dominant male pursues. This behavior will often result in prolonged chasing. Morris (1954) observed that when the dominant bird is attack, it assumes a horizontal posture, pointed in the direction of the target (Figure 3, left). This threat posture does not always result in an attack, but it can also serve to intimidate the other bird (Morris 1954). When two male zebra finches of relatively equal dominance come into con-

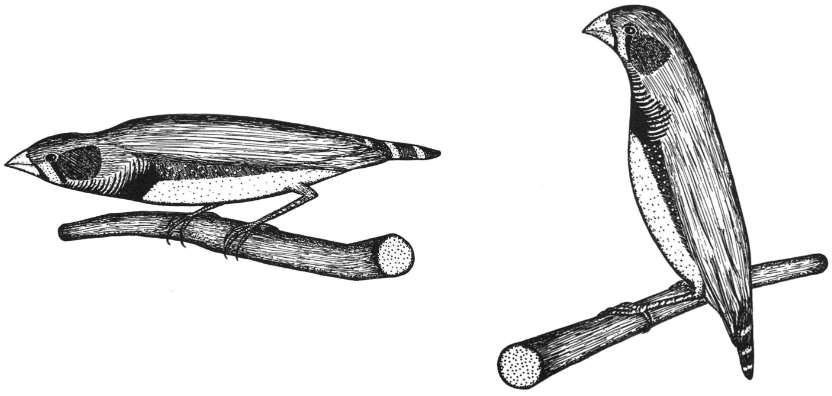

Figure 3. Horizontal posture adopted by male zebra finch expressing dominance, when confronting a rival (left). Vertical posture of male zebra finch is expressed when facing another male and neither bird is dominant (right). From Morris (1954).

flict, they both take on a vertical posture and face each other (Morris 1954; Figure 3, right). The birds will often engage in 'beak-fencing', attacking one another with their beaks closed and aiming for the head (Morris 1954). If one of the birds goes on the defensive, it will often assume a more vertical posture and angle itself backwards, while the attacker will assume a horizontal posture, providing it more balance for continued attack (Morris 1954; Waters 1979).

Zebra finches that take on a passive or submissive role in conflicts will often attempt to flee, or when this is not an option, they will assume submissive postures and gestures. Often males will adopt a gaping display oriented in the direction of the opposing male, to stem attack of the dominant bird and to function as an appeasement display (Morris 1954). The submissive male will crouch low to the ground, tilting his head back and opening his mouth, when the dominant male approaches or courts the female (Morris 1954). Submissive postures serve to appease the attacker and thus minimize harm (Morris 1954). Together, these threat and submissive postures and movements form an “agonistic code” which inform zebra finch interactions.

Courtship behavior

The courtship behaviors of male to female zebra finches have also been well documented by Morris (1954). Both the male and female zebra finch perform courtship displays, however the courtship of the male consists of auditory, static-visual, and dynamic-visual elements, while the courtship of female is solely static-visual.

As described previously, the male advertises his sexual arousal through the repetition of his unique song phrase during courtship. The song can be sung without movements, but it is often performed in sync with a courtship dance. The male also assumes a specific posture when courting a female. Morris (1954) depicts a high intensity version of the posture (Figure 4, left), showing the upright positioning of the bird's body while fluffing out his spotted flank feathers. This posture allows the male bird to display all of his distinctly male markings--white abdomen, black and white barred breast, spotted flanks, chestnut brown ear-patches, black and white banded tail--to the female (Morris 1954; Waters 1979). In a combination of singing and posturing, the male will make advances towards the female in a courtship dance (Morris 1954; Figure 5).The male will move along the branch in the direction of the female, by pivoting his body from left to right, showing off his male markings and unique song.

