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„Quivering Response of Female Ring Doves (*Streptopelia risoria*) to Unimodal and Multimodal Courtship Playback"

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Abstract

In many bird species, courtship features a combination of multimodal auditory and visual signal components. Whether separate unimodal signal components carry redundant or non-redundant information, and whether a combination of those signal components leads to a stronger response in the receiver, remain however elusive. We investigated the effect of unimodal and multimodal courtship signals on the behavioural response of the receiver by exposing 21 female ring doves (*Streptopelia risoria*) to male courtship recordings for a period of seven days. Females were split into three playback conditions and exposed either to multimodal audio-visual, or unimodal audio or video playback of male courtship clips in which the respective other sensory channel was occluded using familiar stimuli. Manual scoring was used to quantify the effect of courtship playback on three sexual behaviours of interest (tail quivering, wing quivering and wing flipping). We additionally aimed to quantify quivering behaviours by training an automatic tracking algorithm to automatically recognize the position of body parts of the female doves, but the quivering response was too subtle for automated analysis. We found that, while the duration of wing flipping was significantly higher in playback intervals than during breaks, the immediate presence of male courtship was not necessary to induce a quivering response in the females. Moreover, playback condition had no measurable effect on the number nor duration of quivers over experimental days. Overall, the behavioural response strongly depended on female identity, and future hormonal analysis could help to uncover the reasons for this inter-individual variability between females.

1. Introduction

A multimodal (or multisensory) signal is composed of different elements that are transmitted via different sensory modalities (Guilford and Dawkins, 1991; Partan, 1999). Across the animal kingdom, multimodal communication is widespread, especially in the context of courtship. Examples range from jumping spiders (*Habronattus dossensus*) performing precisely coordinated visual and seismic courtship displays (Elias, 2003), over iguanas (*Lilolaemus pacha*) combining headbob and tongue-flip displays with chemical pheromone cues (Vicente and Halloy, 2017), to all kinds of male birds showing off their beautiful plumage during singing and dancing (Dalziell et al., 2013; Ullrich et al., 2016). The prevalence of complex multimodal signals in nature raises questions about their evolutionary advantage over unimodal signals, as their production and detection might be more energetically expensive and potentially increases the predation risk for both the sender and receiver (Partan and Marler, 2005).

Multiple explanations on the evolution and function of multimodal signals exist (Guilford and Dawkins, 1991; Mitoyen et al., 2019), but most are centred around two non-mutually exclusive hypotheses based on redundancy or non-redundancy of single signal components. According to the “Multiple message hypothesis”, each component of the multimodal signal carries unique information and can thus trigger differential behavioural responses by the receiver (Moller and Pomiankowski, 1993; Johnstone, 1996). For instance, different components of a multimodal signal can be directed to different receivers. In territorial female Red-winged Blackbirds (*Agelaius phoeniceus*), the same type of visual display is used both in an aggressive context and in a courtship context, but distinct types of song are produced in each context (Beletsky, 1983). Alternatively, different components of a courtship signal might indicate different aspects of male quality. In male satin bowerbirds (*Ptilonorhynchus violaceus*), plumage coloration and bower quality predict different aspects of male quality related to body size and general health (Doucet and Montgomerie, 2003).

The “Back-up signal hypothesis” on the other hand states that the components of multimodal signals carry redundant information, acting as a back-up to ensure a more efficient information-transfer to the receiver (Moller and Pomiankowski, 1993; Johnstone, 1996). In environments with high temporal and/or spatial variability, multimodal signalling can improve signal transmission, as well as signal detection and discrimination by the receiver. For example, in Anurans, vocal sac inflation during courtship calling is

hypothesized to have evolved to improve female detection of male signals in noisy environments (Starnberger et al., 2014).

During sensory integration of the signal by the receiver, multiple components of a signal may interact in very complex ways depending on if redundancy ("Back-up signal hypothesis") or non-redundancy ("Multiple message hypothesis") of the components is assumed. According to Moller and Pomiankowski (1993) and Johnstone (1996), exposure to redundant signal components should elicit equivalent or enhanced behavioural responses by the receiver, while exposure to non-redundant components should trigger different behavioural reactions in the receiver. Combining multiple non-redundant components can either result in each component exerting a unique effect (independence), one component dominating over (domination) or changing (modulation) the effect of the other component, or the creation of an entirely new effect (emergence) (Partan and Marler, 1999).

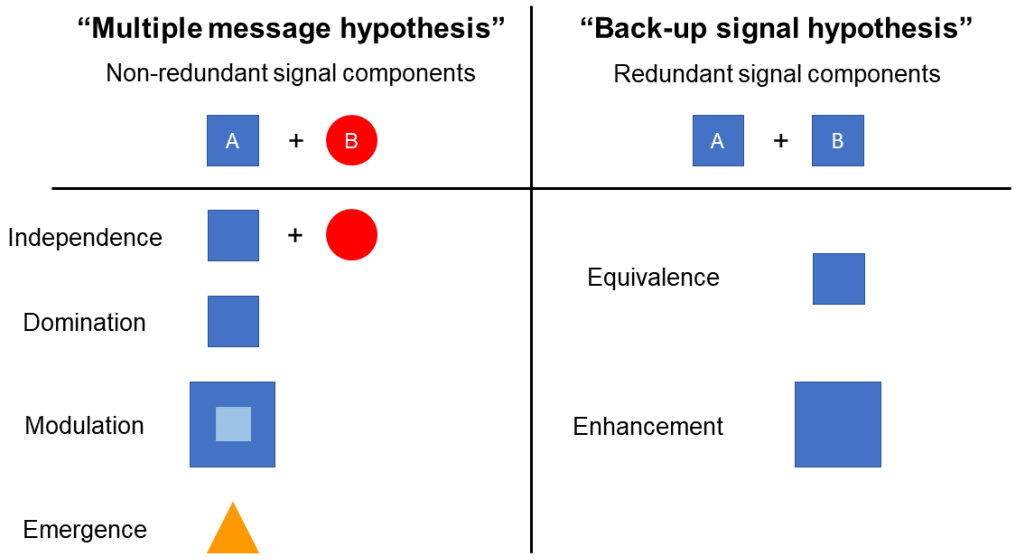

Figure 1: Potential responses to two separate non-redundant ("Multiple message hypothesis") or redundant ("Back-up hypothesis") components of a signal. The geometric shapes below the horizontal line represent the response to the signal components when A and B are presented together. The same geometric shape indicates an equivalent response, and a different shape indicates a differential response. The size of the shape indicates how strong the effect of the response is. Adapted from Partan and Marler (1999).

Traditionally, multimodal courtship signals have been investigated in the laboratory using "cue isolation experiments". During these types of experiments, stimuli are presented either separately (e.g. auditory only or visual only) or combined with each other (e.g. audio-visual) and female preference (Uetz and Roberts, 2002) and/or physiological

responses (Crews, 1975) are assessed. This approach is however often insufficient to draw reliable conclusions about proximate and ultimate mechanisms that are involved in shaping these complex signals.

Mitoyen et al. (2019) argue that isolating certain cues from other stimuli that accompany them in a natural situation might be responsible for a decreased response by the receiver. For example, presenting a female ring dove with video playback of a male dove performing a bow-coo display, while blocking the auditory channel without any ecologically valid reason, might create an unnatural stimulus. To my knowledge, only very few studies offer an ecologically valid presentation of signals in the unimodal condition. One notable example for the importance of this is a study by Lambe and Erickson (1973), in which female ring doves were exposed to either auditory-only stimulation or auditory stimulation and the shadow of a courting male. Firstly, this study allowed to disentangle female response due to preference of specific morphological features of the male from the response to the mere presence of a male. Secondly and more importantly, it was found that none of the females responded to the audio-only stimulus, while half of the females exposed to the shadow laid eggs, indicating that even a marginal visual cue is sufficient to create a more natural courtship situation.

Furthermore, cue isolation studies often disregard the interaction between different signal components. Despite numerous examples of a simple enhanced (or additive) response resulting from the combination of two or more redundant signal components (Rybak et al., 2002), multisensory integration can also result in the emergence of a new percept that is different from the sum of its parts (Halfwerk et al., 2019). In túngara frogs, two types of male calls, whines and chucks, are normally only perceived as attractive by the females if they are produced in a precise temporal pattern, one relative to the other. If the visual cue of vocal sac inflation in between a whine-chuck call is added, the males will be perceived as more attractive even if the inter-call duration is modified (Taylor and Ryan, 2013). This finding suggests that empirical research should not only focus on the effect of isolated signal components, but also how relative variation of the temporal/spatial properties of signal components impacts how they interact with each other during integration by the receiver.

Nowadays, playback studies are used as the main tool to investigate multimodal signalling in animal communication. Playback studies enable the presentation of acoustic, visual, vibrational, or electric stimuli to the animals while quantitatively manipulating certain components of the signal and assessing how the receivers respond. The main advantage

of playback studies is that the receivers are exposed to the same standardized playback stimuli under controlled conditions, making the response of the receivers more comparable and reliable (Rosenthal, 2019). One major drawback of playback experiments is however, that the video display equipment that is used is specifically designed for the human visual system and might not be suited for other species. Most non-human species have visual systems with very different photoreceptor absorption functions and can perceive wavelengths outside the human range of perception (e.g. ultraviolet wavelengths), so colours and consequently the conspecifics in a playback video might be perceived differently (D'Eath et al., 2002). In animals with high temporal resolution visual systems (e.g. diurnal birds), one additional problem may arise if the screen refresh rate (frequency with which frames are renewed on the screen) of the monitor is lower than the critical flicker-fusion threshold (CFF) specific for that species. The refresh-rate of the monitor should be above the CFF, so that the footage is perceived as continuous by the animals (Oliveira et al., 2000). A playback study using pigeons (*Columba livia*) revealed that the image presentation rate (IPR; rate at which the images are sampled and displayed on the screen) had a significant effect on the behavioural response of the observer. Male and female pigeons presented with videos of the opposite sex showed the highest courtship frequency at an IPR of 60 frames per second, while the response declined with decreasing IPR's (Ware et al., 2015).

Despite these limitations, video playback has been used successfully for several species (invertebrates: Clark and Uetz, 1990; fish: Kodric-brown and Nicoletto, 1997) including birds. Evans and Marler (1991) pioneered in the field of animal social behaviour by demonstrating that male chickens (*Gallus domesticus)* show alarm calling behaviour in response to video playback of female hens threatened by a predator similarly to that shown in the presence of a live female being threatened. Since that point, numerous studies indicated that multiple bird species, including zebra finches (*Taeniopygia guttata*; Galoch and Bischof, 2007) and rooks (*Corvus frugilegus*; Bird and Emery, 2008) can perceive their conspecifics through video playback and react to them in an appropriate manner. This includes studies of courtship. Partan et al. (2005) exposed female pigeons (*Columba livia*) to multimodal video and audio playback of male conspecifics and found that the combined multimodal playback elicited a stronger behavioural courtship response in the females than the different signal components presented in isolation.

In experiments involving measurement of animal behaviour, the use of high-speed video recordings with temporal resolutions of hundreds or even thousands of frames per second allows very precise quantification of animal behaviours, but it also creates the need

for tools that allow to efficiently process the large datasets which are generated (Janisch et al., 2021). One promising tool for automated data analysis in animal behaviour are machine learning algorithms. These algorithms (or models) are trained on a set of manual annotations of specific visual features (e.g. body parts of animal) in representative video frames chosen by an experimenter. The model is then able to make predictions on previously unseen data by drawing inferences from certain patterns in the training data. Apart from studying complex behaviours, machine learning can also be used to automatically classify vocalisations, species or individuals into different groups (Valletta et al., 2017). Depending on the research question, motion of the whole animal (object detection), movement of specific points of the animal's body (keypoint estimation) or movement and configuration of different body parts relative to each other (pose estimation) can be tracked (Janisch et al., 2021). In a recent experiment, Mitoyen et al. (2021) investigated different elements of the male courtship bowing display in ring doves. To quantify the vertical amplitude of a male bow, automated tracking of the eye was used and the distance between the lowest and the highest pixel-coordinate value of this keypoint on the y-axis was calculated. However, in some cases, for subtle behaviours such as the "tail quivering" behaviour of female ring doves described in this thesis, manual coding by a human coder might be more efficient than automated tracking (Janisch et al., 2021).

Ring doves (*Streptopelia risoria*) are a domesticated and socially monogamous bird species that form pair bonds which can last over several reproductive cycles (Morris and Erickson, 1971). Their relatively simple and stereotyped courtship behaviour makes them the ideal model species to study multimodal communication in a sexual context. The typical ring dove courtship cycle usually begins with the male chasing the female, followed by the male performing a bow-coo display, a repetitive bowing movement oriented towards the female accompanied by a bow-coo vocalisation. In the next stage, the male selects a nest site where he performs a nest soliciting display during which he sits in a particular posture, producing small vibratory movements of the wing tips called wing flips (Lovari and Hutchison, 1975). This display is accompanied by vocalisations which have a structure that is very similar to bow-coos, called nest coos (Miller and Miller, 1958). The nest coos are generally produced for 3 to 4 days or until the female starts nest-cooing. The behaviour of the male and female is synchronized in a way so that the frequency of nest cooing by the male only starts declining when the female responds with nest-cooing behaviour herself. After this point, the male starts gathering nesting material for the female to build the nest and after nest building, copulation usually takes place. Between seven to eleven days after the start of courtship, the female will lay her first egg and a few hours later her

second. Both parents alternate incubating the eggs until they hatch approximately two weeks later (Lehrman, 1964).

This whole behavioural cycle is regulated by hormone secretion, which in turn is controlled by external stimuli such as repeated exposure to a courting male and the presence of nesting material (summarized by Lehrman (1964)). The cycle starts with androgen secretion mediating the initiation of courtship behaviour in the male (Erickson and Lehrman, 1964). Male courtship behaviour then stimulates oestradiol secretion via gonadotropins in the female, leading to the female displaying behaviours which are typical for the first stages of the breeding cycle such as wing flipping and nest cooing. After oestradiol secretion has reached its peak, it starts to decline while progesterone levels start to rise. The combined effect of oestradiol and progesterone secretion finally leads to the initiation of nest soliciting behaviour including nest-cooing, nest building and incubation of the eggs (Cheng, 1979). The nesting cycle can be broken by removing the eggs or the young at any point after egg laying, resulting in the dove starting to perform courtship behaviours once again (Cuthbert, 1945).

Early experiments have shown that no contact stimulation by a male is necessary to induce ovulation in female ring doves. Females physically separated from males via a translucent barrier laid eggs upon only hearing and seeing male courtship stimuli (Erickson and Lehrman, 1964). Lambe and Erickson (1973) wanted to know if male vocalisations were sufficient to induce an ovulation in females or whether other structural features of male courtship were necessary. As mentioned earlier, they exposed females to either a combined stimulus of a shadow and vocalisations of a male or to vocalisations alone and found that none of the females responded to the auditory-only stimulus while half of the females laid eggs in the presence of a courting male shadow. These results are supported by another study in which female ovarian development was significantly higher when the females were exposed to multimodal auditory and visual male courtship stimuli, rather than to unimodal auditory stimuli (Friedman, 1977). In the following years, Cheng (1986) was able to disprove Lehrman's original hypothesis that female follicular growth is mediated entirely by male nest coos. Muted females exposed to playback of their own nest coos presented greater follicular growth than those subjected to male nest coos, indicating that the female's own nest coos are stimulating her pituary-hypothalamus-ovarian axis, culminating in egg laying. Follicular development is however greater when the female is exposed to male vocalisations rather than her own because an intact female usually produces significantly more nest coos when being courted by a male rather than when hearing her own or other female's nest coos. Moreover, other visual and tactile aspects of

male courtship also play a role in eliciting female behavioural and physiological changes (Cheng, 2003).

In a recent experiment performed at the dove laboratory of the University of Vienna, Mitoyen et al. (2021) investigated the effect of multimodal bow-coo courtship signals on immediate behavioural responses of female ring doves and whether male variability impacts these responses. Tail quivering, a behaviour previously associated with sexual stimulation in zebra finches (*Taeniopygia guttata*; Witte, 2006) and canaries (*Serinus canaria*; Amy et al., 2015) was exclusively shown in the presence of a male, but never occurred when the females were exposed to an empty cage. Moreover, tail quivering behaviour was observed much more frequently when the female interacted with a courting rather than a non-courting male, further confirming that this behaviour is also related to sexual stimulation in female ring doves. This behaviour was additionally linked to the fundamental frequency of male calls, the number of courtship bouts as well as the duration of courtship, and was therefore highly dependent on which male courted the female.

In a follow-up study by Mitoyen et al. (submitted), female ring doves were exposed to audio-visual playback of male courtship displays differing in their audio-visual timing. A higher number of female sexual behaviours, including tail quivering, was recorded in the stimulus intervals (exposure to male courtship video) compared to the playback breaks (exposure to empty cage video). Additionally, female sexual behaviours (except tail quivering) and oestradiol levels increased over the whole duration of the exposure to male courtship, demonstrating that playback is an efficient tool to elicit similar behavioural and physiological responses in the females as live courtship. Audio-visual timing had no effect on the occurrence of sexual behaviours apart from chasing, observed significantly more often in the control condition. Interestingly, tail quivering behaviour in females was negatively correlated with oestradiol concentration, and it was the only behaviour that occurred consistently over the whole duration of the experiment, leading the authors to hypothesize that tail quivering behaviour is a sign of immediate sexual interest displayed in response to courtship. Consequently, they concluded that tail quivering behaviour should decrease with increasing oestradiol concentrations during subsequent phases of courtship.

In this study, we aimed to investigate how different components of multimodal courtship signals affect female behavioural and physiological responses related to sexual stimulation by using cue occlusion rather than cue isolation. This Master's thesis is based on videos of female ring doves acquired from a previous experiment performed by D.

Biegler for her Master ‘s thesis. In this experiment, female ring doves were exposed to high-quality audio-visual recordings of male courtship for seven consecutive days. A total of 21 females were split into 3 playback conditions: A “normal” Audio-visual (AV) condition and a Visual (V) and Audio (A) condition, in which the respective other sensory channel was occluded using an ecologically valid stimulus (foliage; vacuum cleaner sound). The resulting videos recordings of the females viewing the playback material were then analysed for female behaviours associated with sexual stimulation between playback intervals and playback breaks and across conditions. Additionally, blood oestradiol concentrations were measured before and after testing to assess the physiological response of the females to the male courtship (Biegler, 2021). This thesis focuses on the following three female sexual behaviours: Tail quivering, wing quivering and wing flipping.

Preliminary results (for half of the available recordings) indicated that female ring doves do respond to playback of male courtship, as chasing and preening was significantly higher during playback intervals than during playback breaks (Biegler, 2021). Playback condition had no effect on most behaviours of interest, except preening, which was observed significantly more often in the AV condition than in the A condition. Since behaviours such as chasing were observed equally as much in each condition, it is very likely that the unimodal courtship signals in ring doves carry redundant information, but no definitive conclusions can be drawn until the full dataset is available. Perch coos, which are known to occur more often when individuals are isolated (Davies, 1974), were observed more frequently in the A condition than in the AV or V condition, suggesting that females might have vocalised more to attract the male to their location when they could not see him. Nest coos which are usually produced at later stages of the reproductive cycle (Cheng, 1979), on the other hand, occurred more often in the V than in the AV condition. They were however never observed in the A condition, indicating that only hearing the male might be insufficient to elicit nest coo behaviour in the females. Against the predictions, pre- and post-experimental blood oestradiol concentrations did not differ significantly from each other (Biegler, 2021).

Based on previous results from Mitoyen et al. (submitted) and the Master’s thesis of Biegler (2021), we hypothesized that playback of multimodal male courtship signals can elicit sexual behaviours in ring doves. Consequently, we predicted that the sexual behaviours of interest (tail quivering, wing quivering and wing flipping) would occur more often during stimulus playback intervals than during playback breaks. Moreover, we hypothesized that female response to male courtship playback differs depending on which sensory modality can be used to access male courtship. We expected that exposure to

the multimodal audio-visual playback rather than to unimodal audio or visual playback would lead to an enhancement effect of the behavioural response (Friedman, 1977; Lambe and Erickson, 1973; Partan et al., 2005). This would indicate that components from different modalities carry redundant information ("Back-up signal hypothesis"). Alternatively, we expected that (tail) quivering behaviour is mainly driven by auditory rather than visual input, since tail quivering behaviour was dependant on the fundamental frequency of male calls in the study of Mitoyen et al. (2021). Thus, the quivering response of females in the audio and audio-visual condition should be similar, suggesting that signal components from different modalities carry non-redundant information ("Multiple message hypothesis").

