



Female behaviour is differentially associated with specific components of multimodal courtship in ring doves



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Courtship displays are typically conspicuous, elaborate and composed of concomitant signals occurring in different sensory modalities. Although multimodal signals have received increasing attention over the past years, technical issues, in particular the lack of appropriate recording and analytical methods, have long restricted large-scale systematic study of their function. Here, we investigated in detail in 100 male–female dyads whether the multimodal, audiovisual courtship signals of the male ring dove, *Streptopelia risoria*, are associated with different immediate behavioural responses by the female, and whether such responses depend on the courting individual. We used synchronized high-speed video and audio recordings followed by semiautomatic image- and audio-processing techniques to precisely quantify variation in male courtship and female response. In particular, we investigated the structure of acoustic and visual courtship components, as well as aspects related to multimodal synchronization. We found that the fundamental frequency of male calls, as well as the total courtship duration and the duration of courtship bouts, influenced female tail-quivering behaviour, confirming that this behaviour is a sign of sexual interest in doves. On the other hand, some courtship variables frequently investigated in the literature, such as courtship rate, did not affect any aspect of female response. Additionally, we demonstrated an effect of repeated encounters with an individual of the opposite sex both on male courtship variables and on female sexual response to courtship. Females also responded differently to different males, that is, we found variation in female behavioural response to courtship variables related to identity and courtship effort. Further empirical studies are needed to assess how different male courtship elements influence female behaviour and ultimate mating decisions.

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Many social signals are elaborate and composed of elements occurring in different sensory modalities (Candolin, 2003; Higham & Hebets, 2013; Rowe & Halpin, 2013). These signals are referred to as multimodal (or multisensory), and they have received increasing interest over recent years. Sexual signalling and courtship behaviour are particularly interesting cases, as courtship displays are among the most elaborate, diverse and conspicuous signals described in the animal kingdom, for example the impressive visual-acoustic courtship of some tropical bird species (Frith & Frith, 1997; Fusani, Giordano, Day, & Schlinger, 2007) or the visual-vibratory display of wolf spiders (Scheffer, Uetz, & Stratton,

1996). Courtship is typically displayed in at least two sensory modalities and considerable theoretical literature exists with several hypotheses being proposed to explain the function of these multicomponent signals (Guilford & Dawkins, 1991; Hebets & Papaj, 2005; Mitoyen, Quigley, & Fusani, 2019; Rowe & Halpin, 2013). The multiple message hypothesis suggests that every component of an elaborate signal carries different information and therefore is responsible for different responses in receivers (Johnstone, 1996; Møller & Pomiankowski, 1993). Alternatively, different courtship traits are redundant and act as a mutual backup (Johnstone, 1996; Møller & Pomiankowski, 1993). A more recent hypothesis proposes that different components interact with each other, leading to the emergence of new information (Hebets & Papaj, 2005; Johnstone, 1996; Møller & Pomiankowski, 1993). Yet relatively few empirical studies have attempted to precisely

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characterize, quantify and explain the proximate and ultimate functions of these multiple components (Wiens & Tuschhoff, 2020).

The classical approach in sexual selection studies has been to investigate courtship behaviour as a whole, examining the effects of gross variables (such as courtship duration) or the occurrence of specific behavioural patterns (circling, jumping, occurrence of a type of call, etc.) on female response or female choice (Candolin, 2003). Some recent studies have examined in a more quantitative way the role of different physical parameters of each component signal in the sexual selection context, for example the speed or amplitude of limb movements or specific acoustic parameters of songs. These studies tested associations between female choice or male reproductive success and specific courtship characteristics such as song structure (Christie, Mennil, & Ratcliffe, 2003), song and call frequency (Nemeth, Kempenaers, Matessi, & Brumm, 2012; Hasegawa & Arai, 2016) or amplitude of courtship display movements (Murai & Backwell, 2006). In laboratory studies, the role of specific courtship components is usually investigated using cue isolation experiments where females are exposed to only a subset of the usual courtship components, and their response recorded in terms of preference (Uetz & Roberts, 2002) or physiology (Crews, 1975). In both cases, the complexity and the multimodality of the courtship display are generally not addressed per se, and there are few or no studies on the effect of every component of the courtship.

Another limitation originates from the way the value of these components is usually assessed. In most studies, the function of signal components is investigated by looking at their effects on the courted sex (typically the female) in terms of preference or choice. Obviously, courtship plays a major role in partner choice and thus has a major effect on individual fitness through reproductive success (Andersson, 1994; Bastock, 1967). However, this approach, though useful, does not always allow a direct investigation of the mechanistic function of the whole signal. According to the multiple message hypothesis, the diversity of responses triggered by various components can potentially provide valuable information about the function of these complex signals and should not be overlooked. For instance, females could respond behaviourally to some courtship components signalling species or sex identity and physiologically (Pedroso, Barber, Svensson, Fonseca, & Amorim, 2013; Vicente & Halloy, 2016) to other components carrying information about male quality. In fact, the signal could even carry information about undesired features such as high aggressiveness, low parental investment or kinship, which may adversely influence the choice made by the receiver (Burley, 1986; Borgia & Coleman, 2000; von Hippel, 2000). Choice and preference experiments with only two possible outcomes or investigating only one female response (time spent next to a male or latency to approach for example) therefore cannot grasp the potentially conflicting effects or simply the different responses associated with diverse signal elements. The diversity of sensory modalities involved in the courtship interaction, the relation between these different sensory components but also the different responses from the receiver all contribute to courtship signalling. Trying to investigate as many of these aspects as possible in a more comprehensive way could increase our understanding of the evolutionary mechanisms at play in the sexual selection context (Halfwerk et al., 2019; Mitoyen et al., 2019).

In this study, we aimed to investigate in more detail how interindividual variability in male courtship components and their interaction affect female responses. To do so, we focused on the courtship display of the ring dove, *Streptopelia risoria*. The ring dove is a domesticated, socially monogamous species that forms strong pair bonds over consecutive years (Morris & Erickson, 1971). Its courtship is relatively simple, making it a good model for the study of multimodality in controlled laboratory conditions. Males typically court a female for several days before a pair forms, and

courtship is repeated before every copulation. The first and very typical courtship phase consists of the male performing a bowing display, where he bends repeatedly towards the floor, facing the female. A call, the bow-call, always accompanies the bowing, creating a multimodal 'stereotyped' courtship display (see Supplementary Video S1). Even when the pair is formed, courtship continues to occur and successful reproduction depends on a successful reciprocal courtship (Cheng, Porter, & Ball, 1981; Dios, 2015). This indicates that female doves do not use male courtship only to choose their partner; for example, courtship could also have a role in consolidating pair bonds (Dios, 2015; Morris & Erickson, 1971). Additionally, in doves the multimodal aspect of courtship is important, as physiological stimulation is higher when females are exposed to audiovisual courtship rather than unimodal auditory courtship lacking the visual component (Friedmann, 1977). Finally, we know that male interindividual variability exists for several courtship elements (Davis, 1970; Fusani, Beani, Lupo, & Dessi-Fulgheri, 1997), suggesting that these traits undergo sexual selection by female choice or may carry information about individual qualities and/or physical condition.

We hypothesized that variation in visual and auditory components of the ring dove courtship display is associated with variation in female responses. We focused on one male courtship display that is predominant during the first phases of courtship, the bowing display. We investigated a range of courtship variables falling into three main categories: temporal and spectral structure of bow-calls, temporal structure of the bowing movements and synchronization between vocal and visual signals. We performed a cross-over experiment in which each of 10 females encountered 10 males repeatedly (three times). High-speed video and audio recordings of these interactions, associated with semiautomatic movement tracking and analysis, allowed us not only to describe the structure of the displays and their multimodal synchronization, but also to precisely quantify the variability of all these components. In addition, the experimental design allowed us to assess how female response and male courtship changed over repeated encounters between the same birds and throughout the experiment. It is well known that both female (Barfield, 1971; Cheng, 2008) and male (Lovari & Hutchison, 1976) behavioural and physiological responses change after several days of courtship and sexual interaction. Lastly, we asked whether some individual males consistently induced stronger responses in most of the females they encountered, in an attempt to search for male behavioural variables that reflect objective courtship values.

METHODS

Study Species

The ring dove is probably the domesticated form of the African collared dove, *Streptopelia roseogrisea*, a native species of northern Africa (Baptista, Trail, & Horblit, 1997; van Grouw, 2018). The ring dove is usually sexually active between April and October, and several clutches can be laid every year. The birds studied here came from different breeders across Austria and most of them were sexually naïve and had never completed a breeding cycle when we acquired them. All birds were between 10 months and 2 years old. The birds were housed in single-sex groups prior to experiments to reduce any effect of familiarity on behaviour (Erickson & Morris, 1973). To ensure that the males were sexually motivated and thus ready to perform courtship during testing, we housed them indoors in individual cages (50 × 38 cm and 60 cm high) from 1 week before and throughout the experiment (Hutchison, 1970). Males were visually but not auditorily separated from each other. We maintained the housing room under a light regime of 14:10 h

light:dark. We housed females in groups of six in two outdoor aviaries (2.95×1.20 m and 2.80 m high).

Ethical Note

This work was approved by the local ethics committee of the Faculty of Life Sciences, University of Vienna, and by the national committee of the Austrian Federal Ministry of Education, Science and Research (BMFWF permit 66.006/0042-WF/V/3b/2017). It adheres to the ASAB/ABS Guidelines for the Use of Animals in Research and the ARRIVE Guidelines. All efforts were made to limit stress before and during the experiment; the doves were tame and used to being handled. Prior to the experiment, doves underwent a habituation phase with regular handling and exposure to the experimental set-up, which was done daily for several weeks. When not in the set-up, birds had constant access to seed mix, grit and water ad libitum. Males housed in individual cages were never socially isolated from each other, as this species relies on acoustic communication, and the use of pre-experiment visual separation is standard in this species (Hutchison, 1970; Lovari & Hutchison, 1976). We monitored birds throughout the experiment for any signs of acute stress, which was never observed during testing.