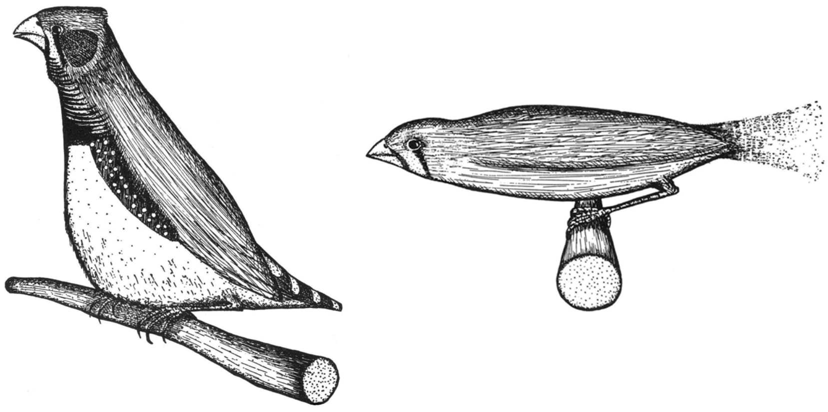

Figure 4. Male zebra finch upright courtship posture (left). Female finch tail quivering response indicates receptiveness (right). From Morris (1954).

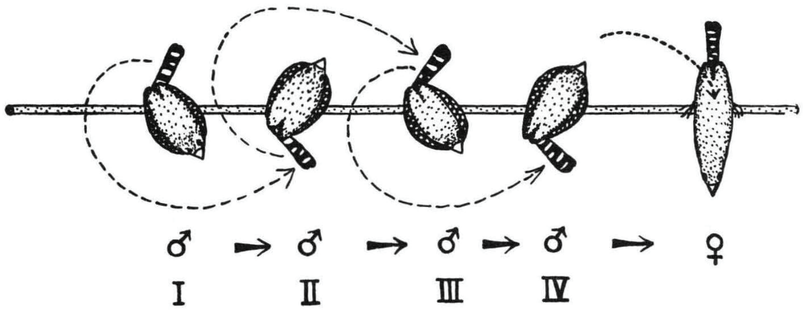

Figure 5. Male zebra finch courtship movements directed at female. From Morris (1954).

The courtship of the female zebra finch is much less complicated than that of the male. Often enough, the female will ignore or avoid the courtship of a male by creating distance between them. However, when a female is responsive to a male's advertising, she holds her position, perpendicular to the branch, as the male advances (Morris 1954; Waters 1979). The female will display her arousal by lowering her body horizontally and vibrating her tail rapidly (Morris 1954; Waters 1979), inviting the male to mount and copulate (Figure 4, right).

Predictions

Despite having a basic pattern, each male zebra finch develops a unique song phrase used to stimulate courtship in females. Specific elements of the male song structure, such as motif and song duration, repertoire size and variation, have been shown to be more effective in eliciting a positive female response to courtship. Therefore, we would expect to see that males with more complex songs are interacting and spending more time with the females. However, in the presence of other males, agonistic behaviors may also inform female mate preference. If female mate selection is fundamentally determined by song preference, we would expect to initially observe less male-to-male interaction and a larger amount of male-to-female courting interactions and singing behavior. If female mate selection is more complicated than simple song preference, initially we would expect to observe more agonistic interactions between males as they compete for dominance, and later would expect to see an increase in the dominant male singing and interacting with the female. Determining the relative importance of male song and behavior on female mate selection is important for understanding how sexual selection interacts with song and behavioral expression.

Methods

Subjects

The zebra finches (*Taeniopygia guttata*) used in this study were acquired from the Keck Science Department's breeding colony and Magnolia Bird Farm, a commercial supplier in Riverside, CA. The birds were housed in an indoor aviary with other zebra finches and kept on a 12-hour light/dark cycle. Their diets consisted of dry finch seed and water, which were replenished as needed, and occasionally supplemented with millet.

Data was collected from six male finches, with no previous exposure to females, and one adult female. The song of each male was first analyzed for complexity before they were paired and placed in a competitive mate choice experiment. This experimental design was approved by the Animal Care and Use Committee (IACUC) of the Keck Science Department.

Song recordings

The directed songs of each male were recorded in a soundproof chamber using a Sure SM94 directional microphone at a sampling frequency of 44,100 Hz and processed using Sound Analysis Pro 2011 (Tchernichovski et al. 2000).