2. Materials and Methods

In a playback experiment performed by my colleague D. Biegler (Biegler, 2021), 21 female ring doves were split into three playback conditions (audio-visual, audio, visual) and exposed to respective audio-visual recordings of male courtship for seven consecutive days. Female behavioural and physiological responses were then assessed between playback- and break intervals and across playback conditions. I carried out the manual scoring of three specific behaviours potentially related to sexual stimulation in female ring doves (tail quivers, wing quivers and wing flips) and ran the statistical analysis of the data. To assess whether automated analysis of the (tail) quiver behaviour could be implemented, I additionally trained an automated tracking model to recognize specific body parts of the female ring doves by annotating selected frames of videos from different doves.

2.1. Playback experiment

The playback experiment, that this Master's thesis is based on, was conducted on two experimental groups of female ring doves: Nine females were tested from August 25 to August 31, 2020, and the remaining twelve females were tested from September 8 to September 14, 2020. The experiment was conducted with the approval of the local ethics committee of the Faculty of Life Sciences, University of Vienna, and of the Austrian Federal Ministry of Education, Science and Research (BMWFW permit 066.006/0042-WF/V/3b/2017). On each experimental day, females were additionally filmed in their isolation cages by GoPro Cameras (hero4 model) for 30 minutes before the start and after the end of the experiment. During this period, female vocalisations were scored by my colleague A.T..

During the testing, females were exposed to 10 pre-recorded courtship videos of 2 stimulus males. The male courtship videos were recorded at 120 frames/second in order to make them seem as natural as possible to the female doves (Ware et al., 2015). Moreover, audio was sampled at 48Hz and synchronized with the video recordings. Playback consisted of stimulus intervals of 5 randomly ordered courtship clips (8-14 seconds, 4-6 bow-coos) separated by short breaks of 0.5-1.5 seconds, followed by a longer break of 40 seconds. In the breaks between playback intervals (playback break), the set-up with an empty cage was shown. The total playback duration varied between

~15.5-16 min depending on male ID, but females were exposed to the same number of bow-coos. Moreover, no courtship clip was shown twice in a row (**Figure 2C**).

21 Females were randomly assigned to 3 groups of seven birds, each group experiencing a different playback condition. Additionally, they were randomly assigned to one of the two stimulus males and experimental weeks, and the assignment was balanced over conditions. In the audio-visual (AV) condition, females were exposed to both the video and audio recordings of the male courtship. In the unimodal audio-only (A) condition, females were only exposed to the audio recordings of male courtship, while a still image of a bundle of ivy was shown on the screen. In the unimodal video-only (V) condition, the video recordings of the male courtship were shown on the screen, but the corresponding audio was replaced by the sounds of a vacuum cleaner. Instead of just isolating the different sensory modalities, the image of the ivy and the sounds of the vacuum cleaner were used to mask the corresponding visual or auditory part of the male courtship (**Figure 2A**).

During the testing, behavioural and auditory responses of the females to the male courtship were recorded using one microphone (Sennheiser ME66 K6, directional head with battery powered K66 module) and three cameras (Basler acA1920-155uc; Figure **2B**). The recordings of the three cameras, one recording from the side, one from above and one recording the monitor displaying the playback material, were synchronised. The video material filmed by the third camera allowed to distinguish between the stimulus intervals and playback breaks in each video. For a detailed description of the experimental setup see Biegler (2021).

For analysis of the endocrinological response to the playback, blood samples were taken of each female one day before the start of the experiment and one day after the experiment ended. Oestradiol levels were measured in each blood sample resulting in one pre-test baseline E2 value and one post-test E2 value for each female. Blood sampling was performed by Cliodhna Quigley and G.S., and hormone analysis was conducted by V.C.. Moreover, faecal samples were taken on each experimental day, as well as on the day after the experiment. Analysis of oestradiol concentrations in the faecal samples is still pending.

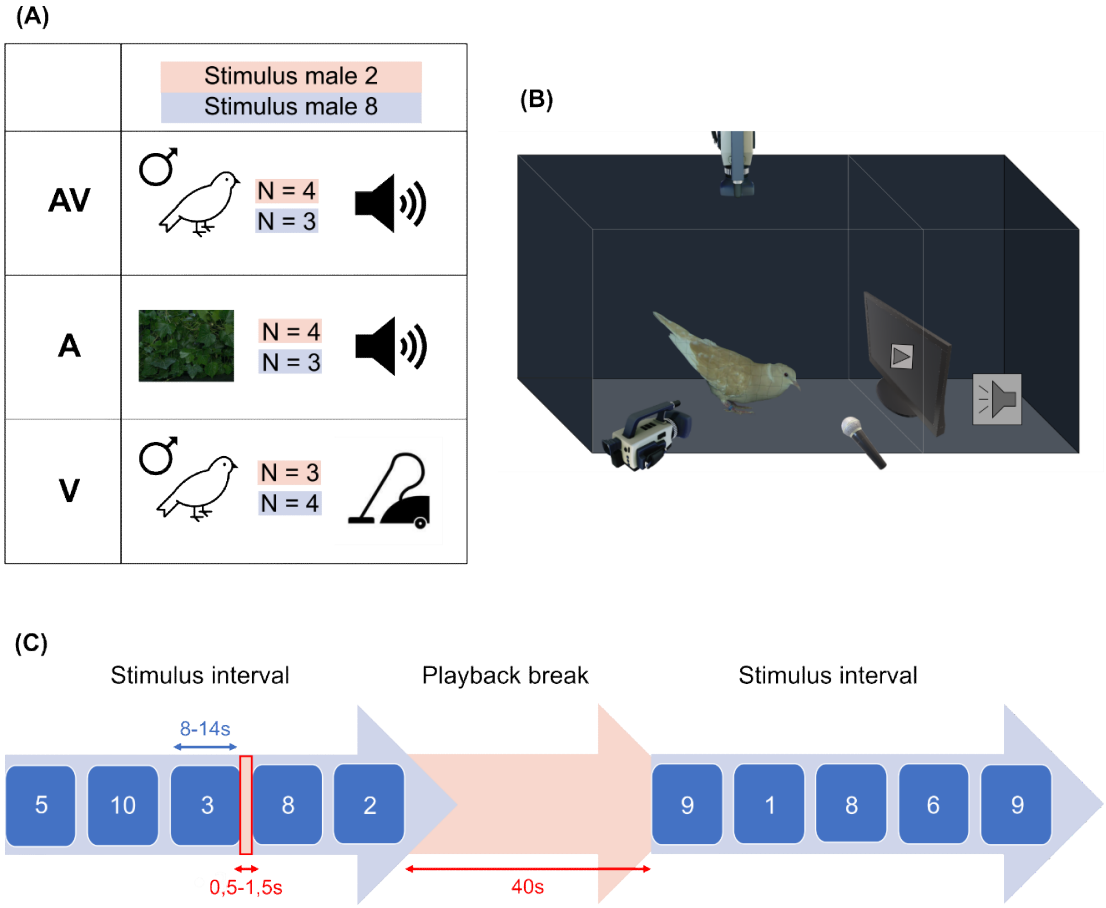

Figure 2: Experimental design (A) 21 females were divided into three groups of seven birds with each group exposed to a different playback condition: 1) Multimodal audio-visual (AV) male courtship stimuli, 2) Unimodal audio (A) male courtship stimuli; the visual channel was occluded by an image of ivy on the screen, 3) Unimodal visual (V) male courtship stimuli; the audio channel was occluded using vacuum cleaner sounds. Females from different conditions were balanced across two different stimulus males. (B) Experimental setup (camera filming the monitor not shown). (c) Playback design. Playback consists of multiple stimulus intervals containing five randomly ordered male courtship clips separated by short breaks (0.5-1.5 sec) and followed by longer breaks (40 sec) showing silent video playback of the empty cage.

2.2. Video analysis

Video coding was performed using the manual coding software Loopy (http://loopb.io/, loopbio GmbH, Vienna, AUSTRIA). Behavioural scoring was only performed on videos recorded from the side-view camera. Two specific female dove behaviours previously associated with sexual stimulation in other dove species, tail- and wing quivering (Amy et al., 2015; Witte, 2006), were scored for the entire catalogue of 147 recorded videos. One additional behaviour called “wing flips” was added to the scoring, as it often occurred alternating with tail quivers. A detailed definition for each of these behaviours, as well as a list of previously scored and analysed behaviours for D. Biegler’s

Master's thesis (Biegler, 2021) can be found in **Table 1**. For each behaviour, the start and stop frame, as well as the duration of its occurrence was recorded. The occurrence of these behaviours of interest could then be assigned to playback breaks or stimulus intervals.

Table 1: Ethogram of all the scored behaviours relevant for analysis (adapted from D. Biegler's ethogram)

Category	Behaviour	Definition
Quivering	Wing quiver	The dove rapidly shakes her closed wings, but not her tail, within a small range of motion, similar to a vibration movement. Can be observed in a motionless (standing or sitting), as well as in a walking dove. Also scored during vocalisation.
	Tail quiver	The dove shakes her tail, and sometimes her wings, within a small range of motion, similar to a vibration movement. The shake can be very clear as well as subtle. Can be observed in a motionless (standing or sitting), as well as in a walking dove. Also scored during vocalisation.
Body movements	Wing flip	The dove jerks her shoulders repeatedly at a frequency of approximately 2 flips per second, producing a wing flip movement. Most of the time both wings are flipped simultaneously, but single wing flips can also occur. For this movement to count as a wing flip, multiple flips must be repeated and breaks in between flips shouldn't be longer than half a second. The scoring of one bout of wing flips starts with the first wing flip and ends with the last. This behaviour is almost exclusively performed with the head held low and tail held high. Wing flips are occasionally also interspersed with tail quivers.
	Step	The dove lifts one of her feet off the ground and sets it down again immediately. One step counts as one behavioural event.
	Chase	The dove moves by setting one foot in front of the other while assuming a distinct body posture. The typical body posture may be more or less clear between birds, but in general, her back is slightly hunched, her head tucked down, and her wing feathers are slightly spread (on one or both wings). She may drag her wings and/or tail on the ground as she walks. Her head may bob back and forth along a horizontal axis. She may move in a circular pattern, but this is not a must.
Preening	Wing preening	The dove touches her beak to any part of her wings or runs her beak along her wing feathers.
	Underside preening	The dove touches her beak to the feathers or skin of the underside of her body.
	Back preening	The dove touches her beak to the feathers or skin of her back, i.e. the region between her wings and above her tail.
	Tail preening	The dove touches her beak to the feathers or skin of her tail, i.e. the region distal of the cloaca.
Vocalizations	Nest coo	The dove makes a cooing sound while assuming a distinct body posture: She holds her tail high; her head may be lowered so that her head and her tail are in one line, but she may also move her head up and around. Both feet are on the ground, but she may stand only on the tips of her feet. Her wings may twitch. Her neck may inflate and deflate with the coos.
	Perch coo	The dove makes a cooing sound while in perch position, i.e. with her tail higher than her head and both feet planted on the ground; her neck may inflate or deflate with the coos.

2.3. Automated tracking

In an attempt reduce the scoring time, we additionally aimed to quantify tail (and wing) quivering behaviour by training a deep learning algorithm in Loopy (http://loopb.io/, loopbio GmbH, Vienna, AUSTRIA) to automatically track body parts of interest. Loopy software includes a tool called pose detection, that uses machine learning to automatically track certain keypoints of interest. For optimal results, a pose detection model must be trained with a dataset of at least 800 manually annotated frames. For the tail detector model, a total of 881 frames taken from 24 side-view camera videos of the female doves were annotated. Manual annotations were performed by me and my colleague A.T.. For each of the selected videos, the following 11 annotation classes were used:

1) ID_0 – Beak (keypoint)
2) ID_1 – Tail_tip (keypoint)
3) ID_2 – Right_eye (keypoint)
4) ID_3 – Left_eye (keypoint)
5) ID_4 – Right_foot (keypoint)
6) ID_5 – Left_foot (keypoint)
7) ID_6 – Right_wing_tip (keypoint)
8) ID_7 – Left_wing_tip (keypoint)
9) ID_8 – Right_wing_crease (keypoint)
10) ID_9 – Left_wing_crease (keypoint)
11) ID_10 – Animal (rectangle)

For an example frame showing the exact position of these annotation classes on the doves' body, see **Figure A1** in the Appendix. Keypoints were marked as "not visible" in a frame when the corresponding body part was occluded from view.

Videos used to choose frames to train the pose detector model were selected in a way to maximize variety of morphological types of doves (different colours and patterns), positions, behaviours, and locations in the cage. For each morphological type, two videos with the highest step count, and preferentially containing different behaviours of interest, were selected using previous manual scoring data. A high step count usually reliably indicates that the bird covered a large area of the cage during one testing trial. If possible, videos of females of a different ID were used for the annotations. For females of a unique

morphological type (e.g. dove with dark grey-brown plumage) two videos of the same female from a different testing day were used. After first analysis of automated tracking results by means of viewing prediction videos including tracked keypoints, frames from two additional videos were added to the training data. Some videos were split in half or parts cut out due to the occurrence of unwanted behaviours in too many frames (i.e. flying or sitting for a longer period of time). In the final annotation project, between 16 and 41 frames (mostly between 39 and 40 frames) of each of the selected videos were used to train the model. A detailed list of the videos and the corresponding number of frames used can be found in **Table A1** in the Appendix.

The pose detection model called “Tail detector” was trained on the 881 annotated frames using the default settings in Loopy (Network stride: 8, Network Size: 968x608, Training duration: 50k). Accuracy of the model was determined by manually confirming that the keypoints of interest were tracked correctly in a sample of prediction videos including automatically tracked keypoints. The changes in the x and y pixel coordinates of the “tail” keypoint over the whole duration of the experiment were then visually compared to the scorings of the “tail quiver” behaviour. The aim of this visual comparison was to find some kind of temporal pattern in the y pixel coordinates during the tail quivering behaviour (e.g. larger changes in amplitude) that could help to automatically predict this behaviour in the future. Unfortunately, the changes in amplitude of the y-axis pixel coordinates were too small to reliably predict tail quivering behaviour (see Results, **Figure 3**). Therefore, only manually scored quivers could be used for data analysis.

2.4. Data Analysis

The statistical analysis of the data was performed in R version 4.0.3 (R Core Team, 2020). The figures and plots contained in this Master’s thesis were produced using the R package “ggplot2” (Wickham, 2016) and edited in Inkscape (Inkscape Project, 2020. *Inkscape*, Available at: https://inkscape.org). Model random effects were visualized using the package “sjPlot” (Lüdecke, 2021). For data wrangling, the R packages “dplyr” (Wickham et al., 2021), “tidyr” (Wickham, 2021a) and “forcats” (Wickham, 2021b) were used. The threshold for significance levels was set to 0.05.

As previously reported by Biegler (2021), there is a slight variation in the total duration of playback sessions (15.56min-16.31min) due to the duration of courtship clips varying by male identity (summed duration ~104.2 seconds for stimulus male 1, ~111.6

seconds for stimulus male 8). However, I decided against using relative measures (i.e. number of events per minute and proportion of the total length of an experimental session) for the quiver and wing flip behaviours, as the overall rareness of the behaviours would lead to very small proportions. Moreover, the two stimulus males were balanced over conditions (see Materials and Methods, **Figure 2A**).

In a first step, the raw manual scoring data was pre-processed in a way that each occurrence of a behaviour of interest (tail quivers, wing quivers and wing flips) was assigned to a particular female-day combination and the duration of this behaviour was calculated. Behaviours that occurred before the start and after the end of the playback experiment were eliminated from the scoring data. Additionally, information about the playback condition, country of origin, stimulus male and week of testing of each female was added to the pre-processing table. To assess whether a certain behaviour was occurring during a stimulus interval or a playback break, behavioural scoring data was combined with data containing information about the playback on/off-timing of each recording session and each behavioural occurrence was classified into a “playback on” or “playback off” category. Due to the overall sparseness of wing quivering behaviour and overlap of tail quivering and wing quivering in the ethogram definition (i.e. if wing- and tail quivers occur at the same time, they are classified as tail quivers), both behaviours were combined for further statistical analyses.

To assess whether playback influences a behaviour, the frequency and duration of each behaviour of interest was summed by female and experimental day split into behaviours occurring during playback intervals or during breaks, resulting in total of 2×147 values for both the number of events and the duration of quivers and wing flips (two values by female-day combination). Because overall duration of stimulus intervals (~8.74-9.37min) was longer than overall duration of breaks (~6min), we scaled the number and duration of behaviours performed during playback intervals by multiplying them with a scaling factor (Duration PB “off”/Duration PB “on”). Paired Wilcox signed-rank tests for nonparametric data were used to assess whether there was a difference in female behaviour during the playback intervals compared to the breaks. For reasons of simplicity, only the long breaks (40s) in between stimulus intervals were considered as playback breaks, while the short breaks between courtship videos were included in playback intervals (0.5-1s).

For analysis of the frequency of the behaviours by condition, the data was not separated into “playback on”- vs. “playback off” behaviours since excluding behaviours

performed during playback breaks could conceal a potential effect of condition. Models were fitted using the r-package “lme4” (Bates et al., 2015) and “MASS” (Venables & Ripley, 2002) and the effect of playback condition was tested using model comparisons of full to reduced models via likelihood ratio tests. Full models (except post-hoc analysis) include the interaction of condition and experimental day as fixed effect variables. As random effects, we had intercepts for female identity and by-female random slopes for the effect of experimental day. Stimulus male, country of origin and experimental week were not added as random effects since these variables were counterbalanced in the experimental design. Reduced models have the same structure but lack the fixed effect “condition”.

First, we aimed to investigate whether the number of quivers performed by a female over experimental days is dependent on the condition that the female is assigned to. As the quiver count data contained many zeros (>40% of all values), a two-part model approach was used. In the first step, we investigated whether the presence and absence of quivering behaviour by a specific female on a given experimental day was guided by playback condition. The quiver data was converted into presence or absence of quivers for a particular female on a given day and a binomial GLMM (generalized linear mixed model) with logit-link was fitted. To test the effect of condition, the full model was compared to a reduced model lacking the variable “condition”. The full “quiver presence/absence”-model had the following structure:

$$Presence\ of\ quivers \sim condition * experimental\ day + (1 + experimental\ day|femaleNr)$$

In the second part, we analysed how playback condition influences the number of quivers, if there were any quivers on a given day for a female. Since the count data contained many zeros, only non-zero quiver values were used to fit the model. To account for overdispersion of the count data (the variance is bigger than the mean) which prevented use of a Poisson model, we fitted a negative-binomial GLMM. To test the effect of condition, the full model was compared to a reduced model lacking the variable “condition”. The full “quiver count”-model had the following structure:

$$Number\ of\ quivers \sim condition * experimental\ day\ + (1 + experimental\ day|femaleNr)$$

In an effort to find the most parsimonious model structure to explain the count data, further exploratory model comparisons were performed (see **Table A2** and **A3** in the Appendix for the different model structures that were compared).

In post-hoc analysis, I investigated how condition impacts the total number of quivers performed by each female over the whole duration of experiment (21 values). A negative-binomial GLM was fitted, and a full-reduced model comparison was performed (with and without "condition"). In the full "total quiver count"-model, only condition was entered as a fixed effect:

$$Total\ number\ of\ quivers \sim condition$$

The "DHARMa" (Hartig, 2021) package was used to evaluate the assumptions of the selected models (see model diagnostics plots **Figures A3, A4, A5, A6** and **A7** in the Appendix). Analysis of the duration of quivering was excluded from this thesis due to convergence issues of our models, as more complex modelling was beyond the scope of this Master's thesis. However, Kruskal-Wallis rank sum tests were used to test if there was difference in the total number and duration of quivers between conditions.

In a separate exploratory data analysis step, I investigated whether quivering, specifically tail quivering, is simply a (maybe involuntary) by-product of cooing. During pre-processing, quivers and wing flips were classified into 3 different categories depending on if they were co-occurring with one of the selected types of coos ("nest coo", "perch coo") or occurring on their own ("silent"). According to our definition, a coo is classified as coincident with a quiver if it satisfies at least one of the following three criteria: (1) A coo happens during a quiver, meaning that the start-frame of the quiver is smaller than or equal to the start-frame of the coo and the stop-frame of the quiver is greater than or equal to the stop-frame of the coo, (2) A quiver starts during a coo, meaning that the start-frame of a quiver is greater than or equal to the start-frame of a coo, but the start-frame of the quiver is smaller than or equal to the stop-frame of a coo, (3) A quiver stops during a coo, meaning that the stop-frame of a quiver is greater than or equal to the start-frame of a coo, but the stop-frame of a quiver is smaller than or equal to the stop-frame of a coo. To avoid misclassifications into the "silent"-category due to small differences in the timing, a match tolerance of 0.1 seconds was added at either end of the coo allowing for an earlier start or later end of a quiver. The number of quivers assigned to each of the three categories ("nest coo", "perch coo", "silent") was then summed by female and experimental day.

