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RESEARCH ARTICLE

Three-dimensional movement of the beak during seed processing in domestic canaries

Maja Mielke* and Sam Van Wassenbergh

ABSTRACT

Many songbird species rely on seeds as a primary food source and the process of picking up, positioning, cracking, dehusking and swallowing seeds is one of the most sophisticated tasks of the beak. Still, we lack understanding about how granivorous songbirds move their beak during the different phases of seed processing. In this study, we used multi-view high-speed imaging to analyze the 3D movement of the beak in feeding domestic canaries. Our analysis focused on the correlation of the upper and lower beak, the frequency of mandibulation and the direction of mandible movement in 3D space. We show that the correlation of maxilla and mandible movement differs among the phases of seed processing. Furthermore, we found that the beak moves at extremely high frequencies, up to 25 Hz, which resembles previously reported maximal syllable rates in singing canaries. Finally, we report that canaries use specific 3D mandible movements during the different phases of seed processing. Kinematic parameters do not differ between male and female canaries. Our findings provide an important biomechanical basis for better understanding the beak as a functional tool.

KEY WORDS: Beak movement, Feeding, Kinematics, Seed cracking, Songbirds

INTRODUCTION

In granivorous songbirds, efficient cracking and dehusking of seeds is essential for meeting the high energy demands and minimizing exposure to predators by reducing foraging times. The ability of birds to successfully crack certain types of seeds is largely determined by beak size and shape, as has been mostly described in Darwin's finches (Grant, 1981; Grant and Grant, 1995; Herrel et al., 2005), but also in other songbirds (Kear, 1962; Ziswiler, 1965; Smith, 1987). In seed-cracking birds, not only the outer shape but also the inner beak morphology is adapted to a granivorous diet, showing specialized grooves and edges on the horny palate aiding in seed immobilization (Ziswiler, 1965) and an adapted trabecular bone within the bony part of the beak improving mechanical resistance during biting (Genbrugge et al., 2012). For very hard seeds, birds need a sufficient bite force, which is related to beak morphology (Herrel et al., 2005), skull morphology (van der Meij and Bout, 2008) and jaw muscle size (van der Meij and Bout, 2004; Genbrugge et al., 2011). Furthermore, feeding on hard seeds requires the beak and skull to withstand demanding loading regimes to avoid fractures (Soons et al., 2010, 2015).

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Different songbirds show different efficiency and techniques in cracking the same type of seeds (Kear, 1962; Grant, 1981; van der Meij and Bout, 2006). Small-billed birds need longer for processing large and hard seeds. Large-billed birds, however, are also less efficient and less successful in handling small seeds (Abbott et al., 1975; Smith, 1987), presumably because they have difficulty positioning small seeds for cracking (Abbott et al., 1975). To meet their high energy demands, small-billed birds need to compensate for their inability to crack large seeds that exceed their bite force by a high frequency of consumption of small seeds (Benkman and Pulliam, 1988). Furthermore, they use seed size characteristics to predict seed hardness and selectively choose seeds that are in the range of their biting ability (van der Meij and Bout, 2000).

Adaptations to produce high bite forces and crack hard seeds impose constraints on beak movement in general. Kinematic analyses of beak movement during singing have revealed that songbirds with a high bite force move their beak at lower frequencies and produce songs of lower frequency bandwidths than birds with low bite force (Podos, 2001; Huber and Podos, 2006; Herrel et al., 2009). In contrast, small-billed songbirds with low bite forces, such as domestic canaries (Fringillidae, 'true finches'), move their beak extremely fast (up to 30 Hz) during singing (Drăgănoiu et al., 2002). Such high beak agility should be beneficial for successful feeding on small seeds in canaries. In order to test this, kinematic analysis of beak movement during feeding is needed, which will help to reveal whether feeding and singing behavior share similar performance constraints.

In contrast to mammals, in birds, both the upper jaw (maxilla) and the lower jaw (mandible) contribute to gape opening. The elevation of the maxilla is called cranial kinesis, which can occur via rotation around the naso-frontal hinge (prokinesis) or via bending zones along the dorsal bar of the maxilla (rhynchokinesis; Bock, 1964). Maxilla elevation is induced via a forward rotation of the quadrate bones, which protract the jugal bones and the pterygoid–palatinum complex, and is thus only possible because the avian cranium is highly kinetic. In some species, a coupling mechanism has been described that induces maxilla elevation when the mandible is depressed, resulting in a synchronized movement of the mandible and maxilla (Bock, 1964; Zusi, 1967). However, the exact mechanism of coupling is still not fully resolved. Bock (1964) has suggested that inextensible ligaments such as the postorbital ligament (PO ligament) or functionally equivalent ligaments induce coupled kinesis via restriction of mandible depression. Zusi (1967), however, has shown in domestic chickens and evening grosbeaks that the coupled coordination of the upper and lower beak does not require a PO ligament and can even be induced by activation of the M. depressor mandibulae alone. In zebra finches, the PO ligament has been shown to contribute only 20% of total resistance force (Nuijens and Bout, 1998), which makes a central role of this ligament in coupled kinesis unlikely in that species. Furthermore, in white-throated sparrows, maxilla and mandible

movement can be completely independent (uncoupled kinesis), although a PO ligament is present (Hoese and Westneat, 1996). Many studies on beak kinematics during singing or feeding do not allow for an analysis of coupled kinesis, because only gape distance and/or gape angle is recorded (e.g. Westneat et al., 1993; Bout and Zeigler, 1994; van der Meij and Bout, 2008; Neves et al., 2019). To analyze whether maxilla and mandible movement are coupled or not, movement of the upper and lower beak needs to be recorded separately relative to a common reference frame (cf. van der Meij and Bout, 2006). Such an analysis, combined with anatomical knowledge of the species, is needed to identify how specific cranial morphologies relate to the coupling of beak movement.