Recording Apparatus

To record the male courtship and the associated female response in controlled conditions, we built a two compartment (each $50 \times 50 \times 50$ cm) recording apparatus in which one male and one female could interact (Fig. 1). For each compartment, a camera (Basler acA1920-155uc) recorded a side view of the bird. We placed two microphones (Sennheiser ME66 directional head with a battery powered K6 power module) behind black fabric walls on each side of the apparatus to record vocalizations. The microphones were chosen for their broad (40–20 000 Hz) and reasonably flat frequency response. Care was taken with hardware and software audio settings and the placement of the microphone in the set-up to avoid clipping of audio recordings. We separated the two compartments by a net to prevent birds from entering the partner's compartment, but the birds could see and hear each other without restriction. A movable partition was placed between the two

compartments before recording to prevent birds from seeing each other prior to testing. For optimal video recording, we placed 10 LED lights above each compartment (powered by a DC power supply to eliminate flicker, set at 0.30 V and 0.26 A), as well as one 30 W LED panel behind each camera to suppress shadows. We fixed acoustic foam on the walls to suppress echoes, and a double layer of thick curtains was additionally used for acoustic dampening and to separate the birds from the experimenter. Video acquisition was done using a Motif Video Recording System (loopbio gmbh, Vienna, Austria). Video recordings were controlled through a web interface, and audio recording was controlled using Audacity (Audacity, <http://audacity.sourceforge.net>). We recorded video images at 60 frames/s, with an exposure time of 0.015 s. A synchronization signal from the Motif camera system hardware synchronizer (one pulse per frame) was recorded as an audio signal synchronously with the microphone recordings (Allen & Health, ZEDi 10), which allowed us to synchronize audio and video recordings with a precision of ± 1 video frame (16.67 ms). The recording room temperature was between 25 and 28 °C throughout the experiment.

Experimental Design

We used 10 sexually mature males and 10 sexually mature females in a cross-over design experiment where every male was presented to every female resulting in the recording of multiple test sessions between 100 unique dyads (Fig. 1). For logistical reasons, it was not possible to test all individuals each day, so we split males and females into two groups and the experiment was staggered in two blocks in June and August 2018 (Fig. 1).

After we retrieved one female from the aviary and one male from the individual cages, we put both individuals in the experimental set-up with the partition closed to let them habituate. After 10 min, we removed the partition between the two compartments and the birds could freely interact for 5 min. We started video and audio recording 1 min before the removal of the partition and stopped it 5 min after the partition was opened. As a control, we recorded every individual alone in the set-up once a week, following the same timing of partition removal and recording. Instead of encountering another individual, the test bird faced an empty compartment.

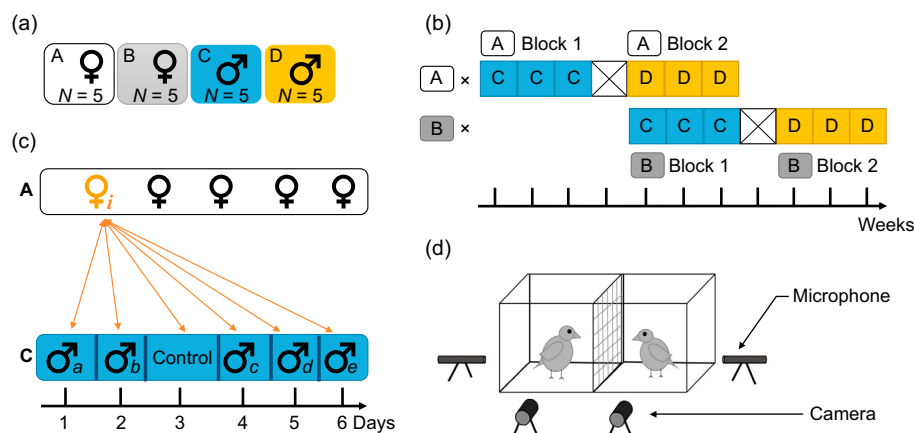


Figure 1. Scheme of the cross-over experimental design. (a) Experimental groups. We divided the birds into four groups of five. (b) Temporal organization of the testing. Every male–female dyad was tested three times (three repetitions) between July and August 2018. For females, each block corresponded to a 3-week period during which they encountered each individual male three times. This led to recordings of 300 test sessions (between 100 unique dyads) and 120 control treatments. Crossed boxes indicate a week without testing. (c) Example of the experiment repetition pattern for one female, over a 6-day period (example for groups A and C). Female *i* (from Group A) meets male *a* (from Group C) on day 1, male *b* on day 2, etc. Female *i* is tested in front of an empty box on day 3 (Control). The same pattern applies for every female with a different random order for each female and for each week of the experiment. At the end of the 6 days, every female had met every male once, and faced an empty box once. (d) A sketch of the recording apparatus.

Behavioural Analysis

Female behaviour

We coded video recordings of female subjects using Solomon Coder v17.03.22 (Peter, 2017). The coding started when the partition separating the two compartments was completely removed and continued for 5 min until the end of the recording. Two coders coded the videos, and we used Cohen's kappa coefficient to assess intercoder reliability on a subset of 20 videos coded by both coders. Depending on behaviour investigated, there was at least 95% reliability between coders ($P < 0.001$). Coding resolution was 0.2 s. We coded female behaviours that had previously been found to be associated with sexual response or sexual interest in doves and other bird species (Amy, 2015; Cheng, 1973; Witte, 2006). These included tail quivering, self-preening, approach to the male and time spent near the male. We additionally coded behaviours related to female activity in general, such as the number of steps taken, number of pecks to the ground or net and duration of periods during which the female had its eyes closed. As females did not vocalize during the experiment, we did not perform any acoustic analysis of female behaviour. A summary of the coded female behaviour is provided in Table 1.

Male behaviour

All male acoustic and visual courtship variables that were measured are illustrated in Fig. 2 and described in Table 2. For every variable calculated (except total courtship duration, total number of calls, number of bouts and call rate), we averaged the values over each test session to obtain only one average value per male per day.

Acoustic Analysis

We recorded sound in wav format with a sampling rate of 44.1 kHz and a bit depth of 16 bits. Analysis of the auditory part of the courtship was performed using PRAAT v6.0.26 (Boersma & Weenink, 2020). We automatically annotated our recordings (function: annotate) by defining a courtship call as being higher than 100 Hz and longer than 0.05 s and by setting a silence threshold relative to higher intensity at -30 dB. We then manually differentiated between the first and the second part of the call (Fig. 2). For the spectral analysis, we used a Fourier transform method (spectrogram settings: frequency range of 0–3000 Hz and window length of 0.05 s). For both element 1 and element 2 of every call of every courtship recording, we extracted maximum, minimum and median fundamental frequency (F0). We also calculated temporal variables of courtship elements by extracting the duration of both call elements. We defined a bout as a courtship display sequence that is separated from other bouts by at least 2 s. We calculated the duration of every courtship bout and the total courtship duration, the number of calls per bout, the total number of calls, the call rate and the number of courtship bouts per test session.

Visual Analysis

Male behaviour was semiautomatically quantified using Loopy (<http://loopb.io>, loopbio gmbh, Vienna, Austria). Loopy software is a suite of image-processing applications including pose detection, which uses machine learning to automatically track user-defined points of interest on the object of interest. It returns frame-by-frame estimates of the pixel coordinates of these points. We tracked either the right or the left eye, depending on which box contained the male (Supplementary Video S2). We manually annotated these reference points on 864 sample frames from three courtship videos from three different males. The model was trained with those annotations and then used to quantify all courtship video recordings. We manually checked the accuracy of predictions on a sample of our recordings to confirm that the points of interest were properly tracked. With the resulting coordinates, we calculated the amplitude of the bows (in pixels). We also extracted temporal variables of the visual courtship, including bowing ascent time, bowing descent time, up time and down time (see Fig. 2 for a description of all calculated postural variables).

Audiovisual Synchronization

Video capture timing was controlled using hardware synchronization. To allow postrecording synchronization of audio and video recordings, the synchronization signal was recorded as an acoustic signal synchronously with the microphone audio tracks. During recordings, the experimenter manually synchronized the video cameras; the index and clock time of the synch frame (i.e. first frame after the synchronization command was executed by the software controlling the video recording) were recorded in the video metadata.

The corresponding audio synchronization track contained a pulse corresponding to each frame of the video. The sync frame pulse was characterized as the first pulse onset after a long period of no pulses. This allowed us to use the same timeline for video and audio. To quantify audiovisual synchronization of male courtship, we calculated the following durations: the difference between the time of the video frame containing the start of the descent movement and the time of the audio sample corresponding to the beginning of part 1 of the call, the difference between the end of the descent movement and the end of part 1 of the call, the difference between the start of the ascent movement and the beginning of part 2 of the call and the difference between the end of the ascent movement and the end of part 2 of the call (Fig. 2).

Statistical Analysis

We performed all statistical analysis using R (version 3.3.2, R Core Team 2013). To assess correlation among same-sex behaviour variables we used a Spearman correlation test with a 0.05 significance level (R package Hmisc v.4.3–1, Harrell et al., 2020). For

Table 1
Female behaviours measured in response to male courtship

Behaviour	Description
Tail quivering	Number of times the female quivered her tail rapidly
Preening	Number of times the female performed self-preening
Approach attempts	Number of times the female approached the male. Defined as the female touching the net to try to go through it and then taking a step back
Time near adjacent box	Proportion of time the female spent in the third of the test cage closest to the adjacent box. This is synonymous to 'Time near male' in the noncontrol condition
Steps	Total number of steps taken by the female
Pecks	Total number of pecks (to ground or net)
Time eyes closed	Proportion of time during which the female remained with its eyes closed

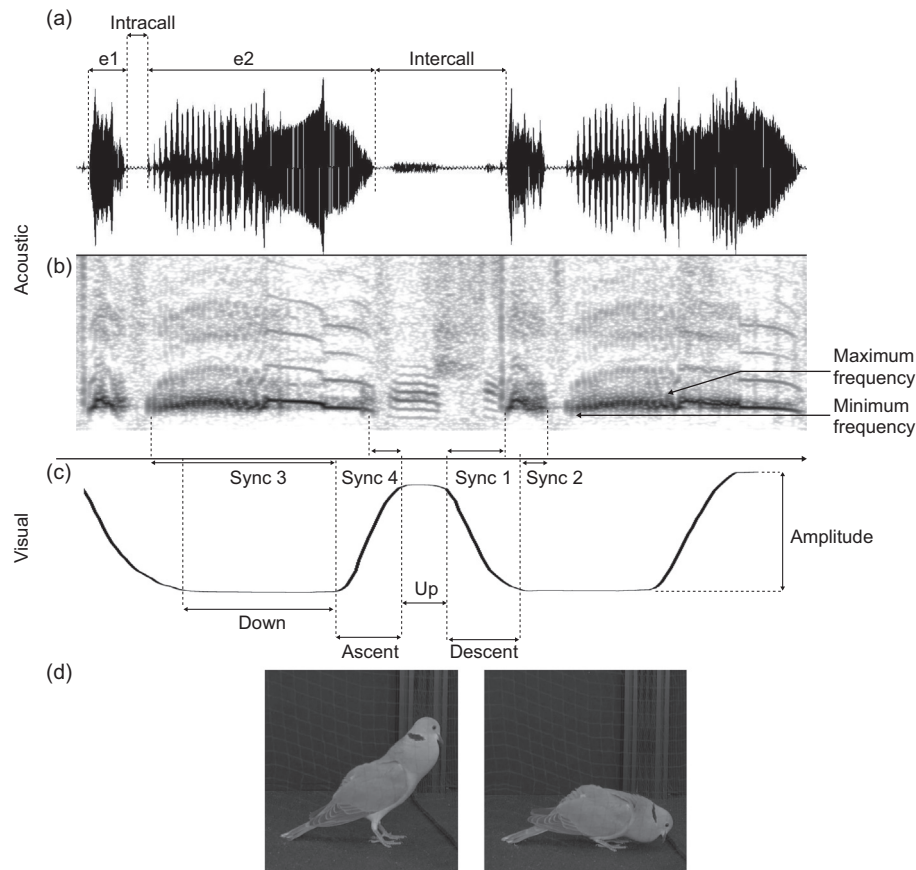


Figure 2. Representation of the two data channels recorded of the ring dove multimodal courtship display and the extracted courtship variables. (a) Oscillogram of the acoustic display, which comprises repetitive calls composed of two elements (e1 and e2). (b) Spectrogram of the acoustic display where the maximum and minimum frequencies of e2 are described. (c) Representation of the visual bowing display. The line is the trajectory of the eye of the dove seen from a side view, during the bowing, and extracted from an automatic annotation. The variables describing the synchronization between acoustic and visual display are shown. (d) Pictures of a male in the experimental apparatus in the up and down positions of the bowing display.

every female behaviour considered, the duration of the behaviour was highly correlated with the number of occurrences of the same behaviour ($r > 0.98$). We used MANOVA followed by Tukey HSD post hoc tests to examine whether repetition number, experimental block and male identity affected male courtship variables (separated between structural, spectral, visual and synchronization variables).