Song analysis and cutting syllables

A free software program, Sound Analysis Pro (SAP) 2011, was used to analyze male zebra finch vocalizations (Tchernichovski et al. 2000). This program is able to record and determine the acoustic features of a song and compare the structure across multiple songs. SAP provides basic song structural analyses such as the isolation of syllables, calculation of amplitude, pitch, frequency modulation, and entropy (Tchernichovski et al. 2000).

Amplitude is one of the main criterion used for automatic syllable segmentation. It denotes the peak of the sound wave, which correlates with the sound power and intensity. However, the measurement of amplitude is not used outside of syllable extraction and between comparisons, because it can be affected by factors unrelated to the bird song i.e. distance and orientation of microphone relative to the bird.

The function to automatically identify syllables in SAP uses amplitude as its main criterion, which can lead to problems such as misreading cage noise as a syllable or the premature shortening of syllable ends. As such, syllable types were manually segmented, outlined, and extracted in SAP. A syllable, or song note, was defined as "a continuous sound bordered by either a silent interval or an abrupt transition from one frequency pattern to a different one" whereas a song motif consists of unique notes repeated in a specific order (Figure 2) (Tchernichovski et al. 2000). Motif length was manually highlighted and outlined using the open-source program Audacity. The number of syllables per motif was visually determined and then compared against the syllable duration and start times within the outlined motif length. If an identical syllable was repeated at least two times within a single motif, only the first one was counted towards the number of original syllables.

Song complexity index

In order to determine song complexity or 'attractiveness' of each male, three related features of song were analyzed: 1) motif length, 2) the number of syllables per motif, and 3) the number of original syllables per motif. The analysis of these three specific song features was used to predict which male the female was likely to pair with, based on song alone. The average of each song feature was calculated for each male and then assigned a weight. Weights were determined by dividing a male's feature average by the male's feature average with the highest recorded complexity, such that the male with the most complex song feature had a score of 1.00. The weighted measurements were then totalled to create a song ‘sexiness index’ or score for each male. These scores could then be used to determine the relative attractiveness of a male’s song in relation to the other males. The male with the highest score of 3.00 indicates that his song is the most complex, and thus he would be the most preferable mate based on song alone.

Cage construction and video recording

To capture the interactions and behaviors of the finches, a 36 in. x 7 in. x 24 in. cage was constructed through which video could be recorded (Figure 6). The cage design was based off of a model used in Branson et al. (2009), that could be used to video-record interactions between the finches and then analyze their behavior with a movement tracking program. The cage had a transparent acrylic sheet front, through which video was recorded, and twin-wall plastic sheet backing, through which two 20W portable LED floodlights could be shown. This backlighting could provide a bright background that would effectively silhouette the animals for optimized tracking analysis. However, we did not use tracking programs in our analysis, but the cage remains for future studies. Bird movement and behavior was recorded using a Logitech C920 Pro Webcam and processed using Logitech Webcam Software.

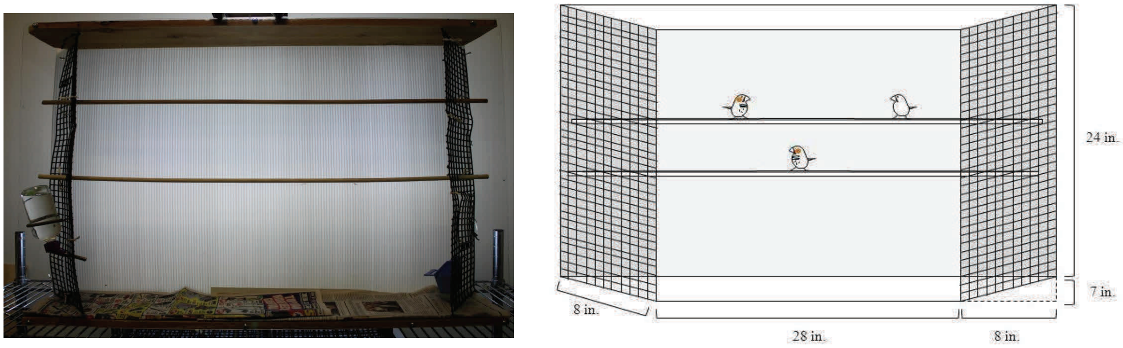

Figure 6. Photograph (left) and diagram with dimensions (right) of cage used for video recording interactions between zebra finches.