During seed processing, the beak can move in a way that goes far beyond a mere open–close mechanism. In particular, for efficient positioning, cracking and dehusking of seeds, the lower beak can perform medio-lateral movement, as has been described in different fringillids (Ziswiler, 1965; Nuijens and Zweers, 1997; van der Meij and Bout, 2006), but the extent to which medio-lateral movement is used depends on the species and cracking techniques. It has been suggested that the absence of a PO ligament can facilitate medio-lateral movement of the mandible during seed dehusking (Nuijens and Zweers, 1997; Nuijens and Bout, 1998). This would be in line with the findings of comparative studies on two families of granivorous passerine birds, Fringillidae and Estrildidae. These studies showed that medio-lateral movement occurs in fringillid species, which do not have a (fully developed) PO ligament, but does not occur in estrildid species, which have a PO ligament (e.g. Nuijens and Zweers, 1997; van der Meij and Bout, 2006). Most studies on beak kinematics, e.g. of singing, are only based on lateral recordings, allowing for an analysis of movement in the sagittal plane only (but see, for example, Gussekloo et al., 2001, for an *ex vivo* study of 3D kinematics). Consequently, *in vivo* medio-lateral movement of the mandible has only been described qualitatively so far, if at all (but see van der Meij and Bout, 2006, for a preliminary analysis). 3D video recordings of beak movement are necessary to assess beak movement in all planes. Analyzing how songbirds utilize the full range of motion of their beak during tasks such as seed cracking will contribute to our knowledge on the functional adaptation of the beak and the underlying cranial system.

In this study, we used multi-view high-speed videography of feeding domestic canaries to test the following hypotheses about the 3D movement of the beak in a granivorous songbird. Firstly, we hypothesized that upper and lower jaw movement are coupled, because this has been reported for another fringillid songbird before (Zusi, 1967). Secondly, we hypothesized that maximal beak movement frequencies during feeding are similar to those during the fastest song fragments. This is expected because of the sexual selection favoring fast songs in domestic canaries (Drăgănoiu et al., 2002). Thirdly, we hypothesized that domestic canaries utilize medio-lateral movement of the mandible during seed processing. This is especially expected for cracking attempts (cf. van der Meij and Bout, 2006) and during seed husk removal (cf. Ziswiler, 1965).

Our results provide novel insights into the beak kinematics of a granivorous songbird and help us to understand how they are functionally adapted to a mechanically demanding and sophisticated task like seed cracking.

MATERIALS AND METHODS
Study species
In this study, six individuals (three females, three males) of domestic canaries, *Serinus canaria domestica* (Linnaeus 1758), Fife Fancy breed, were used. Birds of the genus *Serinus* are small

granivorous passerine birds that belong to the finch family Fringillidae. The domestic canaries used for this study were from a lab-bred population of the Behavioral Ecology and Ecophysiology Research group at the University of Antwerp, Belgium. The birds were born in 2016 and experiments were performed between March and October 2021. Birds were housed individually in cages (60×50×40 cm) with water and standard seed mix available *ad libitum*. All experiments described in this study were approved by the Ethical Committee for Animal Testing of the University of Antwerp (ECD code: 2020-40).

Recording setup and experimental procedure
Video recordings were made at 500 frames s^{-1} using four synchronized high-speed cameras (FASTEC-IL5, Fastec Imaging, San Diego, CA, USA) with 35 mm f1.7 lenses and a shutter speed of 100 µs. The four cameras filmed the birds from four different angles in 2×2 arrangement. The feeder was positioned such that the birds, while looking ahead, faced the center of the 2×2 camera setup (Fig. 1A). Illumination was provided by near-infrared LED spots. Videos were stored as series of tiff images (936×1024 pixels). During recording, birds were in a glass cage containing bird sand, a long perch, water and a feeder with a short perch for sitting on while feeding. The feeder was positioned so that when the birds came to sit on the short perch, their beak was probably positioned in the center of the small focus volume. Before and after each recording session, 20–30 calibration images of a self-made 3D-calibration fixture were recorded. This allowed us to later reconstruct the beak movement in 3D space.

Nineteen hours prior to the recording session, birds were transferred to the glass recording cage where they were deprived of food but had access to water *ad libitum*. Before starting the recording, markers were applied with a fine black ink pen on the maxilla, mandible and head of the birds. Seven and five markers were applied on the maxilla and mandible, respectively, and six markers on the head (drawn on a piece of adhesive tape that was attached to the crown of the birds; see Fig. 1B,C). The canaries were offered hemp seeds (*Cannabis sativa*) in a small Petri dish (35×10 mm) on the feeder. Whenever the bird hopped on the short feeder perch and started feeding, video recording was started manually via an external trigger, and it was paused whenever the bird left the feeder. In addition to feeding, short sequences of the birds with a closed beak were recorded to determine the reference position of maxilla, mandible and head markers relative to each other. The session was stopped when the bird stopped feeding or after a maximum duration of 1 h, after which animals were returned to their home cage.