Since the link between condition and quivers coincident with coos was clear and hard to model (almost all the quiver-coo type combinations in one condition: perch coo-quivers in A condition and nest coo-quivers in V condition, see Results page 46), we only investigated how playback condition impacts the number of quivers which are produced independently of coos ("silent-quivers"). To test the effect of condition on the number of "silent-quivers" (including zero-values), we fitted a GLMM with a Poisson distribution and performed a comparison of the full model to a reduced model lacking the predictor "condition". The full model includes the interaction of condition and day as fixed effect and intercepts for female identity and by-female random slopes for experimental day as random effects (same structure as the "quiver-count"-model, see Materials & Methods, page 25). An additional Kruskal-Wallis rank sum test was performed to assess if there is a significant difference in the number of "silent-quivers" between playback conditions.

In a supplementary step, I checked whether coos are always accompanied by quivers or if they can also occur independently from them using stricter criteria. According to our definition, a quiver is classified as coincident with a coo if the following criteria are met: The quiver starts after the coo starts, meaning that the start frame of a quiver is greater than or equal to the start-frame of a coo (1) and the quiver stops before the coo ends, meaning that the stop-frame of a quiver is smaller than or equal to the stop-frame of a coo (2). Again, a match-tolerance of 0.1s was added at to the start- and stop-frame of a coo. Preliminary analysis suggests that quivers produced coincidentally with nest or perch coos are very short (0.58% of nest coo-quivers and 0% of perch coo-quivers shorter than 1 second), so a quiver produced coincidentally with a coo should fall within the limits of the start- and stop frame (plus the match-tolerance) of a coo. Once more, the number of coos assigned to each of the two categories ('yes' = coincident with a quiver, 'no' = not coincident with a quiver) was then summed by female over experimental days.

For the analysis of the coincidence of wing flip and coo behaviour, the approach used to classify quiver-coo coincidence was not appropriate, since wing flip duration was coded for a bout of wing flips (see ethogram definition in **Table 1**) rather than a single wing flip event. Thus, the duration of one wing flip bout (median = 2.333s) is generally longer than the duration of one coo (median = 1.45s), making it more likely that multiple coos fall within one wing flip bout. For a coo to be defined as coincident with a wing flip, it must satisfy at least one of the following criteria: (1) A coo happens during a wing flip, meaning that the start-frame of a wing flip is smaller than or equal to the start-frame of a coo and the stop-frame of a wing flip is greater than or equal to the stop-frame of a coo, or (2) the wing flip starts during a coo or (3) ends during a coo (see previous criteria for quiver-coo

coincidence on page 26). Since the temporal pattern of cooing and wing flipping was often an alternating sequence with no direct overlap rather than a precise temporal match (personal observation), a larger match-tolerance was added at either end of the coo. A match-tolerance of 0.7s was selected arbitrarily since it represents approximately half of the average length of a single coo event. In a final step, the number of wing flips assigned to each of the three categories was summed by female and experimental day. Kruskal-Wallis rank sum tests were used to test whether there was a significant difference in the number and duration of wing flips between conditions.

According to the literature, wing flips and nest coos generally happen at the same stage of the reproductive cycle (Cheng, 1973; Lovari and Hutchison, 1975; Miller and Miller, 1958). Thus, I additionally quantified the coincidence of wing flipping- and cooing behaviour by summing the number of events of each behaviour on each experimental day and classifying them as either behaviour occurring on their own (“coos-only”, “wing flips-only”), simultaneously (“both”) or both not present (“none”) on a given day. Then, coos were further sub-divided into nest coos and perch coos and co-occurrence with wing flips on a given day was quantified.

3. Results

3.1. Automated tracking

As a side project, I trained a deep-learning algorithm to automatically track the position of different body parts of female doves over time. This was done to investigate whether subtle behaviours such as the tail quiver could be automatically scored rather than manually scored by a human observer. During exploratory data analysis, I focused on the pixel dynamics of the "tail" keypoint over the vertical and horizontal coordinate axes with the expectation that I would observe larger deflections of this keypoint across the y-axis during a tail quiver movement. To test my hypothesis, I chose two prediction videos of females performing regular and stereotypic tail quivers (ring N°6 and N°700) and visually confirmed that the tail keypoint was accurately tracked in a large proportion of the frames by viewing the video with superimposed tracked keypoints. The number of up-and-down vibrations during a specific tail quiver bout was counted by observing the video and then visually compared to the prediction of the tail keypoint across the vertical axis during the same period of time (data depiction similar to **Figure 3**). Unfortunately, no clear deflection of the tail keypoint across the y-axis could be identified in either of the two prediction videos, even though possible sources of error were strongly reduced by analysing only those videos in which the "tail" keypoint was accurately tracked (see **Figure 3** for an example). For examples of problems arising during the tracking of specific keypoints in prediction videos see **Figure A2** in the Appendix.

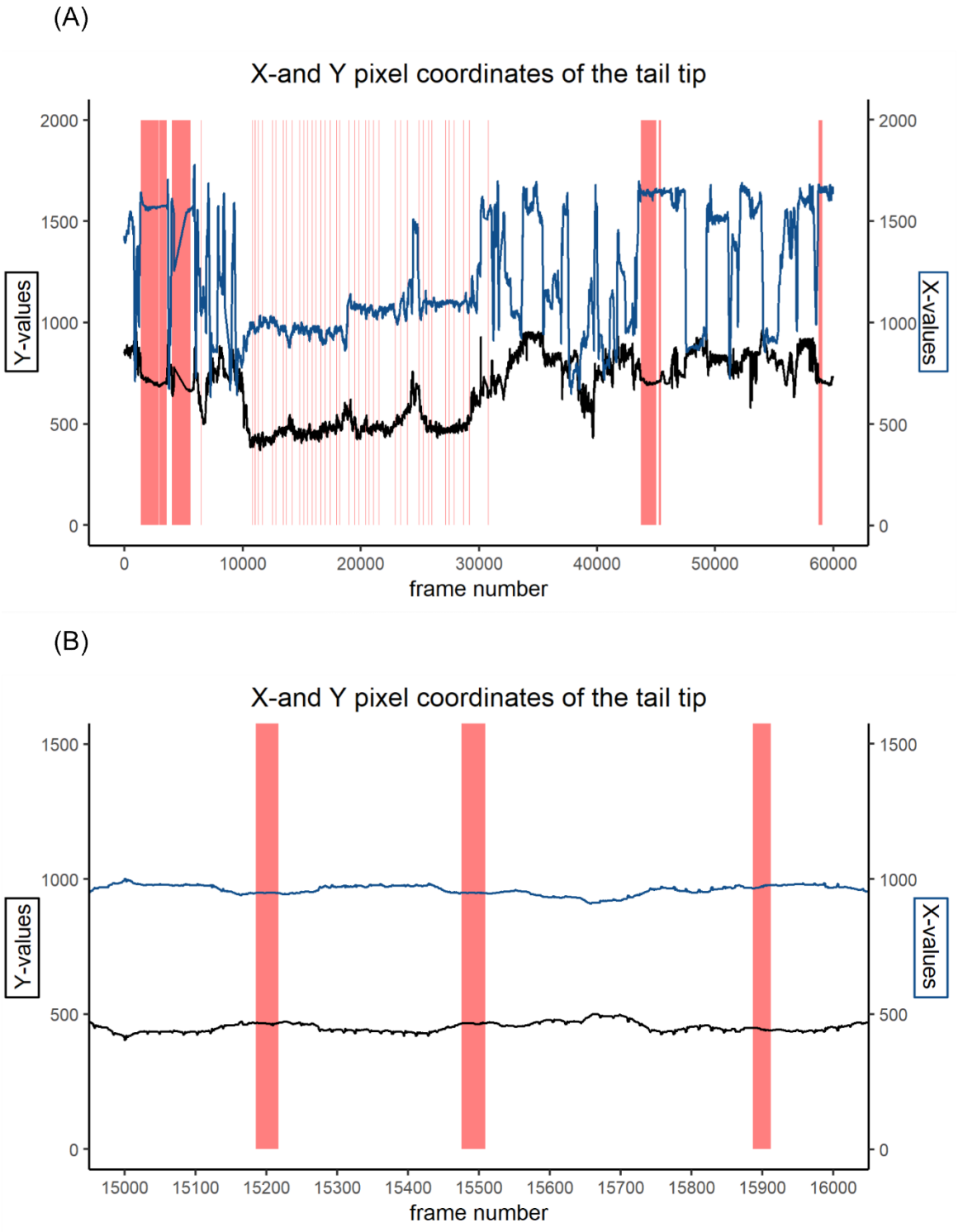

Figure 3: (A) Automated tracking results of "tail" keypoint of dove N°700 on day 4. The blue line represents the change of the horizontal pixel coordinates, and the black line represents vertical change over time. The total duration of the prediction video is approximately 16.7 minutes (60000 frames). Areas marked in red represent periods during which the dove quivered, according to manual scoring by a human observer (B) A more time-resolved representation (frames 15000-16000) of individual quiver events.

3.2. Behavioural scoring

In total, 147 recorded videos, coming from 21 female ring doves over seven experimental days, were scored and analysed for three behaviours of interest. Out of the 147 experimental sessions, females displayed tail quivering in 74 sessions (1190 occurrences), wing quivering in 31 sessions (82 occurrences) and wing flipping in 20 sessions (482 occurrences). As already mentioned in the methods section, tail- or wing quivers were combined for statistical testing. For detailed information on the total number and duration the two separate quivering behaviours by female and over experimental days see **Figures A8, A9, A10** and **A11** in the Appendix.

Quivering was performed by 19 out of the 21 females, but there was quite a large variation between females ranging from 0-393 quivers during the whole duration of the experiment (group mean = 60.57, group median = 29.00, SD = 106.051, N = 21) (**Figure 4A**). Wing flipping was observed in only 7 out of 21 females, with a large interindividual variation of 0-317 wing flips over the whole experimental period (group mean = 22.95, group median = 0.00, SD = 70.245, N = 21) (**Figure 4B**).

Quiver duration was also very heterogenous between females with values ranging from 0-592.217 seconds (group mean = 65.748s, group median = 28.850s, SD = 128.585, N = 21) over the whole experimental period (**Figure 5A)**. For wing flip duration, the range is even larger with values from 0-898.250 seconds (group mean = 117.780s, group median = 0.000s, SD = 280.715, N = 21) (**Figure 5B**).

Median duration of individual quiver events was 0.4 seconds. Maximum quivering duration was 77.183 seconds and only 10% of quivers lasted longer than 1 second. Wing flips were scored as bouts of individual wing flip events, but the median duration of one individual bout was still low with 2.333 seconds. Maximum wing flip duration was 89.817 seconds and most wing flip bouts (91,91%) were shorter than 10 seconds.

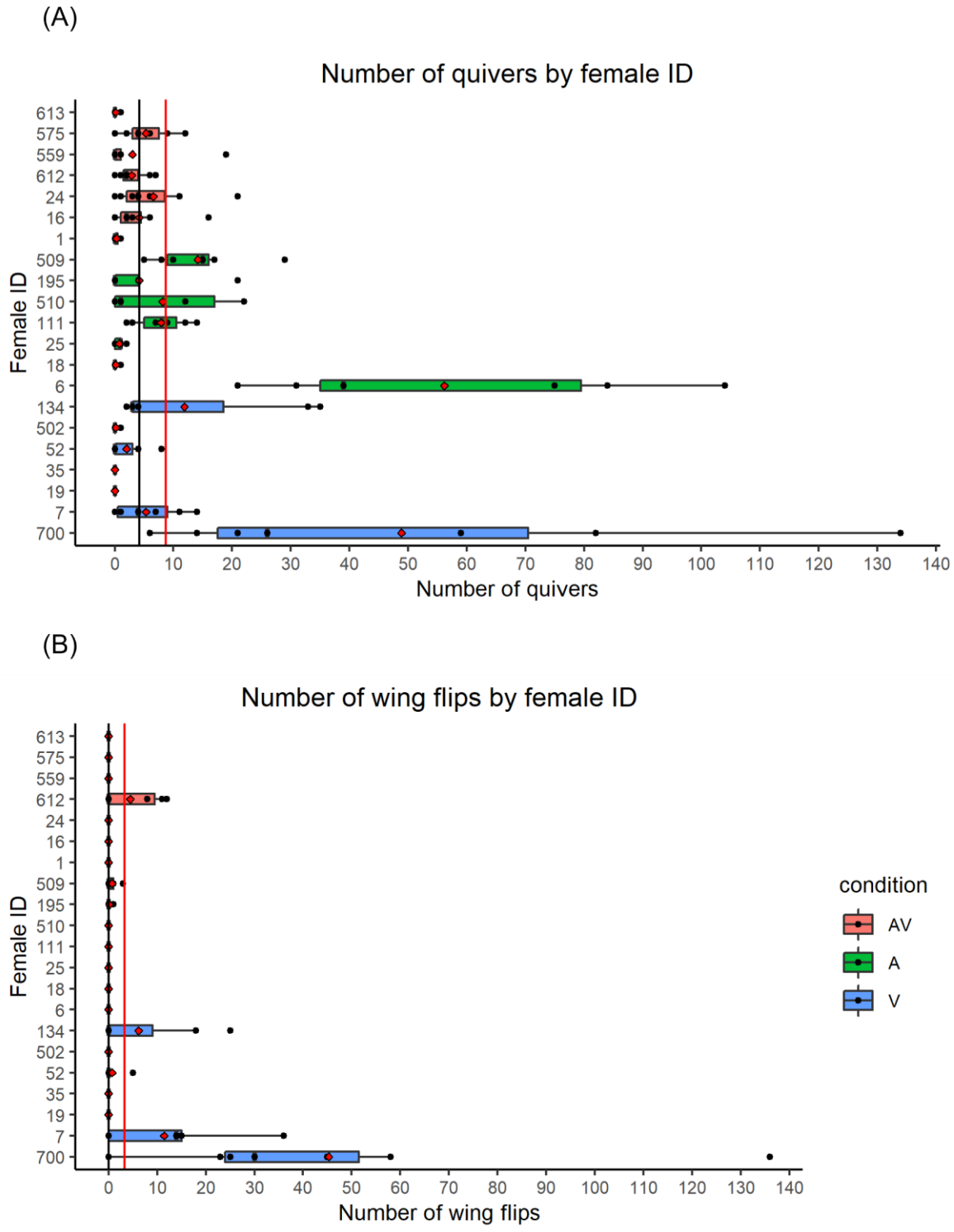

Figure 4: Number of quivers (A) and wing flips (B) by 21 females over seven experimental days. Each datapoint represents the sum of behavioural events performed by one individual (ring number on the y-axis) on one experimental day. Boxplots display the median (small black line) and upper and lower quartiles of daily behavioural events from each female. Red diamonds indicate the mean of daily behavioural events for each female. The two large vertical lines represent the group median (black) and mean (red) of daily behavioural events for all the females. Note that the group median number of wing flips is zero.

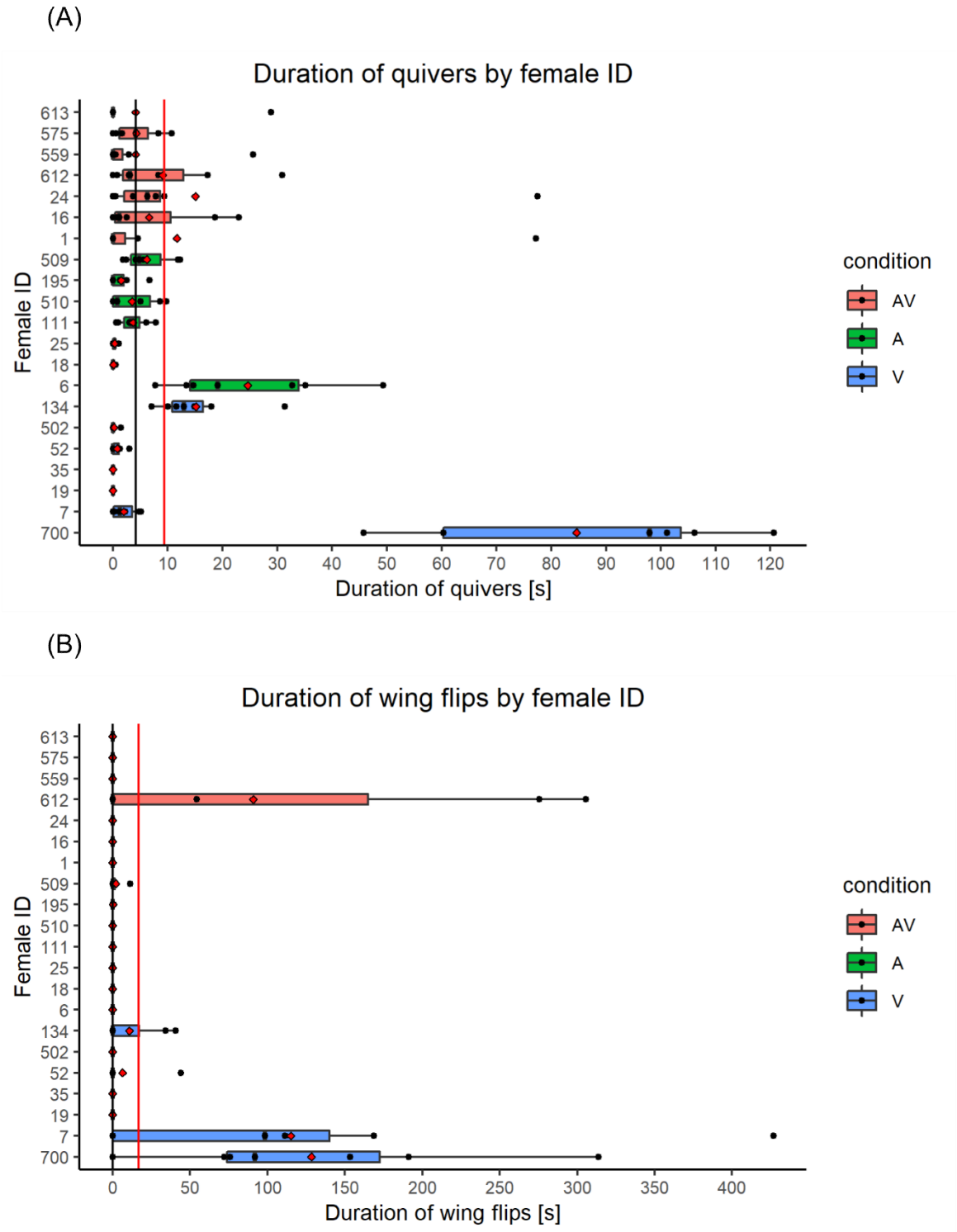

Figure 5: Duration (in seconds) of quivers (A) and wing flips (B) by 21 females over seven experimental days. Each datapoint represents the summed duration of daily behavioural events by individual. Boxplots display the median (small black line) and upper and lower quartiles of the daily duration that a behaviour was performed by each female. Red diamonds indicate the mean of the daily duration that a behaviour was performed per female. The two large vertical lines represent the group median (black) and mean (red) of daily behavioural durations for all the females. Note that the group median duration of wing flips is zero.

3.2.1. Female response to courtship stimulus intervals vs. playback breaks

In a first step, the effect of male courtship playback on female behaviours was analysed without considering differences across playback conditions. Due to large inter-individual differences between females, average values for the number and duration of quivers refer to median rather than the mean. For the number and duration of wing flips, only mean values are given, since the median is always zero.

The relative number of quivers did not differ significantly between the stimulus intervals and playback breaks (Wilcoxon signed-rank test, V = 101, p-value = 0.825). On average, females quivered 9.59 times during stimulus intervals (group mean = 18.04, SD = 26.437, N = 21) compared to 7.00 times during playback breaks (group mean = 31.33, SD = 71.334, N = 21) (**Figure 6A**). Moreover, the presence of male courtship playback did not impact the relative number of wing flips (Wilcoxon signed-rank test, V = 13, p-value = 0.933) with a mean number of 8.3 wing flips performed during playback intervals (SD = 23.493, N = 21) compared to a mean number of 9.24 wing flips performed in playback breaks (SD = 31.388, N = 21) (**Figure 6B)**.