Competitive mate experiments

Using five competitive mate experiments, male courtship and agonistic behaviors were observed and analyzed in relation to female mate preference:

Experiment 1: green164 male, green162 male, red041 female
Experiment 2: green170 male, white273 male, red041 female
Experiment 3: green169 male, white276 male, red041 female
Experiment 4: green164 male, green170 male, red041 female
Experiment 5: green162 male, white273 male, red041 female

For each experiment, six males were paired up and placed in the cage with a female, where they were video-recorded for a period of 60 minutes. The birds were given a 5-minute adjustment period, providing them time to get acclimated to their new environment, before we began counting the number of courtship and agonistic displays observed for each male.

Courtship behavior

The total number of courtship displays performed by each male was calculated for the first three experiments, to determine the most attractive male finches based on their behavior alone. Within each experiment, the total number of courtship displays were counted for each male. A courtship display was counted if the male directed his song at the female, exhibited characteristic courtship posture (Figure 4, left), or performed courtship dance (Figure 5). The behavioral attractiveness of each male was determined by assigning a weight to each male’s average display, and adding the three weighted averages together to produce a numerical index that was used to determine the relative behavioral attractiveness of each male. The male with the highest score indicated that he was performing the most courtship behaviors and thus was spending the most time with the female, making him the most preferable mate. This behavior score was then compared against the previously determined song complexity scores, to determine whether song was the most important factor in mate selection or if there were other elements of courtship behavior at play.

Experiments 4 and 5 paired two males with closely ranked song complexities--two higher ranks and two lower ranks--in a mate competitive experiment, to determine whether courtship behaviors played a more significant role in determining mate preference when the competing males had similar complexities.

Agonistic interactions

The relative importance of agonistic interactions between males on female mate selection was determined using the first 3 competitive mate experiments. The video recording for each experiment was analyzed for both the total number of courtship behaviors displayed and the total number of agonistic interactions observed with two 30 minute time frames. Courtship displays were counted if the male directed his song at the female, exhibited characteristic courtship posture, or performed courtship dance. Agonistic behaviors between males were counted if either male chased or fled from the other, pecked at each other, performed a submissive gaping behavior, adopted a sleek horizontal posture (Figure 3, left), or performed displacement preening. The total number of courtship and agonistic displays between all three experiments were added up and separated between whether they occurred within the first 30 minutes of video recording, or the last 30 minutes. Before statistical analysis was performed, the data was log-transformed in order to meet assumptions of normality and equality of variances. Two paired-sample t-tests were run in order to determine if there was any significant difference between the mean number of agonistic and courtship displays within the first 30 minutes or last 30 minutes of recording.

Results

Song complexity and courtship behavior

In order to determine the overall song complexity for each male zebra finch, the average motif length, number of syllables per motif, and number of original syllables per motif was calculated and then assigned a weighted value for each feature. The sum of the three weighted values produced a ‘sexy song index’ score for each male, that indicated his song attractiveness in relation to the other males. Male green170 had the highest overall song complexity score of 3.00, indicating that he would be the preferred male based on song alone (Table 1). The other males were listed from highest overall song complexity to the lowest, in descending order, with white276 ranked with the lowest overall song complexity of 1.85 (Table 1).

Table 1. Ranked song complexity of six male zebra finches as an indicator of song attractiveness.

