

## RESEARCH ARTICLE

# Flexibility of feeding movements in pigs: effects of changes in food toughness and stiffness on the timing of jaw movements

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## ABSTRACT

In mammals, chewing movements can be modified, or flexible, in response to changes in food properties. Variability between and within food in the temporal characteristics of chewing movements can impact chewing frequency and rhythmicity, which in turn may affect food breakdown, energy expenditure and tooth wear. Here, we compared total chewing cycle duration and intra-cycle phase durations in pigs chewing on three foods varying in toughness and stiffness: apples (low toughness, low stiffness), carrots (high toughness, low stiffness), and almonds (high toughness, high stiffness). We also determined whether within-food variability in timing parameters is modified in response to changes in food properties. X-ray Reconstruction Of Moving Morphology (XROMM) demonstrates that the timing of jaw movements are flexible in response to changes in food properties. Within each food, pigs also exhibited flexibility in their ability to vary cycle parameters. The timing of jaw movements during processing of high-toughness foods is more variable, potentially decreasing chewing rhythmicity. In contrast, low-toughness foods result in jaw movements that are more stereotyped in their timing parameters. In addition, the duration of tooth–food–tooth contact is more variable during the processing of low-stiffness foods compared with tough or stiff foods. Increased toughness is suggested to alter the timing of the movements impacting food fracture whereas increased stiffness may require a more cautious control of jaw movements. This study emphasizes that flexibility in biological movements in response to changes in conditions may not only be observed in timing but also in the variability of their timing within each condition.

**KEY WORDS:** Flexibility, Stereotypy, Chewing, Kinematics, XROMM, *Sus*

## INTRODUCTION

The interaction between an organism and its environment is fundamental to the process of evolution. In organisms that interact with a narrow range of ecological pressures or external stimuli, behavioral responses may be specialized, if not adapted, to fulfill the task(s) that enable them to perform optimally (Futuyma and Moreno, 1988). However, most organisms face significant variation in these external stimuli or pressures, which may require them to

alter or engage in a totally different behavior. Significant differences in organismal performance and behavior in response to changes in a given stimulus is called flexibility (Wainwright et al., 2008). The degree to which an organism can be flexible may be crucial to its fitness and survival.

Among the myriad examples of ecological pressures that affect how an organism performs in its environment are those associated with diet, and in this context, an example of a potentially flexible behavior is intra-oral processing. During intra-oral processing in most vertebrates, the physical and mechanical properties of the food item are modified during the gape cycle by the teeth, palate and/or tongue. Compared with other vertebrates, intra-oral processing in mammals, or mastication, is more specialized in that food is finely fragmented between occluding upper and lower teeth to increase digestion and gut passage rates to sustain high metabolic rates (e.g. Clauss et al., 2009; Fritz et al., 2009; Karasov et al., 1986).

In mammals, active modulation by oral and periodontal mechanoreceptors enables rapid response of the masticatory system to alter not only occlusal forces but also jaw movements within and between chews (e.g. Trullsson, 2006, 2007; Trullsson and Johansson, 2002). Whereas between-food differences in masticatory kinematics reflect flexibility in feeding performance to match particular food properties, within-food variability reflects the extent to which an animal's movements are stereotyped in response to the same food (Wainwright et al., 2008). Between-food differences in food processing have been demonstrated at the muscular and/or kinematic level in a number of mammalian species, including bats (De Gueldre and de Vree, 1984, 1988), shrews (Dotsch, 1986; Dotsch and Dantuma, 1989), rabbits (e.g. Weijs and de Jongh, 1977; Weijs et al., 1989; Yamada and Yamamura, 1996), goats (De Vree and Gans, 1976), opossums (Thexton and Crompton, 1989), carnivorans (Gorniak and Gans, 1980; Davis, 2014), non-human primates (e.g. Hylander et al., 2004; Iriarte-Diaz et al., 2011; Reed and Ross, 2010; Thexton and Hiiemae, 1997; Vinyard et al., 2008) and humans (e.g. Agrawal et al., 1998; Anderson et al., 2002; Koyama et al., 2002; Woda et al., 2006). However, most studies compare food items that vary in multiple mechanical properties, and only a few studies successfully isolate the effects of changes in specific mechanical properties.