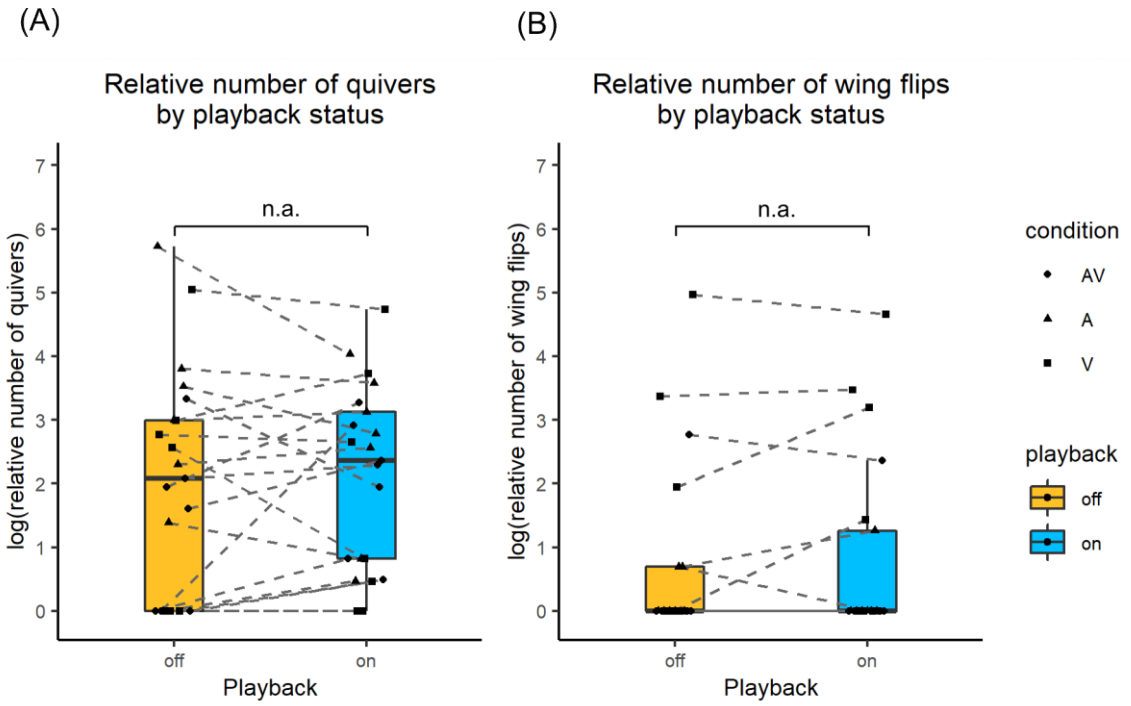

Figure 6: Scaled number of quivers (A) and wing flips (B) during playback breaks ("off") vs. playback intervals ("on"). Each datapoint represents the log-transformed relative number of behavioural events performed by one female over a period of seven days. Connected datapoints represent the same female. Different playback conditions are depicted using different symbols, but condition differences were not analysed.

Likewise, the immediate presence of male courtship playback had no significant effect on the relative duration of quivering (Wilcoxon signed-rank test, V = 54, p-value = 0.103). On average, female quivering behaviour was observed for a duration of 10.549 seconds during playback intervals (group mean = 31.363s, SD = 66.542, N = 21) and 3.267 seconds during playback breaks (group mean = 14.652s, SD = 32.761, N = 21) (**Figure 7A**). On the other hand, females spent more time wing flipping when they were exposed to male courtship playback (mean = 55.670s, SD = 130.394, N = 21) compared to break intervals (mean = 25.259s, SD = 70.419, N = 21) during which no playback occurred (Wilcoxon signed-rank test, V = 1, p-value = 0.035) (**Figure 7B**).

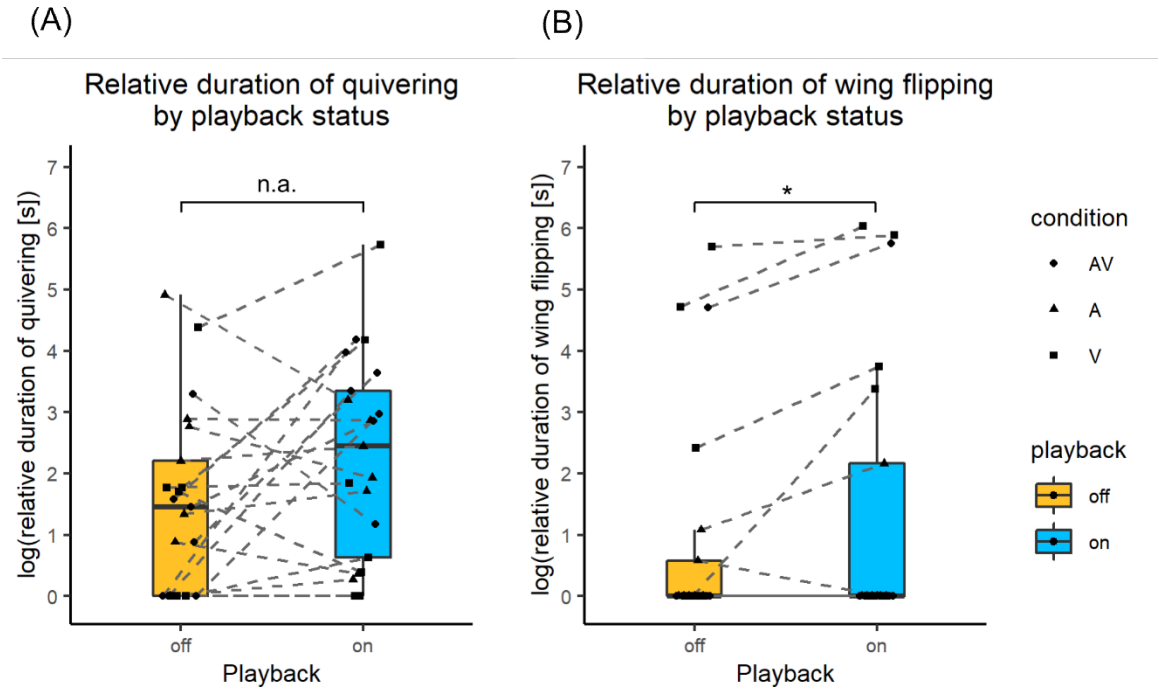

Figure 7: Scaled duration of quivers (A) and wing flips (B) during playback breaks ("off") vs. playback intervals ("on"). Each datapoint represents the log-transformed relative duration (in seconds) of a behaviour performed by a female over a period of seven days. Connected datapoints represent the same female. Different playback conditions are depicted using different symbols, but condition differences were not analysed.

3.2.2. Female response by condition over experimental days

I. Quivers

To investigate the role of the multimodal components present in male courtship signals on female quivering, we assessed whether there was a difference in the number of quivers depending on which playback condition a female was assigned to. For analysis of female response by condition, we decided to additionally include behaviours occurring

during playback breaks since there was no significant difference in the number and duration of quivers during “playback on” vs. “playback off” (see **Figure 6A** and **Figure 7A**). Again, average values for both the number and duration of quivers refer to the median, since outlier values have a strong effect of the mean.

Quivers were observed in each of the three playback conditions. The average number of quivers was the highest in the A condition (group median = 55.00, group mean = 91.29, SD = 137.271, N = 7), followed by the AV condition (group median = 21.00, group mean = 22.29, SD = 16.81, N = 7) and the lowest in the V condition (group median = 14.00, group mean = 68.14, SD = 124.442, N = 7) (**Figure 12**). Moreover, each female from the A and AV condition produced at least one quiver, whereas only 5 out of 7 females from the V condition performed quivers. The total number of quivers in each condition appeared to be relatively stable across experimental days, with the number of quivers increasing, decreasing, or staying stable depending on female identity (**Figure 8** and **Figure 9**).

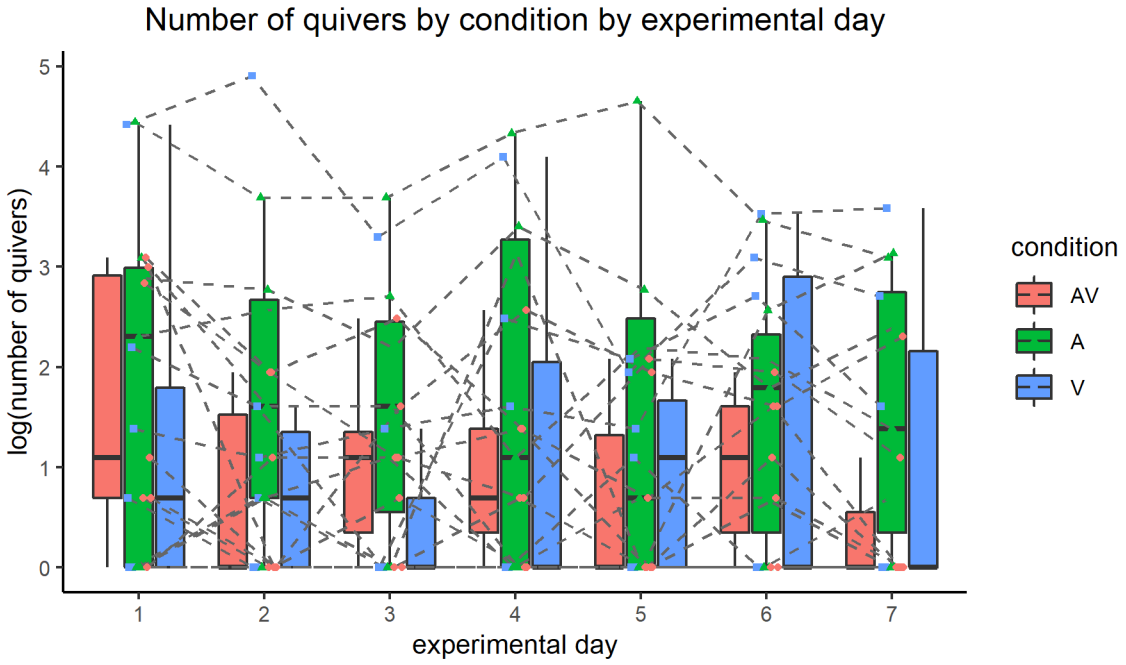

Figure 8: Number of quivers by condition over seven experimental days. Each datapoint represents the log-transformed number of quivers performed by one female on a given day. Connected datapoints represent the same female. See **Figure A8** in the Appendix for the separate number of tail and wing quivers over experimental days.

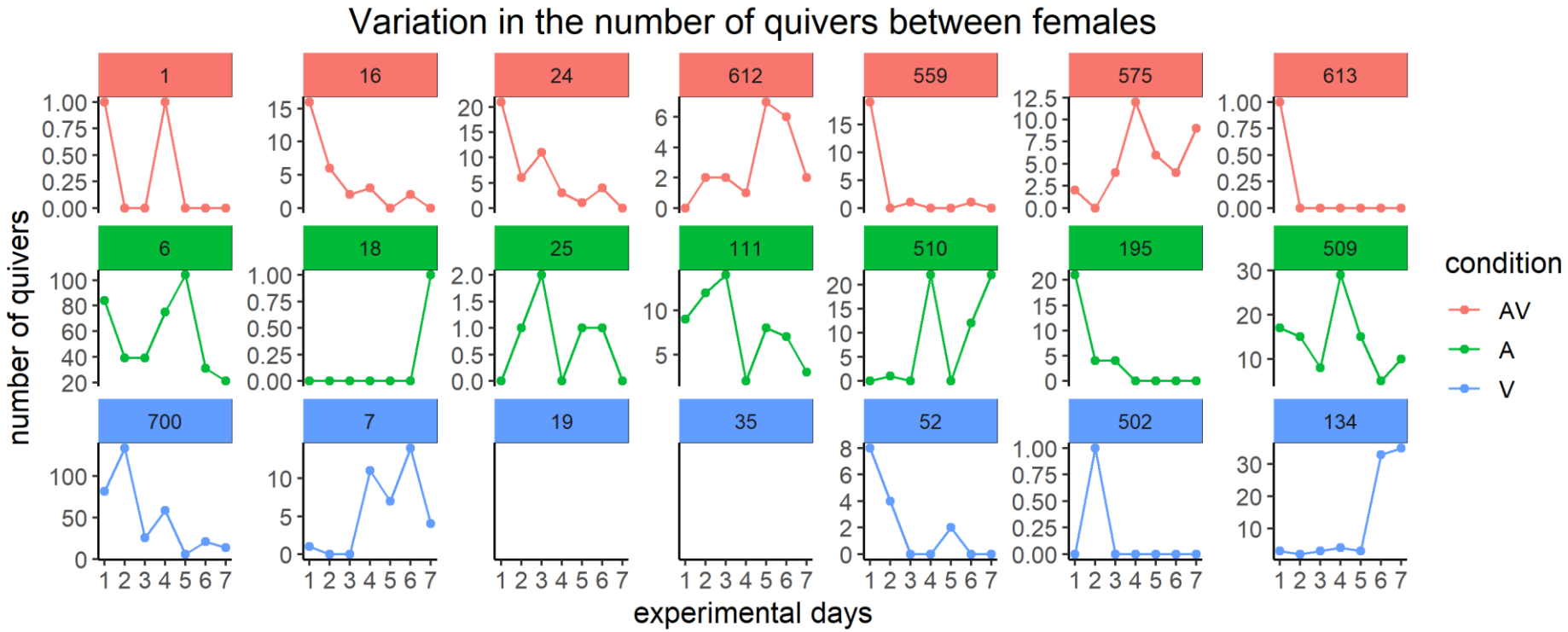

Figure 9: Variation in the number of quivers over experimental days by female identity. Each panel represents the number of quivers by a different female (ring number indicated above panel) over seven days. Plots also include zero values from females that quivered at least once; females 19 and 35 did not quiver during the whole duration of the experiment.

In a two-part model approach, we first tested the effect of condition on the presence or absence of quivering over experimental days (**Figure 10**). The presence-absence model was selected based on a comparison of a full model including the interaction of condition and experimental day to a reduced model lacking the fixed effect condition. The full-reduced model comparison suggests that condition has no measurable effect on the presence or absence of quivers on a given experimental day (χ^2 = 2.911, df = 4, p = 0.573).

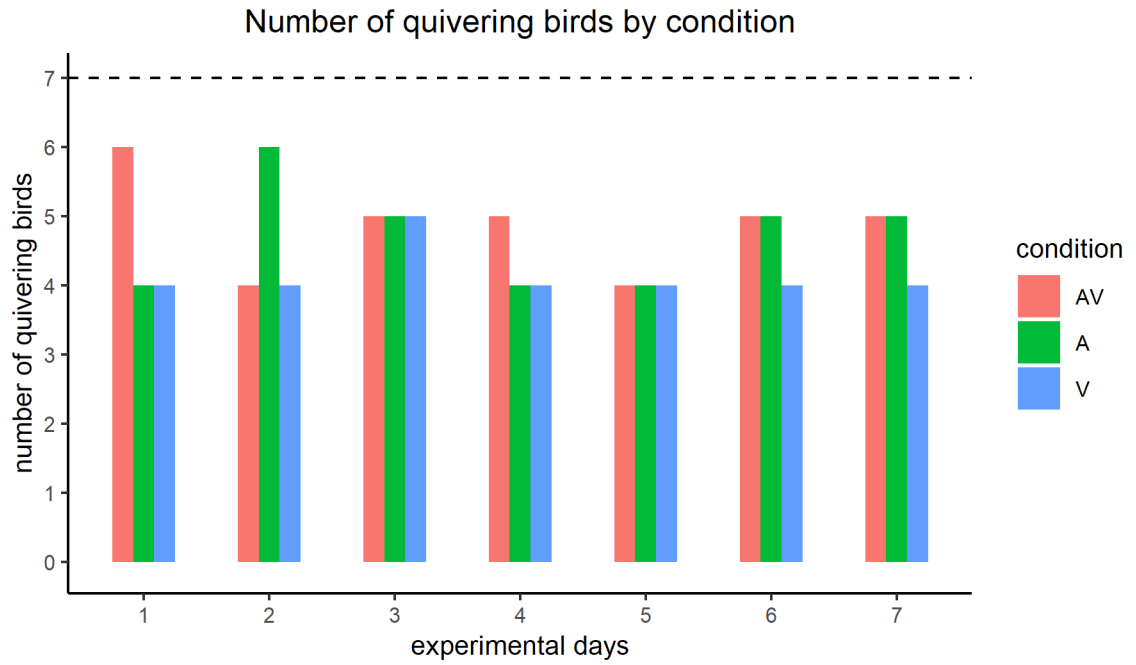

Figure 10: Number of birds that quivered on a given experimental day, divided by playback condition. Differently coloured bars indicate different conditions. The dashed horizontal line represents the maximum number of birds assigned to each condition (n = 7).

In a second step, we investigated how condition affects the number of quivers after excluding the zero values from the count data. The results from a full-reduced model comparison suggest that, if a female produced quivers on a given day, the number of quivers was not impacted by the playback condition that the female was assigned to (likelihood ratio test, χ^2 = 5.418, df = 4, p = 0.247). Additional model comparisons (see **Table A2** and **A3** in the Appendix) revealed that the model that explains the data best is a null model (lacking the fixed effects condition and experimental day) that allows the slopes of the relationship of the number of quivers and experimental day (i.e. the number of quivers can increase, decrease or stay stable over experimental days) as well as intercepts (i.e. the average number of quivers over days) to vary by female identity (**Figure 11**). The null model had the following structure:

$$Number\ of\ quivers \sim (1 + experimental\ day|femaleNr)$$

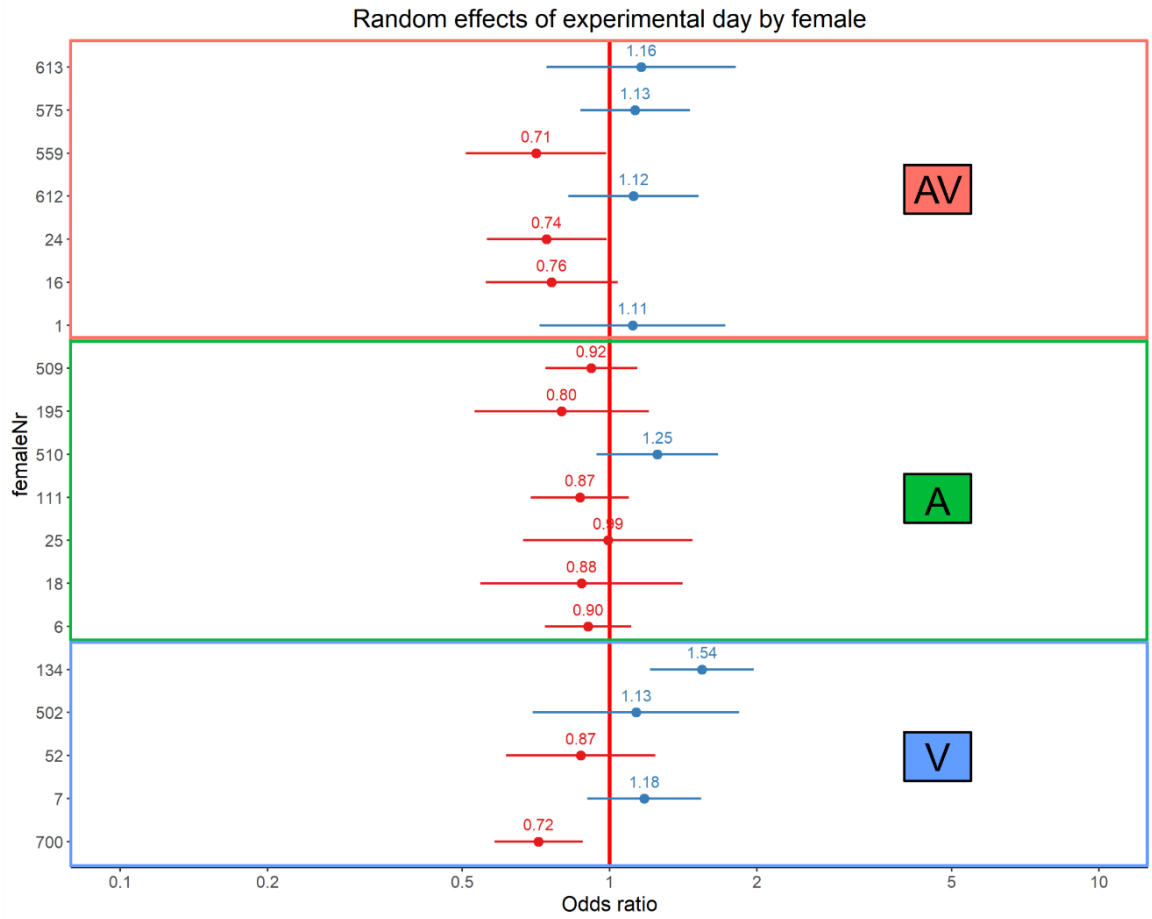

Figure 11: Forest plot displaying the by-female random effect slopes of experimental day from the null model. Numbers over 1 on the odds ratio scale indicate that the number of quivers increases (blue) and numbers under 1 on the odds ratio scale indicate that the number of quivers decreases (red) over experimental days for a specific female. Horizontal lines represent the error bars of an estimate; if the line crosses the value 1, then the effect of experimental day (increase/decrease) is not significant. Differently coloured panels represent the different playback conditions that the females were assigned to.

