Courtship Dynamics and Factors Influencing Female Choice of Mates in Zebra Finches

A thesis submitted towards partial
fulfilment of BS-MS Dual Degree
Programme

by

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For the study conducted under the supervision of

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at the

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Certificate

This is to certify that this dissertation entitled "**Courtship dynamics and factors influencing female choice of mates in zebra finches**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Vrushali Rao Gumnur**, at **IISER, Pune** under the supervision of **Dr. Raghav Rajan, Department of Biology, IISER, Pune** during the academic year **2017-18**.

Raghav Rajan

Signature of Student

Signature of Supervisor

Date: 20/03/2018

Declaration

I hereby declare that the matter embodied in the report entitled “**Courtship dynamics and factors influencing female choice of mates in zebra finches**” are the results of the work carried out by me at the **Department of Biology, Indian Institute of Science Education and Research, Pune** under the supervision of **Dr. Raghav Rajan**, and the same has not been submitted elsewhere for any other degree.

Raghav Rajan

Signature of Student

Signature of Supervisor

Date: 20/03/2018

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ABSTRACT

Extensive studies have been conducted on mate-choice and courtship behaviour in animals. It has been established that certain traits have an evolutionary advantage in terms of getting selected by the opposite sex. Previous work with the model organism, zebra finch has shown that females tend to prefer male traits such as specific song features, bright beak colour and such like. But we don’t have much clarity on the exact dynamics of the courtship ritual. Though our study we address this very question of preference establishment in female zebra finches. We conducted preference assays using live males in a choice chamber setup to explore the events that occur during preference development, the parameters that can be used to judge this preference and the factors that could influence the same. Also, we tested the effect of varying the distance between participating females and introducing a peer on a female’s preference. We found multiple parameters that can be used to judge female preference, the most robust one being the time spent next to a certain male. Next we moved on to show that preference dynamics can be described in terms of female activity, male-female interactions and evolvability of preference. Lastly, we demonstrated that reducing the distance between participating individuals or, introducing a pair female led to an increase in female activity and delay in preference establishment. Also, we found that in the presence of males, any existent social hierarchy between the females is revealed, by one female being dominant over the other. But she need not necessarily have an influence on preferences of the submissive female. Collectively, our findings paint a picture of the details involved in the process of mate-choice decision making and the influence of external conditions on female preference in zebra finches.

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ACKNOWLEDGEMENTS

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INTRODUCTION

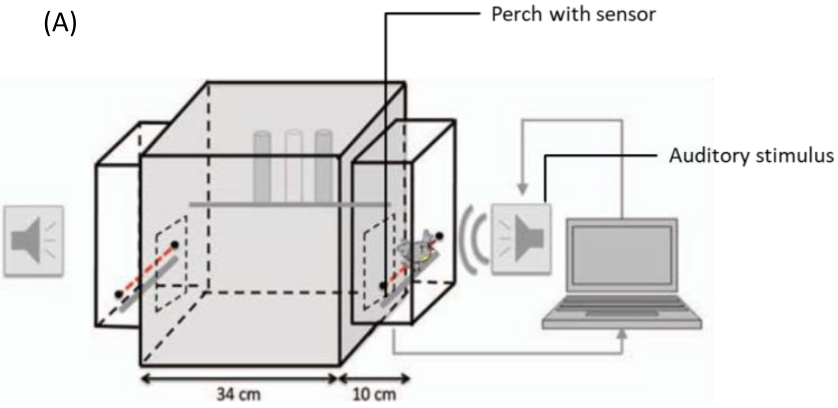
Every animal species strives towards ensuring that its future generations to come are healthy. A large part of this effort is put into choosing a mate that displays good traits and in turn a good genetic makeup (Weatherhead and Robertson, 2015). Thus, the process of intersexual selection is a carefully designed process, modelled by biological evolution to enable the choosing of a desired mate by filtering out unsuitable mates through a courtship ritual. The sex that displays, (usually the male) can send courtship signals either in the form of directed actions towards the recipient or as mere traits on the displayer. In turn, recipient animals display certain communication cues and behaviours to display their preference while choosing a mate, which can either be multi-sensory or behavioural in nature (Guilford and Dawkins, 1991). Thus, proceedings of their interaction make for an intriguing question in the field of animal behaviour.

One of the widely used model system to study mating behaviour is the Zebra finch or *Taeniopygia guttat*a. This species of finch is a native of central Australia and belongs to the general category of songbirds (order Passeriformes) and family Estrildidae, which are passerines found throughout tropical and subtropical parts of Africa, Southeast Asia, and
Australia. Being highly social beings, zebra finches live in huge flocks and follow a granivorous diet. These birds have been observed to be monogamous in nature and show distinct sexual dimorphism in various aspects such as size, colour, body patterning and vocalisations. While the females have plain coloured feathers, adult males carry bright orange patches on their cheek and dark stripes over their throat and chest (Dunn and Zann, 1996). Only the males are able to produce song, which is a learned behaviour acquired from the father bird (long memory), similar to speech acquisition in humans (auditory experience). Other vocalisations include calls which both males and females produce. The song produced being sung in the presence of a female differs in some properties from the songs sung in isolation (Dunn and Zann, 1996) .

FIG 1. Zebra finch male (left) and female (right)

A variety of tests have been used to conduct mate-choice related experiments. For example, operant conditioning tests wherein females are trained to respond to recorded songs or dummy male birds are done to check the ability of females to discriminate between courtship signals (FIG. 2A)(Riebel et al., 2002). For our study, we chose to go ahead with a well-known test, using a choice chamber which has been used previously to a large extent to conduct preference assays (FIG. 2B) Choice chambers cages have artificial/live stimuli on opposite ends and the female is free to move to the side with the preferred stimulus. Unlike the conditioning experiments, this setup doesn't involve any learning, but tests basic preferences that could be made by females. With lesser artificial intervention through the use of live males, results of the choice chamber test could better represent natural conditions and have been shown to be fairly reliable (Forstmeier and Birkhead, 2004).

(A)



(B)

FIG 2. (A) Setup for operant conditioning based preference assay (Hernandez et al., 2016)

(B) Tri-compartment choice chamber for preference assays

Previous work on mate choice among zebra finches has shown that females show highly selective behaviour while choosing a mate, which could be based on the male's physical appearance or song(Coopersmith, 1987; Collins and tenCate, 1996). The available literature describes experiments that tested for the final choice made by the female in the form of a correlation between female preference cues and certain male traits such as beak colour and familiarity of song. There was increased preference shown for males with high song rate and bright orange beaks (Collins et al., 1994). The results from literature such as the aforementioned data reveal that particular traits that might be largely considered desirable in a male, however, the exact dynamics involved in the phenomenon of courtship isn't clear. Hence, an entire area of questions related to nuances in mate-choice interaction opens up for investigation. The following project mainly aims to explore the details of the courtship interaction between male and female zebra finches that leads to a decision taken by the female.

It has been hypothesized that mated pairs show increased bonding even after a period of separation, demonstrating that females are capable of recognising their chosen mate. Females were found to choose males producing familiar songs than novel ones (Amelio et al., 2017). A possible extension of this hypothesis would be to verify if naïve females establish and maintain preferences among novel males introduced to them. Additionally, whether this preference is subject to change with repetitions (with different male pairs) and through the time course of the experiment is yet to be investigated thoroughly, although since earlier studies report the choice chamber test to be fairly repeatable with the same pair of birds. The work by Fostermier and Birkhead found but significant consistency (around 70%) in a female when tested twice with the same set of males (Forstmeier and Birkhead, 2004).

To judge the female’s interest, the time spent by her next to a certain male and the behavioural responses shown by her (copulation solicitation displays) have been considered important parameters in most of the previously carried out experiments (Mark E. HauberA, C, 2010). Available literature describes the time spent by females next to males as a highly reliable cue of preference towards that male, and thus is the opted observable in a number of choice chamber experiments (Wales, 2007; Witte, 2010). In addition, actions such as hopping, calls, puffing up, beak rubbing etc. shown by the females can be considered secondary cues of preference. Through our study, we aim to add to the set of parameters than can be used to quantify and judge female preference.

Though male traits and female-specific variability mainly drive a selection procedure, other factors such developmental factors (Buchanan and Catchpole, 2005) and external conditions have been shown to affect mate choice. The work by Brumm and Slater states one such factor to be the distance between the participant zebra finches, which causes rate of the directed male songs to change (FIG 3). Also, change in acoustic traits (such as an increase in song amplitude) with increase in distance from the female was found, demonstrating that cues can be adapted to conditions such as proximity to receiver (Brumm and Slater, 2006)(Suri and Rajan, 2018). Likewise, along with the male’s courtship signals, varying distance might also have an impact on the female’s behavioural responses and decision making dynamics en route to establishing a preference towards the male. Thus, in this study we performed our preference assays with two different cage lengths (long and short) to probe to understand the role of proximity in courtship dynamics and verify if any present effects are in accordance with previously demonstrated change in male vocal traits.

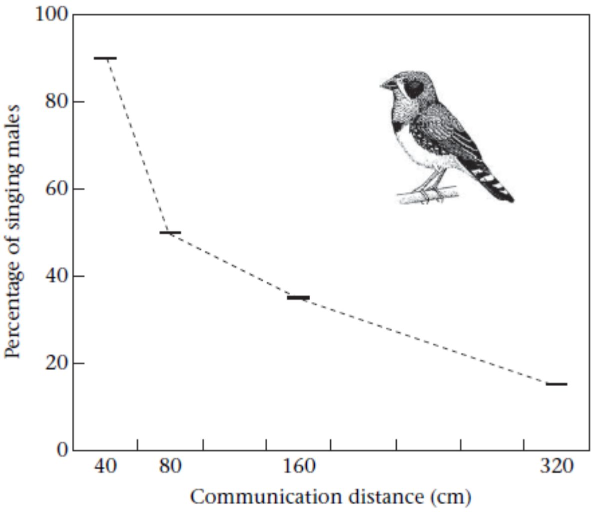


FIG 3 . Singing responses of male zebra finches to a female at different distances (Brumm and Slater, 2006)

Additionally, there have been extensive studies carried out on learning through imitation among social animals, with mate choice copying as suitable example in this context (Galef and White, 1998). Earlier work on courtship in zebra finches also demonstrates mate choice copying among females. Females, who did not have any prior preference for an adornment on males, developed such a preference after observing a model female with an adorned male thus suggesting a role for conspecific model individuals (Kniel et al., 2015, 2017) . Proceeding in this direction, we intend to shed light upon the influence of the presence of a female peer on the participating female's mate selection strategy, since little is known about whether or not peer presence and possible social hierarchy among females is a crucial influential factor for mate choice.

By virtue of choice chamber aided preference assays, we hope to further our knowledge about the process of mate choice decision making among female zebra finches by determining trends, if any in behavioural cues that play a role in shaping this dynamic process and could be potentially employed in experimental manipulations to replace live birds by conducting choice chamber preference assays with live males. Additionally, we intend to verify the effect of male-female proximity and peer-influence on the preference establishment by varying the size of the choice chamber and introducing a peer female respectively. Using the established courtship parameters in future experiments to successfully to obtain desired preference from females would in part, connote a fair understanding of sexual selection and help comprehend courtship signal values.

OBJECTIVES AND HYPOTHESES

The study aims to explore the details process of decision making in female zebra finches during mate choice and understand how preference for a male is established. We intend to determine the parameters that can be used to judge female preference, verify the evolvability of these parameters and preference, and investigate causal factors. Additionally, we aim to diagnose the influence of varying the distance between the individuals and, the presence of a peer on the preference dynamics of a female.

We hypothesize that:

- There could be general trends and behaviours that all females show en route to making a preference.
- Male song traits could be influential factors for female choice.
- Varying the distance and introducing a peer may cause a change in the dynamics of male-female interaction and final choice made by females.

METHODS

All experiments were approved by the Institutional Animal Ethical Committee (IAEC), IISER Pune and performed according to the guidelines of the Committee for the Purpos of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

A total of 35, healthy zebra finches were used in this study, of which 20 were female and 16 were male. All birds were aged at least 150 days post hatch. The birds were reared in the colonies of our laboratory or were bought locally. These birds were provided with ad-libitum food and regularly supplemented with cuttlebone, sprouts, and boiled eggs. They were maintained in a bird colony where they are exposed to light from 6:00 AM to 8:00 PM (14 hours a day). 5 of the birds were kept were in individual cages instead of colony, but yet in the presence of conspecifics. Four of the birds had been used before for breeding purposes, either successfully or unsuccessfully. However, we ensured a gap of at least 10 months between the previous the breeding attempt and our experiments and these birds were treated as equals with the naïve birds.

Acclimation

For experiments with individual birds, all chosen females were subjected to a 3-day acclimation period before experimentation, during which they would be isolated into the choice chamber cages accompanied by seed and water cups (placed in the centre compartment) for 2-3 hours. Male were isolated only for 1 hour before commencement of the experiment. Females were exposed to the male only during experiment. The male cages were placed on either side of the choice chamber and visually blocked from the female until recording was started.

In the case of experiments done to test presence of a peer, two random females from the same colony were chosen. Each of these females underwent acclimation separately and was used for individual experiments prior to pair experiments. The females were observed beforehand in the colony to check for any kind of aggressive reactions towards each other and those without any were chosen to avoid fatalities during the course of experiments.

Video Recordings and experimental design

The experiments were conducted in rooms away from the bird colonies (incomplete auditory isolation) with minimal human movement in the surroundings, to minimise fear caused error in performance. All video recordings were done using either Logitech Webcam C270 HD or Ipad 4, both with frame rate 30 fps. Detailed acoustic analysis of songs was out of the scope of this project and hence microphones for recording audio exclusively weren't used.