For all the models described hereafter, we ran separate models with each female behaviour of interest as the response variable (number of tail quivers, number of approaches, number of times female self-preened, number of steps, number of pecks, proportion of time female spent near the adjacent box).

We first investigated whether female behavioural response depended on social context, that is, when encountering a male versus facing an empty box. We modelled the impact of social context on female behaviour using generalized linear mixed models. When female behaviour was expressed as counts, we fitted the models with a negative binomial distribution that deals with overdispersion. We fitted the models with a beta distribution when investigating behaviour expressed as percentage (time spent near male). We fitted the models using the glmmTMB function from the glmmTMB packages (version 1.0.0, Brooks et al., 2017). Full models included male identity, female identity and male–female dyad as random effects. We included the interaction between repetition number and female experimental block (Fig. 1) as a control. Social context (empty box versus male) was included as a fixed effect. To

test the effect of social context, we used a full-null model comparison approach. The null model lacked the social variable, but the random effects and the controls remained. We used the DHARMa package (v. 0.2.7, Hartig, 2020) to check our model assumptions. We then investigated whether female behaviour would depend on whether they faced a courting or a noncourting male. We used the same approach to model the effect of presence of courtship behaviour per se on the number of female behaviour events. As a fixed effect, we replaced social context by courtship status (yes/no). The data set for the latter models only comprised the test sessions in which females encountered a male ($N = 295$).

To further investigate the influence of male courtship variables on female behavioural responses, we ran additional models. As explanatory variables (fixed effects), we used 14 uncorrelated male courtship variables (see Results). We standardized the data by scaling each continuous variable beforehand by subtracting the variable mean and dividing by its standard deviation. The resulting z-scores were used in the model. We included the interaction between dyad repetition and experimental block, as well as the side of the experimental apparatus (left/right) where the male was placed. We tested the effect of explanatory variables using the drop1 function with a likelihood ratio test (stats package v.4.0.1, R Core Team 2013). To assess effect size, we used the function r.squaredGLMM from the package MuMIn (v.1.43.15, Barton, 2019). We used the emmeans function from the emmeans package (v.1.4.5, Lenth, 2020) to perform post hoc tests.

Table 2
Variables measured from courtship of male ring doves

Courtship parameters	Description
Call: temporal structure	
Total courtship duration	Total courtship display duration (s) during each session (5 min encounter)
Number of calls	Total number of calls during the session
Number of bouts	Number of courtship bouts during the session
Call rate	Average call frequency within bouts (no./s)
e1 duration	Average duration of element 1 of the call (s)
e2 duration	Average duration of element 2 of the call (s)
Intracall duration	Average duration of the interval between the end of element 1 and the beginning of element 2 (s)
Intercall duration	Average duration between the end of element 2 of a call and the beginning of element 1 of the following call (s)
Call: spectral structure	
e1 max F0	Average maximum fundamental frequency of element 1 (Hz)
e1 min F0	Average minimum fundamental frequency of element 1 (Hz)
e1 median F0	Average median fundamental frequency of element 1 (Hz)
e2 max F0	Average maximum fundamental frequency of element 2 (Hz)
e2 min F0	Average minimum fundamental frequency of element 2 (Hz)
e2 median F0	Average median fundamental frequency of element 2 (Hz)
Bow	
Bowing amplitude	Average vertical distance between the eye position before ascent start and the eye position when ascent ends (pixels)
Up duration	Average time the bird spends in the up position of the bow (s)
Descent duration	Average time between up position and down position (s)
Down duration	Average time the bird spends in the down position of the bow (s)
Ascent duration	Average time between down position and up position (s)
Audiovisual synchronization	
Sync 1	Average difference between onset of e1 and onset of descent movement (s)
Sync 2	Average difference between offset of e1 and offset of descent movement (s)
Sync 3	Average difference between onset of e2 and onset of ascent movement (s)
Sync 4	Average difference between offset of e2 and offset of ascent movement (s)

Apart from total courtship duration, call number, bout number and call rate, all other variables were averaged for each 5 min test session, yielding one number per male per day.

To investigate whether female behaviour was influenced by repeated encounters with males (one repetition per week in each block), we fitted a model where we included the repetition number, the experimental block and their interaction as fixed effects. Female identity, male identity and dyad were included as random effects. Finally, to investigate whether specific males triggered stronger behavioural responses from females we fitted a final model where male identity was entered in the model as a fixed effect, and we used female identity as a random effect. The null model only comprised female identity as a random effect and repetition number and experimental block as controls. Female behaviour was strongly influenced by the experimental block (see Results) and owing to our experimental design, males of group C were only tested in female block 1 and males of group D only in female block 2 (see above). Therefore, for each female behaviour we ran two models: one comprising the males from group C and the other comprising the males from group D. The data set comprised all the dyad test sessions ($N = 295$), not only the ones in which males were courting.

RESULTS

We recorded 300 male–female test sessions. Four sessions could not be analysed because of technical issues, and one session had to be stopped because the male managed to intrude into the female's compartment. Our final data set thus comprised 295 sessions with male–female interactions. Males courted females in 253 sessions, whereas in the remaining 42 sessions males did not perform any courtship. We also recorded 60 control sessions in which both females and males faced an empty box. As no male ever courted the empty box, no male data were collected from these sessions.

Females' Response to Social Interaction and Courtship

Of 295 recorded test sessions where males and females interacted, females displayed preening in 159 sessions, tail quivering in

133 sessions and attempted to approach the male in 78 sessions. Every female displayed tail-quivering behaviour even though there was a large variation between females regarding the number of tail quivers, ranging from only three over the whole experiment for one female to 285 for another. We observed the same pattern for approach behaviour (range across females 1–275) and self-preening (3–904). The numbers of steps and pecks were positively correlated with every coded behaviour, apart from the time with eyes closed, with which they were negatively correlated (steps: $r_s = -0.32$, $P < 0.001$; pecks: $r_s = -0.19$, $P < 0.001$). The durations of tail quivering, preening and approach were highly positively correlated with their respective number of occurrences ($r_s > 0.99$, $P < 0.001$ for the three behaviours). We therefore decided to use only the latter in further analyses. Because of the small number of females closing their eyes during testing, and the negative correlation of this variable with step and peck number, we did not investigate this behaviour further and only kept steps and pecks as a measure of activity.

The number of tail quivers was impacted by social context (whether a male was present in the other box or not; full-null model comparison: likelihood ratio test: $\chi^2_{11} = 33.57$, $P < 0.001$). On average, females quivered their tail 2.33 ± 5.19 times (mean \pm SD, $N = 295$, range 0–34) when a male was present and never if they faced an empty box (Table 3, Fig. 3). The number of pecks was also influenced by male presence ($\chi^2_1 = 4.51$, $P = 0.03$). Females pecked significantly more often in front of a male (1.32 ± 4.10 times, $N = 295$, range 0–45) than an empty box (0.62 ± 2.48 times, $N = 60$, range 0–18). Females also showed a difference in the number of steps depending on whether they faced a male or not ($\chi^2_1 = 5.69$, $P = 0.01$), taking more steps during encounters with males (84.88 ± 112.07 times, $N = 295$, range 0–529) than in front of an empty box (52.75 ± 84.73 times, $N = 60$, range 0–283; Fig. 3). The number of times the female self-preened was not related to male presence ($\chi^2_1 = 2.22$, $P = 0.13$), nor was the number of approach attempts ($\chi^2_1 = 0.33$, $P = 0.56$) or the

Table 3
Summary of female behaviour when facing different contexts

Female behaviour	Context		
	Control (N = 60)	Noncourting male (N = 42)	Courting male (N = 253)
Proportion of time spent near adjacent box	0.26 ± 0.39	0.24 ± 0.31	0.34 ± 0.38
Preening	2.38 ± 4.18	3.5 ± 5.79	12.24 ± 26.84
Approach attempts	3.33 ± 10.80	0.62 ± 1.94	2.37 ± 7.24
Tail quivering	0 ± 0	0.57 ± 1.17	2.62 ± 5.53
Steps	52.75 ± 84.74	52.74 ± 74.14	90.21 ± 116.44
Pecks	0.62 ± 2.48	2.17 ± 5.36	1.18 ± 3.86

Means are shown ± SD.

proportion of time females spent next to the adjacent box ($\chi^2_1 = 1.29$, $P = 0.25$).

The number of tail quivers was related to the presence of courtship ($\chi^2_1 = 4.41$, $P = 0.03$; Fig. 3), with females showing more tail quivering during encounters with a courting male than in front of a noncourting male (Table 3). Females also took more steps when males were courting than when males were not courting ($\chi^2_1 = 3.96$, $P = 0.04$; Fig. 3). The number of times the female self-preened was not related to courtship ($\chi^2_1 = 0.91$, $P = 0.33$), and neither was the number of approach attempts ($\chi^2_1 = 0.22$, $P = 0.63$), number of pecks ($\chi^2_1 = 1.10$, $P = 0.29$) or the proportion of time female spent near the adjacent box ($\chi^2_1 = 0.35$, $P = 0.55$).