Since no measurable effect of condition on the number of quivers over experimental days could be confirmed, I summed the number of quivers for each female over seven days (yielding 21 values) and investigated whether condition would influence the total number of quivers performed over the whole experimental period rather than individual days (**Figure 12**). Model comparison of the full model to the reduced model supported that condition has no measurable effect on the total number of quivers produced by a female (Likelihood ratio test, Likelihood ratio test statistic = 2.801, df = 2, p = 0.246). A Kruskal-Wallis rank sum test further confirmed that condition doesn't affect the total number of quivers per female (χ^2 = 1.925, df = 2, p = 0.382).

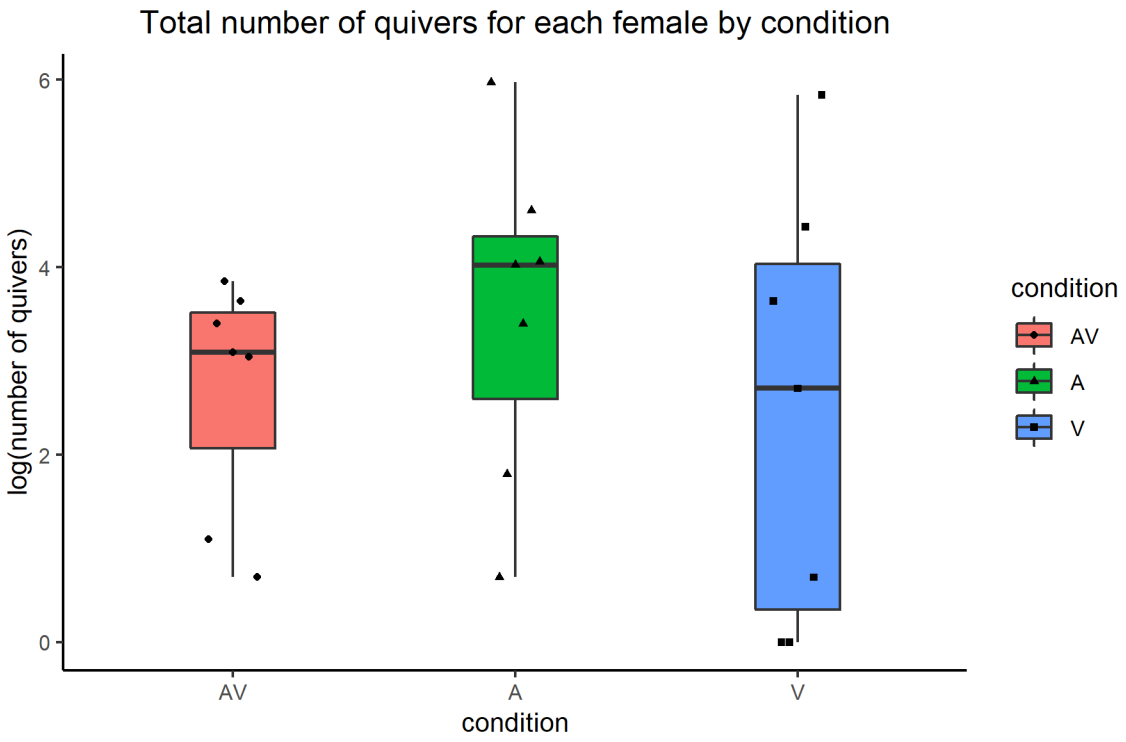

Figure 12: Summed number of quivers by female and condition. Each datapoint represents the log-transformed total number of quivers performed by one female over seven days (7 values in each condition). See **Figure A9** in the Appendix for the total number of tail and wing quivers depicted separately.

In contrast to the number of quivers, the average duration of quivers was the highest in the AV (group median = 46.030s, group mean = 54.870s, SD = 29.919, N = 7), followed by the A (group median = 24.133s, group mean = 39.719s , SD = 60.303, N = 7) and finally V condition (group median = 5.233s, group mean = 102.655s, SD = 219.237, N = 7) (**Figure 15**). Similarly, to the number of quivers, the average duration of quivers by condition seemed to be relatively stable over experimental days, but how the duration of quivers varied with progressing experimental days appeared to depend strongly on female identity (**Figure 13**).

Modelling of the duration of quivers was beyond the scope of this thesis because our models had convergence issues, but visual inspection of the descriptive plots **Figure 14** and **Figure 15** reveals that an effect of condition on the duration of quivering cannot be ruled out. A Kruskal-Wallis rank sum test suggests however that playback condition had no measurable effect on the total duration of quivering by female (χ^2= 3.572, df = 2, p = 0.168).

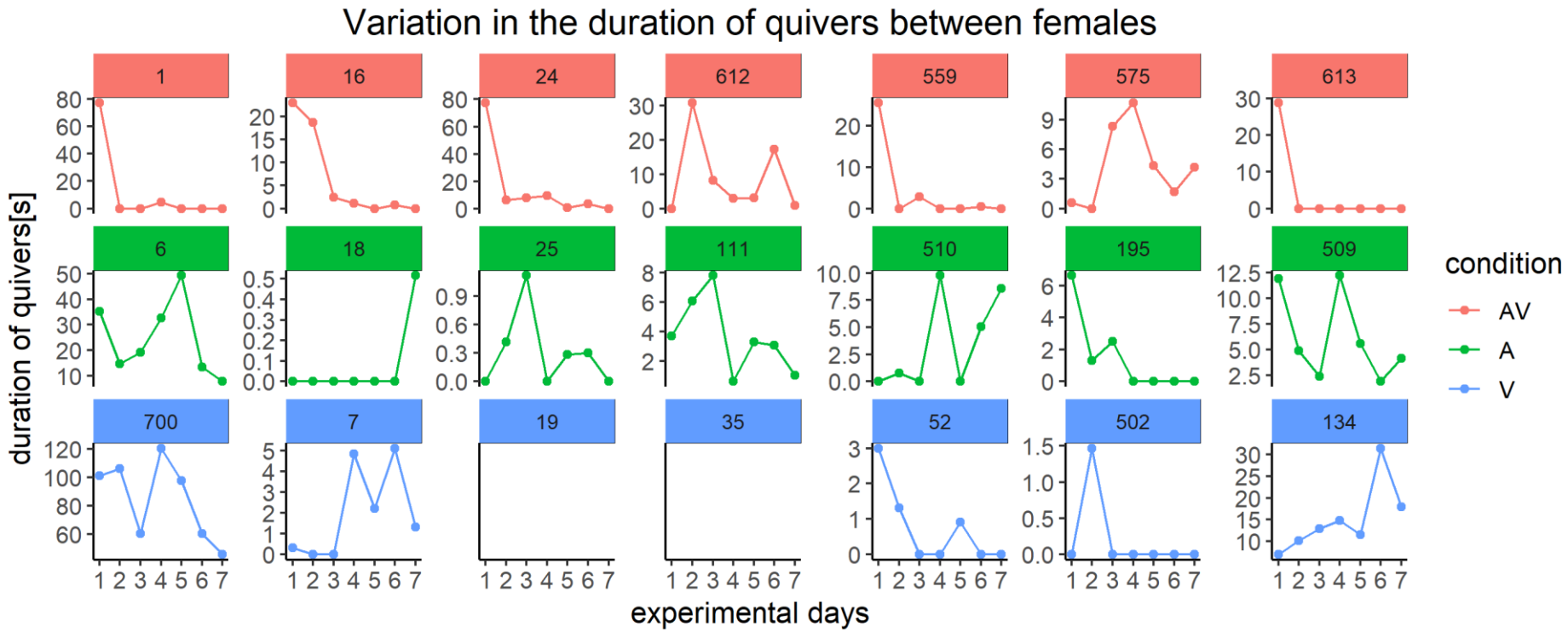

Figure 13: Variation in the duration of quivers over experimental days by female identity. Each panel represents the duration of quivers by a different female (ring number indicated above panel) over seven experimental days. Plots also include zero values from females that quivered at least once; females 19 and 35 did not quiver during the whole duration of the experiment.

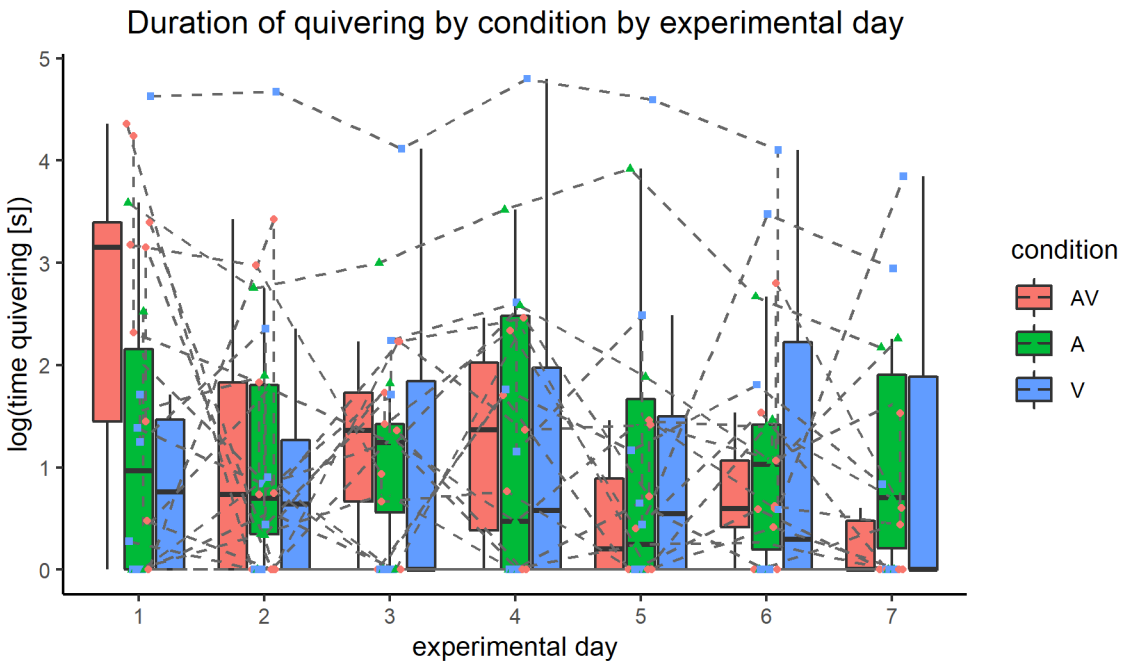

Figure 14: Duration of quivering by condition over seven experimental days. Each datapoint represent log-transformed duration (in seconds) of quivers performed by one female on a given day. Connected datapoints represent the same female. See **Figure A10** in the Appendix for the duration of quivers over experimental days separated into tail and wing quivers.

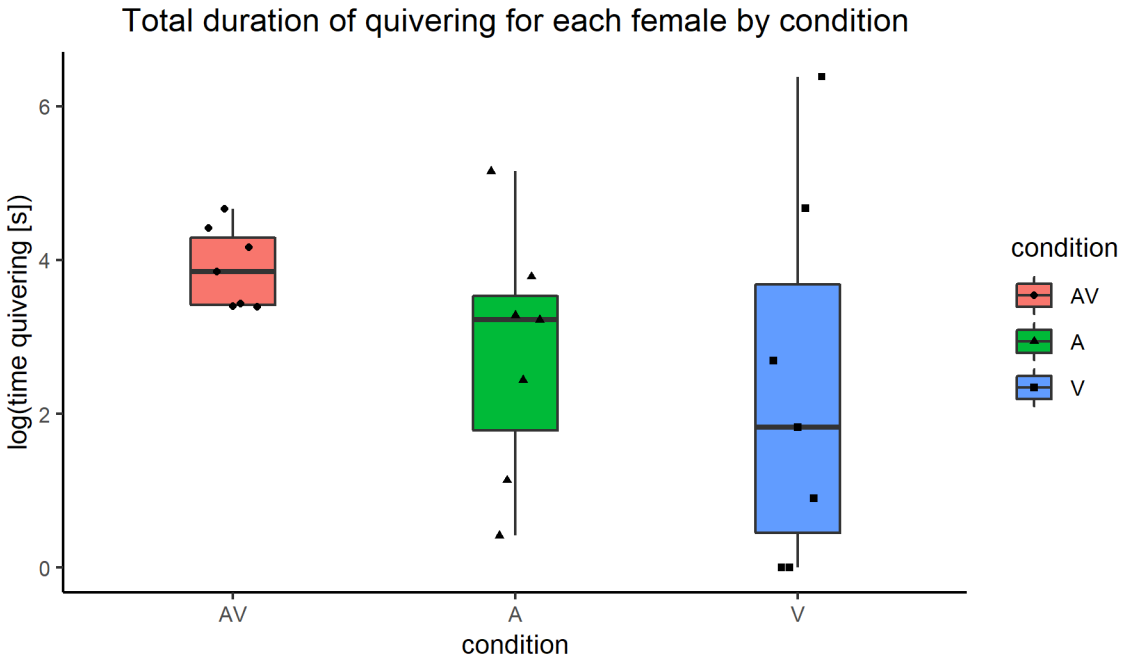

Figure 15: Summed duration of quivering by female in each condition. Each datapoint represents the log-transformed total duration of quivering performed by one female over seven days. See **Figure A11** in the Appendix for the total duration of tail and wing quivering depicted separately.

Interestingly, the duration of individual quiver events rarely surpasses 1 second in the A (11.11% of quivers > 1s, median = 0.417s, mean = 0.435s, SD = 0.223, N = 7) and V (10.9% of quivers > 1s, median = 0.367s, mean = 1.507s, SD = 5.303, N = 7) condition, but almost half of the quivers are longer than 1 second in the AV condition (45.51% of quivers > 1s, median = 0.883s, mean = 2.462s, SD = 7.740, N = 7) (**Figure 16**).

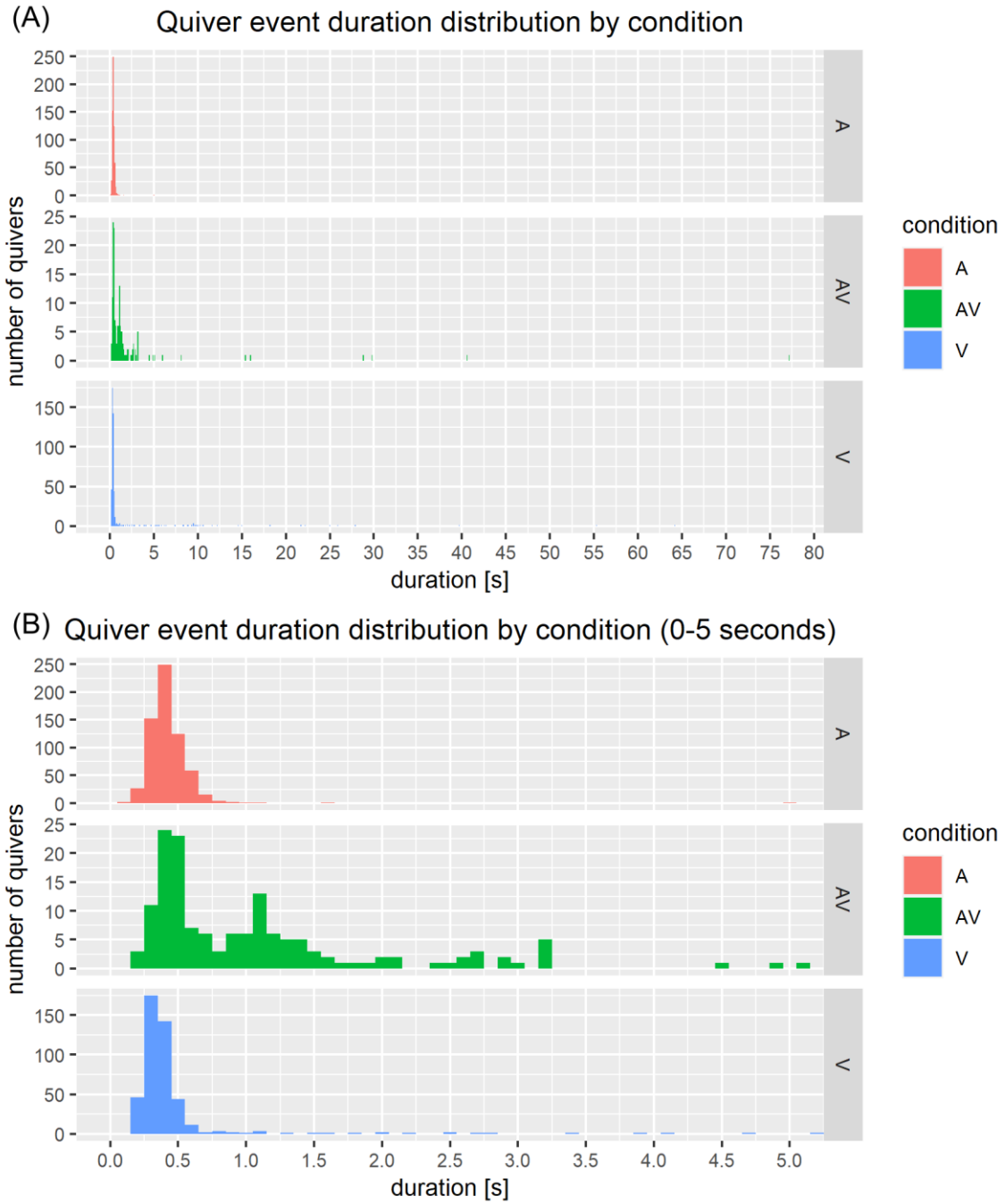

Figure 16: (A) Distribution histogram of the duration (in seconds) of individual quiver events by condition. (B) Zoomed in quiver duration histogram reaching from 0-5 seconds. Note the difference in y-axis scales between conditions.

II. Wing flips

For the analysis of wing flips, I decided against modelling the number and duration by condition as a very small number of females from the sample (7 out of 21) performed any wing flips at all, resulting in an excess of zero values (86.39%) in the data. As for the analysis of quivers by condition, both wing flips happening during playback intervals and during playback breaks were analysed across conditions.

Almost all of the recorded wing flips were produced by four females from the V condition (92.32% of the total number of wing flips, mean = 21.19, SD = 70.475, N = 7). Only one female from the AV condition (6.43% of the total number of wing flips, mean = 1.48, SD = 6.765, N = 7) and two females from the A condition (1.24% of the total number of wing flips, mean = 0.29, SD = 1.102, N = 7) produced the remaining wing flips. Statistical testing via a Kruskal-Wallis rank sum test indicates that there is no detectable effect of playback condition on the total number of quivers performed by a female (χ^2 = 3.922, df = 2, p = 0.141). In the AV condition, wing flips were only observed during the last three experimental days, whereas in the A and V condition no clear behavioural pattern could be observed throughout the experimental week (**Figure 17A**).

Mean wing flip duration over the whole experimental period was the highest in the V condition with 86.8 seconds (SD = 255.426, N = 7), followed by the AV condition with 30.27 seconds (SD = 138.713, N = 7) and finally the A condition with a very low mean duration of 0.71 seconds (SD = 3.088, N = 7). As for the number of wing flips, no effect of condition on the total duration of wing flipping by female could be confirmed (χ^2= 3.705, df = 2, p = 0.157) (**Figure 17B**).