A schematic of the experimental design is as shown in FIG 4.A. Each female was recorded with the same male pair 3 times, each being considered as a single experiment and collectively forming a set. Each set consisted of 2 experiments with the male cages in one orientation and 1 experiment with the cages laterally exchanged (to account for any existent bias towards one side). We chose to keep the exchanged cages in the 2nd experiment out of the 3. In this manner, each female was recorded with 3 pairs of males, and hence the number of experiments per female totalled to 9, forming 3 sets. The mean duration of all recordings was maintained around 12.5 minutes. We recorded one experiment per female per day between 9 a.m. and 4 p.m. (activity levels were observed to be almost identical throughout this period). Additionally, control recordings were done without any males and, with only one male on the side of the choice chamber

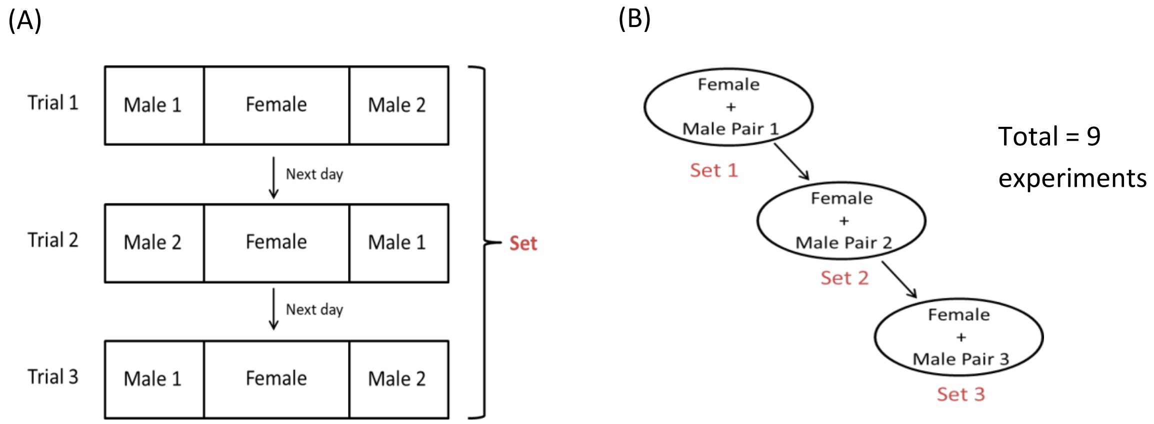


FIG 4. (A) Experiments in a single set

(B) Experiment structure for each female

Individual female experiments - The first group of experiments (individual) was done using the 3-compartment long (60cm x 20 cm x 20cm) choice chamber (FIG 5.A). 19 females were paired with 16 males (8 pairs) and the total number of experiments performed was 170, with 58 sets. The next group of recordings (individual) were done with a 1-compartment short cage (20cm x 20cm x 20cm) choice chamber (FIG 5.B) with a subset of only previously used females, consisting of 14 birds. The number of males used was also reduced to 12 males (6 pairs) due to the death of 4 birds.

Paired female experiments -The third group of experiments (peer experiments) was done by introducing 2 females in the choice chamber together. These experiments were done with both sizes of cages, long and short. In both categories, the 12 males (6 pairs) used in the individual category were maintained for peer experiments. However, we used 14 females (7 pairs) for short cage peer experiments out of which 1 female died after a single set of recordings and had to be discarded for most analyses. The long cage peer experiments followed with 12 females (6 pairs).

A summary of number of experiments in each category is given in Table 1

	Individual Experiments	**Peer Experiments**
Long Cage	170 exp ; 58 sets (19 females)	54 exp ; 18 sets (6 pairs)
Short Cage	114 exp ; 38 sets (14 females)	63 exp ; 21 sets (7 pairs)

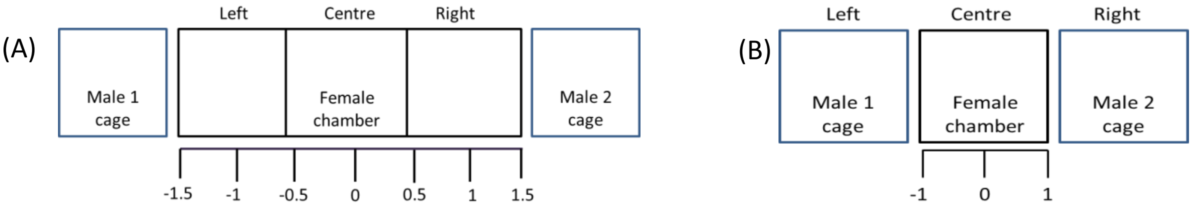
TABLE 1.Total number of experiments in each category

Video Scoring

The videos were scored manually for the following aspects of male and female behaviour:

(i) Female position

The choice chambers were divided into sections of equal length with markings shown as below in FIG 3.A and 3.B. We noted the time points when the female changed her location from one mark (-1.5, -1, -0.5, 0, 0.5, 1 or 1.5) to the other, enabling us to calculate the time spent at that former location. The raw data of female position was plotted in the form of trajectory graphs (FIG 3.C).



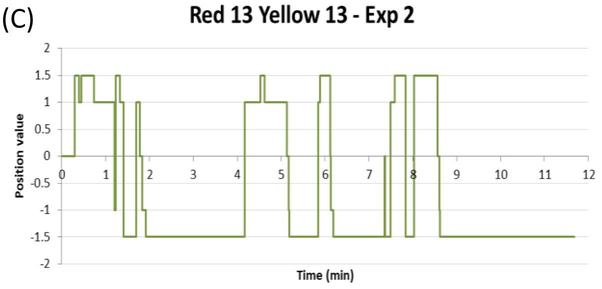


FIG 5.1 (A) Position marking and cage designation for long cage

(B) Position marking and cage designation for short cage

(C) Trajectory graph showing female position

(ii) Time spent

The time spent in each compartment was calculated from the position data and plotted in the form of pie charts for convenience.

(iii) Male song

We noted the time points when a male starts singing, which was scored -1 or 1 if the left or right male sang respectively (FIG 4.A). The raw data was plotted in the form of singing vs time graphs (FIG 4.B).

(A)

Left Male singing	-1
No singing	0
Right male singing	1

(B)

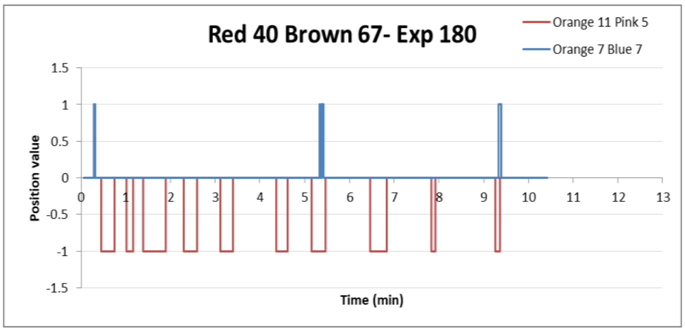


FIG 5.2 (C) Song scoring

(B) Male Song graph

(iv) Copulation solicitation displays (CSDs)

The female shows behavioural cues such as hopping, puffing, calls etc. as copulation solicitation displays (CSDs) which are indicators of preference towards a male. We observed the video recording for 3 major CSDs, namely, tail quivering, hopping and tail pointing, and counted the number of occurrences for each (for each male)

(v) Darting behaviour or number of darts

In peer experiments, we noticed that there were instances of one female chasing the other. We counted the number of times a female made a darting action towards the other female (number of darts) and considered it as a proxy for peer interaction.

Data analysis and Statistical analysis

All analyses were done using the XLSTAT statistical analysis, an add-in software for Microsoft Excel 2013. We conducted statistical analyses using non-parametric tests. Significance estimation and comparisons between sets, cage size and individual-peer categories were carried out through Kruskal-Wallis ANOVA and the alpha value was set to α = 0.05. All correlations were computed using Spearman's correlation coefficient.

RESULTS

SECTION 1 – Individual long cage experiments

We conducted the first groups of preference assays with a single female placed in a three-compartment choice chamber. There were 19 females tested against 8 different male pairs in this category experiments. We analysed the dynamics in terms of male-female interaction in terms of female cues/behaviours that could point towards female preference and factors contributing to preference. Also, repetitions of an experiment (trials in a set) were performed thrice to check whether preference and female-given cues pointing to it remain constant. A change in these features across a set of experiments could indicate either strengthening or habituation depending on the direction of change.

(i) *Choice or preference* – A female's choice was evaluated based on the amount of time that she spent in the compartment adjacent to a particular male. A male was considered to be preferred/chosen during an experiment if the female spent most of her time (>2 min) next to the male.

(ii) *Stayed in the centre* – The female stayed in the centre for more than 4 min during the experiment

(iii) *Female was undecided* – No significant change (<2 min) between time spent with each male.

(iv) *Preference strength (|L-R|)* – The absolute value of the difference in time spent by the female in the left (L) and the right compartment (R), which value could be considered to be proportional to the extent of preference shown towards one male.

(v) *Crossovers* –A jump made by the female from one compartment to the other was considered as a crossover. The number of crossovers made by a female could possibly show the female's activity level.

(vi) Interaction – The instance where a male's song initiation and the female's arrival on his side occur simultaneously (precedence decided by 5 sec gap; two song bouts were considered separate if separated by 3 seconds).

The following section presents results from individual female data which were analysed with non-parametric tests.

I. A) In the presence of males on either side, the female mostly chose to stay next to one particular male for the majority of her time.

To check where the female spends most of her time in the choice chamber, we made comparisons between the numbers of experiments in which the female chose to stay next to a male (|L-R| > 2 min), stay undecided (|L-R|< 2 min) and in the centre(>4 min). We observed that the time spent in the three compartments (left, right and centre) differed significantly across all experiments (n=170, p = 0.0001, range = 0 to 12.751 minutes). Secondly, females spent significantly more time next to any male as compared to an empty cage (control experiments), (n = 12, p = 0.0021, Kruskal-Wallis test).

Thus, in most experiments, the female chose to stay next to one male over the other than being undecided or in the centre. (FIG. 6.B)

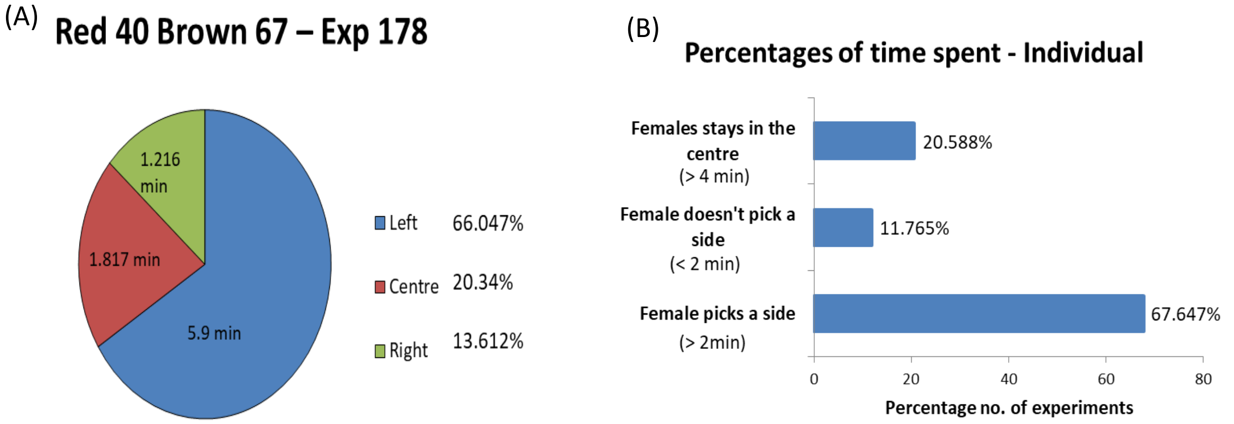


FIG 6. Percentages of time spent in each compartment by individual females

(A) An example graph of time spent by female in a long cage experiment. Each colour denotes different o compartments.

(B) Female location plotted against percentage of experiments for long cage experiments(n = 170)

I. B) Repeated interactions/familiarity to male pair did not lead to strengthening of female preference .

The absolute differences between time spent by females next to either male or their preference strength (|L-R|) was calculated. To verify whether there is any change in the preference strength with repetitions, we calculated the differences between values of |L-R| in consequent trials (T_1-T_2-T_3). There was no significant change in preference strength across trials in a set (n=54, p = 0.289, Kruskal-Wallis test) (FIG

7.A). The number of females showing no significant increase was more in number than those that did. Additionally, we found no change in the mean |L-R| through a set values for all experiments (p = 0.0001)(FIG 7C). This can be interpreted preference for a male to be constant and not strengthening or, getting habituated.