Intra- and Intermale Variability in Courtship Structure

To avoid multicollinearity issues, we only kept a set of 14 relatively uncorrelated variables as explanatory factors for our models which were chosen on the basis that they would grasp a representative spectrum of male audiovisual courtship parameters (call temporal structure, call spectral structure, bow parameters and audiovisual synchronization; see Variation in courtship structure after repeated interactions below and Table A1). The intracoo duration was strongly negatively correlated with the duration of the first element of the coo-call (e1: $r_s = -0.82$, $P < 0.001$): the longer the e1, the shorter the interval between the two elements. The total duration of the coo, however, was highly positively correlated with the e2 duration ($r_s = 0.90$, $P < 0.001$). Together, these results indicate that call duration was dependent not on the interval between the two elements, but rather on the e2 duration. We therefore discarded the intracoo duration from our models but kept both coo durations and intercall duration as they better represented temporal acoustic aspects of courtship. Median F0 of both

e1 and e2 were highly positively correlated with the maximum (e1: $r_s = 0.84$, $P < 0.001$; e2: $r_s = 0.61$, $P < 0.001$) and minimum F0 of both elements (e1: $r_s = 0.88$, $P < 0.001$; e2: $r_s = 0.94$, $P < 0.001$). We therefore only used the median frequencies as explanatory factors as they better represented how high pitched the calls were on average. The amplitude of bowing was moderately negatively correlated with the intercoo duration ($r_s = -0.52$, $P < 0.001$), that is, the larger the bowing movements, the shorter the interval between two consecutive bows. We kept the bowing amplitude in the models as this was the only estimation of courtship movement that we measured. Total courtship duration and bout duration were highly correlated with the total number of coos ($r_s = 1$, $P < 0.001$) and the number of coos within bouts ($r_s = 1$, $P < 0.001$). We chose to use durations as explanatory factors as they better represent the courtship effort to which a female is exposed. The total courtship duration was also positively correlated with the number of bouts ($r_s = 0.58$, $P < 0.001$). Finally, all audiovisual synchronization timing values were strongly positively correlated ($r_s > 0.74$, $P < 0.001$) and we only used the timing difference between the start of the descent movement and the start of part 1 of the call in our models. The strongest correlation between temporal characteristics of bowing was between time in the down position and ascent time and was only -0.32 ($P < 0.001$); therefore, we integrated all these variables in our models. All calculated male courtship variables are displayed in Table A1. As explanatory factors for our models, we discarded highly correlated variables and kept 14 relevant courtship variables (see Variation in courtship structure after repeated interactions below).

Males were statistically different from each other for dependent variables describing courtship acoustic temporal structure (Pillai's trace = 3.30, $F_{81,2187} = 15.69$, $P < 0.001$), acoustic spectral structure (Pillai's trace = 3.12, $F_{36,972} = 95.80$, $P < 0.001$), bow variables

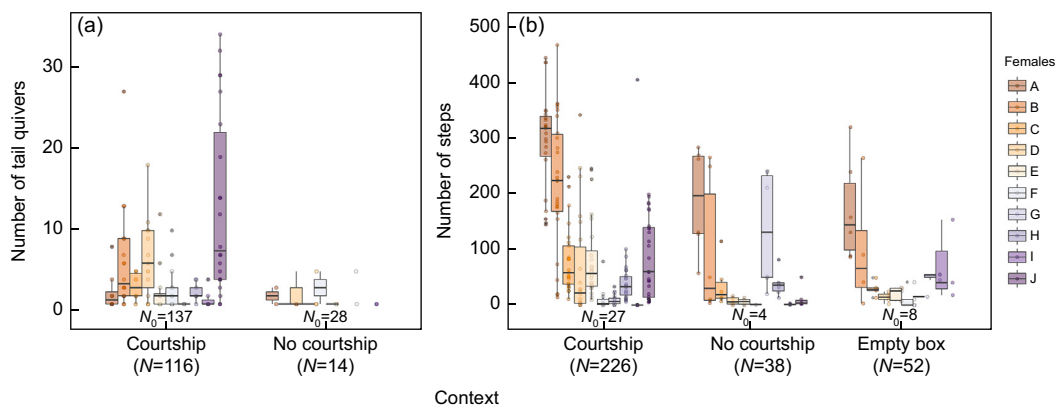


Figure 3. Number of behavioural events recorded from females in different social contexts. For clarity, we did not plot observations where numbers of tail quivers and steps were equal to zero. N_0 : number of sessions with zero events for each social context; N : number of observations where females showed the behaviour at least once. (a) Number of tail quivers in the presence or absence of courtship. Females never quivered their tail in front of an empty box. (b) Number of steps taken in the three behavioural contexts. Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range. Dots represent individual observations.

(Pillai's trace = 2.32, $F_{45,970} = 15.18$, $P < 0.001$) and audiovisual synchronization (Pillai's trace = 0.85, $F_{36,652} = 4.95$, $P < 0.001$). A set of one-way analyses of variance revealed that males differed for each variable of the courtship (Figs A1, A2, A3, A4, Table A2).

Variation in Courtship Structure after Repeated Interactions

We asked whether male behaviour was affected by repeated exposure to females by assessing whether it was consistent throughout the experiment. We investigated male behaviour over repeated encounters with the same female (repetition number), as well as over a larger timescale, between the two experimental blocks (separated by a week-long pause). None of the male behaviours changed across the 3 weeks (repetition number) during which they encountered the same females. For instance, element 1 duration differed significantly between males ($F_{9,243} = 185.85$, $P < 0.001$) but not between repetitions ($F_{2,250} = 0.65$, $P = 0.51$; Fig. 4a). However, some aspects of the courtship structure changed between the two experimental blocks (one block corresponds to the 3-week period during which a bird was tested with the same group of opposite-sex birds). There were changes in the acoustic temporal structure (Pillai's trace = 0.17, $F_{8,244} = 6.46$, $P < 0.001$) and visual structure (Pillai's trace = 0.07, $F_{4,168} = 3.01$, $P = 0.01$), but not acoustic spectral structure (Pillai's trace = 0.01, $F_{4,248} = 1.03$, $P = 0.39$) or synchronization between the acoustic and the visual components of the courtship (Pillai's trace = 0.03, $F_{4,168} = 1.28$, $P = 0.28$). One-way analysis of variance revealed that the call rate was higher ($F_{1,251} = 10.51$, $P = 0.001$; Fig. 4b) and the duration of the element 2 of the call longer ($F_{1,251} = 9.70$, $P = 0.002$) during the second block. Courtship duration was shorter in the second block ($F_{1,251} = 7.81$, $P = 0.005$), as was coo duration ($F_{1,251} = 13.79$, $P < 0.001$) and duration of courtship bouts ($F_{1,251} = 16.35$, $P < 0.001$). Regarding variables characterizing visual courtship, the time the bird spent in the up position of the bow was shorter in the second than the first block ($F_{1,251} = 13.79$, $P = 0.002$).

Link Between Male Courtship Traits and Female Response

After discarding highly correlated courtship variables to deal with multicollinearity issues (see Intra- and intermale variability in

Table 4

Result of the generalized linear mixed model predicting the number of tail quivers

Fixed effects	Estimate	SE	z	P
(Intercept)	-0.067	0.557	-0.121	0.904
Bout duration	-0.334	0.170	-1.968	0.042
Total courtship duration	0.436	0.142	3.071	<0.001
Coo rate	0.059	0.257	0.230	0.936
e1 duration	-0.250	0.168	-1.484	0.271
e2 duration	0.191	0.234	0.818	0.276
e1 median F0	0.208	0.237	0.876	0.273
e2 median F0	-0.502	0.201	-2.492	0.016
Intercoo duration	-0.321	0.253	-1.265	0.152
Up time	0.193	0.227	0.854	0.452
Descent time	0.082	0.180	0.454	0.568
Ascent time	-0.208	0.178	-1.168	0.198
Down time	-0.207	0.259	-0.800	0.336
Synchronization	0.081	0.152	0.533	0.561
Bowing amplitude	0.330	0.230	1.435	0.174
RN 2	0.294	0.398	0.740	0.460
RN 3	-0.141	0.413	-0.342	0.732
EB 2	-0.355	0.667	-0.532	0.595
Displaying box	-0.038	0.285	-0.132	0.895
RN 2 * EB 2	0.158	0.684	0.231	0.817
RN 3 * RN 2	0.966	0.659	1.467	0.142

Male courtship variables are fixed effects. Repetition number (RN), experimental block (EB) and displaying box were used as controls in the models. Random effects included female and male identity, as well as the male–female dyad (variance = 1.480, 2.17e-11 and 2.34e-08, respectively). Estimates are from the full models and are z transformed (scaled). P values are from a likelihood ratio test of a reduced model lacking this specific effect compared with the full model. Significant P values are in bold.

courtship structure below), we defined the following as predictor variables: the difference between the start of the descent movement and call part 1 (synchronization), bowing amplitude, total courtship duration, average bout duration, ascent time, descent time, up time, down time, e1 median F0, e2 median F0, e1 duration, e2 duration, intercoo duration and call rate (Table A1). Several of these variables impacted tail quivering in females (full-null model comparison: likelihood ratio test: $\chi^2_{14} = 24.04$, $P = 0.04$). The effect size of this model for the fixed effects was $R^2 = 0.24$ and for the whole model including random effects $R^2 = 0.69$, meaning that individuals' identities were responsible for a large proportion of the variation in tail quivering. In particular, the variability between

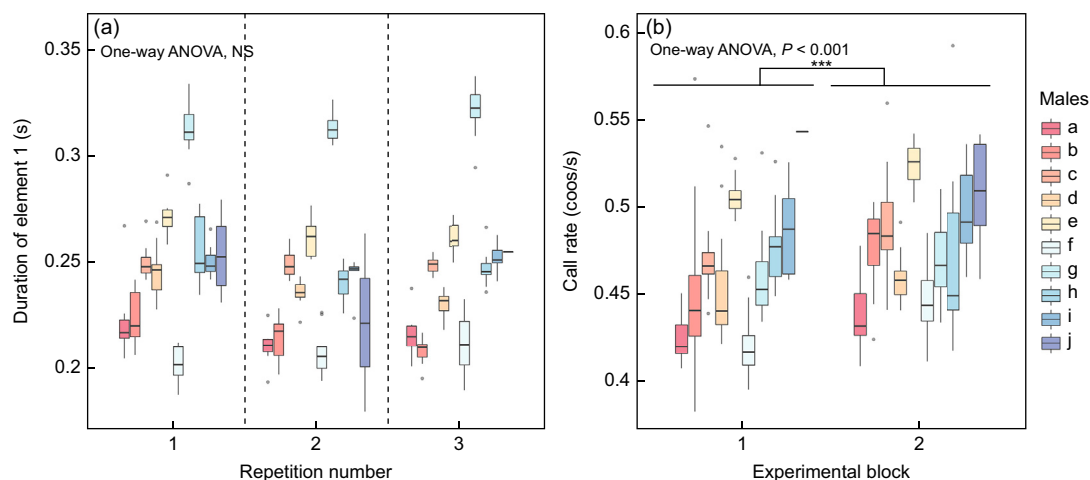


Figure 4. Changes in male behaviour over the course of the experiment. (a) Box plot of the individual duration of the first element of the coo call in the three sessions (repetition number) in which males encountered the same female. Values are averaged over experimental blocks. The result of the ANOVA testing the difference between repetition numbers is shown. (b) Box plot of the individual call rate during the two experimental blocks. Values are averaged over repetition number. The result of the ANOVA testing the difference between experimental blocks is shown. Asterisks represent significance between experimental blocks ($P < 0.001$). Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range and dots are outliers.

individual females was higher (1.480; Table 4) than interindividual variability for males (2.17e-11) or for the dyad (2.34e-8). The median F0 of element 2 of the call was negatively associated with tail quivering ($\chi^2_1 = 5.806$, $P = 0.016$; Fig. 5, Table 4). Courtship structure was also associated with female tail quivering, with the number of tail quivers being higher for high values of total courtship duration ($\chi^2_1 = 11.873$, $P < 0.001$) and low values of courtship bout duration ($\chi^2_1 = 4.117$, $P = 0.042$).