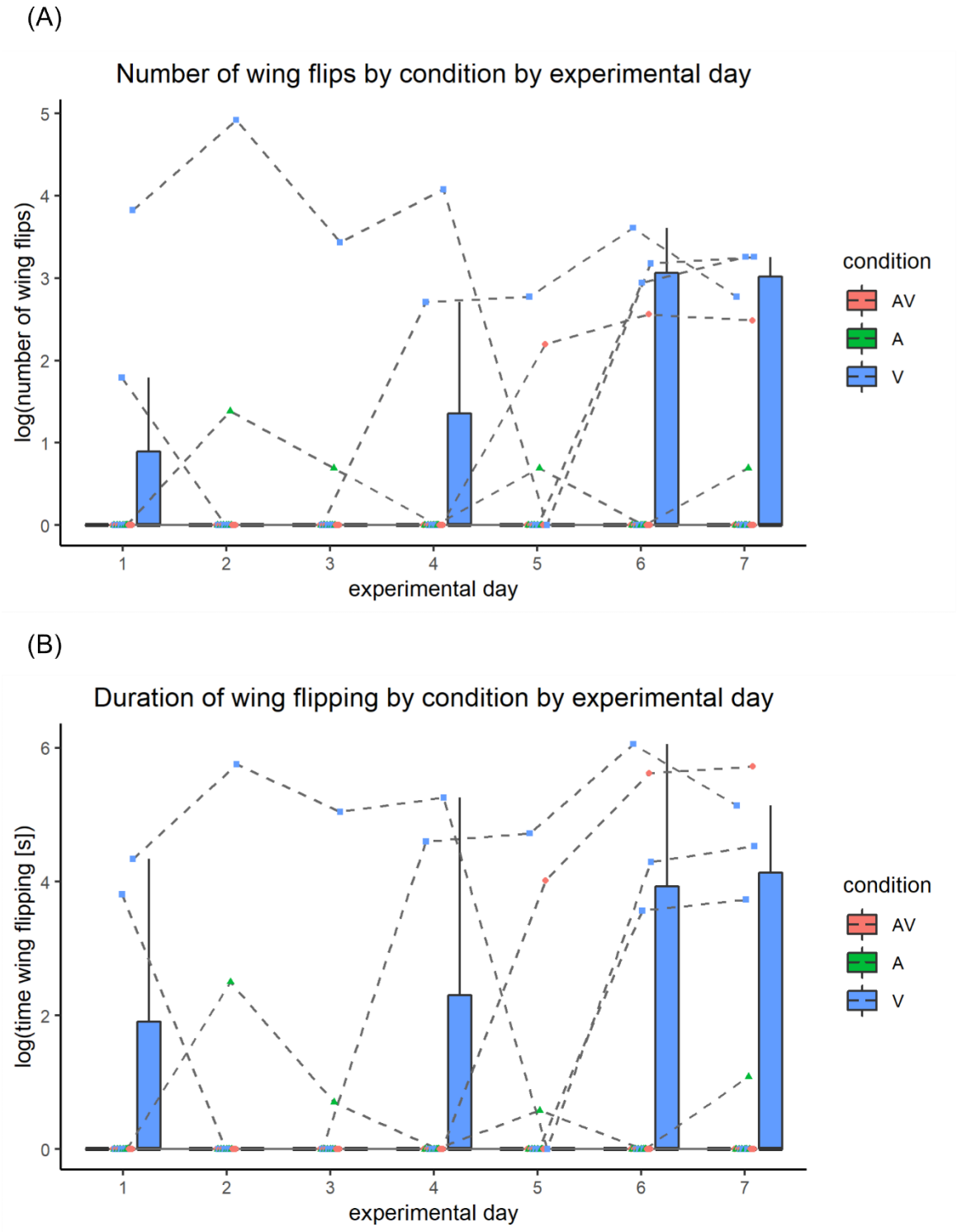

Figure 17: Number (A) and duration (B) of wing flips by condition over seven experimental days. Each datapoint represent log-transformed number (A) or duration (B) of wing flips performed by one female on a given day. Connected datapoints represent the same female.

3.2.3. Link between female cooing and quivering behaviours

In a next step, I investigated whether female quivers co-occur with coo vocalisations, and if so, during which type of coo (nest coo or perch coo) they are produced. Out of a total of 1272 female quivers, 425 were produced simultaneously with nest coos (33.42% of all cases) and 632 simultaneously with perch coos (49.69% of all cases). According to our definition (see Materials & Methods, page 26), 215 quivers occurred independently from coos (16.90% of all the cases).

Previous results from D. Biegler's thesis show that nest coos were predominantly recorded in the unimodal video condition and perch coos mostly in the unimodal audio condition (Biegler, 2021) (**Figure 18**). The results from my quiver-coo analysis are consistent with these findings: quivers co-occurring with nest coos are produced most frequently in the visual condition (96.00%, 337 out of 425 "nest coo-quivers"), while quivers co-occurring with perch coos dominate in the audio condition (97.31%, 615 out of 632 "perch coo-quivers"). Quivers occurring on their own ("silent-quivers") are produced in all three conditions with the highest number in the AV (58.14%, 125 out of 215 "silent-quivers") followed by the V (30.70%, 66 out of 215 "silent-quivers") and A (11.16%, 24 out of 215 "silent-quivers") condition. Overall, almost each type of quiver-coo was represented in each condition. However, there were no nest coo-quivers in the A condition, which is logical since no nest coos were observed in the said condition (**Table 2**, **Figure 19**).

Individual quivers produced in combination with coos present a very different duration to those occurring independently from coos. While none of the perch coo-quivers (median = 0.417s, mean = 0.422s, SD = 0.111) and only 0.58% of the nest coo-quivers (median = 0.350s, mean = 0.364s, SD = 0.101) were longer than 1 second, approximately 58% of "silent"-quivers surpassed this duration (median = 1.117s, mean = 4.460s, SD = 9.762).

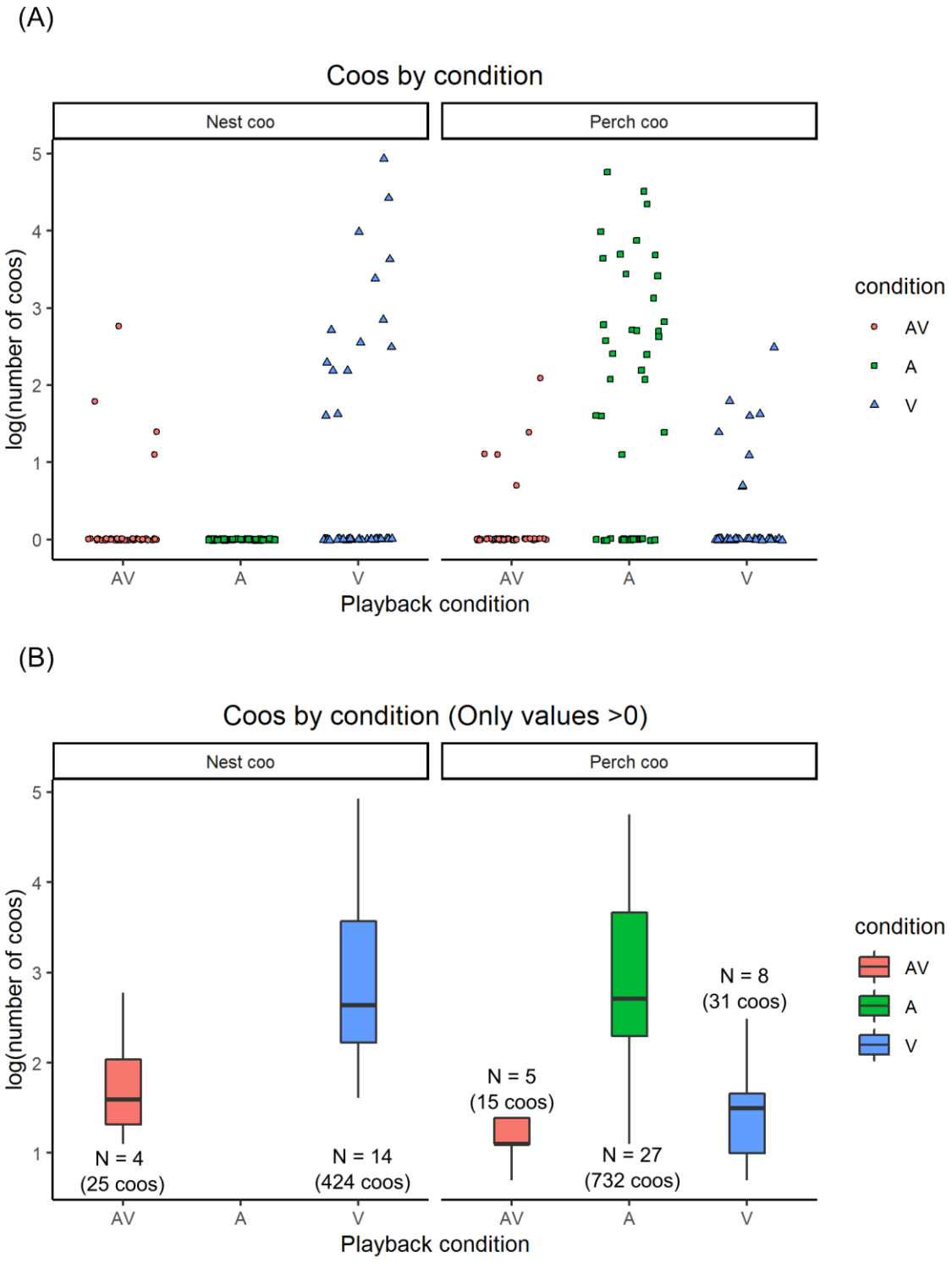

Figure 18: Number of coos produced during the whole experimental period separated by coo type and playback condition. (A) Scatter plot showing total number of coos produced by female and day (N = 147). Each datapoint represents the log-transformed number of coos that were produced by one female during one experimental session. (B) Boxplots displaying the median and upper and lower quartiles of the number of coos produced in each condition (excluding zero values). The total number of samples (N: female-day combinations) and the number of coos produced in each condition is indicated at the bottom of the boxplot.

Table 2: Total number of quiver types that were produced by each female over the whole duration of the experiment. Bold numbers indicate the predominant type of quivers produced in one condition.

Female Nr°	Condition	Nest coo	Perch coo	Silent
1	AV	0	0	**2**
16	AV	2	0	**27**
24	AV	0	0	**46**
612	AV	11	0	**9**
559	AV	0	8	**13**
575	AV	0	6	**31**
613	AV	0	0	**1**
6	A	0	**374**	19
18	A	0	**0**	1
25	A	0	**0**	5
111	A	0	**52**	3
510	A	0	**38**	19
195	A	0	**29**	0
509	A	0	**92**	7
700	V	**236**	0	106
7	V	**33**	1	3
19	V	**0**	0	0
35	V	**0**	0	0
52	V	**12**	2	0
502	V	**0**	0	1
134	V	**56**	0	27

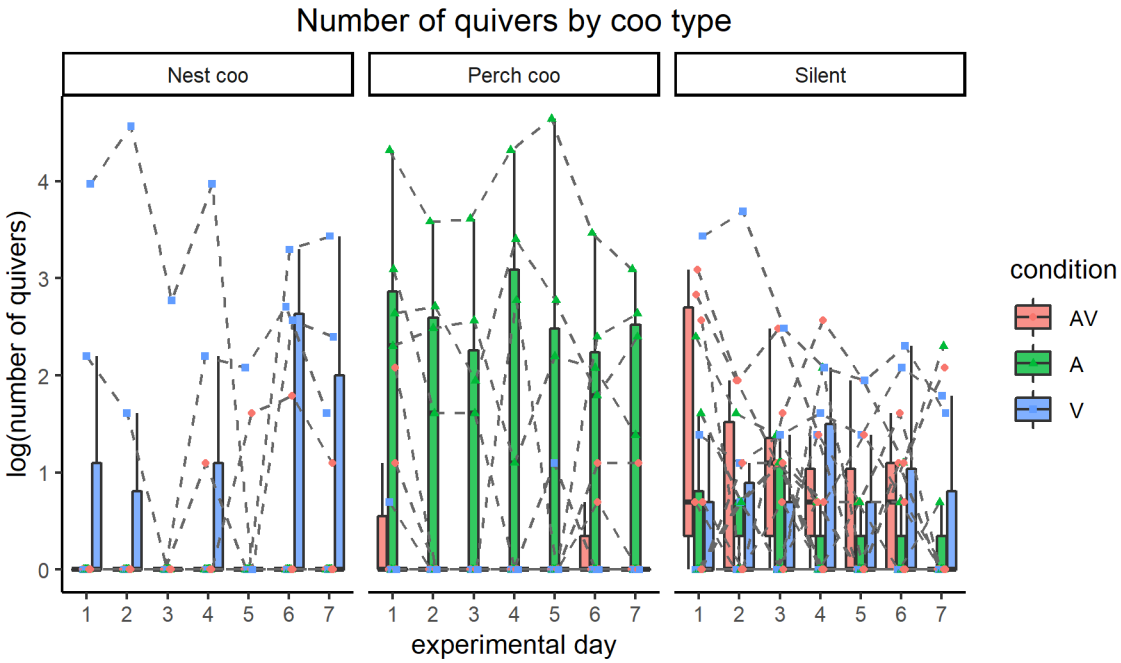

Figure 19: Number of quivers that are produced coincidentally with nest coos or perch coos, or occur independently from coos ("silent"). Each datapoint represents the log-transformed number of quivers performed by one female on a given day. Connected datapoints represent the same female.

Next, we investigated how condition affects the number of quivers that were produced on their own ("silent"-quivers). A full-reduced model comparison suggests that the total number of "silent"-quivers by a female on a given day (including zero values) was not measurably influenced by playback condition (likelihood ratio test, $\chi^2 = 6.692$, df = 4, p = 0.153). As a follow-up question, I investigated whether condition would influence the summed number of "silent"-quivers by each female over the whole duration of the experiment (including the females that didn't produce any "silent"-quivers). Once more, no measurable effect of condition on the total number of quivers by female could be confirmed (Likelihood ratio test, Likelihood ratio test statistic = 4.448, df = 2, p = 0.108). This was further supported by a Kruskal Wallis rank-sum test ($\chi^2 = 2.572$, df = 2, p = 0.276).

In a supplementary step, I assessed in a reverse way whether quivering is an obligatory by-product of cooing. This is clearly not the case, as out of a total of 1227 coos produced during the whole experimental period only 282 (22.98% of the total number of coos) were coincident with a quiver, while the other 945 coos (77.02% of the total number of coos) occurred independently of quivers.

3.2.4. Link between female cooing and wing flipping behaviours

As nest coos and wing flips are often described as being performed coincidentally during the nest solicitation display in female ring doves (Cheng, 1973; Lovari and Hutchison, 1975; Miller and Miller, 1958), I investigated whether wing flips and coos (specifically nest coos) occurred in a closely spaced temporal sequence, one relative to the other, during the experiment. Indeed, most wing flips occurred either during or immediately before or after a nest coo (66.60%, 321 out of 482 wing flips) and only very few coincidentally with a perch coo (0.41%, 2 out of 482 wing flips). However, according to our definition (see Materials & Methods, page 27), a large proportion of wing flips also happened independently of coos (32.99%, 159 out of 482 wing flips) (**Figure 20**).

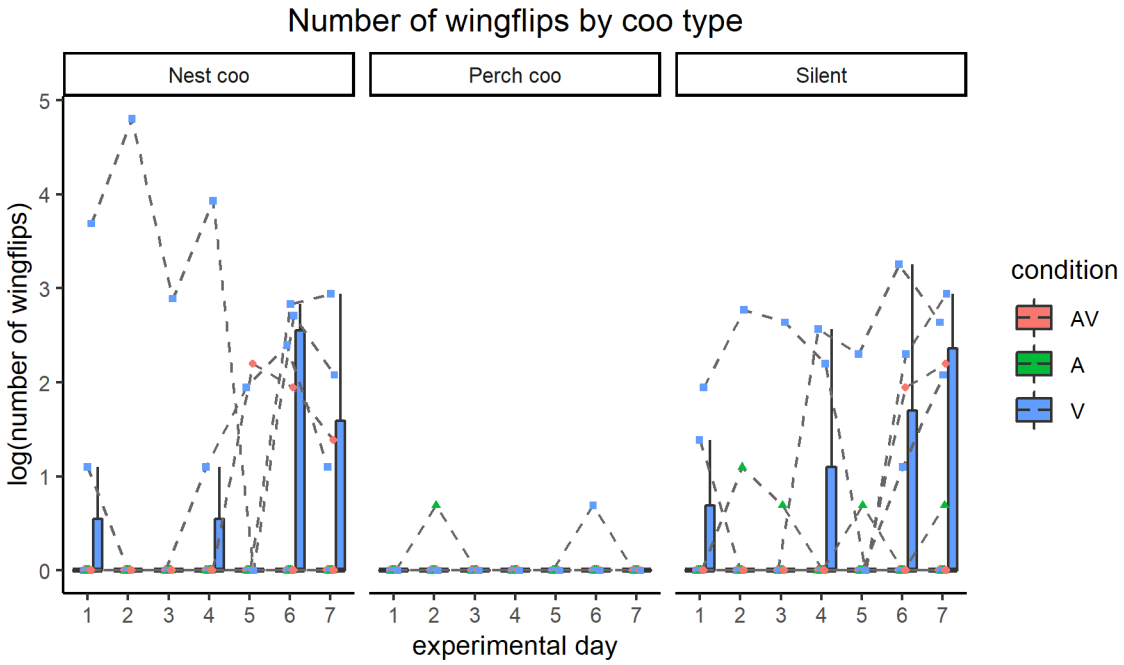

Figure 20: Number of wing flips that are produced coincidentally with nest coos or perch coos or occur independently of coos ("Silent"). Each datapoint represents the log-transformed number of wing flips performed by one female on a given day. Connected datapoints represent the same female.

To assess whether wing flips are exclusively produced on days on which a dove is cooing, we compared the number of wing flips occurring on their own to the number of wing flips occurring in conjunction with either nest- or perch coos on a given experimental day. Out of the 20 days on which wing flips were produced by seven females, on 19 days wing flipping occurred on the same day as cooing (95% of days on which wing flips were produced). Wing flips were produced without cooing only once. Wing flips occurred in total on 16 out of the 18 days (88.89%) during which nest coos were produced by a female. On

the contrary, they occurred only on 6 out of the 40 days (15%) during which perch coos were produced by a female.

4. Discussion

The main aim of this study was to investigate how different components of male multimodal courtship signals affect female behavioural responses related to sexual stimulation. 21 females were assigned to three playback conditions (audio-visual, audio and visual), and quiver and wing flip behaviours were scored for a period of seven consecutive days of exposure to male courtship playback. We expected that females would display a higher frequency and longer duration of sexual behaviours when they were exposed to male courtship playback rather than courtship playback breaks. Additionally, we predicted that exposure to a combination of auditory and visual male courtship playback components would elicit an enhancement of the female quivering response, compared to exposure to unimodal auditory or visual components only. As an alternative outcome, we suggested that female quivering might be mainly driven by the auditory component of male courtship, which would result in a similar quivering response in the audio and audio-visual condition.

Against our predictions, female sexual behaviours of interest, with the exception of the duration of wing flipping, were not impacted by the presence or absence of male courtship playback. This finding is in contradiction with previous results from Mitoyen et al. (submitted) that showed, that females performed more tail quivers when they were exposed to audio-visual playback of male courtship compared to videos of an empty cage. Moreover, the playback condition that the females were assigned to, had no measurable effect on the presence and absence nor number of quivers performed by the females. Overall, the average number of quivers did not increase over experimental days with repeated exposure to male courtship, further confirming that female quivering is an immediate rather than a delayed response to male courtship (Mitoyen et al., submitted). Analysis of the temporal coincidence of quivers and coos revealed that quiver displays are not only a simple by-product of female vocalisations since quivers and coos can occur independently of each other. Interestingly, quivers co-occurring with coos were almost entirely produced in the unimodal conditions, with nest coo-quivers being most prominent in the visual (V) condition and perch coo-quivers dominating in the audio (A) condition. We couldn't confirm however, that the number of quivers occurring independently from coos ("silent"-quivers) was impacted by playback condition, even though it was numerically the highest in the audio-visual (AV) condition. Wing flips were almost exclusively observed in females from the V condition, and they mostly occurred temporally interleaved with nest coos but were also often produced independently from coos. Additionally, analysis of the

daily co-occurrence of wing flips and coos revealed that if there were wing flips on a day, they almost always co-occurred with coos (in particular nest coos) on the same day.

In zebra finches and canaries, tail (and wing) quivering acts as a mate solicitation display showing the willingness of the female to copulate with a male (Amy et al., 2015; Witte, 2006). To my knowledge, literature on quivering behaviours in dove species is quite rare, which might be related to the inconspicuousness of these small vibratory movements. A recent study from the dove lab in Vienna suggests however that female tail quivering is a sign of sexual interest in ring doves, as this behaviour was only shown in the presence of a male. Moreover, the females in that experiment reacted with producing more tail quivers in response to a courting compared to a non-courting male (Mitoyen et al., 2021). Assuming that tail quivers are an immediate behavioural reaction to male courtship, one would expect a similar behavioural response of female ring doves exposed to audio-visual playback courtship clips of males. This assumption was supported by a follow-up playback study by Mitoyen et al. (submitted), showing that the frequency of tail quivering in female doves was higher when females were exposed to audio-visual courtship playback compared to control videos showing the empty set-up. In the present study however, a differential effect of courtship playback material on the female quivering response couldn't be confirmed, although both the number and duration of quivers was slightly higher during stimulus intervals than during playback breaks. One possible explanation for the absence of the expected effect of male courtship on female quivering might be that the experimental design of this study was inappropriate to answer our question. It is very likely that the switch from "playback on" to "playback off" intervals was too sudden or the "control" breaks in between stimulus intervals were too short (40s) for the focal subject to return to its baseline behavioural state. For instance, a female dove assigned to the visual condition performing tail quivers facing away from the monitor might fail to react if the male playback video is switched off. Alternatively, female quivering in comparison to other sexual behaviours (e.g. sex crouching or nest cooing) might be the result of continuous sexual arousal after first presentation of a courting male, rather than a sexual signal specifically directed to the male.