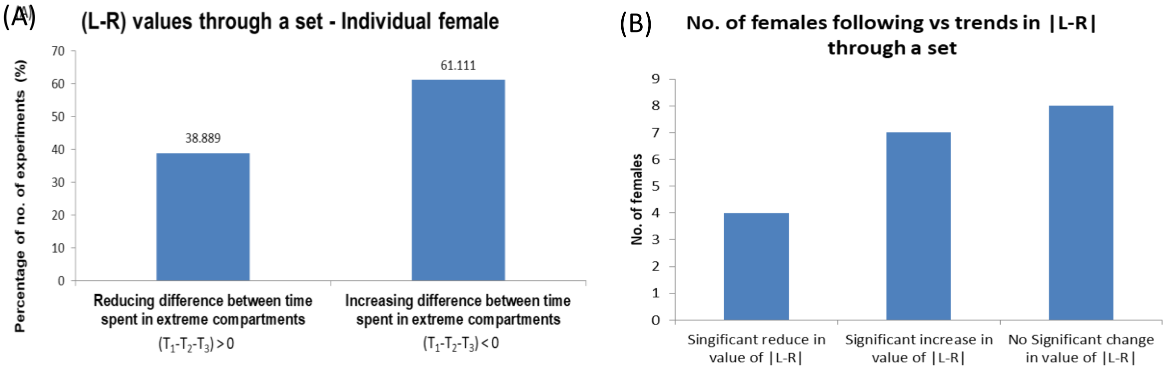


FIG 7. Change in preference index |L-R| with consecutive trials in a set – Individual females

(A) Bars showing the percentages of experiments that shows whether |L-R| changes with repeated trials (n=54, p = 0.289)

(B) No. of females showing specific trends in change in preference strengths, represented by each bar

I. C) Females chose to stay next to the same male twice out a set of three trials.

We determined the number of times females chose to stay next to a given male in a set of three repeated trials. It was observed that when looking at adherence of a female to a certain male, the number of sets showing females staying next to the same male in two out of three trials was the greatest (FIG8) (n=54, p=0.048, Kruskal-Wallis Test). Thus, the female mostly displayed preference for the male only for two trials. However, we did not take the order of trials into account. If the female repeated her choice of male in the last two trials, it could suggest that stable preference develops only at a later stage.

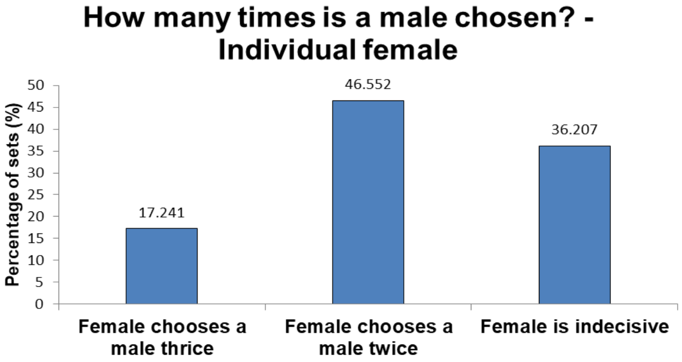


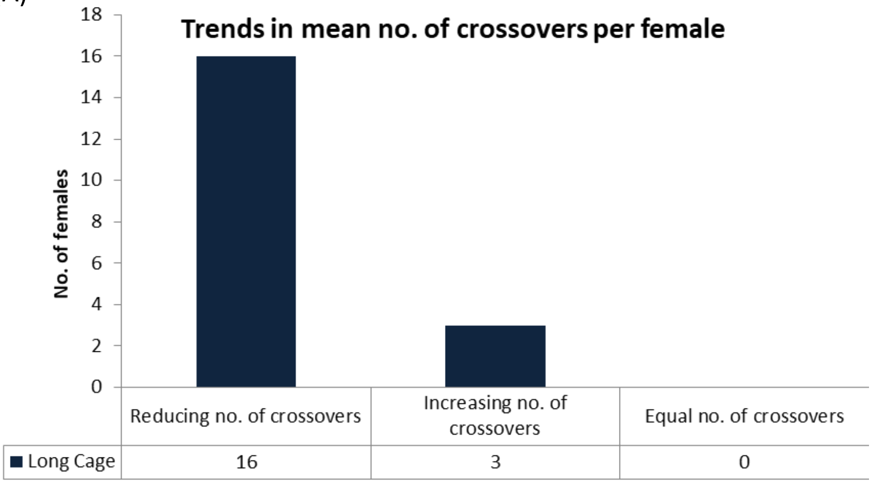
FIG 8. Percentage of sets showing the number of times a male is chosen
Indecisive: Time spent next to the male < 4 min – Individual females

II. A) Female sampling activity reduced as the interaction proceeded.

We noted the number of crossovers made by the female (from one compartment to another) in every 3 min time interval for an experiment and calculated the difference the number made in the first and second half of the video.16 out of 19 females showed the more crossovers in the first half of interaction than the second, i.e. reducing trend with time (FIG 9.A)(No. of crossovers taken as threshold to differentiate between trends was 3, the minimum no, of crossovers made by any female). A few of them have been plotted in FIG 9.B. We think this shows that the female samples both stimuli initially, then establishes the preferred one, or simply stops by getting tired. We think this shows that the female samples both stimuli initially, and then reduces movement to establish the preferred one.

However, not all females showed a significant decrease in mean (over all their experiments) number of crossovers made with time (n=19, p = 0.112, p = 0.193, Kruskal-Wallis test) (FIG 9.C). This might be due to the individual differences in females, the values of which are given in table 1.

(A)



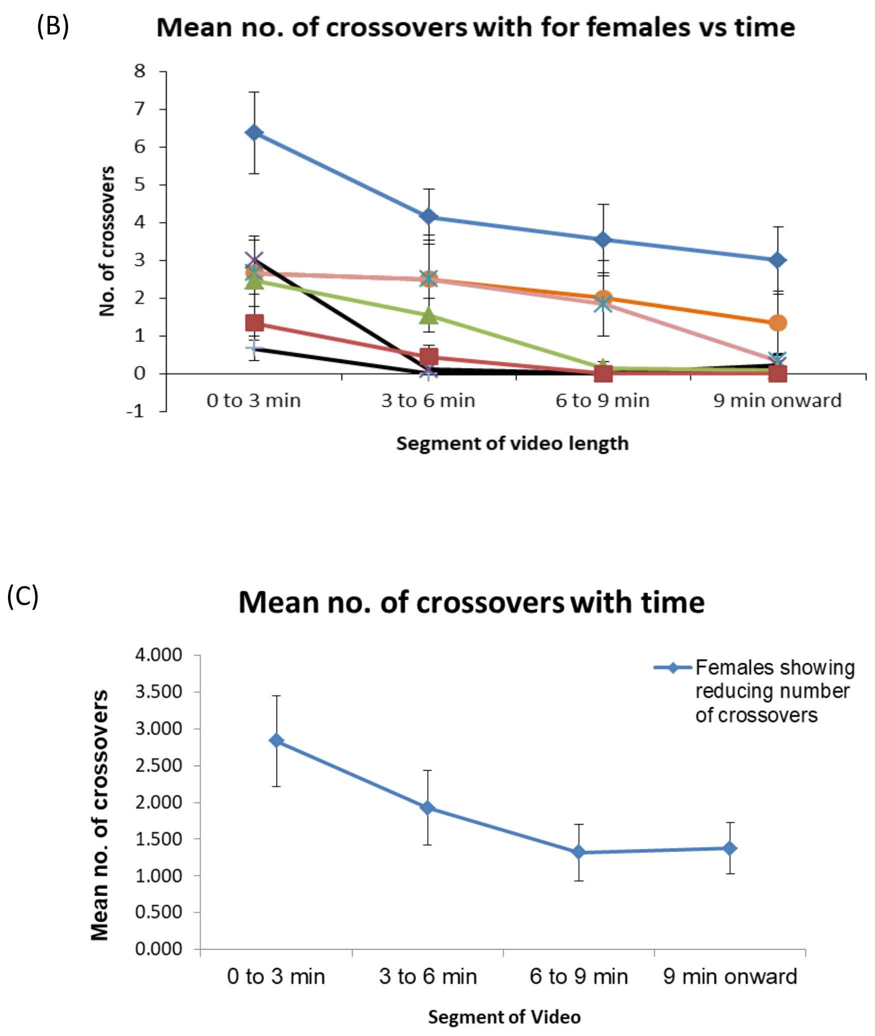
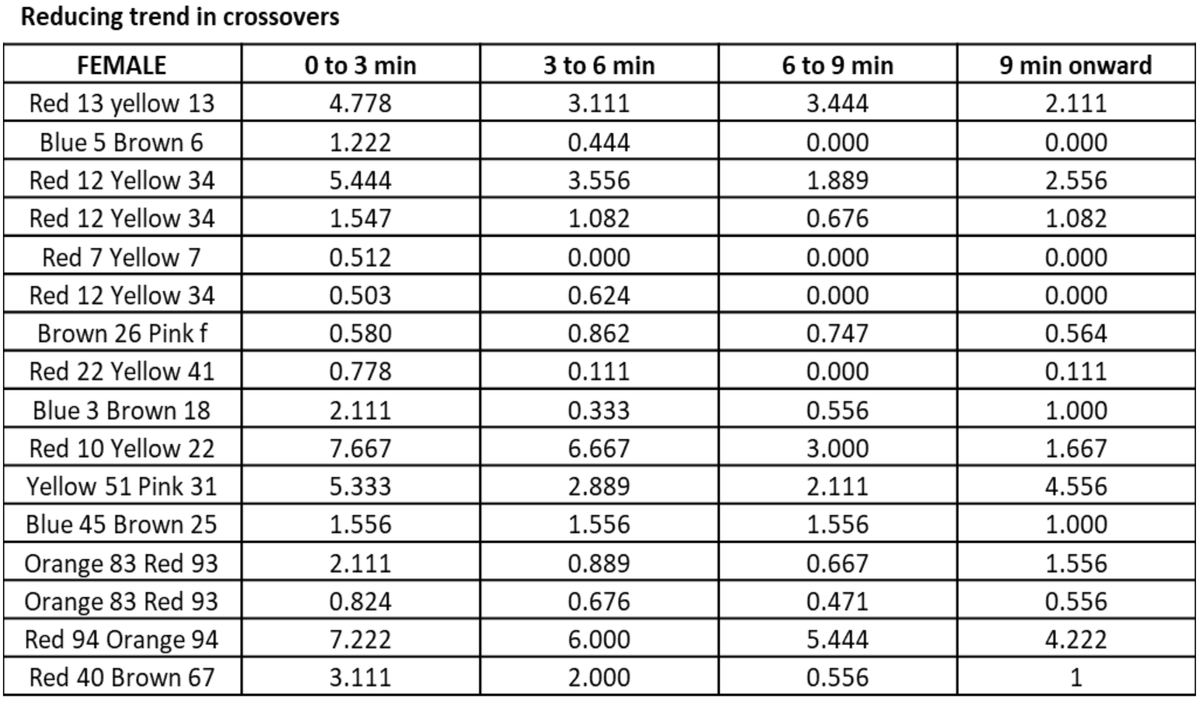


FIG 9. Trends in number of crossovers for individual experiments

(A) No. of females plotted against the category of crossover trend they follow (n =19)
Reducing - Crossovers made in first half>second half;
Increasing - Crossovers made in first half<second half

(B) Mean no. of crossovers plotted for some females. Each coloured line represents a different female (n = 6) and each point represents no. of crossovers made

(C) Mean no. of crossovers by all females plotted with time (n=16, p = 0.114, Kruskal-Wallis test.
Error bars represent standard error



Reducing trend in crossovers

FEMALE	0 to 3 min	3 to 6 min	6 to 9 min	9 min onward
Red 13 yellow 13	4.778	3.111	3.444	2.111
Blue 5 Brown 6	1.222	0.444	0.000	0.000
Red 12 Yellow 34	5.444	3.556	1.889	2.556
Red 12 Yellow 34	1.547	1.082	0.676	1.082
Red 7 Yellow 7	0.512	0.000	0.000	0.000
Red 12 Yellow 34	0.503	0.624	0.000	0.000
Brown 26 Pink f	0.580	0.862	0.747	0.564
Red 22 Yellow 41	0.778	0.111	0.000	0.111
Blue 3 Brown 18	2.111	0.333	0.556	1.000
Red 10 Yellow 22	7.667	6.667	3.000	1.667
Yellow 51 Pink 31	5.333	2.889	2.111	4.556
Blue 45 Brown 25	1.556	1.556	1.556	1.000
Orange 83 Red 93	2.111	0.889	0.667	1.556
Orange 83 Red 93	0.824	0.676	0.471	0.556
Red 94 Orange 94	7.222	6.000	5.444	4.222
Red 40 Brown 67	3.111	2.000	0.556	1

TABLE 2. Mean no. of crossovers for females with time

II. B) No. of crossovers did not show significant change with repetitions.

We looked at the difference in the number of crossovers by the female made in repeated trials (T_1-T_2-T_3). Though many sets showed an increase in no. of crossovers through consecutive trials, this change wasn't significant (n=54) (p=0.539, Kruskal-Wallis test). This again suggests that the females are unable to establish a preferred male and neither do they get habituated even with repeated experiments with the same males.

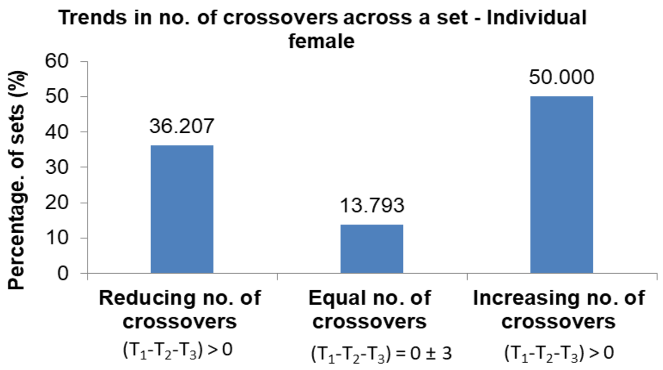


FIG 10. Change in the number of crossovers across a set – Individual females
Percentage of sets plotted with categories of possible crossover trends for long and short cages.