Video selection and digitization
From the raw video material, only successful feeding attempts were extracted (where the seed was cracked, dehusked and swallowed). The start of a feeding sequence was defined as the moment when the beak starts to open to pick up the seed and the end was defined as the moment when the seed is fully swallowed (see Movie 1). Because the head markers were used to define the reference frame of maxilla and mandible movement, videos were discarded when head markers were not fully visible. Camera calibration and marker tracking were done in XMALab (Knörlein et al., 2016). For marker tracking, dot detection was used, which successfully detected the markers on a great majority of frames. Where necessary, tracking was corrected manually, using reprojection errors and marker-to-marker distances as indicators for incorrectly tracked markers. The final mean reprojection error was 0.34 pixels. Each marker was tracked in at
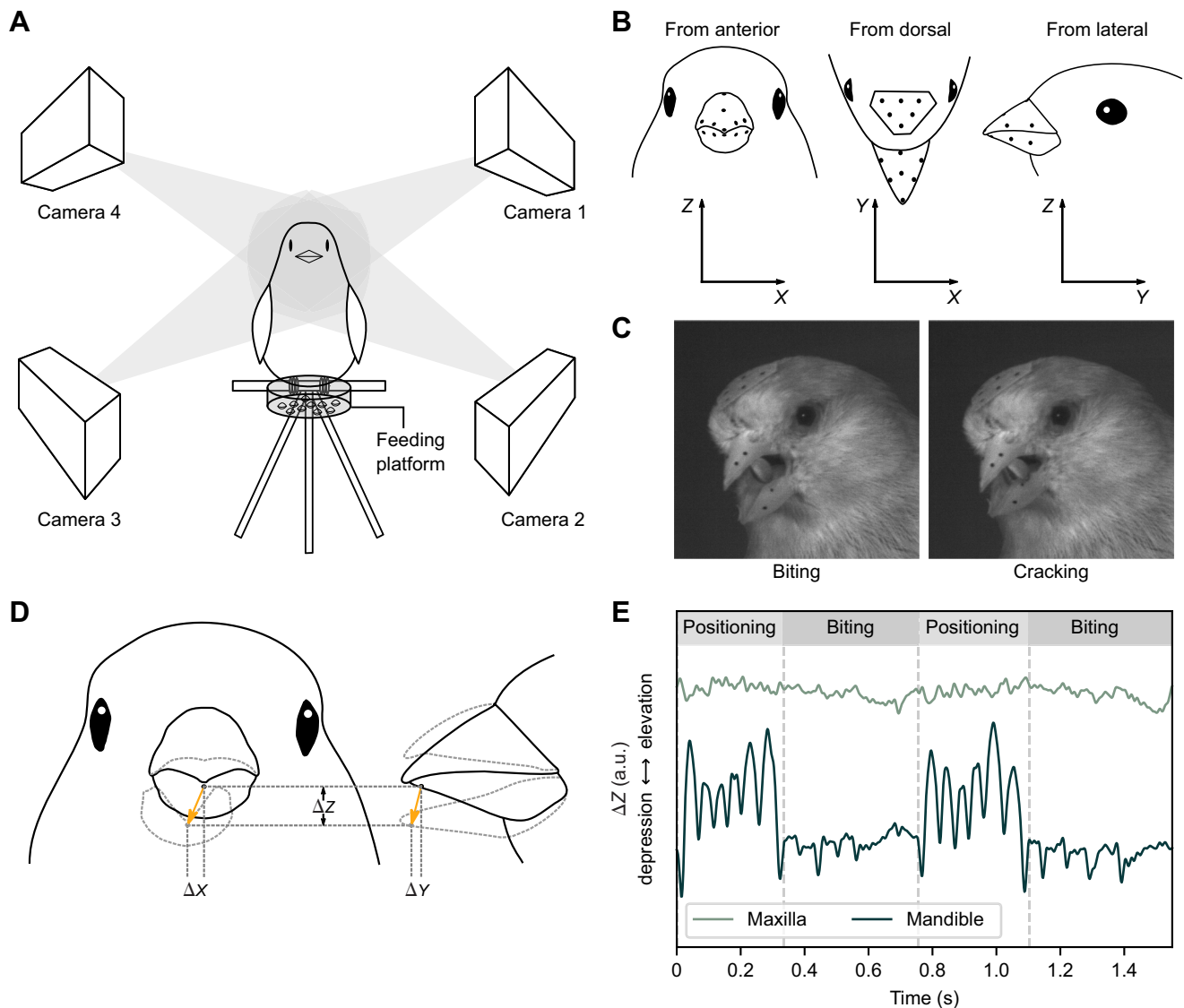
Fig. 1. Experimental setup, marker positions, definition of the reference coordinate system and displacement of the beak relative to the closed beak.
(A) Schematic overview of the experimental setup, showing the position of the four cameras relative to the bird. (B) Position of the markers on the maxilla, mandible and head. The dorsal and ventral markers on the maxilla and mandible, respectively, were used to define the Y–Z plane of the reference coordinate system. The head markers defined the X–Y plane. (C) Example frames of a video showing a bird shortly before (left) and after (right) cracking a hemp seed. Images were cropped to the head region. (D) During feeding, each marker moves relative to its reference position on the closed beak in all three dimensions, as exemplified for the ventral marker on the mandible tip. (E) Example trajectory for the dorso-ventral movement ΔZ (in arbitrary units, a.u.) of the maxilla and mandible during alternating positioning and biting phases. Data were low-pass filtered (threshold 60 Hz). See Movie 1 for a full example recording.

least two camera views to allow calculation of 3D coordinates. Additionally, the different phases of each feeding event were marked in XMALab. We distinguished the four phase types 'positioning', 'biting', 'dehusking' and 'swallowing' (Movie 1). During positioning, the seed lies rather loose in the beak and is rotated by the tongue to bring it into the right position for a cracking attempt. During biting (the actual cracking attempt), the seed is fixed in an upright position between the maxilla and mandible, with the natural seed margins aligned with the edges of the upper and lower beak. In a successful trial, positioning and biting phases are alternately repeated until the shell is cracked open. Note that each repetition of positioning or biting within one feeding event accounts for a single data point later on. During the dehusking phase, the two major halves of the shell are removed from the seed. The swallowing phase involves removal of small remains of the shell and

swallowing of the seed. The feeding phases, together with the 3D marker coordinates, were exported from XMALab for further processing. In total, 16 feeding events from six individuals (3 males, 3 females) were analyzed. The data include 79 positioning phases, 80 biting phases, 16 dehusking phases and 15 swallowing phases.