All other full-null models testing the effects of male courtship variables on female response were not significant. This concerns the proportion of time females spent next to the male ($\chi^2_{14} = 11.87$, $P = 0.61$), the number of times the female self-preened ($\chi^2_{14} = 17.42$, $P = 0.23$), approach attempts ($\chi^2_{14} = 18.68$, $P = 0.17$), steps ($\chi^2_{14} = 20.96$, $P = 0.08$) and pecks ($\chi^2_{14} = 21.34$, $P = 0.09$).

Effect of Repeated Encounters and Male Identity

The number of steps was linked to repetition number (repeated encounters with the same individual; full-null model comparison, likelihood ratio test: $\chi^2_5 = 11.91$, $P = 0.035$), as was the number of approaches ($\chi^2_5 = 15.92$, $P = 0.007$). In particular, the interaction term between repetition number and experimental block was significant in explaining the number of female approaches ($\chi^2_2 = 9.25$, $P = 0.009$), with females approaching the male less often during the third encounter with the same male of the second experimental block (i.e. the last test session of the experiment). Step number was linked to experimental block ($\chi^2_2 = 9.53$, $P = 0.002$). It was smaller during the second (58.32 ± 85.08 , $N = 147$, range 0–435) than during the first experimental block (100.68 ± 124.48 , $N = 148$, range 0–529) but did not vary with repetition number ($\chi^2_2 = 0.12$, $P = 0.93$).

When testing the effects of male identity, we had to take the inherent asymmetry in the experimental design into account, in that males from group C met females during experimental block 1 whereas males of group D met females during block 2. Additionally, some female behaviours (number of steps and approaches) were found to differ between experimental blocks (see above). We therefore conducted separate analyses for each experimental block, each containing five individual males, that is, males in group C in block 1 and males in group D in block 2. For C males, we found that male identity was linked to preening behaviour (full-null model comparison, likelihood ratio test: $\chi^2_4 = 12.35$, $P = 0.01$; Fig. 6) and peck number ($\chi^2_4 = 9.63$, $P = 0.04$). For D males, male identity was associated with the number of approaches ($\chi^2_4 = 15.75$, $P = 0.003$),

preening behaviour ($\chi^2_4 = 13.17$, $P = 0.01$; Fig. 6) and step number ($\chi^2_4 = 11.24$, $P = 0.02$). The link between male identity and female behaviour remained the same whether the data set we used in our model comprised all the sessions or only the sessions in which males were courting.

DISCUSSION

The aim of this study was to describe and quantify how variation in the structure and synchronization of components of male ring dove courtship was associated with variation in the patterns and strength of the female behavioural response. Rather than investigating female choice for specific characters, we studied whether

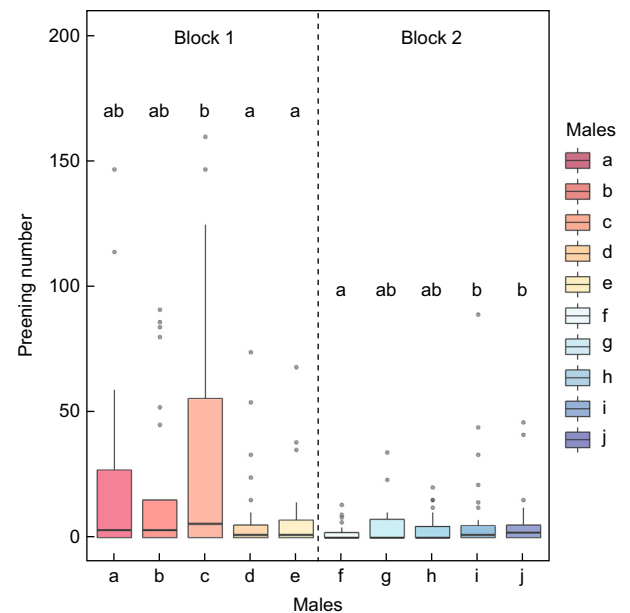


Figure 6. Box plot of number of times the female self-preened when facing individual males. Different letters indicate significant differences between males; these were extracted from post hoc tests run after generalized linear mixed models using a negative binomial distribution. Analyses were performed separately for the two male groups (i.e. the two female experimental blocks). Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range and dots are outliers.

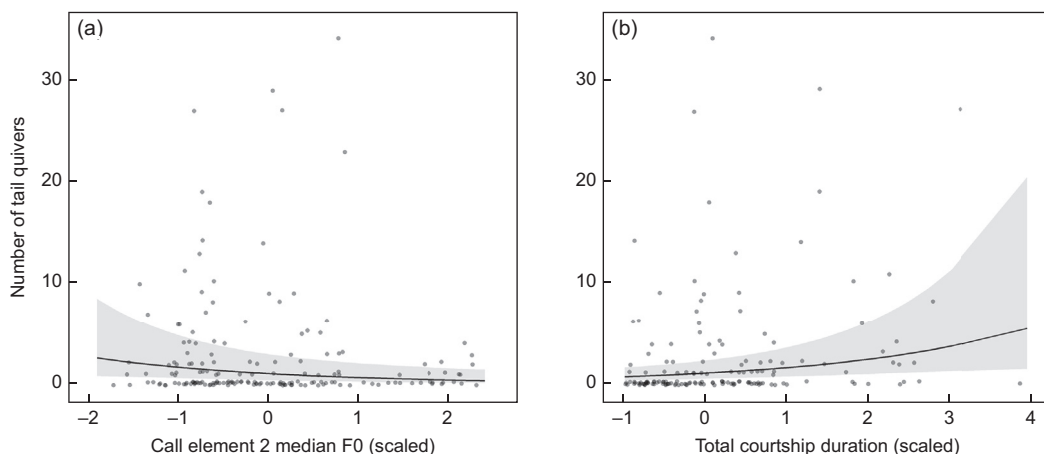


Figure 5. Raw data of the fixed effects with regression line predictions and 95% confidence intervals for the model investigating the effect of male courtship variables on number of female tail quivers. (a) Number of tail quivers versus median fundamental frequency of element 2 of the coo call. (b) Number of tail quivers versus total courtship duration.

different male signals were associated with different female behavioural responses. We hypothesized that different types of female response (which would be overlooked if only mating choice or time spent with a focal male was considered) could relate to different information contained in the male multimodal signal. Overall, tail quivering and number of steps were distinctive responses of females that depended on the presence of male courtship. Individual males differed in most courtship components, both acoustic and visual. Both male and female behaviour changed across the experimental period. The fundamental frequency of the call, number of courtship bouts and the total duration of the courtship were related to tail quivering, which was the only variable of female behaviour that was associated with male courtship traits. Finally, we found that female behaviour depended on which male they encountered.

When investigating female response to male presence, we found that the numbers of tail quivers, steps and pecks were higher when females interacted with a male. Steps and tail quivers were also more frequent during encounters with a courting male than in the presence of a noncourting male. Female activity was therefore positively influenced by male presence as well as male courtship. In pigeons, *Columbia livia*, changes in females' general activity and number of steps are linked to female choice and female sexual interest (Partan, Yelda, Price, & Shimizu, 2005), and in *Drosophila* sp., female movement is needed for the male to keep courting (Tompkins, Gross, Hall, Gailey, & Siegel, 1982), demonstrating a role of female activity for the maintenance of courtship interaction. Additionally, we found that females quivered their tail exclusively when a male was present, and the number of tail quivers was much higher during encounters with a courting male. Rapid shaking of the tail is a sign of sexual stimulation and male acceptance in zebra finches, *Taeniopygia guttata* (Witte, 2006) and song sparrows, *Melospiza melodia* (O'Loughlen & Beecher, 1997). In birds of paradise, wing fluttering seems to be linked to mutual courtship (Scholes, Gillis, & Laman, 2017). In ring doves, the copulation solicitation display that appears after several days of courtship interaction is sometimes also associated with wing fluttering (Zenone 1979). Thus, our results strongly support the notion that tail quivering is a reliable indicator of female sexual response in ring doves.

The courtship variables we analysed were similar to those already described in the dove literature (Davis, 1970; Fusani et al., 1997). We found that males differed greatly from each other in all the courtship variables investigated (acoustic structure, spectral, visual and audiovisual synchronization variables). A previous study (Fusani et al., 1997), however, did not find differences in the temporal structure of the bowing movements. The discrepancy is probably due to differences in statistical power, as in the present study we had a larger number of individuals and more courtship events. Our results are consistent with findings in other species where males show high interindividual and small intraindividual variation in courtship variables (Abs & Jeismann, 1988; Jiguet & Bretagnolle, 2001; Jouventin, Guillotin, & Cornet, 1979). When investigating the role of specific courtship variables, we found that the only female behaviour to be influenced by variation in male courtship variables was the number of tail quivers. We found that total courtship duration was positively associated with the number of tail quivers while mean bout duration had a negative impact. This seems to indicate that females were more stimulated by many shorter bouts than by one long courtship bout. In doves, the bow-call courtship is structurally similar to the aggressive display males use in agonistic contexts (Lovari & Hutchison, 1976). A long, uninterrupted bow-call display could therefore be perceived as more aggressive than attractive and might decrease the level of interest or stimulation in the female, a phenomenon already known from other species (Bastock, 1967; Patricelli, Uy, Walsh, & Borgia,

2002, Ophir, Persaud, & Galef, 2005, but see Borgia & Coleman, 2000 for the opposite effect).