	Average complexity			Weighted complexity			Overall complexity
Bird	Motif length (s)	Number of syllables per motif	Number of original syllables per motif	Motif length (s)	Number of syllables per motif	Number of original syllables per motif	‘Sexy song index’
green170	0.77 ± 0.2	6.4 ± 1.3	6.4 ± 1.3	1.00	1.00	1.00	3.00
green164	0.69 ± 0.2	5.9 ± 1.3	5.5 ± 1.0	0.90	0.92	0.85	2.67
green169	0.61 ± 0.2	5.1 ± 1.0	4.9 ± 0.8	0.79	0.80	0.76	2.35
white273	0.64 ± 0.1	4.4 ± 0.5	4.0 ± 0.0	0.83	0.68	0.62	2.13
green162	0.66 ± 0.3	3.7 ± 1.3	3.5 ± 0.9	0.85	0.58	0.55	1.98
white276	0.51 ± 0.1	4.3 ± 0.5	3.4 ± 0.5	0.65	0.67	0.53	1.85

To determine which male was interacting the most with the female and thus was the preferred mate, the total number of songs directed at the female, courtship posture displays, and courtship dances performed were recorded for each male in experiments 1-3. The total number of each courtship display was weighted for each male, and added together to create a ‘sexy behavior index’ score used to indicate a male’s behavioral attractiveness in relation to the other males. Male green169 scored the highest in terms of overall courtship behavior with a score of 2.69, followed by green164, and green170 (Table 2). When these attractive behavior rankings were compared with the song complexity scores, the order in which the males ranked was observably different. This would indicate that different elements of male courtship are important in determining female mate preference.

Table 2. Ranked courtship behaviors of six male zebra finches from experiments 1-3 as an indicator of behavioral attractiveness.

	Total courtship behavior			Weighted courtship behavior			Overall courtship behavior
Bird	Number of song displays	Number of posture displays	Number of dance displays	Number of song displays	Number of posture displays	Number of dance displays	‘Sexy behavior index’
green170	12	6	3	0.86	0.38	0.6	1.83
green164	7	16	3	0.5	1	0.6	2.1
green169	14	11	5	1	0.69	1	2.69
white273	0	0	0	0	0	0	0
green162	0	0	0	0	0	0	0
white276	0	0	0	0	0	0	0

As no courtship behaviors were observed from males white273, green162, and white276, two additional competitive mate experiments were performed to better elucidate the role that song complexity played in determining female mate preference when the competing males had similar complexity scores. An analysis of the overall courtship behavior score for the two males with the highest scored song complexities--green170 and green164--showed no difference in the order of their ranking from their scores measured in experiments 1 and 2 (Table 3; Table 1); however the difference between their scores was much smaller when they were competing against each other in the same experiment. When the two males with lower song complexities were paired up--white273 and green162--they performed courtship displays, although a noticeably lower fraction than the males with higher ranked song complexities (Table 3). In comparison to green162, white273 scored higher in the attractiveness of his overall courtship behavior (Table 3), which was inline with their attractiveness rankings determined by song complexity (Table 1).

Table 3. Ranked courtship behaviors of four male zebra finches from experiments 4-5 as an indicator of behavioral attractiveness.

	Total courtship behavior			Weighted courtship behavior			Overall courtship behavior
Bird	Number of song displays	Number of posture displays	Number of dance displays	Number of song displays	Number of posture displays	Number of dance displays	‘Sexy behavior index’
green170	28	0	2	1	0	0.29	1.29
green164	18	1	7	0.64	0.2	1	1.84
white273	0	5	0	0	1	0	1
green162	1	2	2	0.04	0.4	0.28	0.72

Agonistic interactions and courtship behavior

By analyzing the proportion of agonistic behaviors between males to courtship displays between males and females within the first three competitive mate experiments, we were able to determine the relative importance of agonistic behaviors on female mate selection. In both the first and last 30 minutes of observation, there appeared to be a larger amount of male-to-female courtship interactions, however upon statistical analysis this difference proved nonsignificant (Figure 7). There was no significant difference between the mean number of male-to-male agonistic interactions and male-to-female courtship interactions within the first 30 minute time period (paired t-test, t = -2.304, df = 2, p = 0.148). Additionally, there was no significant difference between the mean number of agonistic behaviors and courtship displays within the later 30 minute time period (paired t-test, t = -1.059 df = 2, p = 0.401). Because there was no significant difference measured between the number of agonistic and courtship behaviors, this would suggest that both types of interactions could play a role in influencing female mate preference.