Preprocessing of 3D marker coordinates

The 3D marker coordinates of closed beak and feeding events were imported into Python (http://www.python.org) where all the subsequent analyses were performed. First, in order to be able to assess beak movement along the anatomical axes of the head, the 3D marker coordinates needed to be adjusted accordingly. We used the point cloud of the 18 reference markers from the closed beak recordings to define the coordinate system. To do so, we rotated this closed beak marker point cloud such that the six head markers were

in the X–Y plane; the Y–Z plane was defined by the dorsal and ventral markers of the maxilla and mandible, respectively (Fig. 1B). In order to bring the 3D data of the feeding events into this coordinate system, we aligned the six head markers of each frame with the head markers of the aligned closed beak reference via Procrustes superimposition (Rohlf and Slice, 1990). By doing so, the head movement of the birds was removed from the data and all the remaining signal was only the beak movement. Furthermore, this alignment allowed us to analyze maxilla and mandible movement separately by calculating the displacement of maxilla and mandible markers from their reference positions on the closed beak (see Fig. 1D). Because of the definition of the reference coordinate system, displacement in the X, Y and Z direction then corresponds to medio-lateral, antero-posterior and dorso-ventral displacement, respectively. By plotting ΔZ (Fig. 1E) of the maxilla and mandible over time, one can visualize qualitative gape change during the different phases of the feeding event. Displacement data (ΔX, ΔY and ΔZ) were low-pass-filtered using a Butterworth filter (threshold 60 Hz). For each frame of a phase (shown in the upper row of Fig. 1E), we averaged the displacement of only those markers that were visible during the whole duration of that phase to omit artifacts due to the appearance or disappearance of individual markers. The filtered and Procrustes-aligned beak movement data (ΔX, ΔY and ΔZ of the maxilla and mandible of all feeding events) are available from Dryad (https://doi.org/10.5061/dryad. zw3r2289z). Note that, because the absolute values of these averaged ΔZ partly depend on the number and position of visible markers during that phase, this analysis does not allow for a calculation of actual gape distance, but instead provides information about the qualitative beak movement over time. As a result of averaging of the displacement of several markers, we were left with only one ΔX, ΔY and ΔZ value per rigid body (maxilla or mandible) per frame interval. These averaged displacement values were used for the calculation of further beak movement parameters (see below).

Calculation of beak movement parameters

We focused on three main questions when analyzing the displacement data (ΔX, ΔY and ΔZ). (1) How strongly are maxilla and mandible movement correlated during the different phases of seed processing? (2) How fast does the beak move, i.e. how many oscillations occur per second? (3) What percentage of displacement per frame interval occurs in medio-lateral, antero-posterior and dorso-ventral direction and do percentages differ among the phases of seed processing?

To assess correlation, we calculated the correlation coefficient C of maxilla and mandible displacement in the dorso-ventral direction (ΔZ) for the individual feeding phases. Negative correlation coefficients indicate opposite movement (e.g. maxilla elevation coincides with mandible depression), positive values indicate parallel movement (e.g. maxilla elevation coincides with mandible elevation). The higher the absolute value, the higher the synchronization in both cases.

To assess the number of beak oscillations per second, we calculated the frequency F of mandible movement in the dorso-ventral direction (ΔZ) using the Welch method [*signal.welch()* function of the SciPy library; Virtanen et al., 2020] in Python. This was only done for the positioning phases, because here the beak moves fastest, with the highest amplitude and most regularly compared with the other phases.

To assess the relative displacement of the mandible in the X, Y and Z direction (i.e. to quantify 3D movement direction), we calculated

for each frame f the percentage M of X, Y and Z displacement relative to frame f−1. M was calculated as follows:

$$M\begin{pmatrix} \Delta X_f \\ \Delta Y_f \\ \Delta Z_f \end{pmatrix} = \frac{\delta \cdot 100\%}{\sum_i \delta_i} \qquad (1)$$

with

$$\delta = \begin{pmatrix} \Delta X_f - \Delta X_{f-1} \\ \Delta Y_f - \Delta Y_{f-1} \\ \Delta Z_f - \Delta Z_{f-1} \end{pmatrix}. \qquad (2)$$

Note that $M(\Delta X_f)$, $M(\Delta Y_f)$ and $M(\Delta Z_f)$ always add up to 100% and are independent of gape angle, because M considers displacement relative to each previous frame rather than relative to the closed beak reference position. Values of $M(\Delta X_f)$, $M(\Delta Y_f)$ and $M(\Delta Z_f)$ were averaged for each individual phase of a feeding event. All calculated beak movement parameters (correlation coefficients, frequency and relative mandible displacement direction) are available from Dryad (https://doi.org/10.5061/dryad.zw3r2289z).

Statistical analysis

Statistical analysis was conducted in Python using the probabilistic programming package PyMC (version 4.0.0; Salvatier et al., 2016). For the correlation coefficient C, mandible frequency F and relative mandible displacement direction M, we tested different models and performed model comparison with leave-one-out cross-validation (PSIS-Loo; see Vehtari et al., 2017) to assess which model best fits the data (see Table 1). The Python code of the modeling is available from Dryad (https://doi.org/10.5061/dryad.zw3r2289z).

As correlation coefficients C are restricted to the interval [−1,1], we selected a continuous distribution function with lower and upper limits for the modeling process. Given a transformation of the correlation coefficients to the interval [0,1] [$C_{trafo}=(C+1)/2$], the logit-normal distribution serves this purpose. We compared five different models (Table 1). Model C_{pool} fitted the logit-normal function to the pooled data of all correlation coefficients. Model C_{sex} included 'sex' as a categorical variable, model C_{ph} included 'phase' as a categorical variable, and model $C_{sex,ph}$ included both 'sex' and 'phase' as categorical variables. Model $C_{sex/ph}$ was an interaction model, considering all combinations of 'sex' and 'phase' as categorical variables.

For analyzing the frequency of dorso-ventral mandible movement, we fitted a Rice distribution, which is a continuous distribution function limited to positive values. The Rice distribution scored slightly better in model comparison than a Gamma function, which would be limited to positive values as well. Because frequency was calculated solely for the positioning phases, only the models F_{pool} and F_{sex} were evaluated (Table 1).