II. A) The number copulation solicitation displays (CSDs) given by a female differed with the type of CSD.

Out of the different kinds of CSDs that can be given by a female to a male, we scored three vastly shown responses which were hopping, tail-pointing and tail-quivering (Wales, 2007) with hopping being the most common (>50% for both sizes of cages). The relative percentage occurrences were seen as shown in (FIG 11).

Tail-quivering responses were given to select males. Out of the 6 males maintained in common throughout the course of the project, only 1 male received tail-quivering responses from multiple females (10/19 females).

Thus different CSDs could show varying levels of preference, tail quivering having the highest intensity.

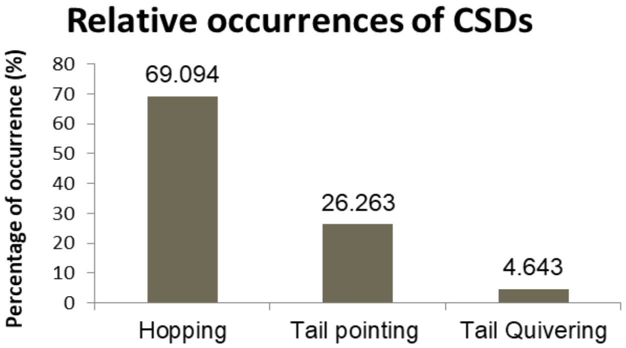


FIG 11. Number of CSDS given as a response towards male – Individual females
Bar graph showing percentage of occurrence of types of CSDs (n = 170)

III. B) The number of displays didn't change with repetitions.

We looked at change in the number of CSDs given out by a female (FIG 7.A) through subsequent trials in a set (T_1-T_2-T_3) and found was no significant change in the total numbers of CSDs or the mean no. of CSDs calculated over all sets for a female (n = 58, p = 0.985, Kruskal Wallis test) (FIG 7 B). Additionally, we did not find significant changes when mean no. of CSDs given through a set was plotted per female either (n=19, p = 0.720) (FIG 7.B)

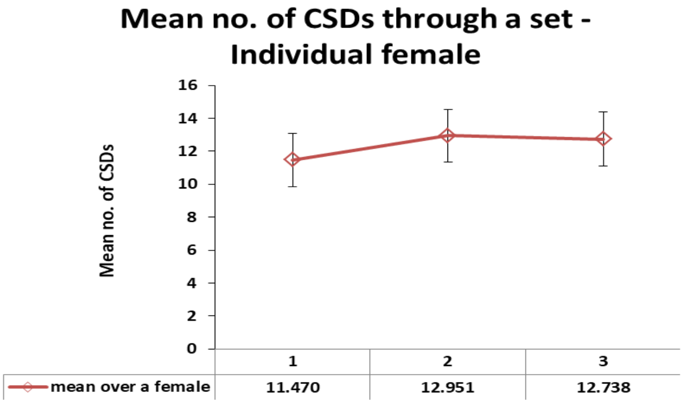


FIG 12. Change in the total and means number of CSDs given in consecutive trials in set – Individual females
Mean no. of CSDs over all sets (n = 58, p = 0.985) and over each female (n=19, p=0.720) plotted against trial number.
Error bars represent standard error.

IV. A) All signals of preference didn't always agree with each other.

Among the parameters identified to judge preference, it was observed that females gave all signals of preference to the same male in a fair percentage of experiments (~30%). The percentages of signal agreement have been shown below (FIG 13). All the three signals, i.e. CSDs, spending time next to preferred male and settling down next to the preferred male seem to be compliant with each other across all experiments in (n = 170). Thus, among the behavioural cues, time spent by a female by settling down next to the male seem to be more robust indicators of preference. The no. of CSDs may be subject to individual variability.

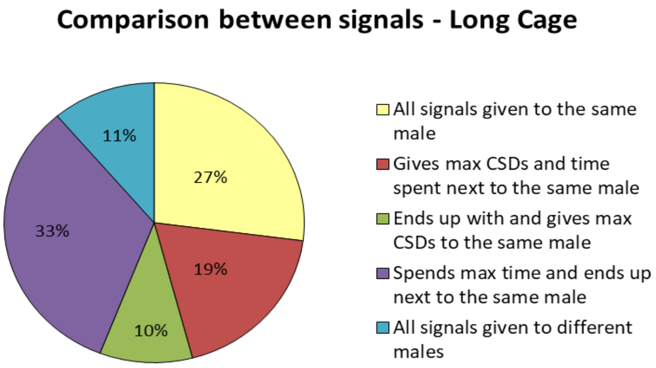


FIG 13. Percentage of experiments showing agreement between female behavioural cues as preference indicators, with each colour representing the extent of agreement. – Individual females

V. *Male song duration and song rate (bout frequency) were not correlated with the time female spends next to him.*

Song traits have been shown to influence female mate choice heavily(Holveck and Riebel, 2007). We compared observable song traits of males with the amount of time of time spent by a female next to him to verify some of the possible contributors to preference establishment.

We plotted the mean time spent by females next to a male averaged over experiments with each pair of males, i.e. according to male indices (The variable values were normalised to the video duration and plotted as ratios)(n=16, r_s = 0.0034, Spearman's rank correlation)(FIG 14) . There was no significant correlation found between the two variables.

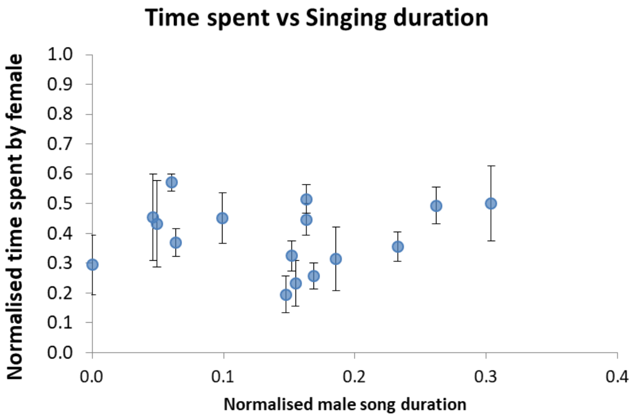


FIG 14. Time spent by female next to male vs the amount of singing – Individual females
Mean female time spent vs mean song duration plotted as ratio indices for each male.
(n= 16, r_s = 0). Each point represents a male
Error bars represent standard error.

Similar to singing duration, we plotted mean time spent by females next to each male against the mean rate of song bouts which gave no significant correlation (n=16, r_s=0.079, spearman's correlation rank test) (FIG 15.A). Though the male traits of song rate and song duration correlate differently with female preference, the traits themselves they are mutually positively correlated (n=16, r_s=0.843, spearman's correlation rank test). This suggests that in addition to song duration and rate, other male traits contribute to female preference.

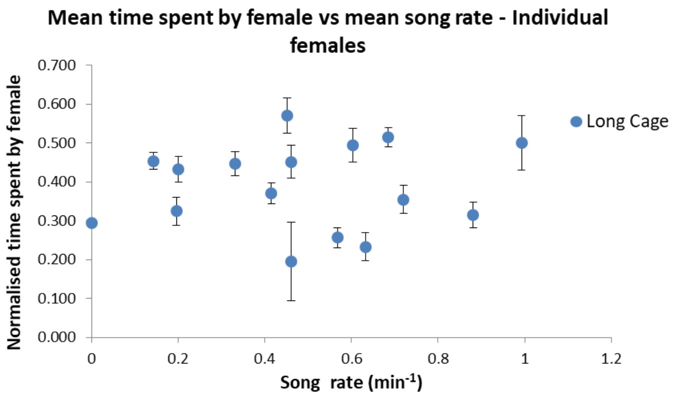
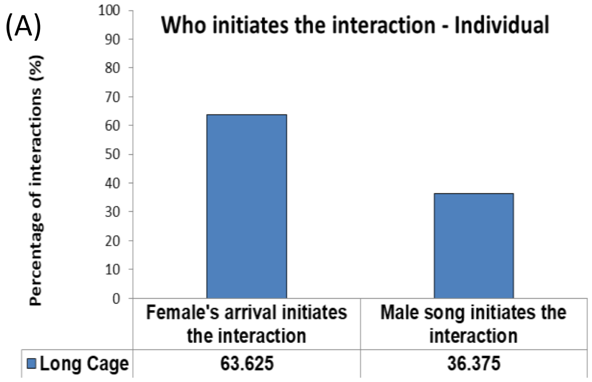


FIG 15. Mean song duration and mean song rate plotted as ratio indices for each male. (n= 16, r_s = 0.843) – Individual females
Error bars represent standard error

VI. A) Females initiated the interaction by arriving next to a male causing them to sing.

Since distance between male and female has been shown to affect male song rate (Brumm and Slater, 2006), it could be a factor influencing the dynamics of interaction. We compared the number of times the male started singing with the female in close proximity (female initiated interaction) vs. when the female was on the opposite side to(male initiated interaction). We observed that most of the cases showed the males start their song only when female arrived in the compartment adjacent to him. Consequently, most male-female interactions (co-occurrence of female arrival and male song) were initiated by female arrival (FIG 16.A, 16.B). Thus dynamics of the interaction could be female activity driven.



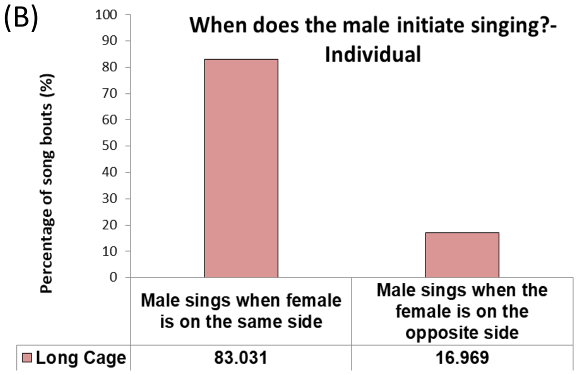


FIG 16. Implicit factors affecting interaction dynamics – Individual females

(A) Bar graph showing percentages of male-female interactions comparing cases where song was started with female on the same with opposite side.

(B) Bar graph showing percentages of male-female interactions comparing cases where the interaction was male initiated with female initiated

VII. B) No. of bouts sang by the males reduced with the duration of interaction, like female crossovers.

Extending the above result, the song rate produced by a male could be shaping female movement/activity. We counted the number of song bouts sung in every 3 minute interval of the experiment video and found significant difference in the mean number of bouts over all experiments (FIG 17.A) and mean number of bouts per male (FIG 16.B) in every interval of time (n = 170, p=0.0000, Kruskal Wallis test). The number of bouts sung decreases with time. This reducing trend is in keeping with the reducing trend of crossovers between compartments made by the female.

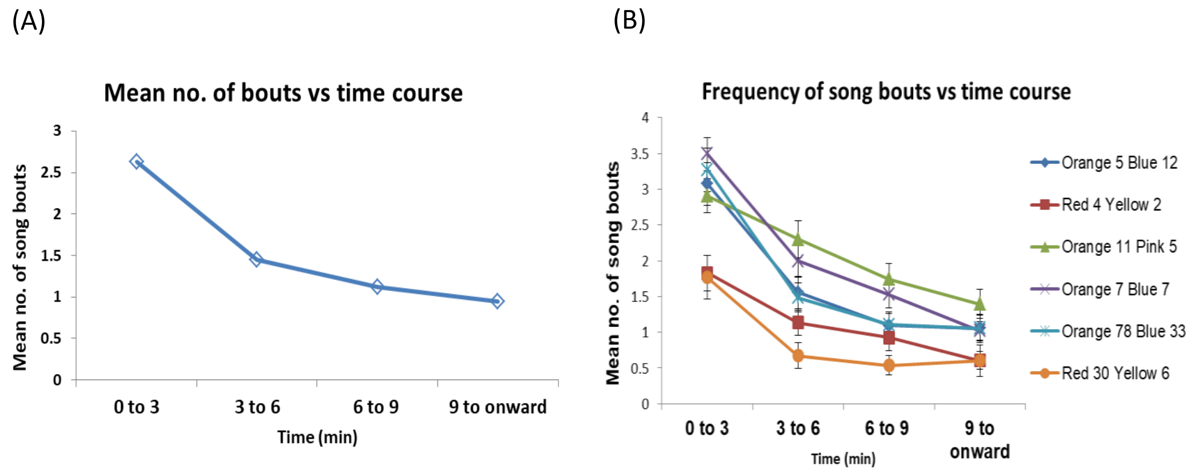


FIG 17. Mean no. of song bouts sung during equal time interval of each experiment (n=170) - Individual

(A) Mean no. of bouts sung over all experiments plotted against time (p=0.512)

(B) Mean no. of bouts sung by some male (averaged over all experiments) plotted against time (p>0.05 for each). Each colour represents a different male

SECTION 2 – Comparing short and long cage individual experiments

As stated in literature, distance between participant birds has been known to affect male song (Brumm and Slater, 2006; Suri and Rajan, 2018). Thus we wanted to verify if distance affects female preference as well. We performed the preference assays with an individual female placed in a short (1-compartment) choice chamber using a subset of the females and males previously chosen (n = 14, n= 6). The following section holds results that compare the data from long cage and short cage experiments to determine any present effects of cage length/proximity between participant birds. We have listed the significant changes seen with change in cage size

I. A) The female mostly chose to stay next to one particular male for the majority of her time for short cage experiments as well.