The other variable that affected tail quivering was the median fundamental frequency of the second element of the call: a lower-pitched call triggered more tail quivers. As the median frequency depends on both the maximum and minimum frequency of the call, this variable captures how high pitched the call was on average. In birds, preference for higher- or lower-pitched vocalizations is found in a number of species (Cardoso, 2012). For instance, female Japanese barn swallows, *Hirundo rustica*, seem to prefer high-frequency male calls (Hasegawa & Arai, 2016), and the same is true in rock sparrows, *Petronia petronia*, Nemeth et al., 2012). On the other hand, Miyasaki and Waas (2003) found that females were more likely to respond to low-pitched calls in little penguins, *Eudyptula minor*. In the grey partridge, *Perdix perdix*, females also seem to prefer males with lower formant frequency (Beani & Dessì-Fulgheri, 1995). Birdsong, and especially the spectral properties of vocalizations, can carry honest signals of male physical traits that are associated with mate quality (i.e. size or age) and can be used by females in a partner choice context (Gil & Gahr, 2002). One of the commonly invoked mechanisms for this is the action of androgens on sound frequency production. Indeed, female selection for androgen-dependent traits has been documented in several species (Fusani et al., 1997; Hagelin & Ligon, 2001; Peters, Astheimer, Boland, & Cockburn, 2000). In particular, lower fundamental frequency calls are associated with higher androgen levels in birds (Cynx, Bean, & Rossman, 2005), and low-frequency calls can therefore be used by females as an indicator of male quality (Beani & Dessì-Fulgheri, 1995; Fusani, Beani, & Dessì-Fulgheri, 1994). However, the link between androgen levels and male quality is not unequivocal, as high androgen levels may also affect the immune system (Roberts, Buchanan, & Evans, 2004). Circulating androgens at a given time can affect song parameters, such as call duration (Fusani et al., 1994), call fundamental frequency (Cynx et al., 2005) or call rate (Beani, Lupo, Dessì-Fulgheri, Briganti, & Campanella, 2000). Basal testosterone levels during development can also alter the fundamental frequency of vocalizations by impacting trachea and syrinx morphology as is the case in grey partridges (Beani, Panzica, Briganti, Persichella, & Dessì-Fulgheri, 1995). A link between fundamental frequency and trachea morphology exists in many species, with animals having larger vocal cords usually producing lower-frequency calls or songs (Riede & Brown, 2013). This could also be true for doves, as in this species administration of exogenous androgen does not affect the fundamental frequency of calls nor other aspects of courtship such as duration or structure (Feder, Storey, Goodwin, Reboulleau, & Silver, 1977; Fusani & Hutchison, 2002; O'Connell, Reboulleau, Feder, & Silver, 1981). In our study, call fundamental frequencies were not affected by experimental factors and remained constant for each male across the experiment, suggesting that they are unlikely to depend on contingent external conditions or internal physiological factors. Rather, call fundamental frequency in doves seems to be a marker of androgen levels during development. However, we cannot exclude that call fundamental frequency could also be linked to morphological features such as body size or weight, as is the case in New World doves (Tubaro & Malher, 1998). For instance, in the males used for our experiment, the e2 median frequency was positively correlated with P8 feather length (mean \pm SD = 128.7 \pm 2.07 mm), a common proxy measure of body size in birds, but not with the tarsus (29.1 \pm 0.93 mm) or the wing length (172.5 \pm 4.26 mm; Fig. A5). Temporal structure and spectral frequency of calls are linked to individual identity in some species (Grunst, Grunst, Formica, Gonser, & Tuttle, 2017), including doves (Hutchison, Hutchison, & Fusani, 1997). Like most courtship variables, the fundamental frequency of the second call element was

highly variable between males and had relatively small intra-individual variation, making it a potential marker for male identity.

In addition to the variables with low intraindividual variation mentioned above, our results show that females were influenced by courtship characteristics that can change over time. For example, as already stated, females preferred longer courtship separated into smaller bouts. These features were not consistent for each male, as total courtship duration and bout duration were shorter during the second experimental block. Courtship call rate was also influenced by repeated encounters, as it was higher at the end of the experiment than at the beginning. The correlation values between different courtship elements show that this higher rate was due to shortening of both the duration of the second call element and the time spent in the up position of bows. Courtship duration can be linked to physical condition (Simmons, 1988; Bertram & Rook, 2012) and therefore has been proposed as a way of displaying current mate quality (Seymour & Sozou, 2008). The decrease in courtship duration towards the end of the experiment could indicate increasing fatigue as testing continued. For example, similar patterns exist in the ruffed grouse, *Bonasa umbellus*, where number and rate of displays declined over time in some males, which was suggested to be due to fatigue (Déaux et al., 2020). By preferring long overall courtship duration, females might therefore favour high-performing males. Like courtship duration, display rate is associated with vigour and condition in many different clades (Dunning, Pant, Murphy, & Prather, 2020; Mowles, Jenion, & Backwell, 2017; Mowles & Jepson, 2015; Pellitteri-Rosa, Sacchi, Galeotti, Marchesi, & Fasola, 2011; Takeshita, Murai, Matsumasa, & Henmi, 2018). In doves, the fact that courtship length decreased over multiple test sessions, whereas courtship rate increased might indicate that males perform an energetic trade-off between courtship vigour and courtship time. Such a mechanism occurs in the golden-collared manakin, *Manacus vitellinus*, in which Tobiansky, Miles, Goller, and Fuxjager (2020) recently demonstrated the role of androgen action on male muscles in mediating the trade-off between endurance and speed during courtship.

The time a female spends next to the male is a variable often used as a proxy for female choice and female interest (Wagner, 1998; Witte et al., 2006; Dougherty, 2020). In our study, however, it was not influenced by male courtship variables. It is possible that our experimental set-up was not optimal for measuring sexual interest using the distance between male and female. The apparatus was relatively small and movements within the test box might have been too limited to reflect the extent of female interest. Other aspects of female behaviour that were not affected by variation in male behaviour were general activity and self-preening. However, although these behavioural patterns are linked to sexual interest in some species (Cheng, 1973; Partan, 2005; Tinbergen, 1952), they could also simply be displacement behaviour and reflect discomfort (Delius, 1988; Laurence et al., 2012; Maestripietri, Schino, Aureli, & Troisi, 1992). Regarding approach attempts, our results suggest that this variable does not reflect sexual interest in doves. Although the difference was not significant, females attempted to enter the opposite box more often in the control condition (where no male was present) than when a male was present. This seems to indicate that these behaviours do not reflect female sexual or social interest in doves, but rather arousal or excitement.

The female response depended on the identity of the male within the same experimental block. Approach behaviour, general activity (steps and pecks) and preening depended on which male they encountered. It is not clear, however, what was responsible for these differences. Female response could depend on physical

characteristics, such as the male's size or plumage colours, factors assessed by females in some species in the context of sexual interactions (Hill, 2006), although this is less likely to occur in a monomorphic and monogamous species like the ring dove (Kirkpatrick, Price, & Arnold, 1990). Additionally, other aspects of male behaviour that we did not consider could be the source of this female behavioural variation, such as call frequency modulation (Drăgănoiu, Nable, & Kreutzer, 2002) or call sound pressure level (Ritschard et al., 2010). We measured courtship variables that have been shown to influence female response; however, some non-investigated courtship variables (such as call modulation or velocity of movement), as well as physical characteristics such as body size, might also impact the female response.

Some male variables we suspected would affect female behaviour were not associated with differences in the female response. For instance, females might have been expected to respond differently to different bowing amplitudes, as is the case in the fiddler crab *Austruca perplexa*, in which Murai and Backwell (2006) described female choice for display structure and claw movement amplitude during courtship. Also, temporal synchronization between different modalities and the extent to which individuals manage to coordinate two (or more) different signals in time is linked to female preference in some species. In Túngara frogs, *Physalaemus pustulosus*, for example, females prefer synchronous multimodal courtship signals (Taylor, Klein, Stein, & Ryan, 2011), and the same is true for the brush-legged wolf spider, *Schizocosa ocreata* (Kozak & Uetz, 2016). Although in our study we did not find any effect of movement amplitude, multimodal synchronization or other courtship variables on the female dove's response, this does not mean that these variables are meaningless. The multiple message hypothesis states that different elements of a complex signal can have different impacts on a receiver. Here, we exclusively studied immediate female behavioural responses, but it is likely that variation in courtship variables affects other aspects of the female response. For instance, the females' physiological state can be affected by courtship in arthropods (Rybak, Sureau, & Aubin, 2002), reptiles (Crews, 1975; Kelso & Martins, 2008) and doves (Cheng, Peng, & Johnson, 1998). In doves, we know that the females' physiological state is strongly courtship dependent, as females show a higher neuroendocrine response (measured by oviduct size) when presented with multisensory courtship compared to auditory only (Friedmann, 1977). In the present study we did not measure the physiological response to courtship; however, we would expect that some courtship elements would affect the physiological response in the same way call fundamental frequency or courtship duration did. For example, it is not clear why female behaviour was influenced by the fundamental frequency of the second call element and not by that of the first one. The duration of the call could play a role, as the second element was on average almost five times longer than the first. However, it is also possible that the fundamental frequency of the first element impacted an aspect of the female response we did not investigate. It could be a behaviour that we did not consider, or even a physiological response in the form of hormonal changes influencing ovarian development (Crews, 1975; Friedmann, 1977).

Assessing the extent to which females vary in their response to courtship by measuring variation in individual preference was beyond the scope of this study. Nevertheless, we showed that even in a controlled experimental set-up, females differed greatly from each other in the range and intensity of their responses to courtship and changed their behaviour over time. In fact, a large proportion of response variation to courtship parameters was due to female identity. Females were housed together in aviaries

during the experiment and their last visual interaction with males was weeks before the experiment started. However, the large variation between females seems to suggest that they were not all in the same reproductive state or equally motivated during this experiment, some of them appearing to be more sexually receptive than others. The fact that females showed fewer approach attempts as well as less general activity towards the end of the experiment might indicate that their behaviour was also impacted by repeated exposures to courtship. Whether this was due to the potential aggressive messages contained in repeated courtship signals or simply habituation or disinterest is unknown. However, despite the large interindividual variability, patterns of exposure to courtship and variation in courtship variables were consistent between females. For example, females that showed the greatest number of tail quivers in front of a courting male also quivered their tail even when the male in front of them was not courting. This suggests that the relative increase in sexual behaviour compared to the individual female's precourtship state might be more informative than the absolute frequency of sexual behaviours displayed towards a male when studying the female response to courtship.