Relative mandible displacement direction is another parameter limited to a fixed interval [0,100]. Thus, a logit-normal distribution was used as for the correlation coefficients, after transforming the percentages to the interval [0,1] via $M_{trafo}=M/100$. Following the approach used for correlation C, we compared the models M_{pool}, M_{sex}, M_{ph}, $M_{sex,ph}$ and $M_{sex/ph}$ (Table 1).

Note that, as we recorded only three individuals per sex, our data were not sufficient to statistically quantify the effect of individual variation on beak kinematics. For test purposes, we analyzed the effect of including 'individual' as a variable instead of 'sex'. During model comparison, 'individual' models scored worse than or equally well as 'sex' models. This indicates that the former set of

Table 1. Definitions of statistical models used for the different parameters and results of model comparison

Model	Model description	Rank	LOO±s.e.	d_LOO±s.e.
$C_{sex/ph}$	$C \sim \sum\limits_{sex}\sum\limits_{ph} \nu_{sex,ph}\beta_{sex,ph} + \epsilon$	0	140±10	0±0
$C_{sex,ph}$	$C \sim \alpha + \nu_{male}\beta_{male} + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	1	136±9	4±2
C_{ph}	$C \sim \alpha + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	2	135±9	5±3
C_{sex}	$C \sim \alpha + \nu_{male}\beta_{male} + \epsilon$	3	72±7	68±7
C_{pool}	$C \sim \alpha + \epsilon$	4	72±7	69±7
F_{pool}	$F \sim \alpha + \epsilon$	0	−215±5	0±0
F_{sex}	$F \sim \alpha + \nu_{male}\beta_{male} + \epsilon$	1	−216±5	1±1
$M_{sex/ph}(X)$	$M_x \sim \sum\limits_{sex}\sum\limits_{ph} \nu_{sex,ph}\beta_{sex,ph} + \epsilon$	0	350±9	0±0
$M_{ph}(X)$	$M_x \sim \alpha + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	1	334±10	16±5
$M_{sex,ph}(X)$	$M_x \sim \alpha + \nu_{male}\beta_{male} + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	2	334±11	16±6
$M_{pool}(X)$	$M_x \sim \alpha + \epsilon$	3	271±8	79±7
$M_{sex}(X)$	$M_x \sim \alpha + \nu_{male}\beta_{male} + \epsilon$	4	270±8	80±7
$M_{sex/ph}(Y)$	$M_y \sim \sum\limits_{sex}\sum\limits_{ph} \nu_{sex,ph}\beta_{sex,ph} + \epsilon$	0	335±10	0±0
$M_{ph}(Y)$	$M_y \sim \alpha + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	1	321±10	14±5
$M_{sex,ph}(Y)$	$M_y \sim \alpha + \nu_{male}\beta_{male} + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	2	320±9	15±5
$M_{pool}(Y)$	$M_y \sim \alpha + \epsilon$	3	232±7	103±10
$M_{sex}(Y)$	$M_y \sim \alpha + \nu_{male}\beta_{male} + \epsilon$	4	231±7	105±10
$M_{sex/ph}(Z)$	$M_z \sim \sum\limits_{sex}\sum\limits_{ph} \nu_{sex,ph}\beta_{sex,ph} + \epsilon$	0	280±8	0±0
$M_{ph}(Z)$	$M_z \sim \alpha + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	1	268±9	12±5
$M_{sex,ph}(Z)$	$M_z \sim \alpha + \nu_{male}\beta_{male} + \sum\limits_{ph \neq pos} \nu_{ph}\beta_{ph} + \epsilon$	2	267±8	12±4
$M_{pool}(Z)$	$M_z \sim \alpha + \epsilon$	3	149±6	131±9
$M_{sex}(Z)$	$M_z \sim \alpha + \nu_{male}\beta_{male} + \epsilon$	4	148±6	132±9

The model description column describes the models as they were implemented in the statistical analysis. The rank column shows the rank of the models after model comparison, starting from 0 (best model). The LOO column shows the values of the leave-one-out cross-validation (LOO±s.e.). The higher the LOO, the better the model. The d_LOO column shows the difference (±s.e.) of each model to the best ranking model. Values are rounded. Note that models accounting for phase type ('ph', 'sex,ph', 'sex/ph') always score best and that differences among these 'ph' model variants are low.

C_{pool}, F_{pool} and M_{pool}, statistical models of beak correlation (C), mandible frequency (F) and 3D mandible displacement (M) fitted to the pooled data; C_{sex}, F_{sex} and M_{sex}, statistical models of beak correlation (C), mandible frequency (F) and 3D mandible displacement (M) including 'sex' as a categorical variable; C_{ph} and M_{ph}, statistical models of beak correlation (C) and 3D mandible displacement (M) including 'phase' as a categorical variable; $C_{sex,ph}$ and $M_{sex,ph}$, statistical models of beak correlation (C) and 3D mandible displacement (M) including both 'sex' and 'phase' as categorical variables; $C_{sex/ph}$ and $M_{sex/ph}$, statistical models of beak correlation (C) and 3D mandible displacement (M) including all combinations of 'sex' and 'phase' as categorical variables; C, correlation coefficient; F, frequency of dorso-ventral movement of the mandible; M_x, M_y and M_z, relative displacement of mandible in the x, y and z direction; α, model parameter defining the intercept of the linear models; β, model parameter defining the slopes of the linear models; ϵ, model parameter defining the residuals of the linear models; ν_{male} and ν_{ph}, boolean vectors defining which data rows are from male birds or from the phase 'ph'.

models is less suited to reliably describe the effects seen in the data, which might be due to a true lack of individual variance, but more likely points at limitations with regard to possible model complexity for the given sample size. Thus, the variable 'individual' was not included in the models.

Dissection
Two canaries that had died naturally were used for dissection of the head. The birds were from the same lab-bred population as our study subjects and of the same age. According to the literature (Nuijens and Zweers, 1997), the PO ligament is absent or weakly developed in Fringillidae. However, we had not found evidence about that for domestic canaries in particular. Hence, the goal of the dissection was to confirm that the canaries do not have a PO ligament. Furthermore, we used the cadavers to test the cranium for a coupling mechanism via manipulation of the beak.