Similar to results from long cage experiments, we observed that the time spent by the female on the left, right or the centre of the cage differed significantly across all experiments (n=114, p = 0.0011, range = 0 to 12.101minutes). Most number of experiments showed the female choosing to stay on one side (|L-R|>2) as opposed to be undecided (|L-R|<2) or staying in the centre (>4 min), which was also observed in the case of long cage experiments. (FIG.18.B)

Secondly, females spent significantly more time next to any male as compared to an empty cage (control experiments), (n = 12, p = 0.0001, Kruskal-Wallis test). There wasn't any significant change in these percentages between long and short cage data (p= 0.00002, Kruskal Wallis).

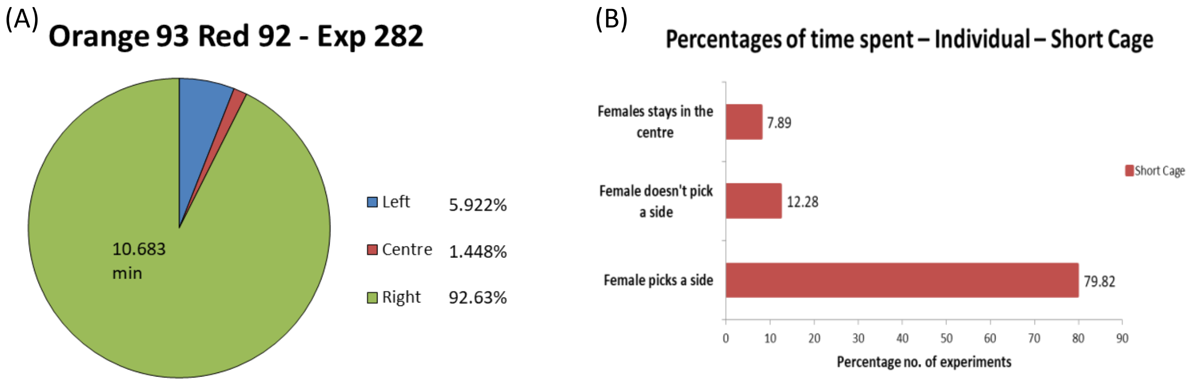


FIG 18. Percentages of time spent in each compartment – Short Cage

(A) An example of a short cage individual raw data showing fractions of time spent by female

(B) Female location plotted against percentage of experiments. n = 114)

I. B) *There was an increase in preference strength with repeated experiments.*

Unlike long cage experiments, there was a significant change in preference strength across trials in a set, i.e. with repetitions which was calculated as the difference between trials (T_1-T_2-T_3) in terms of |L-R| (n=54, p = 0.000, Kruskal-Wallis test) (FIG 19.A). The number of females showed a significant increase (p = 0.003, Kruskal-Wallis test) was the highest (FIG 19.B). Thus we could also be interpreted as the preference not staying stable during repetitions when the distance between participants was reduced.

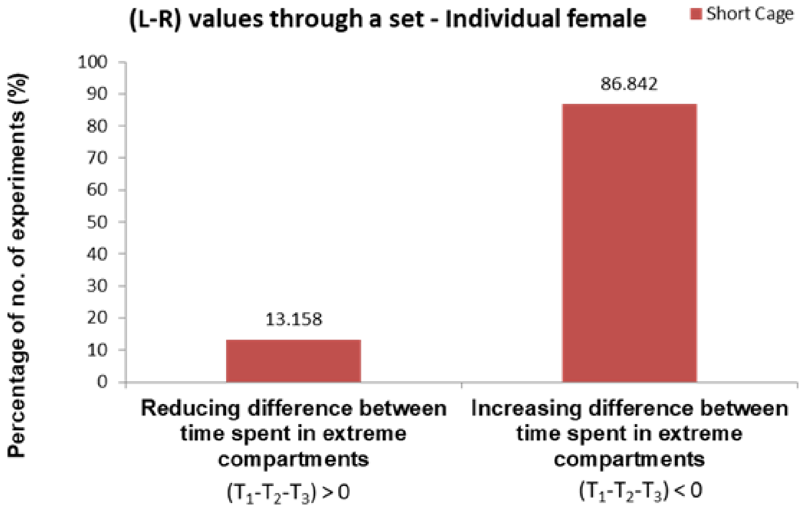


FIG 19. Change in preference index |L-R| with consecutive trials in a set. - Short Cage
Bar graph showing the percentages of experiments that show changes in |L-R| (n=54, p = 0.289)

II. *Female activity increased when distance between birds the birds was reduced.*

Like the long cage experiments, there is a decrease in number of crossovers made by the female with time with reduced cage size and all 14 females showed the trend of reducing crossovers with time (FIG 20.A). We plotted the mean number of crossovers for females against time and found significant changes across time (n=14, p = 0.001, Kruskal-Wallis test). Individual female values are given in Table 3 (FIG 20.B). The total no. of crossovers made by females was significantly higher in the short cage experiments as compared to long cage (p=0.018, Freidman's test) (FIG 20.D). This could mean that the female activity levels during courtship increase with reduce in the distance between participant individuals.

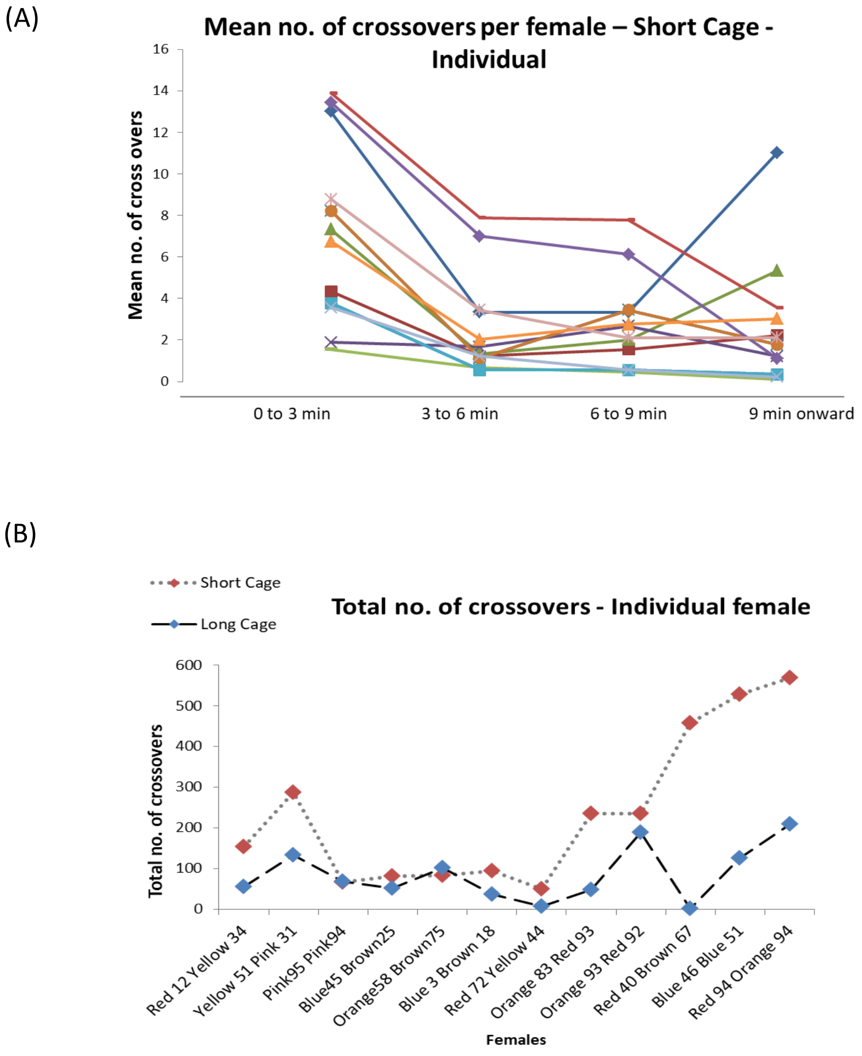


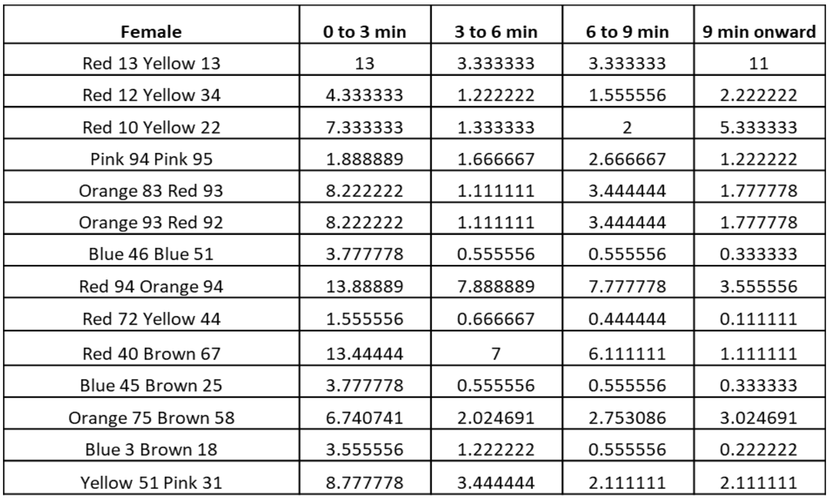
FIG 20. Trends in number of crossovers for individual experiments. - Short Cage

(A) Mean no. of crossovers plotted for females. Each coloured line represents a different female (n = 14) and each point represents the number of crossovers made.

(B) Mean no. of crossovers by all females following a particular trend in number of crossovers plotted with time. Decreasing trend for short cage (n=14, p = 0.001, Kruskal-Wallis test).

(C) Total no. of crossovers compared between cage lengths (n = 12, p = 0.018)

Error bars represent standard error



Female	0 to 3 min	3 to 6 min	6 to 9 min	9 min onward
Red 13 Yellow 13	13	3.333333	3.333333	11
Red 12 Yellow 34	4.333333	1.222222	1.555556	2.222222
Red 10 Yellow 22	7.333333	1.333333	2	5.333333
Pink 94 Pink 95	1.888889	1.666667	2.666667	1.222222
Orange 83 Red 93	8.222222	1.111111	3.444444	1.777778
Orange 93 Red 92	8.222222	1.111111	3.444444	1.777778
Blue 46 Blue 51	3.777778	0.555556	0.555556	0.333333
Red 94 Orange 94	13.88889	7.888889	7.777778	3.555556
Red 72 Yellow 44	1.555556	0.666667	0.444444	0.111111
Red 40 Brown 67	13.44444	7	6.111111	1.111111
Blue 45 Brown 25	3.777778	0.555556	0.555556	0.333333
Orange 75 Brown 58	6.740741	2.024691	2.753086	3.024691
Blue 3 Brown 18	3.555556	1.222222	0.555556	0.222222
Yellow 51 Pink 31	8.777778	3.444444	2.111111	2.111111

TABLE 3 (below). Mean no. of crossovers for females with time for short cage.

III. B) Females gave lesser number of CSDs when the distance between birds was reduced.

Short cage experiments, like long caged ones showed big difference in the relative occurrences of different types of CSDs. Also, there wasn't any change in number of CSDs given with repetitions (n = 38, p = 0.734, Kruskal Wallis test).

Tail-quivering responses were given to select males. Out of the 6 males maintained in common throughout the course of the project, only 1 male received tail-quivering responses from multiple(9/14) females).

Females were observed to be giving to lesser number of CSDs in total (gross and mean both) when the cage size was reduced to a smaller cage (n=6, $p < 0.041$, Kruskal-Wallis test) (FIG 21). We have chosen to present only the mean here.

Decrease in the number of CSDs with reduced distance between birds shows a possible delay in preference making.

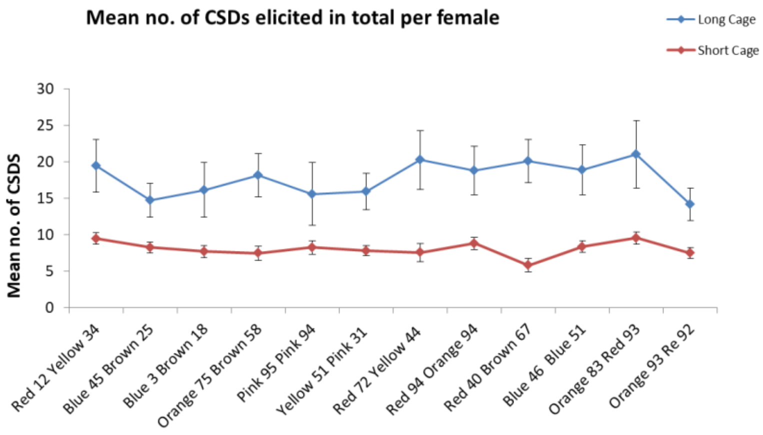


FIG 21. Number of CSDS given as a response towards male - Short Cage
Comparison between long and short cage data for mean number of CSDs across all experiments.
Each point stands for the mean no. of CSDs by the bird.
Error bars represent standard error.

IV. A) *Mean song duration and song rate sung by males were positively correlated with the time spent by the female next to him for reduced distance.*

When we had analysed correlation between male song traits of sing duration and frequency for long cage experiments, we hadn't found any correlation to amount of time spent by the female next to the male.