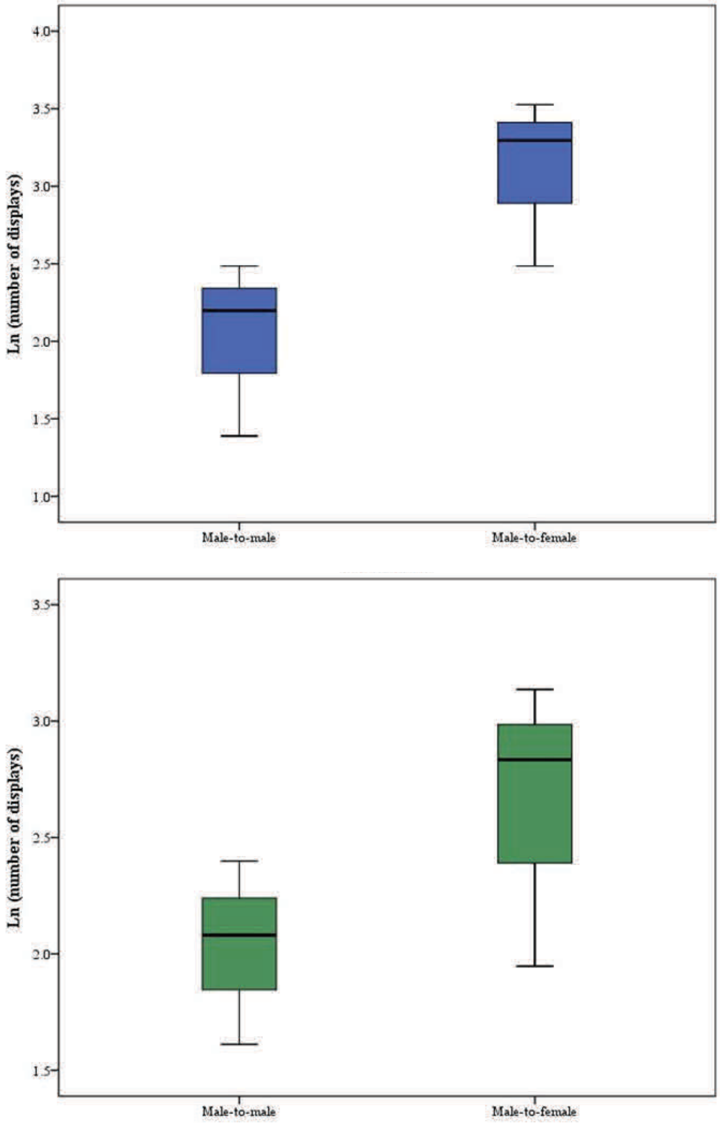

Figure 7. Mean number of displays between male-to-male and male-to-female interactions during the first and last 30-minute time period in experiments 1-3. Blue bars indicate first 30-minutes, green bars indicate last 30-minutes. No significant difference was measured for the mean number of displays between the two types of interactions during the first 30-minute time period (p = 0.148) or the later period (p = 0.401).

Discussion

Overview

In many species of songbirds, song and its social context are important factors affecting female mate preference. A comparison between male zebra finches ranked on their song complexity and courtship display indicated that female mate selection was not determined solely by male song complexity, but other elements of courtship also likely played a role. However, with courtship comes competition for access to potential mates. This intraspecific competition engenders agonistic interactions between males and often results in the establishment of a dominant 'winner' and submissive 'loser', with the 'winner' moving on to court the female. As such, we predicted that agonistic interactions between males, in the presence of a potential mate, would affect female mate selection.

We would expect to see a higher proportion of male-to-female courtship displays and singing bouts during the initial onset of courtship, than agonistic interactions between males, if female mate selection is fundamentally determined by simple song preference or other elements of courtship behavior. On the other hand, if female selection is largely determined by agonistic interactions between males, then we would expect to initially observe a larger number of agonistic interactions than courtship displays. However, our data would suggest that both courtship and agonistic interactions may have an effect on female mate selection, although the root cause of these effects requires further investigation.