RESULTS
Correlation of maxilla and mandible movement depends on the phase of seed processing
In order to assess to what extent the dorso-ventral movement of the maxilla and mandible are correlated, we calculated the correlation coefficient C of their ΔZ trajectories (see Fig. 1E) for the different phases ('positioning', 'biting', 'dehusking' and 'swallowing'). During positioning and swallowing, C was mostly negative (−0.66±0.16 and −0.25±0.21, respectively; Fig. 2). During dehusking and biting, C was more variable, covering both negative and positive values (−0.21±0.34 and −0.11±0.32). Correlation was affected by phase, but not by sex; models C_{pool} and C_{sex} scored equally in model comparison, but C_{ph} scored much better than both (Table 1). Adding 'sex' as a second categorical variable, either independently ($C_{sex,ph}$) or in combination with 'phase' ($C_{sex/ph}$), did not improve the outcome considerably (Table 1). We conclude that in
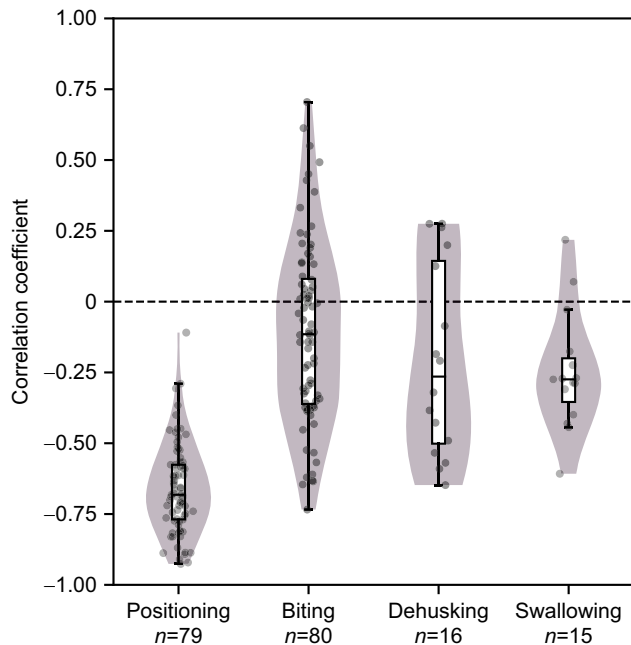
Fig. 2. Coefficient of correlation of dorso-ventral movement of the maxilla and mandible during the different phases of seed processing. Negative values indicate opposite movement, positive values indicate parallel movement. The higher the absolute values, the higher the synchronization. Dots indicate raw data, box plots their medians and quartiles, violin plots their kernel densities. Raw data extracted from 16 feeding events of 6 individuals (3 males, 3 females). Beak movement is mostly negatively correlated during positioning and swallowing. The more variable correlation coefficients during dehusking and biting indicate that coupling of beak movement is not obligate in domestic canaries.

domestic canaries, there is no obligate coupling of beak movement and the amount of correlation depends on the phase of seed processing, but not on sex.

Coupled kinesis is possible without a postorbital ligament
In order to confirm the absence of a PO ligament in canaries and to test for a coupling mechanism via manipulation of the beak, two canaries were dissected. We did not find a PO ligament in the birds. Unrestricted depression of the mandible by pulling the tip of the mandible down did not induce maxilla elevation. However, an upward push at the proximal side of the mandible while pulling it down at the mandible tip induced coupled kinesis of the lower and upper beak. We conclude that, although a PO ligament is missing, coupled kinesis can occur as a result of forces in the dorsal direction applied to the mandible during beak opening.

The mandible moves with a frequency of up to 25 Hz during the positioning phase
In order to assess the number of mandible oscillations per second during the positioning phase, we calculated the frequency of dorso-ventral mandible movement ΔZ. The mandible moved with a frequency of 18 ± 4 Hz during positioning, ranging from 10 Hz up to 25 Hz. Statistical analysis revealed that the model F_{sex} did not score better than the model F_{pool}, indicating that sex did not affect the frequency of mandible movement (Table 1). The observed frequencies lie within the range of reported syllable rates of singing domestic canaries (Drăgănoiu et al., 2002; Fig. 3). We

conclude that domestic canaries move their beak extremely fast during seed positioning, but the frequency does not differ between males and females.

Canaries use specific 3D mandible movements during different phases of seed processing
In order to determine how much the mandible moves in each of the three dimensions, we calculated the percentage of medio-lateral, antero-posterior and dorso-ventral mandible movement for the different phases ('positioning', 'biting', 'dehusking' and 'swallowing') of each feeding event. The canaries used mostly dorso-ventral movement, but to different extents depending on the phase type. The dorso-ventral direction of mandible movement was more dominant during positioning ($64\pm5\%$) and swallowing ($59\pm5\%$) than during dehusking ($49\pm7\%$) and biting ($44\pm7\%$; Fig. 4). This is mainly because during dehusking and biting, more mandible movement was performed in the medio-lateral direction. The percentages of all three dimensions were mainly affected by the phase: the three models which include phase as categorical variables ($M_{sex/ph}$, M_{ph} and $M_{sex,ph}$) scored best in model comparison (Table 1). The differences in score among these three models were low compared with the differences to the models M_{pool} and M_{sex}, indicating that including 'sex' as a categorical variable added little benefit to the model score. We conclude that domestic canaries, independent of sex, use specific 3D mandible movements for the different phases of seed processing.