In ring doves, wing flips (or wing flutters) can be produced by both sexes and usually occur in combination with nest cooing during the nest soliciting display (Cheng, 1973; Lovari and Hutchison, 1975; Miller and Miller, 1958). Generally, it can be assumed that a female dove performing the aforementioned behavioural patterns after repeated exposure to a mate is in an advanced physiological state (Cheng, 1979, 1973). In our

study, only 7 out of 21 doves performed wing flips, and the total duration of wing flip bouts was significantly higher when the doves were directly exposed to male playback compared to playback breaks. The number of wing flip bouts on the other hand wasn't impacted by the immediate presence of male courtship, despite the average number of wing flips being higher during stimulus intervals compared to playback breaks. In fact, coding wing flips as a bout rather than an event might be the cause for the absence of a measurable effect of courtship playback on the number of wing flips. Nevertheless, our results indicate that wing flips are likely to be a direct behavioural response to male courtship and can therefore be considered as sign of sexual stimulation.

Overall, quivering was observed in each of the three playback conditions (audio-visual, audio and visual), and no measurable effect of condition on the presence or absence nor the number of quivers over experimental days could be confirmed. According to the "Back-up signal hypothesis", a similar behavioural reaction by the receiver in the A and V condition indicates that male courtship signal components from the auditory and visual channels carry redundant information. Against our prediction, we were not able to provide support for the hypothesis that a combination of male auditory and visual courtship signal components leads to an enhancement effect of the behavioural response by the female. Each female from the AV and A condition produced at least one quiver, while only 5 out of 7 females from the V condition produced quivers. Additionally, the average number of quivers was the highest in the A, followed by the AV condition and the lowest in the V condition. Modelling the effect of condition on the duration of quivers was unfortunately beyond the scope of this thesis, but a Kruskal Wallis test suggests that there is no difference in the duration of quivering between females. However, the average duration of female quivers was the longest in the AV condition, followed by the A and finally the V condition. All in all, these results indicate that there was potentially a similar quivering response in the A and AV condition, with a higher number of short quivers produced in the A condition and a smaller number of longer quivers occurring in the AV condition. However, it seems that the visual component of male courtship alone might be more ineffective in eliciting the quivering response. This would suggest that the auditory and visual components of male courtship carry non-redundant information, leading to a differential behavioural response of the receiver ("Multiple message hypothesis"). The high number and duration of wing flips observed in the V, compared to the A and AV condition further supports the hypothesis that different components of multimodal courtship signals might carry differential information to the receiver. I am however unable to provide statistical support with my data for either of the two hypotheses ("Back-up signal hypothesis" vs

“Multiple message hypothesis”) due to the large variation introduced by individual differences between test subjects.

In fact, both the number and duration of quivers and wing flips performed over experimental days was strongly influenced by inter-individual variation between females. Additionally, exploratory model comparisons revealed that female identity was the only variable that impacted how the number and duration of quivers (and maybe wing flips) changed with repeated exposure to the stimulus male. These results are consistent with previous results of a behavioural study in ring doves, showing that a large proportion of the variation in the response to different courtship parameters (e.g. fundamental frequency of the male call) could be accounted for by female identity (Mitoyen et al., 2021). This finding combined with the result, that some doves performed behaviours which are characteristic for later stages of courtship (i.e. wing flips and nest coos) during the first two experimental days suggests that they were already in an advanced reproductive state before the start of the experiment. This could potentially also be the reason why Biegler (2021) could not find a significant increase in oestradiol levels after seven days of exposure to male courtship compared to pre-experimental levels. Even though females were isolated for 5-6 days prior to testing to return them to a baseline hormonal state, five females laid eggs a few days before the experiment, suggesting that they were in a sexually advanced stage. As described in (Biegler, 2021), these eggs were however immediately removed and the start of the experiment including blood sampling was delayed for three days, allowing these females to return to their baseline hormonal states (as described in Cuthbert (1945)). Analysis of the faecal samples taken daily during the experimental period might give more information on the hormonal state of different females in the future.

In the present study, we showed that (tail) quivering is not necessarily only a by-product of the vibration produced when a dove vocalises. Likewise, Mitoyen et al. (submitted) observed almost no female coos at all, but found that tail quivering behaviour was nevertheless quite common in response to audio-visual male courtship playback. In the present experiment, individual quivers seem to vary a lot in their duration and style of execution, especially between females. During scoring, I observed that quivers could potentially be sub-divided into multiple groups depending on if one type of quivers was produced on their own (either tail or wing quivers alone; often of a duration of <1second) or if quivers consisted of a mixture of wing and tail quivers performed at the same time e.g. the dove takes on a particular posture resembling a sex crouch and slightly extends its wings, which vibrate continuously while in this particular posture; tail quivers

(sometimes alternating with wing flips) can be observed in the same time interval. Moreover, the duration and type of quivers might also be influenced by whether they were coincident with coos or not. In future experiments, the structure of quivers should be examined more closely, and an ethogram definition should be elaborated for each type of quiver.

Indeed, quivers co-occurring with nest- and perch coos were generally very short (<1s) compared to quivers produced on their own, which were in more than half of the cases longer than 1 second and presented more variability in their style of execution. As already reported by (Biegler, 2021), nest coos were observed more frequently in the V condition, while perch coos clearly dominated in the A condition. On the contrary, coos were very rarely observed in the AV condition. Therefore, it makes sense that nest-coo quivers were also more common in the V condition, whereas perch-coo quivers happened mostly in the A condition. In summary, it seems as if the unimodal audio and visual playback components of male courtship respectively might lack an important feature of courtship to fully stimulate the female, which she tries to compensate for by producing different types of coos. According to my colleague D. Biegler (Biegler, 2021), the frequency of perch cooing in the A condition might be an attempt by the female to direct the male to her location due to the lack of the visual presence of the male (Davies, 1974), while the frequency of nest coos in V condition might serve to compensate for the lack of male nest coos that generally induce ovarian development in the female (Cheng, 1986; Lehrman, 1964).

In summary, our results indicate that multimodal playback of male courtship can be used to successfully elicit sexual behaviours in ring doves. To fully understand how the immediate presence of courtship playback influences a behaviour such as quivering, a different experimental design might be more appropriate e.g. increasing the length of the courtship breaks, so the doves can return to a baseline behavioural state in between courtship intervals. Furthermore, some trends for the effect of playback condition on sexual behaviours could be observed, but it was unclear whether those trends were contingent on condition or simply on female variability. A within-subject study is needed to fully disentangle the effect of multimodal courtship signals on sexual responses, since different females are likely to present a different baseline for certain behaviours. Additionally, a courtship sequence analysis could be used to further investigate whether a lack of contingency during the “interaction” of the male and female is the cause for certain sexual behaviours in the female failing to increase after repeated exposure to male courtship. Analysis of other sexually relevant behaviours (e.g. sex crouching), female coos produced

in the isolation cages and recorded before and after daily experimental sessions, and hormonal values from the faecal samples may help to shed more light on how unimodal and multimodal components of courtship affected the female behavioural and physiological response. Finally, although automated tracking holds great potential for rapid quantification of animal behaviour, using manual scoring by a human coder revealed to be more efficient for quantification of the very subtle quivering behaviour analysed in the present thesis (Janisch et al., 2021). In future analysis, automated tracking of the female dove's head and shoulders from the top view camera videos (angle of the head direction relative to the shoulders) could be used to investigate whether the female is looking at the monitor during playback on/off and across conditions. All in all, both quivers and wing flips seem to reflect sexual stimulation in female ring doves, but these behaviours may vary in their information-content towards the male, as well as in their timing during the courtship cycle.

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Appendix

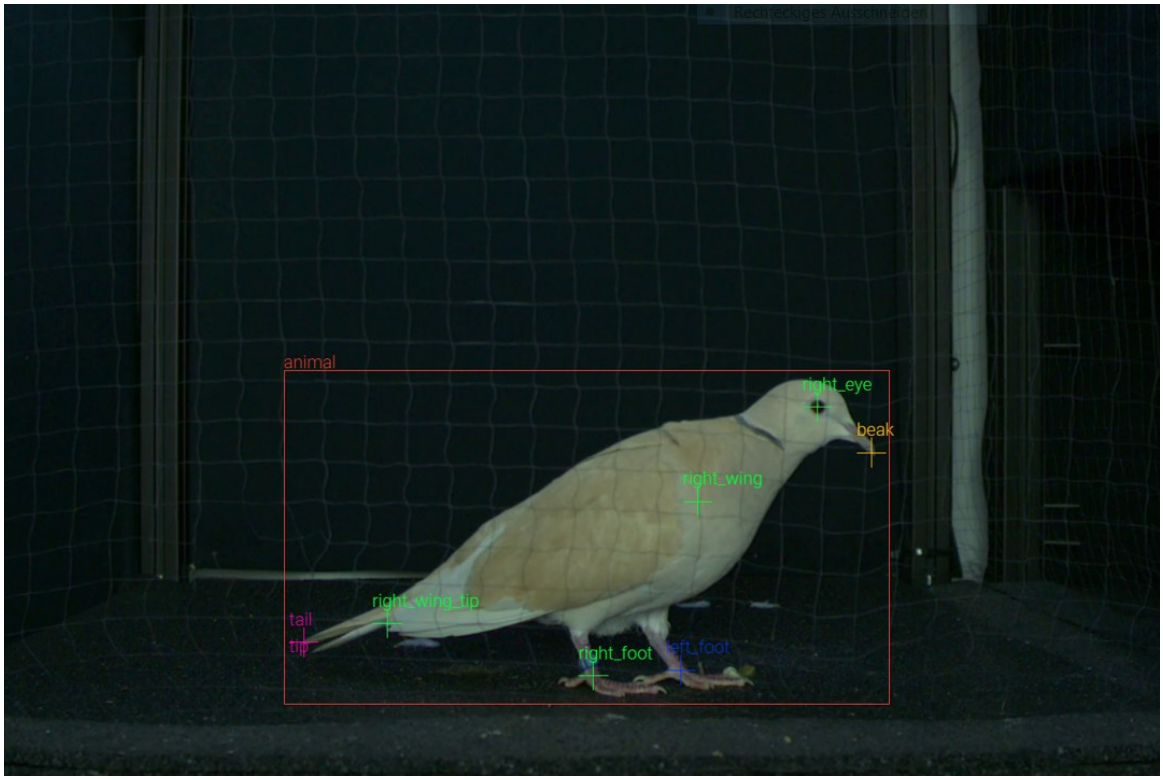

Figure A1: Example frame showing the keypoints that were used to train the automated “Tail-detector” model (left_eye, left_wing_crease and left_wing_tip not visible

(A) (B)

Figure A2.: Problems with misplaced keypoints during automated tracking. (A) Both eye_keypoints are tracked on the same eye because this particular dove has a different eye colour; annotations from this dove were added to train the final “Tail detector” model. (B) Issues of the final “Tail-detector” model to track the wing_tip keypoints. The dove is standing in a specific position during which the wing tips are placed in front of the similarly coloured tail.

Table A1: List of videos and the corresponding number of frames that were used for annotations to train the "Tail-detector" model. Other selection criteria such as the morphological type, step number and behaviours of interest are depicted for each video.

Morphological type	Ring Nr°	Day	Steps	Relevant Behav.	Frame Nr°	Step size
White	1	4	453	nest-coo pos.	40	1500
White	195	2	2063	intrusion cam.	41	1500
White	35	2	212		40	1500
Blonde	7	3	697	circle	42	1500
Blonde	25	5	825	preen	39	1500
Blonde	6	1	690		40	1500
Blonde with marbelish pattern	24	7	66	preen	39	1500
Blonde with marbelish pattern	24	2	35	preen	20	1000
Blonde with marbelish pattern	24	5	34	preen	16	1000
Light blonde (with white wings)	612	6	638	nest-coo pos.	41	1500
Light blonde (with white wings)	613	7	1457	circle	40	1500
Blonde with white tail	52	3	404	nest-coo pos.	41	1500
Blonde with white tail	52	4	277	nest-coo pos.	41	1500
White (very light blonde)	509	1	527	intrusion cam.	39	1500
White with dark spots	700	6	359	nest-coo pos.	40	1500
Reddish-whitish	134	1	587	intrusion mon.	39	1500
Between blonde and reddish	111	1	969	preen	41	1500
Dark Grey-Brown	502	7	49	preen	41	1500
Dark Grey-Brown	502	2	267	preen	11	1500
Dark Grey-Brown	502	2	267	preen	32	1500
Brown with white spots	510	5	94	preen	39	1500
Brown with white spots	510	2	79	preen	39	1500
Reddish with darker belly	599	4	228		41	1500
Reddish with darker belly	599	6	234	circle	39	1500
					TOTAL: 881	

Table A2: Generalized linear mixed model (GLMM) structures used for exploratory model comparison. Models are ordered from the lowest to the highest AIC (Akaike Information criterion). The AIC is used to choose a model that best fits the data, meaning that it explains the greatest amount of variation using the fewest number of independent variables. Lower AIC values indicate a better model fit; a difference in AIC of 2 units indicates a significantly better model fit. The log-likelihood (LogLik) is also a measure of goodness of fit; values closer to zero indicate a better fit of the model.

Model Nr°	Model structure	AIC	LogLik
1	{ null + (1 + e|f) }	585.01	-287.5
2	{ c + (1 + e|f) }	585.16	-285.58
3	{ e + (1 + e|f) }	586.51	-287.25
4	{ e + c + (1 + e|f) }	586.83	-285.41
5	{ e * c + (1 + e|f) }	589.09	-284.55
6	{ null + 1|f }	596.46	-295.23

Predictor variables include condition (c) and experimental day (e). Female number (f) was included as random effect variable.

Table A3: Pairwise GLMM model comparison results via likelihood ratio tests. The first column shows which two models were compared using the numbering in Table A2. Model comparison results are summarized by the chi-square statistic (Chisq), degrees of freedom (Df) and p-value for the test (P-value). Asterisks beside a given p-value indicate significance for the difference in fit between the two models (*: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$).

Model Comparison	Chisq	Df	P-value
5 x 3	54.178	4	0.247
5 x 6	21.363	7	0.003268 **
3 x 6	15.946	3	0.001163 **
1 x 6	15.451	2	0.0004415 ***
1 x 2	38.477	2	0.146
3 x 4	36.847	2	0.1584

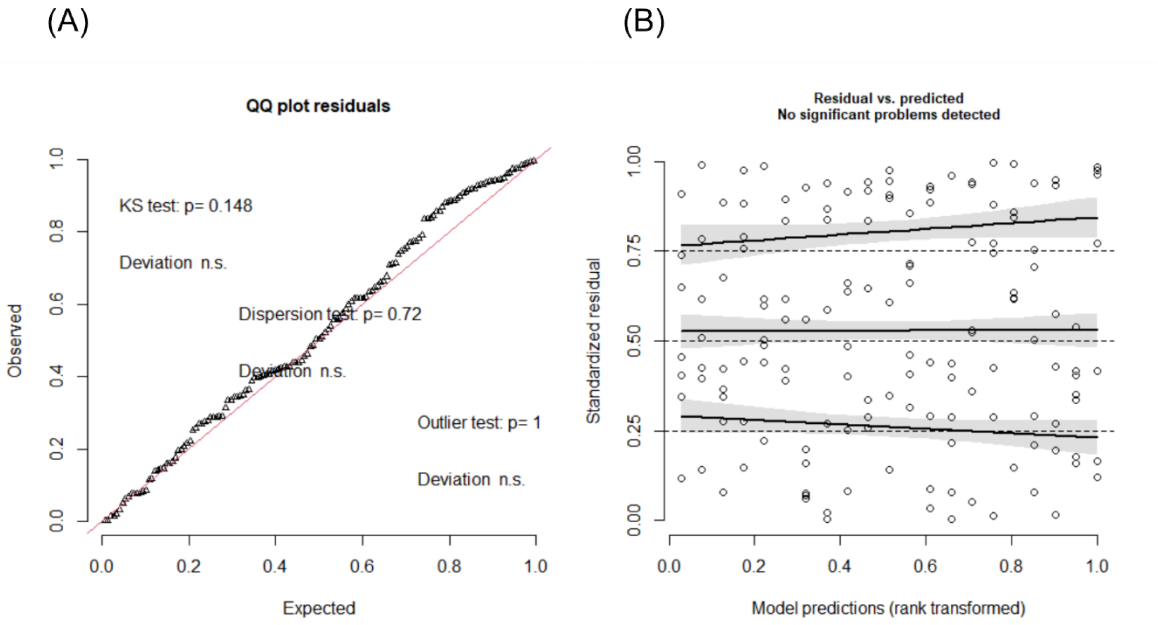

Figure A3: Diagnostic DHARMA plots for the full "quiver presence/absence"-model. (A) QQ-plot depicting overall deviations from the expected distribution, with tests for correct distribution (KS test), dispersion and outliers. If the scaled residuals (triangles) lie perfectly straight on the red line, the simulated data follows the expected distribution. If the data is overdispersed (variance > mean), more residuals are located around 0 and 1 in the tail of the distribution than expected under the fitted model. If the data is underdispersed (variance < mean), more residuals are located around 0.5 and less residuals in the tail of the distribution as expected under the fitted model. (B) Plot depicting model residuals (dots) plotted against predicted values. To visually detect deviations from uniformity of the distribution in the y-direction, the function compares the empirical 0.25, 0.5 and 0.75 quantiles in the y-direction (dashed line) with the theoretical 0.25, 0.5 and 0.75 quantiles in the y-direction (solid line) and gives a p-value for the deviation from the expected quantile.

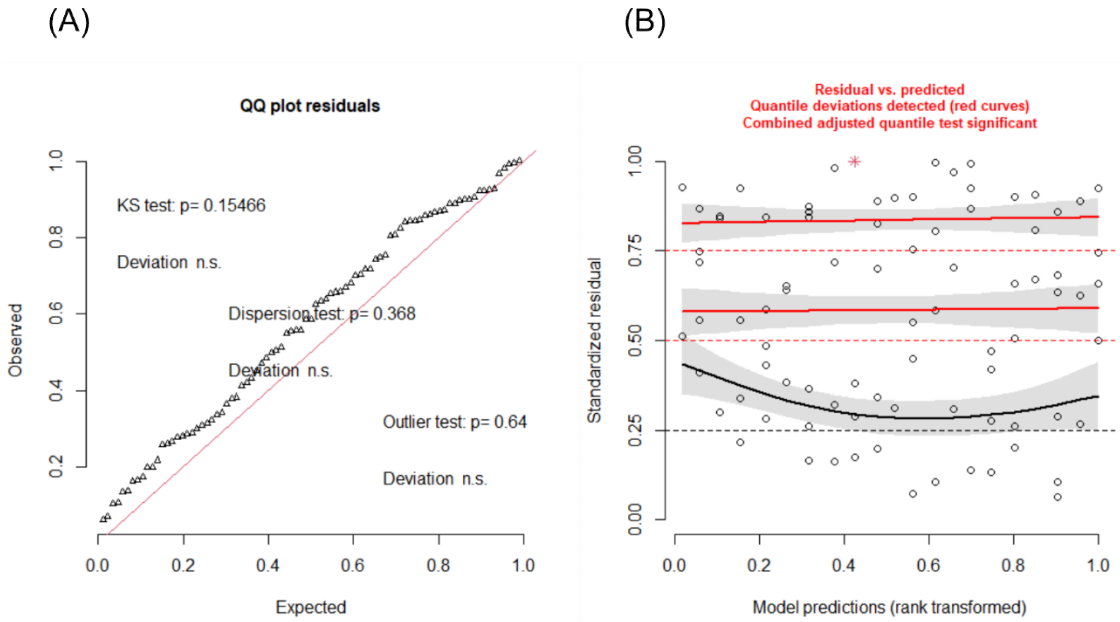

Figure A4: Diagnostic DHARMA plots for the full "quiver count"-model (see Model N°5 in Table A3). For a detailed description of the plots see Figure A3. (A) QQ-plot depicting overall deviations from the expected distribution, with tests for correct distribution (KS test), dispersion and outliers. (B) Plot depicting model residuals (dots) plotted against predicted values. A red star indicates a simulation outlier.