To our knowledge, this study is one of the first to investigate on such a fine scale the influence of small, natural variation in a large number of male courtship elements on different female behavioural responses. By analysing in parallel the effects of component signals, we identified female behavioural responses to male acoustic spectral elements and courtship structure (courtship length and number of courtship bouts, both likely to be markers of courtship effort). Additionally, investigating a wide range of female behavioural responses allowed us to assess possible targets of male courtship variables that might have been overlooked if we had taken only the female's final mate choice into account. We showed that females might be interested in markers of courtship effort, as well as in specific individual males. Ring doves are monogamous, and it is also highly likely that females use courtship not only to make a mating decision, but also to optimize pair matching and reinforce an existing pair bond over several reproduction episodes. Ultimately, the final pairing and mating decision in doves might depend not only on several messages contained in the bow-call display, but also on courtship elements present at later stages of the interaction which we did not explore here. Our results remain correlational, and manipulative studies are needed to specifically test how variation in male behavioural variables affects the female response. Progress in audiovisual technology now allows the creation and display of high-quality video stimuli and these playback techniques can be particularly relevant in the context of sexual communication. Future studies should focus on creating modified courtship stimuli and presenting them to females. This will allow an assessment of how females respond to specific, controlled variation in targeted male courtship variables.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

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Appendix

Table A1

Summary statistics of male courtship variables of interest

Courtship parameters	N	Mean	SD	Minimum	Maximum	Median
Call: temporal structure						
Courtship duration (s)	253	79.13	52.98	5.52	288.95	66.82
Courtship bout duration (s)	253	38.60	29.40	5.52	288.95	31.25
Number of coos	253	35	24	3	136	30
Number of bouts	253	3	2	1	14	2
Coo rate (coos/s)	253	0.46	0.03	0.38	0.54	0.46
e1 duration (s)	253	0.25	0.03	0.18	0.34	0.24
e2 duration (s)	253	1.16	0.10	0.83	1.51	1.14
Intracoo duration (s)	253	0.12	0.07	0.00	0.33	0.11
Intercoo duration (s)	253	0.72	0.13	0.37	1.39	0.72
Call: spectral structure						
e1 maximum F0	253	640.15	35.50	559.47	717.05	635.99
e1 median F0	253	549.29	29.64	472.79	618.22	549.88
e1 minimum F0	253	458.42	32.06	372.23	523.23	454.71
e2 maximum F0	253	597.85	36.03	532.53	681.89	584.51
e2 median F0	253	468.17	45.76	380.89	580.94	464.63
e2 minimum F0	253	338.48	67.52	184.56	483.86	335.51
Bow						
Bowing amplitude (in pixels)	05	200.72	49.02	80.79	294.29	210.33
Up time (s)	204	0.566	0.149	0.167	1.054	0.545
Down time (s)	204	0.694	0.167	0.229	1.176	0.695
Ascent time (s)	204	0.404	0.067	0.229	0.584	0.405
Descent time (s)	204	0.460	0.079	0.254	0.757	0.449
Audiovisual synchronization (s)						
Onset of e1–Onset of descent movement	174	0.144	0.204.76	−0.404	0.785	0.183
Offset of e1–Offset of descent movement	174	−0.070	0.223.97	−0.540	0.953	−0.011
Onset of e2–Onset of ascent movement	174	−0.610	0.257.71	−0.998	0.907	−0.615
Offset of e2–Offset of ascent movement	174	0.027	0.269.33	−0.683	0.930	0.051

Table A2

ANOVA for every courtship variable testing differences between males

Variable	Male					Error		
	Sum of squares	Df	Mean square	F	P	Sum of squares	df	Mean square
Total courtship duration	237559.8	9	26395.54	13.68973	<0.001	468534.9	243	1928.127
Number of calls	10786.39	9	1198.488	8.423826	<0.001	34572.47	243	142.2736
Number of bouts	164.2352	9	18.24835	5.298321	<0.001	836.9348	243	3.444176
Call rate	0.16424	9	0.018249	24.35559	<0.001	0.182072	243	0.000749
e1 duration	0.242555	9	0.026951	185.8483	<0.001	0.035238	243	0.000145
e1 duration	1.46	9	0.162222	37.35151	<0.001	1.055379	243	0.004343
Intracall duration	0.991311	9	0.110146	174.7111	<0.001	0.153198	243	0.00063
Interacall duration	2.361099	9	0.262344	30.4728	<0.001	2.092019	243	0.008609
e1 max F0	286971.5	9	31885.73	272.3804	<0.001	28446.36	243	117.0632
e1 min F0	206139	9	22904.34	96.47826	<0.001	57689.21	243	237.4041
e1 median F0	196664.8	9	21851.64	207.5633	<0.001	25582.31	243	105.277
e2 max F0	294478.7	9	32719.86	247.886	<0.001	32074.92	243	131.9956
e2 min F0	885575.3	9	98397.26	93.02243	<0.001	257040.5	243	1057.78
e2 median F0	450261.3	9	50029.04	160.6316	<0.001	75682.85	243	311.4521
Bowing amplitude	240737.1	9	26748.57	20.91032	<0.001	249444.8	195	1279.204
Up duration	2.334278	9	0.259364	22.82047	<0.001	2.204892	194	0.011365
Descent duration	0.195819	9	0.021758	3.910151	<0.001	1.079497	194	0.005564
Down duration	3.13594	9	0.348438	28.93897	<0.001	2.335845	194	0.01204
Ascent duration	0.43572	9	0.048413	23.81089	<0.001	0.394449	194	0.002033
Sync 1	1.275967	9	0.141774	3.889493	<0.001	5.97789	164	0.036451
Sync 2	1.012865	9	0.112541	2.407702	<0.001	7.66567	164	0.046742
Sync 3	3.536867	9	0.392985	8.103903	<0.001	7.952907	164	0.048493
Sync 4	4.705035	9	0.52278	12.084	<0.001	7.095119	164	0.04326

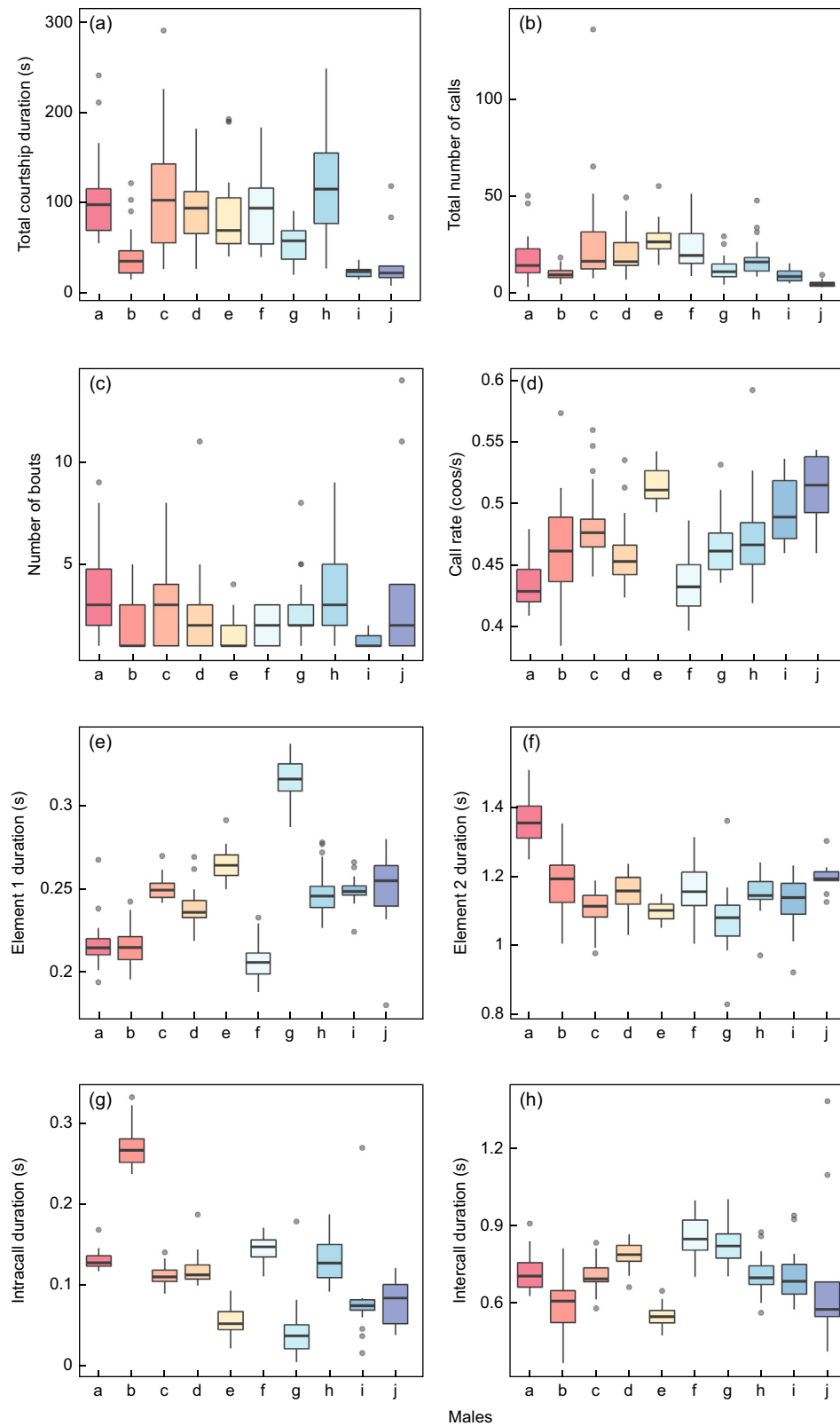


Figure A1. Box plots representing inter- and intramale variability for courtship structure variables: (a) total courtship duration, (b) total number of calls, (c) number of courtship bouts, (d) call rate, (e) call element 1 duration, (f) call element 2 duration, (g) intracall duration, (h) intercall duration. Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range and dots are outliers.