DISCUSSION
Summary of findings
The goal of this study was to test functional hypotheses about beak movement during seed processing in a granivorous songbird. We used multi-view high-speed videography to reconstruct the 3D movement of the upper and lower beak in domestic canaries. Our analysis focused on the correlation of the upper and lower beak, the frequency of beak movement and the 3D directions of mandible movement during seed processing. We have shown that there is no obligate correlation of maxilla and mandible movement. Still, coupled kinesis is possible, given that mandible depression is restricted. The mandible moves at extremely high frequencies of up to 25 Hz, and specific 3D movements are used for the different phases of seed processing.

Mechanisms of cranial kinesis
We have shown that during feeding, maxilla and mandible movement is not always correlated in domestic canaries. While we observed a mostly negative correlation of dorso-ventral movement during the positioning and swallowing phase, the maxilla and mandible moved partly independently during biting and dehusking. Cadaver manipulation revealed that there is probably no obligate coupling mechanism, but coupled kinesis could be induced by restricting mandible depression.

We had hypothesized that beak movement is coupled in domestic canaries. This was based on the finding by Zusi (1967) that coupled cranial kinesis is independent of the presence of a PO ligament and that activation of the M. depressor mandibulae suffices to induce cranial kinesis in anesthetized fringillid finches. This was only partly confirmed in our study; the degree of coupling varied among phase types and unrestricted mandible depression alone did not induce cranial kinesis during cadaver manipulation. Our finding that the correlation of upper and lower jaw movement is not obligate during feeding suggests that domestic canaries can actively vary beak
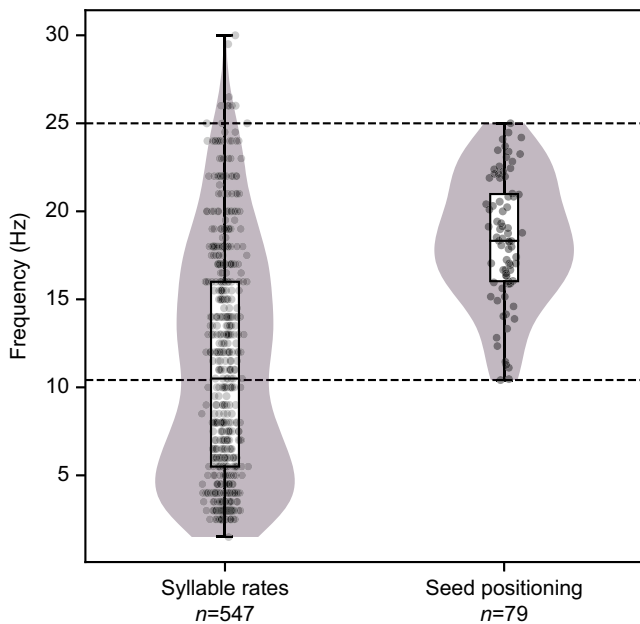
Fig. 3. Beak frequencies of domestic canaries during singing and seed positioning. Syllable rate data are from Drăgănoiu et al. (2002). Dots indicate raw data, box plots their medians and quartiles, violin plots their kernel densities. Seed positioning data (present study) were extracted from 16 feeding events of 6 individuals (3 males, 3 females). Our observed data of mandible frequency during seed positioning lie within the range of reported syllable rates, covering values between the 50th and 98th percentile (dashed lines).

Fig. 4. Percentage of mandible movement in the three dimensions during the different phases of seed processing. See Eqns 1 and 2 for a description of how data were calculated from the 3D coordinates. Data were extracted from 16 feeding events of 6 individuals (3 males, 3 females) and are presented as means±s.d. Dorso-ventral movement direction is less dominant during biting and dehusking, because more movement occurs medio-laterally compared with swallowing and positioning phases.

correlation by adjusting beak movement according to the specific needs of each phase of seed processing.

Interestingly, differences were found among phase types not only in the average correlation coefficient but also in the variation of correlation (Fig. 2), being higher during biting and dehusking. During these phases, the beak has firm contact with the seed, which is necessary for opening and removing the shell. The higher variation of correlation during these phases indicates the ability of the bird to flexibly control beak movement according to the specific size, hardness and position of each individual seed. Note that this higher variation in correlation coincides with a higher amount of medio-lateral mandible movement during biting and dehusking (Fig. 4). During positioning and swallowing, however, the seed lies rather loose in the beak and is manipulated more with the tongue than with the beak itself. Thus, the beak movements are more standardized and correlation is less variable.

The possibility of coupled cranial kinesis in the domestic canary, a fringillid finch, challenges the hypothesis by Bock (1964) that coupled beak movement depends on an inextensible PO ligament. Previous studies have partly questioned this key role of the PO ligament in coupled kineses as well. For example, the resistance force of the PO ligament has been shown to be too low to be able to actually block mandible depression (Bout and Zweers, 2001; Nuijens and Bout, 1998). Furthermore, birds with a PO ligament do not necessarily show coupled kinesis: the maxilla and mandible can move independently in white-throated sparrows, although they have a PO ligament (Hoese and Westneat, 1996). Also, in chickens (*Gallus gallus domesticus*), coupled cranial kinesis does not dominate beak movement during feeding, despite a PO ligament being present. This is because the maxilla is elevated actively via quadrate protractor muscles which become active even before
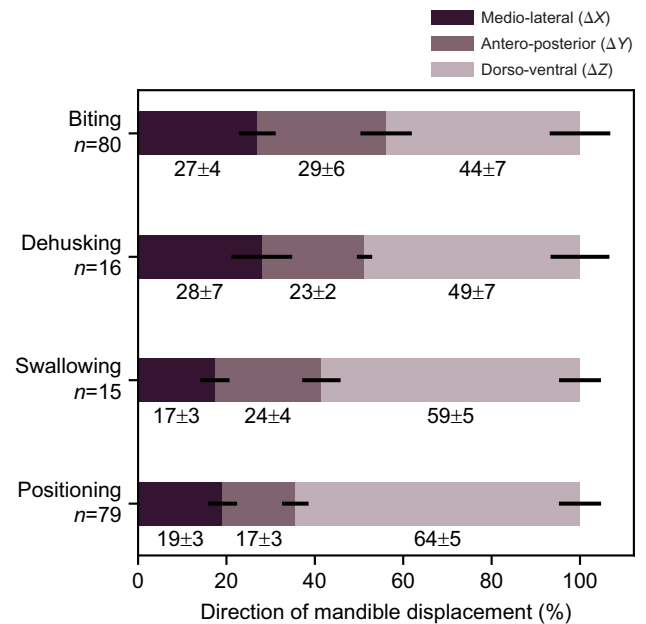
mandible depressor muscles, leaving the PO ligament unloaded (Van Den Heuvel, 1991). Similarly, in mallard ducks (Dawson et al., 2011) and pigeons (Bout and Zeigler, 1994), maxilla elevation precedes mandible depression, deviating from the mechanism suggested by Bock (1964). In conclusion, the presence of a PO ligament does not necessarily imply coupled cranial kinesis.