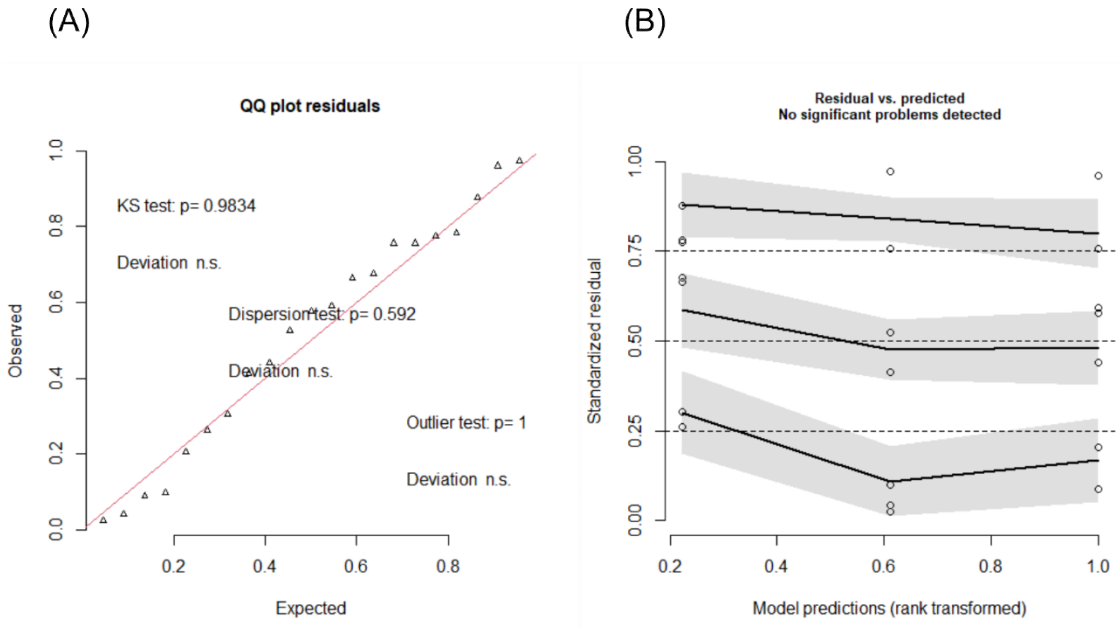

Figure A5: Diagnostic DHARMA plots for the full "total quiver count"-model. For a detailed description of the plots see Figure A3. (A) QQ-plot depicting overall deviations from the expected distribution, with tests for correct distribution (KS test), dispersion and outliers. (B) Plot depicting model residuals (dots) plotted against predicted values

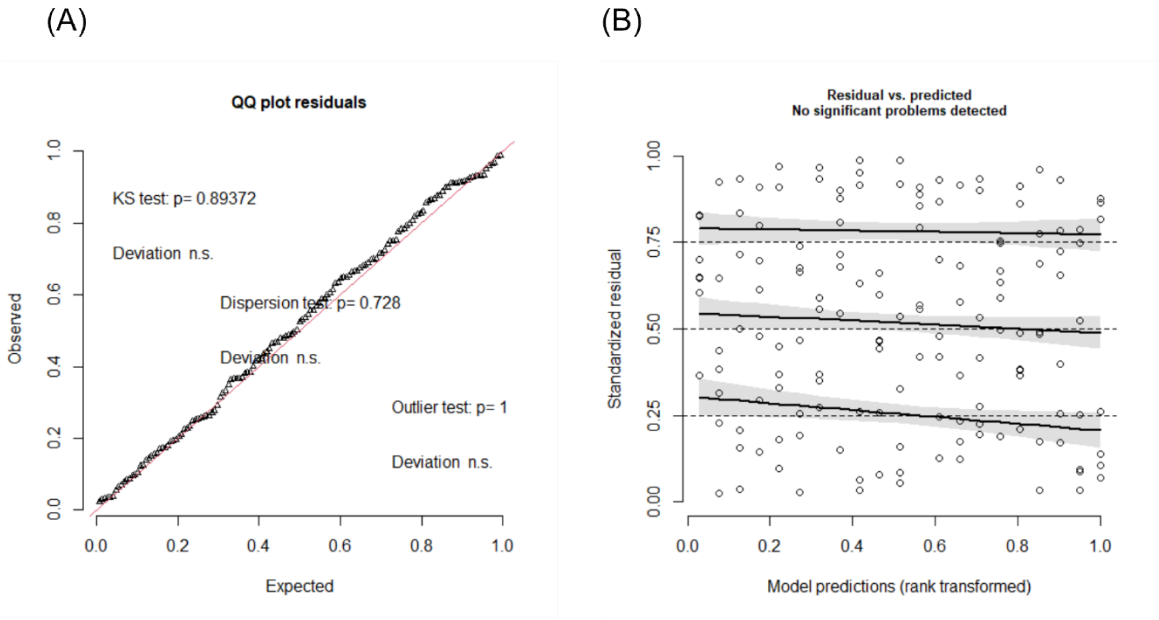

Figure A6: Diagnostic DHARMA plots for the full "Silent-quiver count"-model. For a detailed description of the plots see Figure A3. (A) QQ-plot depicting overall deviations from the expected distribution, with tests for correct distribution (KS test), dispersion and outliers. (B) Model residuals plotted against predicted values.

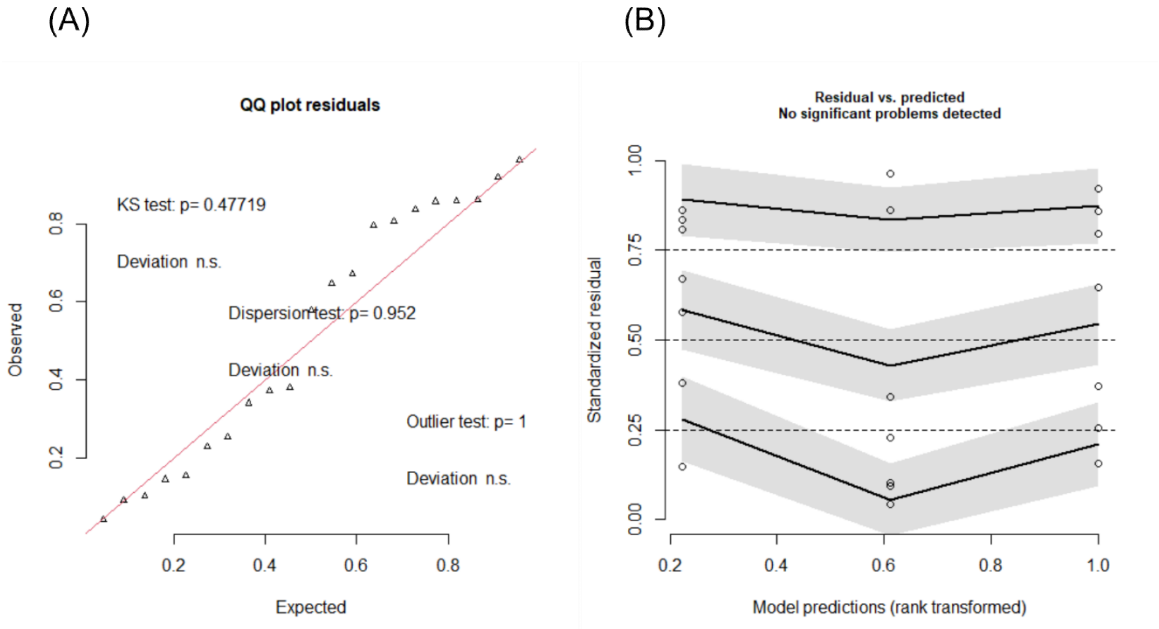

Figure A7: Diagnostic DHARMA plots for the full "Total silent-quiver count"-model. For a detailed description of the plots see Figure A3. (A) QQ-plot depicting overall deviations from the expected distribution, with tests for correct distribution (KS test), dispersion and outliers. (B) Model residuals plotted against predicted values.

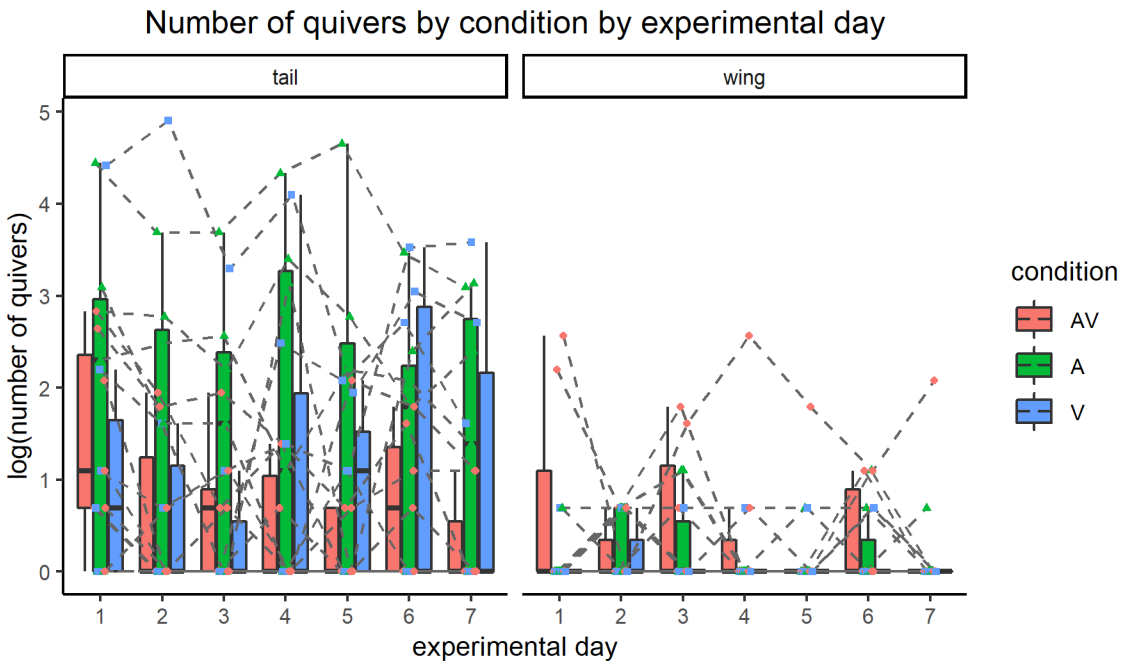

Figure A8: Number of quivers, separated into tail and wing quivers, by condition over seven experimental days. Each datapoint represents the log-transformed number of quivers performed by one female on a given day. Connected datapoints represent the same female.

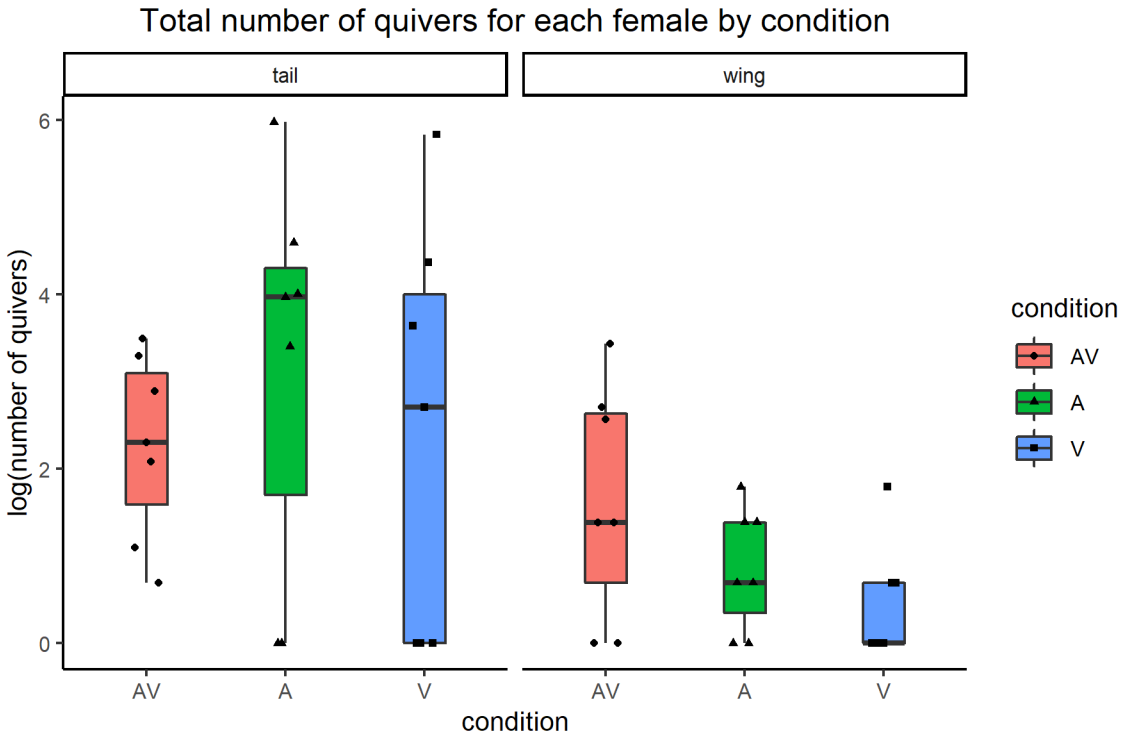

Figure A9: Summed number of quivers, separated into tail and wing quivers, by female and condition. Each datapoint represents the log-transformed total number of quivers performed by one female over seven days (7 values in each condition).

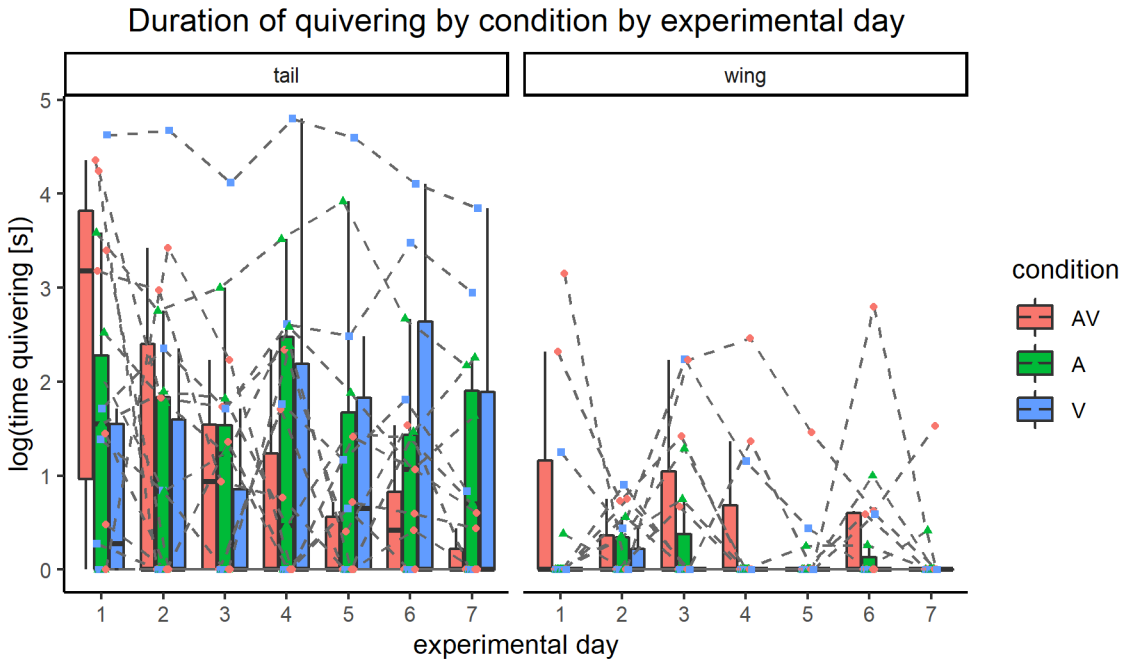

Figure A10: Duration of quivering by condition, separated into tail and wing quivers, over seven experimental days. Each datapoint represent log-transformed duration (in seconds) of quivers performed by one female on a given day. Connected datapoints represent the same female.

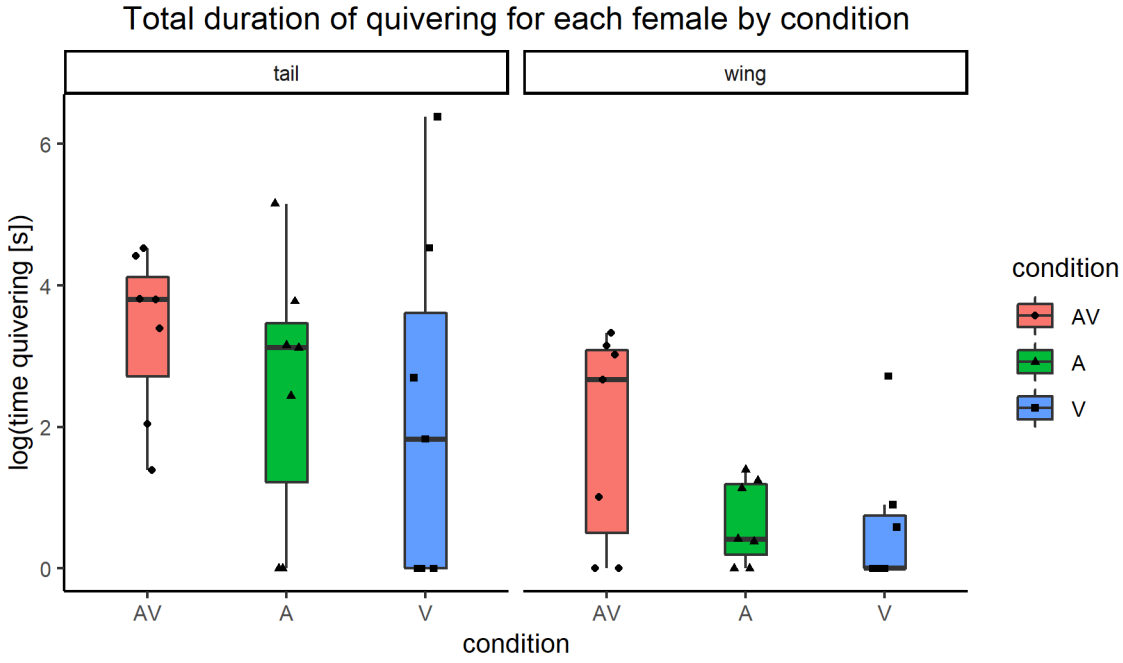

Figure A11: Summed duration of quivering, separated into tail and wing quivers, by female in each condition. Each datapoint represents the log-transformed total duration of quivering performed by one female over seven days.

Zusammenfassung

Bei vielen Vogelarten besteht die Balz aus einer Kombination von multimodalen auditiven und visuellen Signalkomponenten. Ob einzelne unimodale Signalkomponenten redundante oder nicht redundante Informationen enthalten, und ob die Kombination dieser einzelnen Signalkomponenten zu einer stärkeren Reaktion des Empfängers führt, ist jedoch nach wie vor ungeklärt. In dieser Studie haben wir die Wirkung von unimodalen und multimodalen Balzsignalen auf die Verhaltensreaktion des Empfängers untersucht, indem wir 21 Lachtaubenweibchen (Streptopelia risoria) über einen Zeitraum von sieben Tagen Aufnahmen der männlichen Balz präsentierten. Die Weibchen wurden in drei „Playback“-Gruppen aufgeteilt und entweder multimodalen audio-visuellen Reizen, oder unimodalen audio- oder visuellen Reizen männlicher Balzaufnahmen ausgesetzt, wobei der Reize des jeweils anderen Sinneskanals durch alternative bekannte Stimuli verdeckt wurden. Die Wirkung des Balz-Playbacks auf drei relevante sexuelle Verhaltensweisen (Schwanzzittern & Flügelzittern („Quivering“) und Flügelschlagen) wurde durch manuelle Auswertung quantifiziert. Zusätzlich versuchten wir „Quivering“-Verhaltensweisen zu quantifizieren, indem wir einen „Tracking“-Algorithmus trainierten, die Position von Körperteilen der weiblichen Tauben automatisch zu erkennen. Allerdings war das „Quivering“-Verhalten zu subtil für eine automatisierte Analyse. Wir fanden heraus, dass die Dauer des Flügelschlagens in den Playback-Intervallen zwar signifikant höher als während der „Playback“-Pausen war, dass aber die unmittelbare Anwesenheit männlicher Balzreize nicht notwendig war, um eine „Quivering“-Verhaltensreaktion bei den Weibchen auszulösen. Darüber hinaus hatte die „Playback“-Bedingung keinen messbaren Einfluss auf die „Quiver“ Anzahl oder Dauer über den gesamten Versuchszeitraum. Insgesamt waren die verschiedenen sexuellen Verhaltensweisen stark abhängig von der Identität des Weibchens, und künftige Hormonanalysen könnten helfen, die Gründe für diese interindividuelle Variabilität zwischen Weibchen aufzudecken.