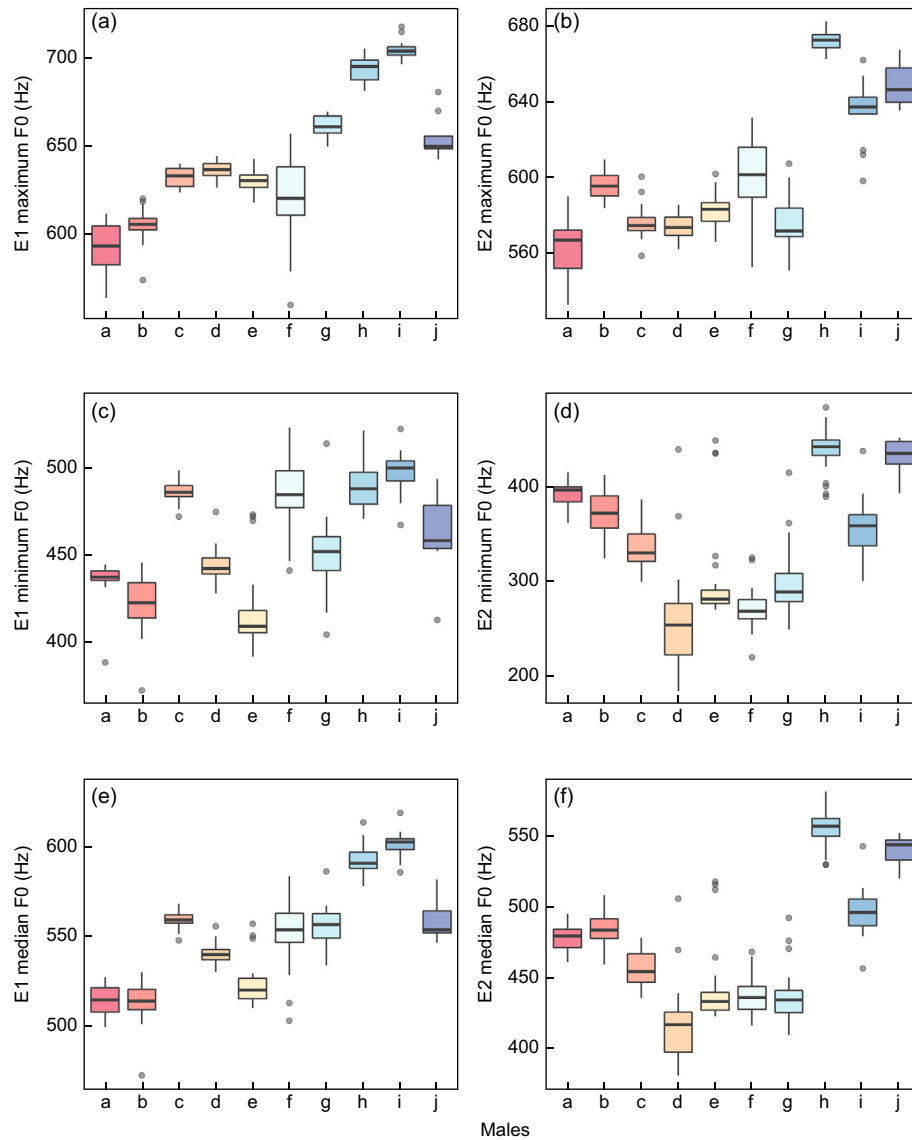


Figure A2. Box plots representing inter- and intramale variability for spectral acoustic courtship variables: (a) element 1 (e1) maximum fundamental frequency (F0), (b) element 2 (e2) maximum F0, (c) e1 minimum F0, (d) e2 minimum F0, (e) e1 median F0, (f) e2 median F0. Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range and dots are outliers.

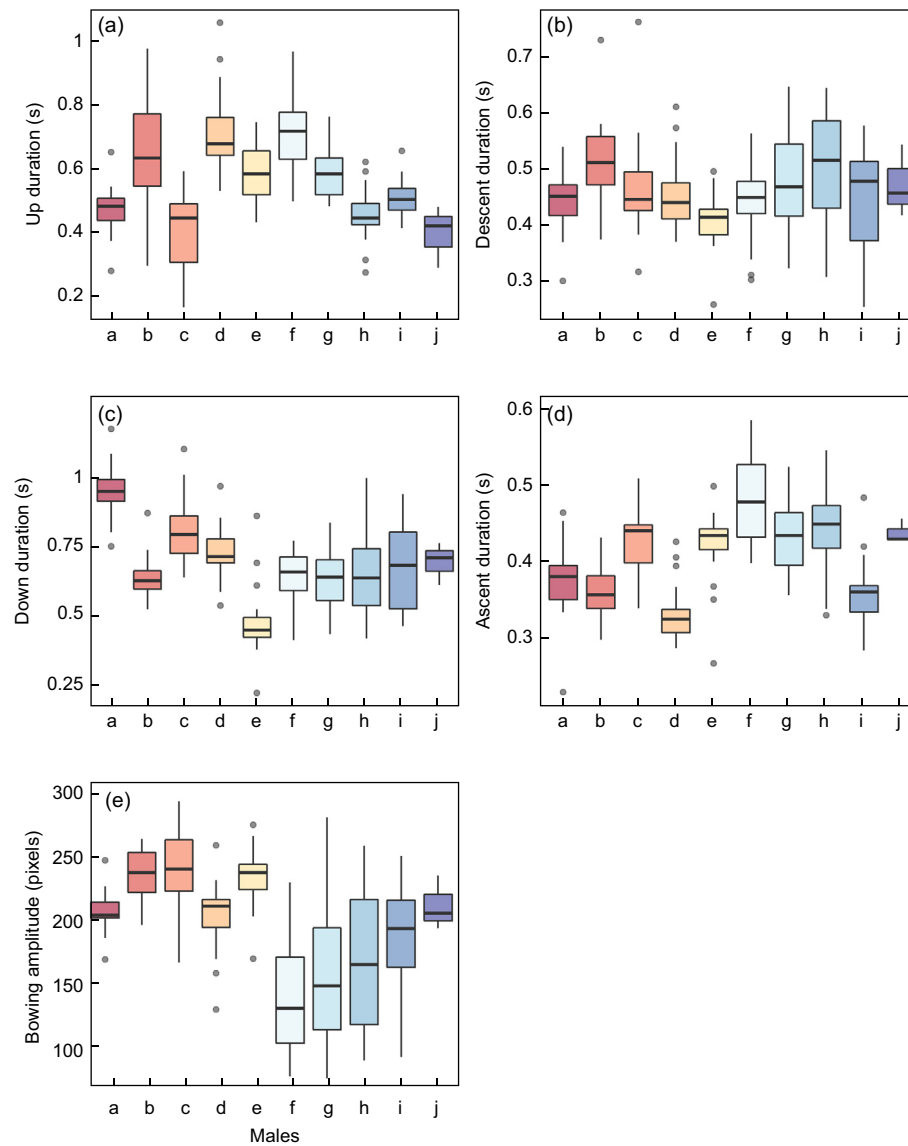


Figure A3. Box plots representing inter- and intramale variability for visual courtship variables: (a) bowing up duration, (b) bowing descent duration, (c) bowing down duration, (d) bowing ascent duration, (e) bowing amplitude. Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range and dots are outliers.

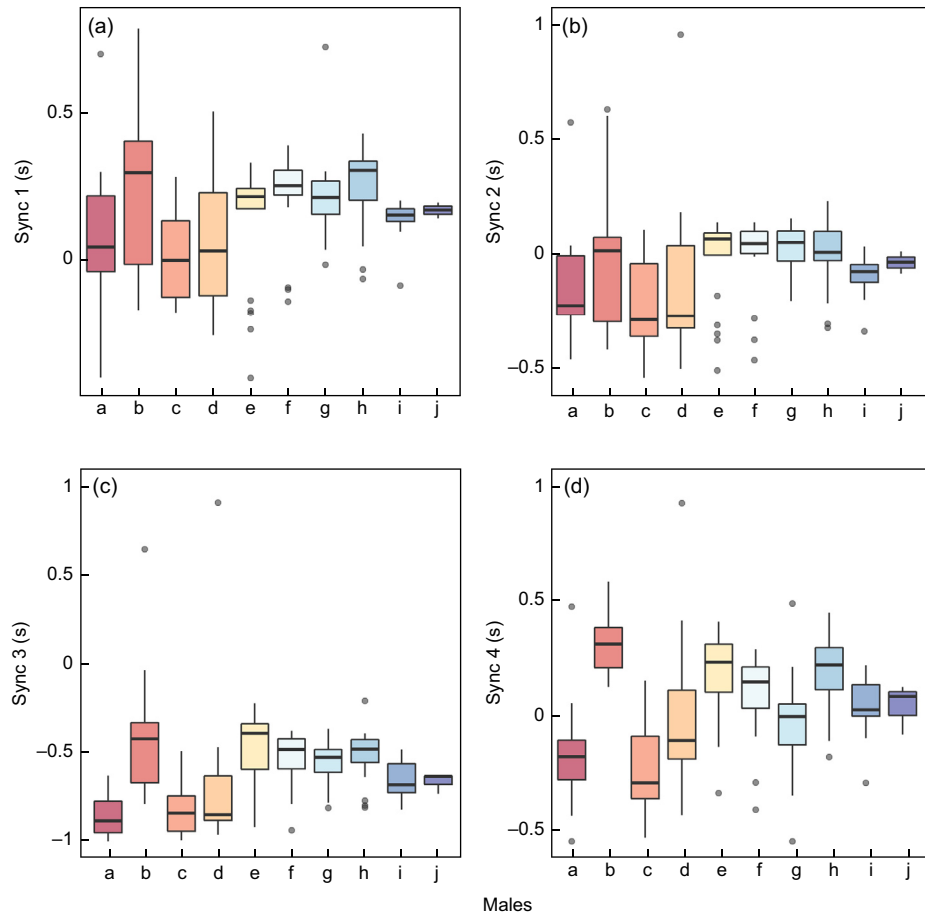


Figure A4. Box plots representing inter- and intramale variability for courtship audiovisual synchronization variables: (a) difference between onset of e1 and onset of descent movement, (b) difference between offset of e1 and offset of descent movement, (c) difference between onset of e2 and onset of ascent movement, (d) difference between offset of e2 and offset of ascent movement. Box plots display the median, lower and upper quartiles. Whiskers represent values within 1.5 times the interquartile range and dots are outliers.

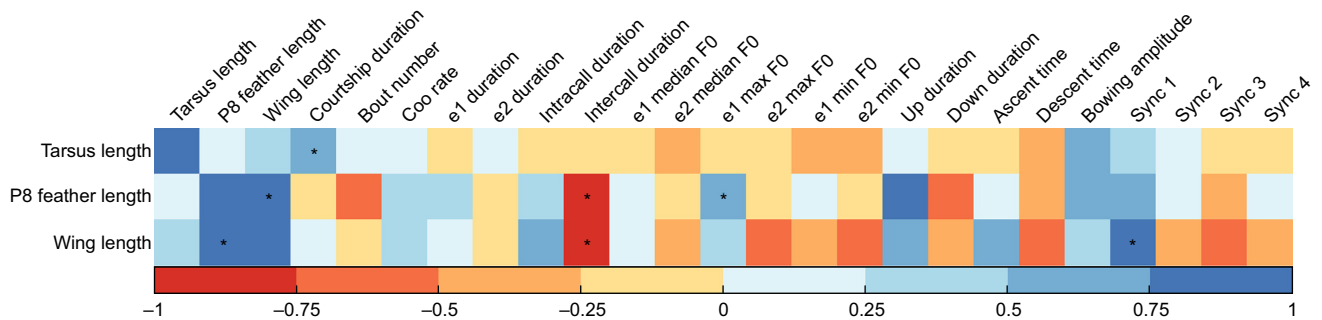


Figure A5. Correlation matrix between body size measurements (tarsus length, P8 feather length and wing length) from nine of the males and their courtship audiovisual parameters (see Table 2 for details). Stars indicate a significant correlation between two variables (Spearman correlation: $P < 0.05$).