Song complexity and courtship behavior

We predicted if female mate selection was largely determined by simple song preference, that the male zebra finches with more complex songs would also be interacting and spending more time with the female; however if other elements of courtship behavior had an impact on female mate selection, then the male zebra finches interacting the most with the female would differ somewhat from the males with the most complex song. This study found that males with more complex song structure, were not the same males who performed more courtship displays and spent more time interacting with females, indicating that female mate selection is influenced by other aspects of male courtship. As male courtship is a combination of song and courtship display, it makes sense that elements of courtship behavior would also have an effect on female mate preference. However, the degree to which song and visual cues play a role in female zebra finch mate choice, is still uncertain.

Female zebra finch mate preference studies have shown that visual cues--such as beak color--and auditory cues--such as song rate--can often be correlated (Collins 1994); thus their independent examination can provide insight into their relative importance in mate selection. It appears that courtship displays play a role in determining female mate choice, though it is possible that there is a hierarchy of choice criteria and we do not know where these courtship behaviors lie in relation to song complexity; ergo, experiments controlling for song complexity could better elucidate the relative importance of courtship displays on female mate selection.

In order to further support our preliminary observations that female mate selection is affected by elements of courtship behavior, future research could measure female response to these displays. Unfortunately, in our studies we did not observe any responsive female behaviors, such as the characteristic tail-quivering described by Morris (1954), and thus could not further reinforce our previous results. A more in-depth study of female response might also be used to identify other elements of courtship behavior that influence mate selection.

Agonistic interactions and courtship behavior

Social dominance hierarchies determine the fitness of an individual, as the more dominant individuals are likely to acquire greater amounts food, posses larger territories, breed more frequently, and thus live longer than submissive individuals (Bonoan et al. 2013). In our studies, we expected to see a larger proportion of male-to-male agonistic interactions within the first 30 minutes of observation, as the males competed for dominance and thus for access to the female, than courtship behavior. Our data appeared to indicate that the mean number of courtship displays observed within the first and last 30 minutes of observation was higher than that of the agonistic interactions, however no significant difference was measured between the mean number of male-to-male and male-to-female interactions within the first 30 minutes ($p = 0.148$) and later time period ($p = 0.401$). This would suggest that both agonistic and courtship behaviors affect female mate selection.

It is possible that the reason why we did not observe more agonistic behaviors within the first 30 minute time period was because the males had a pre-established social hierarchy, and thus did not need to fight to establish dominance because they already knew where they stood in rank. Because all of the males used in this experiment were housed together, they likely already established a ‘pecking order’ (pun intended) before being introduced to the female, which is why we observed such a small number of interactions between the males overall.

This would also explain why three of the birds from first three experiments, did not perform any courtship displays in the presence of another male with a higher song complexity rating (Table 2). In each these experiments, the male with the more complex song was the only one to court the female, while the male with the lower song complexity score would keep to one corner of the cage and never attempted to court the female. However, when we placed two males together with similar song complexity in experiments 4 and 5, a more equal amount of courtship displays were observed for both males. This would further support the notion that there was a pre-established hierarchy between these males, which is why we observed no courtship displays from the males with low song complexity when competing against males with high song complexities. If this explanation is true, then it is also possible that song complexity is used as a factor in determining dominance in zebra finches, and would require further investigation.

Summary

Taken together these experiments indicate that female mate preference is more complex than simple song preference, and is affected by elements of courtship display and dominance hierarchies. Unfortunately, we were unable to observe the responsive behaviors of the female, and as a result, are not completely certain about the accuracy of our conclusions. As such, this study should serve as a foundation upon which further research can be built upon, into the nature of female mate selection and the relative importance of male song. Knowing the degree to which females consider male song when choosing a mate is important for understanding the nature of sexual selection with regards to song expression, courtship displays, and aspects of dominance hierarchies.