But, there was a positive correlation found between mean singing duration and time spent by the female for each male for short cage experiments (n = 6, r_S=0.943, spearman's correlation rank test) (FIG 22)

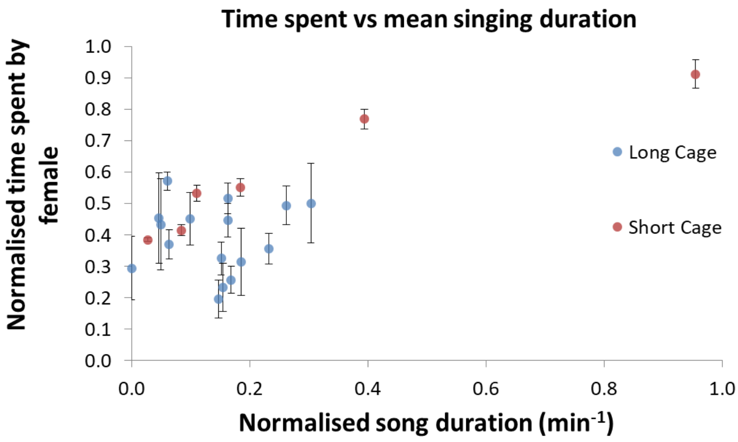


FIG 22. Time spent by female next to male vs the amount of singing - Short Cage
Mean female time spent and mean song duration plotted as ratio indices for each male.
(n= 6, r_s = 0.921, Spearman's rank correlation test.) Each point represents a male
Error bars represent standard error

Similar to singing duration, we plotted mean time spent by females next to each male against the mean frequency of song bouts. In the case of short cage experiments, which wasn't found during long cage experiments (n = 6, r_S=0.943, spearman's correlation rank test)(FIG 23.).

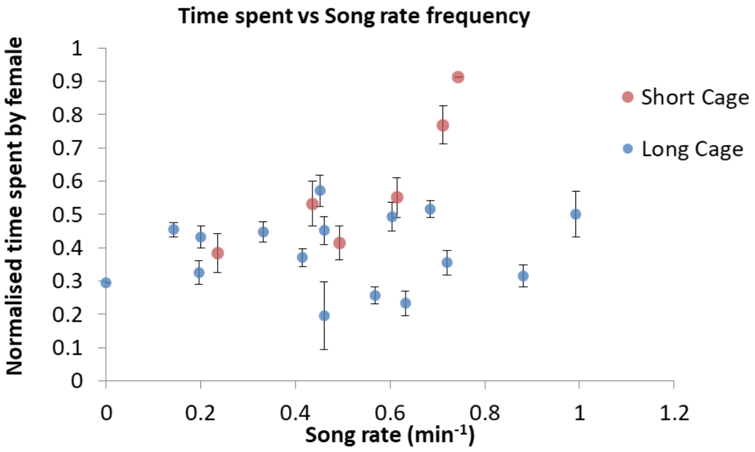


FIG 23 .Mean female time spent and mean song bout frequency plotted as ratio indices for each male - Short Cage
(Short cage, n =6, r_S = 0.943). Each point represents a male

Both the male traits of song duration and bout frequency show correlation with female time spent when the distance between participating birds is reduced, which could indicate that either the female is assessing the songs more carefully for making a preference.

SECTION C – Introduction of a peer female

Pair experiments were carried out in cages of two sizes, i.e. long and short cage, with two females chosen from the same colony. The following section discusses the results pertaining to the effects of introducing of a peer female on female activity, preference strengths and inter-female cooperation. Male song traits weren't analysed for this section due to inconspicuous target females. Results were compared to their individual experiment counter parts according to cage size. In all experiments, the female bird that appeared more active/dominant was considered as female 2 and the submissive birds as female 1 for convenience of analysis. 12 female birds were used for peer experiments, providing a sample set of 6 pairs. We used 6 males (3 pairs).

Definition: Dart – A rapid movement made by a female towards the peer female causing the latter to move from her location and be replaced.

The following results show data from both short and long cage pair experiments that demonstrate significant change between experiments with an individual female and those with a peer female present.

I. A) There was aggressive behaviour seen among peer female in the presence of males. One of the females made more darts than the other.

We found that in some experiments, when present in a pair, the females made darts towards each other in the company of males but showed no such behaviour without males (control experiments had 0 darts being made by both females). For both long ($n = 6$) and short cage ($n = 7$) cases, one of the females (female 2) was observed making significantly more number of darts the other (female 1), in the between the time marks of 3 min to 9 min of the interaction ($p = 0.015$,$p = 0.029$, Kruskal-Wallis test)(FIG 24.A,B). The total number of darts made by females in each segment of time was as stated in TABLE 4. There was no significant change found between the no. of darts made by either female between with change in cage size. ($p = 0.094$, Kruskal-Wallis test).

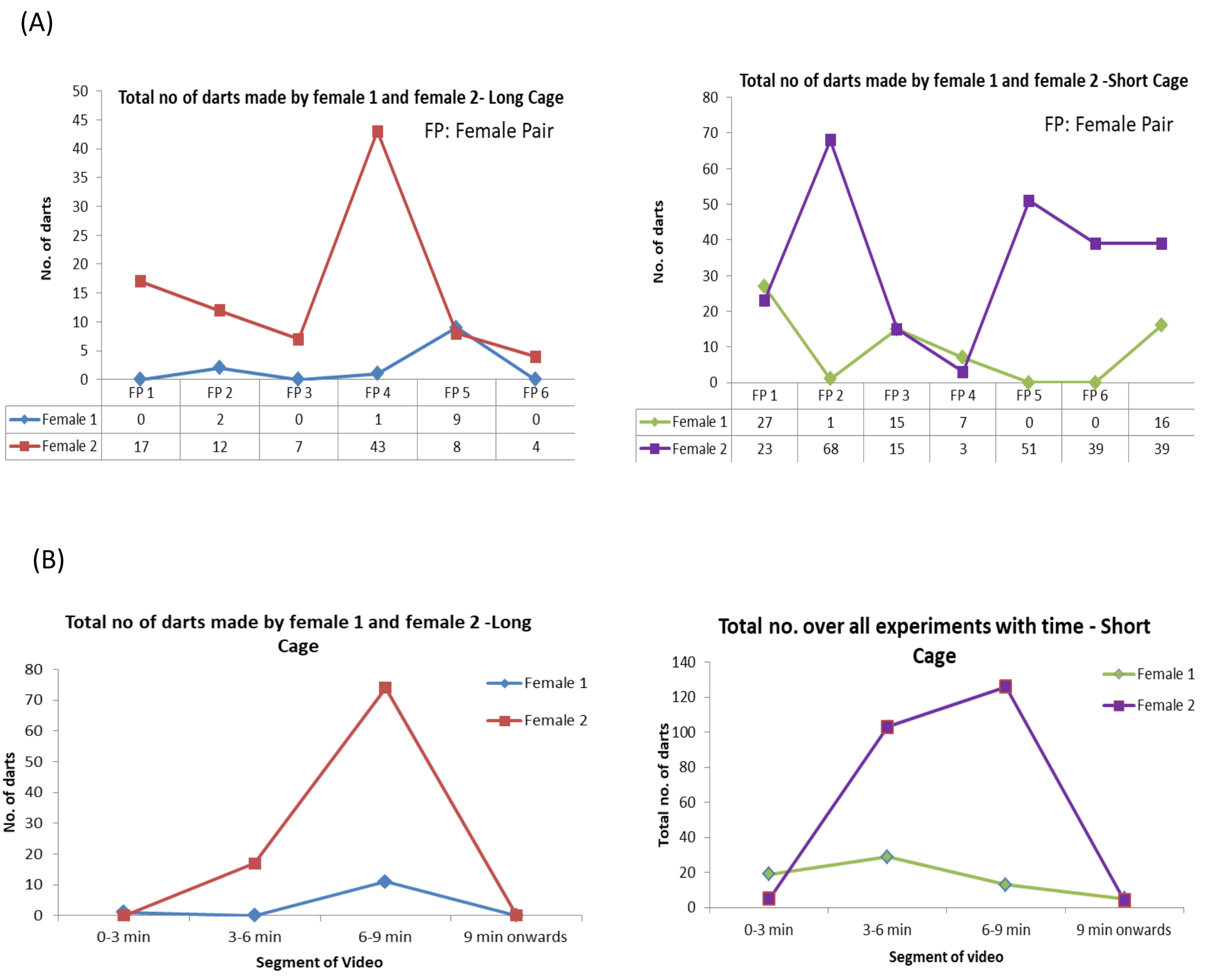


FIG 24. Number of darts made by females through the duration of an experiment – Peer exps

(A) Total number of darts made by each female pair plotted against time. (Long cage, n = 6; Short cage, n = 7)

(B) Total no. of darts plotted against time for all experiments. (Long cage, n = 54; Short cage, n = 63)

Each data point represents the mean no. of darts made by the corresponding female

Peer long cage

Female Pair	Female 1	0-3 min	3-6 min	6-9 min	9 min onward	Total	Female 2	0-3 min	3-6 min	6-9 min	9 min onward	Total
1	Red 12 Yellow 34	0	0	0	0	0	Blue 45 Brown 25	0	7	10	0	17
2	Blue 3 Brown 18	0	0	2	0	2	Orange 58 Brown 75	0	5	7	0	12
3	Orange 83 Red 93	0	0	0	0	0	Orange 93 Red 92	0	0	7	0	7
4	Red 72 Yellow 44	1	0	0	0	1	Blue 46 Blue 51	0	5	38	0	43
5	Red 94 Orange 94	0	0	9	0	9	Red 94 Orange 94	0	0	8	0	8
6	Yellow 51 Pink 31	0	0	0	0	0	Pink 95 Pink 94	0	0	4	0	4

Peer short

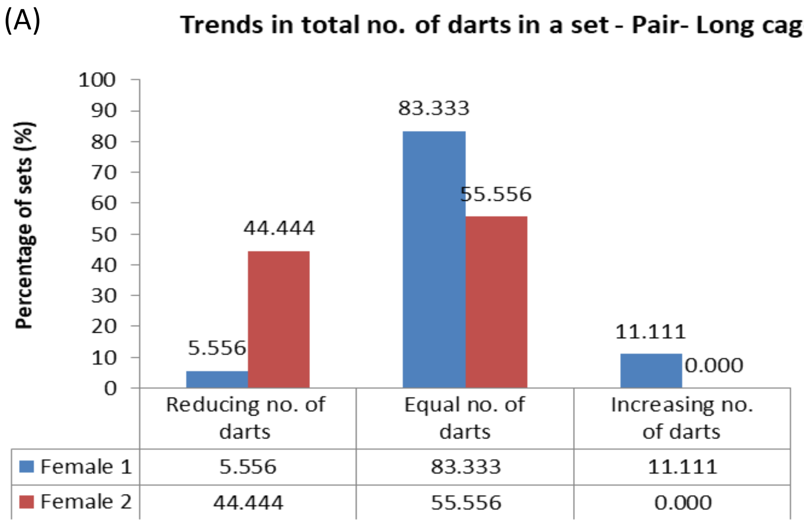
Female Pair	Female 1	0-3 min	3-6 min	6-9 min	9 min onwards	Female 2	0-3 min	3-6 min	6-9 min	9 min onwards
1	Red 13 Yellow 13	2	16	8	1	Pink 95 Pink 94	0	16	7	0
2	Red 12 Yellow 34	0	1	0	0	Blue 45 Brown 25	0	25	43	0
3	Blue 3 Brown 18	7	5	1	2	Orange 58 Brown 75	3	8	4	0
4	Orange 83 Red 93	1	5	1	0	Orange 93 Red 92	0	3	0	0
5	Red 72 Yellow 44	0	0	0	0	Red 72 Yellow 44	0	20	27	4
6	Red 94 Orange 94	0	0	0	0	Red 94 Orange 94	0	9	30	0
7	Yellow 51 Pink 31	9	2	3	2	Pink 95 Pink 94	2	22	15	0

TABLE 4. Total no. of darts made in every time interval by female 1 and female 2 in every female pair for long and short cages

The darts made by the females could stand for non-cooperative behaviour that arises due to the presence of the male (we could rule out space constraint as an issue since the no. of darts doesn’t change significantly with cage size).

I. B) Females reduced their aggressive behaviour with repetitions.

There was a significant drop observed in the number of darts being made by female 2 in consecutive trials in a set for long cage experiments (n = 18, p = 0.014, Kruskal-Wallis test) as well as short cage experiments (n = 21, p<0.006, Kruskal-Wallis test)(FIG 25 A,B). There was no such change in darts for female 1 in either sized cages. The drop suggests that the females get habituated with each other with repetitions/extended exposure to each other.



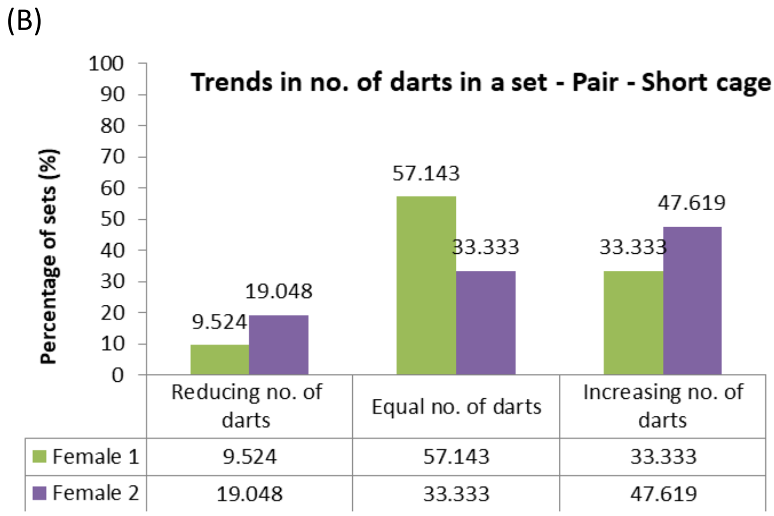


FIG 25. Percentage of sets following certain trends of change in the no. of darts – Peer exps
(A) Different percentage of sets showing change in no. of darts made, in long cage experiments.
(B) Different percentage of sets showing change in the no. of darts made, in short cage experiments.