We have shown that beak movement in domestic canaries can be correlated, especially during seed positioning and swallowing, and that coupled cranial kinesis occurs if mandible depression is restricted. We propose two possible underlying mechanisms that are independent of a PO ligament. Either the maxilla is elevated independently via quadrate and pterygoid protractor muscles in a synchronized way together with mandible depressors (see Hoese and Westneat, 1996), or coupled cranial kinesis is induced via a sufficiently anteriorly directed line of action of the mandible depressor muscles to cause simultaneous quadrate protraction, potentially assisted by resisting forces from the jaw adductors (see Bout and Zeigler, 1994; Van Gennip and Berkhoudt, 1992; Zusi, 1967). As the strongest coupling is observed during the phase in which the highest acceleration of mandible depression is observed, namely the seed positioning phase (Fig. 1E, Fig. 2), such a dynamic effect induced by depressor mandibulae forces seems possible.

In general, however, the complexity of this mechanism demands caution in interpreting kinematic observations. It is likely that a coupling mechanism depends on a variety of factors such as species, gape angle, presence and mechanical properties of ligaments and cranial muscles, morphology and arrangement of cranial bones, etc. This prohibits generic conclusions about this mechanism in songbirds and even fringillids in general. Further research is

needed to reliably identify the actual underlying mechanism of coupled cranial kinesis in domestic canaries.

Impact of force–velocity tradeoff on feeding and mate choice in small songbirds

In this study, we found that domestic canaries move their beak at frequencies of 18±4 Hz, with a maximum of 25 Hz. As hypothesized, this matches the reported frequencies of peak trill rates in singing canaries (Drăgănoiu et al., 2002). The observed frequencies correspond to the fastest trill rates observed in Darwin's finches (cf. Podos, 2001). These observations imply that the force–velocity tradeoff in beak movement described in singing songbirds also affects feeding performance, allowing birds with low bite forces to produce fast syllable rates and to apply extremely rapid and efficient seed processing. This is plausible in the light of sexual selection. Female domestic canaries have a clear preference for elaborate birdsong and use syllable rate as a criterion for mate choice (Drăgănoiu et al., 2002). Based on the 'honest signaling theory', we hypothesize that this might be related to the fact that fast-singing males are also highly efficient in processing small seeds, proving to be able to meet their high energy demands despite their low bite force and to be successful in producing healthy offspring. This hypothesis is based on previous comparative studies reporting that small-billed birds sing faster (Podos, 2001; Herrel et al., 2009) and process small seeds more efficiently (Abbott et al., 1975; Smith, 1987) than large-billed birds.

Relevance of 3D mandible movement

We have shown that domestic canaries use specific 3D mandible movements during the different phases of seed processing. An increased usage of medio-lateral movement during biting and dehusking confirms our hypothesis and is in line with previously reported observations in other fringillid finches (Ziswiler, 1965; Nuijens and Zweers, 1997; van der Meij and Bout, 2006). Using a wide range of motion during seed cracking and dehusking allows for a more efficient way of feeding; the husk is removed skillfully without crushing the seed into pieces, which would involve losing parts of the seed.

Medio-lateral movement of the mandible is not exclusive to feeding. It is also utilized for preening and nest preparation (Kear, 1962), which highlights the importance of a highly kinetic skull for the multiple tasks of a bird beak. The occurrence of medio-lateral movement of the lower beak in birds has been linked to certain morphological characteristics of the cranium, e.g. ligaments and muscle properties (Nuijens and Zweers, 1997). For example, the absence of a PO ligament in Fringillid finches has been suggested to facilitate medio-lateral movement of the mandible (Nuijens and Bout, 1998). The present study provides further evidence for that.

Conclusion

Our analysis of beak movement during seed processing has revealed that there is no obligate coupling of maxilla and mandible movement in domestic canaries. The birds use specific 3D beak movements during the different phases of seed processing, showing variation in the correlation of the upper and lower jaw as well as in the relative directions of mandible movement. During seed positioning, the beak moved at frequencies matching the reported frequencies of peak trill rates in singing canaries, hinting at the joint dependence of singing and feeding on mechanical properties and constraints of the cranial system. Males and females did not show differences in any of the derived parameters, suggesting that there is no sexual dimorphism in beak movement during feeding in

domestic canaries. Further research is needed to identify how the underlying cranial system generates beak movement in domestic canaries and what the effects of the absence or presence of a PO ligament are for beak movement in granivorous songbirds in general.

Author contributions
Conceptualization: S.V.W.; Methodology: M.M., S.V.W.; Software: M.M.; Formal analysis: M.M.; Investigation: M.M.; Resources: S.V.W.; Data curation: M.M.; Writing - original draft: M.M.; Writing - review & editing: M.M., S.V.W.; Visualization: M.M.; Supervision: S.V.W.; Funding acquisition: M.M., S.V.W.

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Movie 1. This is an example movie of one of the analyzed feeding events. It shows a female canary feeding on a hemp seed. First, a slow motion version is shown with captions indicating the different phases of the feeding cycle. Subsequently, the real time version of the same recording is shown.