Future Studies

The results obtained from this study can serve as a basis for the continued study using quantitative behavioral tracking analysis. Video-tracking software programs such as Ctrax (Branson et al. 2009) and idTracker (Pérez-Escudero et al. 2013), can be used to determine the trajectories of multiple finches and calculate descriptive statistics of their individual behaviors and social interactions. The movement of these birds would be analyzed using one of the previously mentioned video-tracking software, that can track the trajectory of and recognize multiple animals in a video (Perez et al. 2013). These programs were designed to accurately track the many individual and social behaviors of *Drosophila*, mice, ants, and zebra fish, occurring in a planar field, while providing a high-throughput, quantitative analysis of their movement. In the context of the present experiment, these programs could be used to record the position, orientation, and movement of the male and female zebra finches, and calculate descriptive statistics of their individual behaviors and social interactions. An analysis of this behavioral data in the context of song complexity, would show the relative importance of the male song in female mate selection.

However, it is also important to consider the main sources of error that are likely to arise, given the nature of the tracking program and the experimental setup. One expected problem will be distinguishing males from females (Branson et al. 2009). In the current cage set-up, the birds would be backlit, making them appear silhouetted, and thus more difficult to distinguish between the sexes. In order to correct for this, additional front lighting could added to provide better visualization of the physiological differences between the two sexes. The second main source of error would be when the identities of individuals were lost or swapped (Branson et al. 2009; Perez et al. 2013). Identity swap can occur when pairs of individuals come into contact with each other, causing the tracking to get confused as to which individual is which when they converge and then separate. In order to detect identity errors made by the tracker, frames would need to be selected in which the individuals touched--as those situations are most likely to result in tracker errors. These frames could then be re-watched in slow motion, focused on the selected individuals, to check for potential mistakes and then manually corrected if an identity swap occurred. In the case of a lost identity, the tracker would not differentiate the individual from the background, resulting in the loss of a reading for the individual entirely. This could occur when an individual remains stationary for a long duration, as the tracking system will fail to detect the individual's movement and separation from the background. This potential error could be minimized by shortening the recording duration, recording for no more than an hour and a half at a time, so that the birds don't get too comfortable in their environment, settling down and stopping their movement.

After much trial and error, I was able to analyze a video-recording of experiment 4 in idTracker and plot the trajectories of each finch within a two-dimensional frame in relation to time (Figure 8). Even from this preliminary data, we can observe that green164 and the female appear to be interacting more as they are located closer together on the x- and y-plane as time passes. This quantitative approach to analyzing animal behavior and interactions will hopefully provide new insight into the nature of female mate selection and the role of sexual selection on male song complexity and other related aspects of courtship.

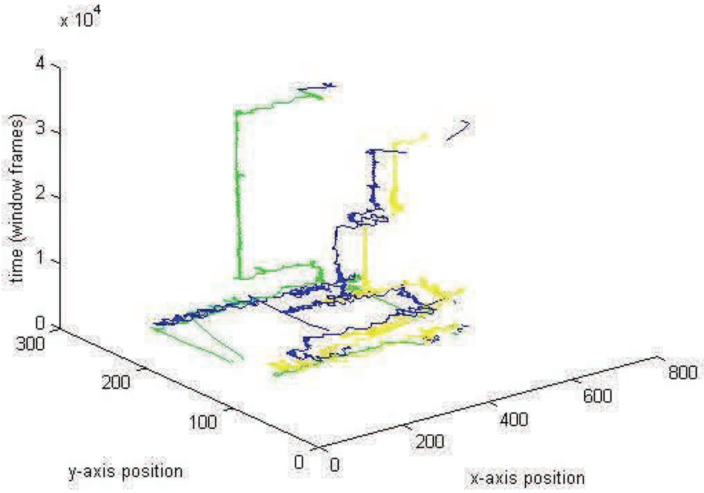

Figure 8. Trajectories of three zebra finches on a two-dimensional axis in relation to time. Green line traces green170, blue line traces green164, and yellow line traces female.

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