II. B) The presence of a peer did not have any influence on the individual choices made by the female in terms of the time spent by the female next to the chosen male.

Given that we found dominance being asserted by one female upon the other, we wanted to verify if one female had any effect on the other, regarding individual preferences established in earlier (individual) experiments. We compared the number of times a female maintained her choice of male with the number of times she didn't, between individual and pair experiments. To compare this, time spent by female was chosen as the only parameter since CSDs have been shown to be highly female subjective and variable signals. It was found that there is no significant difference between the percentages of choices maintained and not maintained (FIG 26) (long cage, n = 54, p = 1; short cage, n = 54, p = 1, Kruskal-Wallis test).

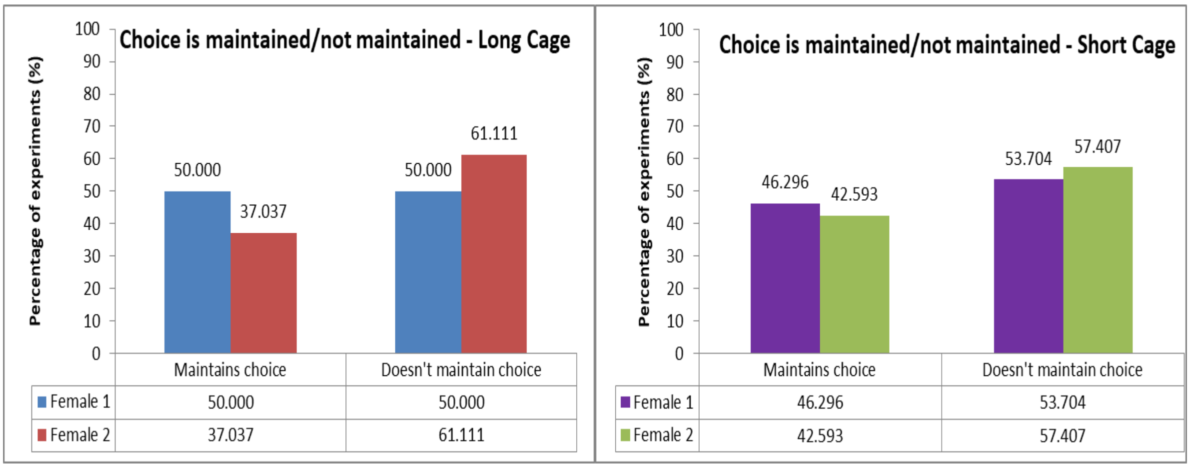


FIG 26. Percentages of sets plotted for the percentage of experiments where the male of choice was maintained – Peer exps
(Long cage, n= 54, p=1; Short cage, n=54, p=1)

Further on, we investigated the different possible scenarios of inter-peer interaction based on whether choices of females had changed from individual choices. There were no visible trends seen which could be characterized as effects of the dominant female's (female 2) presence on the choices made by the submissive female (female 2); this result hold good for long and short cages.

Thus, the females are equally likely to change or maintain their preferred male (according to time spent) in the presence of a peer.

Table 5 summarises the values observed for long cage and short cage pair experiments.

LONG CAGE	Female 1 maintains choice	Female 1 changes choice
Female 2 maintains choice	16.667%	29.630%
Female 2 changes choice	25.926%	27.778%

SHORT CAGE	Female 1 maintains choice	Female 1 changes choice
Female 2 maintains choice	16.667%	25.926%
Female 2 changes choice	29.630%	27.778%

TABLE 5. Table shows percentages of experiments depending upon whether or not female 1 changed her choice due to the presence of female 2 and vice versa
Female 1: Submissive; Female 2: Dominant
(Long cage, n = 54 ; Short cage, n = 54)

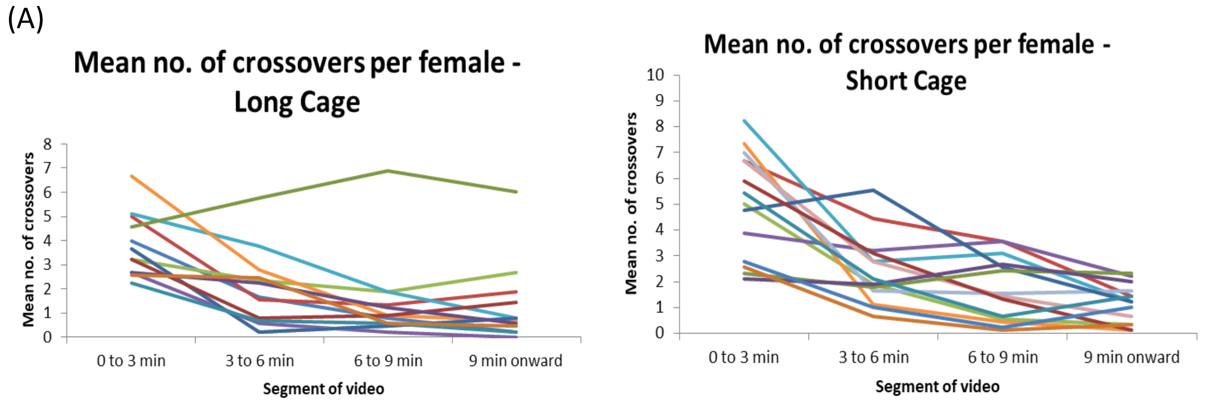
III. *A) Female activity increased in the presence of a peer.*

Most females showed a decrease in the number of crossovers with time during an experiment, which was in accordance with previous results, for cages of both sizes (Long cage: 11 out of 12; Short cage: 10 out of 12). The mean number of crossovers with time for females has been plotted against time in FIG 27.A. Female 1 and female 2 did not differ significantly in the number of crossovers made (p = 0.720, 0.842, Kruskal-Wallis test). But there was a significant increase in the total number of crossovers between compartments made by females in short cage experiments in the presence of a peer female (n =6, p = 0.019, Kruskal-Wallis test)(FIG 27 .B). Long cage experiment did not show any such significant increase (n = 6, p 0.525, Kruskal-Wallis test).

The number of crossover made by both females with time was counted as shown in TABLE 6.

Thus, presence of a peer seems to have an effect on female activity and slows down the preference establishment by making more number of sampling visits.

It could be that female 1 shows the increase as a response to a dart made towards her.



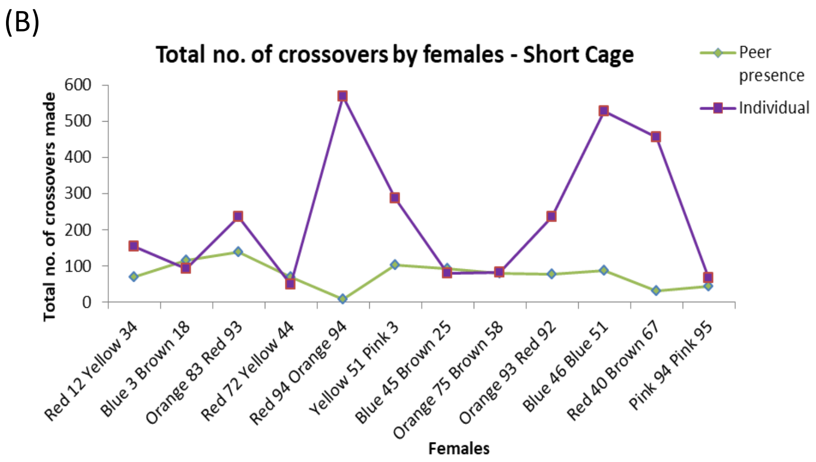


FIG 27. Mean no. of crossovers across all experiments made by females – Peer exps

(A) Mean number of crossovers for each female plotted against time (Long cage, n = 12; Short cage, n = 14).
Each colour corresponds to a different female and each point is the no. of crossovers made

(B) Comparison between number of crossovers made between individual and pair experiments (n = 12). Colours used to differentiate the presence/absence of peer.

Peer Long Cage

Female Pair	Female 1	0 to 3 min	3 to 6 min	6 to 9 min	9 min onward	Female 2	0 to 3 min	3 to 6 min	6 to 9 min	9 min onward
1	Red 12 Yellow 34	4.000	1.667	0.778	0.222	Blue 45 Brown 25	3.667	0.222	0.444	0.778
2	Blue 3 Brown 18	5.000	1.556	1.333	1.889	Orange 75 Brown 58	3.222	0.778	0.889	1.444
3	Orange 83 Red 93	3.222	2.333	1.889	2.667	Orange 93 Red 92	4.556	5.778	6.889	6.000
4	Red 72 Yellow 44	2.667	0.556	0.222	0.000	Blue 46 Blue 51	2.667	2.222	1.222	0.556
5	Red 94 Orange 94	5.111	3.778	1.889	0.778	Red 40 Brown 67	2.222	0.667	0.556	0.222
6	Yellow 51 Pink 31	6.667	2.778	0.889	0.667	Pink 95 Pink 94	2.556	2.444	0.556	0.444

Peer Short Cage

Female Pair	Female 1	0 to 3 min	3 to 6 min	6 to 9 min	9 min onward	Female 2	0 to 3 min	3 to 6 min	6 to 9 min	9 min onward
1	Red 13 Yellow 13	6.667	4.444	3.556	1.444	Pink 95 Pink 94	4.778	5.556	2.556	1.222
2	Red 12 Yellow 34	5.000	1.889	0.556	0.333	Blue 45 Brown 25	5.889	3.111	1.333	0.111
3	Blue 3 Brown 18	3.889	3.222	3.556	2.222	Orange 75 Brown 58	2.333	1.778	2.444	2.333
4	Orange 83 Red 93	8.222	2.778	3.111	1.222	Orange 93 Red 92	2.111	1.889	2.667	2.000
5	Red 72 Yellow 44	7.333	1.111	0.444	0.111	Blue 46 Blue 51	5.444	2.111	0.667	1.444
6	Red 94 Orange 94	7.000	1.667	1.556	1.667	Red 40 Brown 67	2.556	0.667	0.111	0.333
7	Yellow 51 Pink 31	6.667	2.778	1.444	0.667	Pink 95 Pink 94	2.778	1.000	0.222	1.000

TABLE 6. Mean number of crossovers per female (for both females) with time for long and short cage

IV. *A) The total number of CSDs given by dominant female was higher in the presence of a peer than when alone.*

Similar to the individual experiments, we scored relative occurrences of CSDs given by both females in paired experiments to each male. The CSDs were yet again found to differ significantly based on the type given for both sizes of cages (Long cage, n=54, p = 0.704; Short Cage, p = 0.524, n=63)(FIG 28.A). The number of tail quivering CSDs had the least number of occurrences.

However, the total number of CSDs given by female 2 was significantly higher than female 1 for each female pair in short cage experiments but not in the long cage (Long cage, n=6, p= 0.092; Short Cage, n=7, p = 0.002, Kruskal-Wallis test)(FIG 28.B).

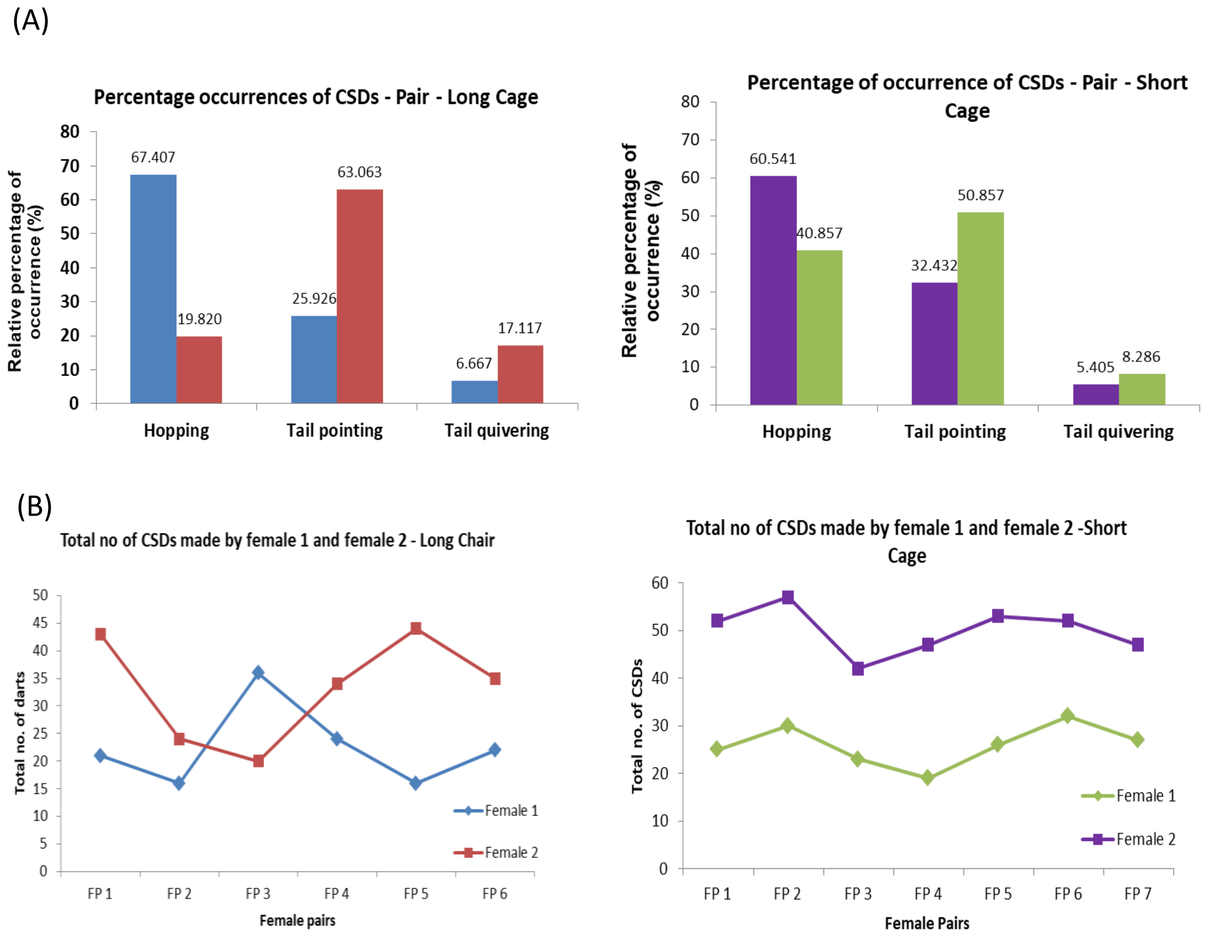


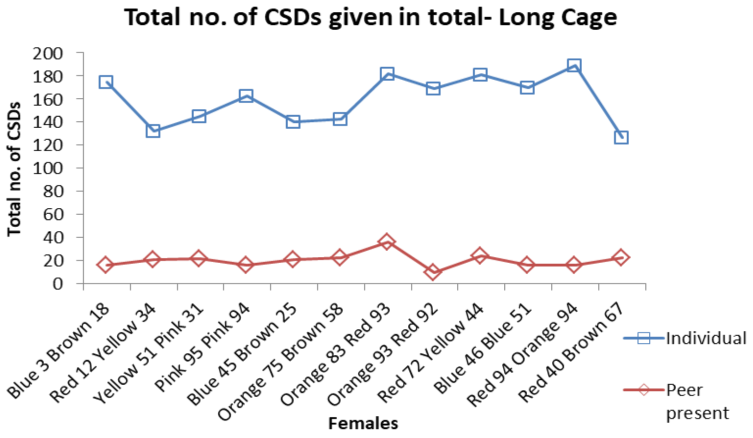
FIG 28. CSDs given by females in pair experiments

(A) Bar graphs showing relative number of CSDs given by both females in long and short cages respectively.

(B) Total no. of CSDs given by female 1 compared with female 2. In long and short cage experiments respectively. Each red-blue data point pair represents a female pair (Long cage, n = 6, p = 0.092; Short cage, n= 7, p = 0.002, Kruskal-Wallis test)

IV. B) Females displayed lower number of CSDs when with a peer than when alone.

We wanted to verify if the number of CSDs differ for peer experiments, since the number of crossovers did show a change. We compared the total number of CSDs shown by females across all experiments in individual and pair experiments and found that the number of CSDs given in the presence of a peer is significantly lower than when alone, irrespective of the cage size (n =12, p = 0.001, Friedman's paired test) (FIG 28). Thus presence of a peer could increase female activity levels and point and perhaps delay the preference decision. Also, the discrepancy between the number of CSDs by female 2 and female 1 shown in the previous subsection (Fig 27 B) could be a result of the dominant-submissive relation between them. The dominant female might have ended up giving more CSDs. As a reiteration of this point, both females were still observed to choose a male in two out of three trials in long (n = 18) as well as short cages (n= 32)



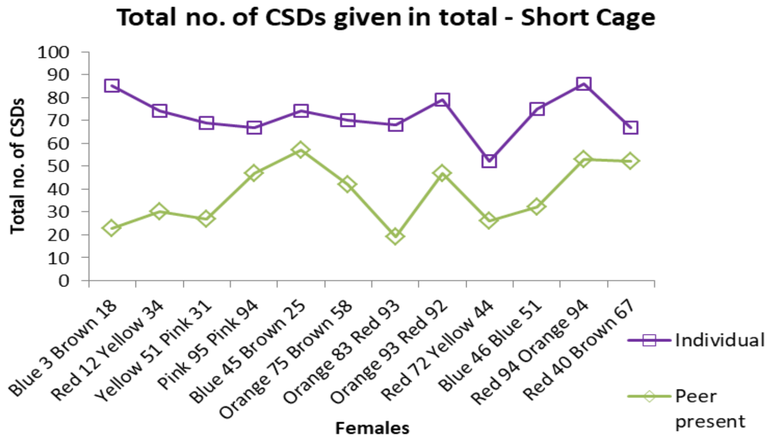


FIG 29. Total no. of CSDs given compared between individual and pair experiments for long and short cage experiments respectively ($p<0.05$).
Each point represents the total number of CSDs shown by a female.

SUMMARY

We conducted preference assays using a choice chamber wherein a female was allowed visual and auditory access, but not physical contact. Also, two variable effects were applied during preference assays, i.e. cage length and presence of a peer. We analysed the female-male interactions in order to understand the process of preference establishment. We found that, in majority of cases females chose to spend most of their time next to one male rather than being undecided between two males or be disinterested (spend time in the centre compartment). This behaviour was seen in experiments conducted with both long and short choice chambers and even when a peer female was introduced. The difference between the time spent next to each male or the preference strength (|L-R|) showed an increase with repeated experiments (in a set) only in single-female experiments with reduced distance between participants.

The number of copulation solicitation displays (CSDs) given by a female to a male were specific to her and differed in number with the type of CSD, with tail quivering responses being the rarest. The total number of CSDs given by all females to males decreased in number when the distance between them was reduced and in the presence of a peer.

Along with CSDs, the number of crossovers made by a female between compartments can also represent levels of female activity and rate of sampling males. We found that the number of crossovers made by a female to the other reduced with time for all categories of experiment, though not significant for all females, implying that she eventually settle down next to a certain male. Also, we found that females made significantly more number of crossovers in experiments with smaller distance between participants, whether alone or with a peer. Thus reducing the distance between participating birds causes an increase in the female's activity.

Thus, the parameters that can seemingly showcase a female’s preference towards a male can either be the amount of time that she spends next to a male, the number of CSDs she gives to a male or if she chooses to settle down next to him. But, we found that females did not always give all three signals of preference to the same male. Total agreement between the signals was found only in 30% of the experiments, regardless of cage size and peer presence. There seems to be a high chance of the female settling down next to the same male she spent most time next to, demonstrating higher agreement between these two signals when compared to CSDs. Thus they could be a more robust and reliable parameter to judge female preference.

To explore to the role played by male song traits on female mate choice, we analysed our video recordings for song duration and frequency of song bouts produced for a male. The time spent by the female next to a male was positively correlated with both these song traits only in the case of only individual-female short cage experiments, when averaged for a male. A general observation across all categories of experiment was that males started their song only when the female was in close proximity causing the female’s arrival to initiate most of the interactions between the two. We did not perform this analysis for peer experiments since there was ambiguity regarding which female the song was being directed to.

In terms of inter-peer interactions during pair experiments, we found only two aspects in which the two females differed. In all cases, one of the females (named female 2) was more active/dominant than the other and made aggressive darts towards her peer, which reduced with repetitions. Female 2 also showed lesser number of CSDs as compared to her peer. We compared preference stability during repetitions of an experiment and in the maintenance of a preferred male, (in terms of time spent) upon comparing individual and pair experiments, but found changes in neither of them. This demonstrated that there was no influence of the dominant female on the choice of the submissive female or vice versa.

DISCUSSION

Through our preference assays conducted in choice chamber experiments, we have shown that there are multiple factors that could affect the process of mate-choice decision making in female zebra finches that could arise either from individual-specific properties or context-dependent properties.

In general, we could point to the three parameters that were useful in indicating a female's preference. The time spent by a female next to a male was the most robust parameter that most females showed a trend in and probably would be suitable in assessing preferences over groups of females. In comparison, CSDs were highly female-specific and could be indicators of individual consistency while making a preference. It could be that each signal was given in response to separate male traits. The difference between preference for visual and acoustic traits of males could be responsible for this discrepancy between female signals, possibly increasing variation in the type of traits that have been reported to be ubiquitously preferred (Collins et al., 1994). Hence, probing this discrepancy by isolating male visual cues from acoustic could be a potential trope for investigation.

In most cases, when tested with two males, females almost always developed a preference for one of the males by deciding to stay next to him rather than distribute her time equally between the two males or be disinterested and stay in the centre. However, the number of cases where the female chose a particular male twice out of three repeated trials was higher than where she maintained her preference for all three. This might point towards females not being consistent in choosing between a pair of males and resonate the conclusions made by earlier work on repeatability of choice (Forstmeier and Birkhead, 2004). However, we did not consider the order of preference in repetitions in our analysis. Thus, it could be that the choice made by the female was the same in the last two repetitions that the first, in which case we could assume that a single instance of interaction wasn't enough for females to make a definite choice in the first trial. Also, the strength of this preference in terms of time allocation by females to each male (|L-R|) did not change with repetitions, possibly indicating no increase in familiarity or response to a male. We expected the preference for an individual to get stronger with exposure, which we failed to observe. Alternatively, this low consistency could have arisen because of insufficient exposure to the novel male.

The second indicator of preference, copulation solicitation displays (CSDs) were found to be highly individual specific. The number of CSDs given varied with the type of CSD with tail quivering responses being the lowest in number which given by females to a male. We speculate that the different type of CSDs could be related to the intensity of preference and thus could be given to both males during an incomplete mate-choice decision. Another general trend that we found to be common across all experiments, irrespective of the category, is the precedence of female arrival over initiation of male song. We found that mostly, males sing only when the female is in close proximity to them, in turn causing more of the interactions to be female-initiated or start with the female's arrival in the male's vicinity, in accordance to the work done by Brumm and Slater (Brumm and Slater, 2006). Thus the dynamics and male display during mate-choice could be driven by the female activity. This could be to ensure specific targeting of the female as a receiver. However, 2 out of the 16 males used showed abnormally high singing rates even with the female on the opposite side and stood out as exceptions.

These results suggest that the process of courtship dynamics is most likely to be affected by the activity levels of the participants. Observations from our study suggest that along with the number CSDs given by a female, the number of crossovers made by her between compartments could be pointers of female activity. We found the number of crossovers to reduce with time as interaction progressed, though the drop was not significant for all females. Multiple crossovers being made in the presence of males could imply that the female might be sampling courtship cues given by both males both before developing any kind of preference. The reducing trend coincided with a drop in male song frequency with time, strengthening the mentioned conclusion. Some females showed more crossovers in total which could be due to individual differences in activeness.

When comparing the results obtained from long cage and short cage experiments, we observed that the total number of crossovers made by the female increases when the distance between participants is reduced. Additionally, the number of CSDs produced by females in the short cage was lesser in number. A possible reason for this change with decreased distance could be that the females take longer to establish preference when the distance between participants is decreased, possibly due to a change in the acoustic traits of the song. This hypothesis is an extension of results from previous work that show change in song properties with distance (Brumm and Slater, 2006; Suri and Rajan, 2018). As an affirmation for this deduction, we found male song duration and song bout frequency to be positively correlated with time spent by the female next to a particular male, though the frequency of male song bouts was not significantly different in long and short cage experiments. Although the number of crossovers made by the female increase in this case, we also found an increase in |L-R| with repeated trials which suggests females are likely to strengthen their preference with repeated exposure to males at a shorter distance, which could again be linked to possible modification of song traits. Collectively, we could conclude that the females might pay more attention to male traits (especially song) with reduced distance between participants, thus delaying her decision. Further analysis of acoustic properties of song with respect to preference dynamics could help validate this conclusion

Lastly, we examined the influence of the presence of a peer female on female activity and maintenance of preference, taking cage length into account as well. We showed that in almost all cases, one of the females was more active than the other and displayed uncooperative behaviour in the form of aggressive darts towards the other female. Thus it is plausible that presence of a male reveals the social hierarchy between the females, if any. Also, the number of darts made did not change significantly when the cage size was varied, ruling out space constraint as a causal factor for aggressive behaviour. However, we observed a drop in the number of darts with repetitions of the experiment, suggesting that the mutual cooperation can develop between the birds with repeated exposure. We observed an increase in the number of crossovers and decrease in the number of CSDs made by the dominant female while in a pair, with most of things effects being prominent for a short cage experiments. This result was similar to the effect that shorter distance between participants had on female activity. We speculate that introducing a peer causes an increase in the female activity of the dominant female but not the submissive female and when combined with cage length, these changes become highly significant.

Finally, we examined the influence of peers on each other's final preferences (according to time allocation) by comparing their preferences in peer experiments vs when alone and found that females no not significantly bias each other's previous choice. Unlike the instances of mate-choice copying (Kniel et al., 2015), females in the physical company of a peer might or might not choose to maintain her preference for a male. A possible explanation for this might be that submissive females would change their previous choice if their dominant peer shares their preference for a male. This observation hints at the possible role of intra-sexual competition in preference establishment. However, our sample size contained females without any prior aggressive attitude towards each other. Perhaps a stronger hierarchy between conspecifics could be effective in driving the submissive female to change her choice.

In conclusion, we surmise that reduced distance between the participants or the company of a peer causes females to be more attentive to stimuli, thus slowing down the decision making process. Furthermore, preferences could evolve over time and need not strengthen with repetitions if enough exposure to courtship stimuli is not provided. Along with shared behavioural trends, courtship dynamics on the whole is shaped by the cumulative effects of individual-specificity and social-context. Probing into individual variability can help better understand perception of signals and their role in moulding interactions in animal systems.

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