A notable exception is the recent research on primate masticatory function focusing on two properties that affect food fracture, the elastic modulus and toughness (e.g. Agrawal et al., 1997, 1998; Iriarte-Diaz et al., 2011; Reed and Ross, 2010). Whereas the elastic modulus, or stiffness, describes the ability of an object to resist elastic deformation, toughness is a measure of the amount of energy absorbed prior to failure. Leading up to fracture, stiffness may be the most important property influencing jaw movements because it reflects the compliance of the food material. However, once fracture has been initiated, toughness may be the most important property because this dictates how easily cracks propagate through the material (Lucas, 2004).

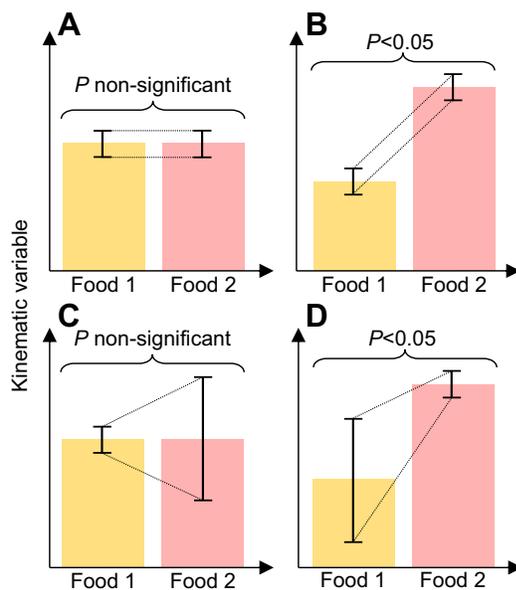
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Given the importance of stiffness and toughness for comminution, we investigated their influence on the temporal dynamics of jaw movements during mastication in pigs by assessing between-food variability (i.e. flexibility) in gape cycle duration and intra-cycle phase durations. We also determined whether within-food variability in timing parameters is modified in response to changes in food mechanical properties. Pigs were chosen as the animal model for this study because they are typical omnivorous mammals that feed on a wide variety of foods differing in their mechanical properties. They also provide a different mammalian model for investigating variability in chewing dynamics in mammals because most of the research available to date is heavily focused on primates (e.g. Ross et al., 2010; Iriarte-Diaz et al., 2011; Reed and Ross, 2010; Thexton and Hiiemae, 1997; Vinyard et al., 2008; but see Ross et al., 2007b).

The overarching hypothesis of this study is that gape cycle duration is similar (inflexible *sensu* Wainwright et al., 2008), when processing different foods, as well as stereotyped within each food (Fig. 1A). Low within-food variability, similar to what is observed in primates (Reed and Ross, 2010; Ross et al., 2010), would



**Fig. 1. Hypothetical patterns of interaction between flexibility and within-food variability in a kinematic variable associated with jaw movements during chewing on foods varying in mechanical properties.** (A) Kinematic variable is not significantly different between foods, and variability in the kinematic variable is low and similar within each food. In this example, movements are stereotyped for each food, inflexible in response to changes in food properties, as well as inflexible in the level of stereotypy associated with each food. (B) Kinematic variable shows a significant difference between foods but each food is characterized by similarly low levels of variability. In this example, movements are stereotyped within each food, flexible in response to changes in food properties but inflexible in the level of stereotypy associated with each food. (C) Kinematic variable is not significantly different between foods but within-food variability differs between each food: low variability in food 1 versus high variability in food 2. In this example, movements are inflexible in response to changes in food properties but flexible in within-food variability, i.e. the level of stereotypy, as feeding movements are stereotyped for food 1 but highly variable for food 2. (D) Kinematic variable shows a significant difference between foods, and so does variability within each food (i.e. high variability for food 1, low variability in food 2). In this example, feeding movements are flexible in response to changes in food properties and flexible in stereotypy as feeding movements are highly variable for food 1 but stereotyped for food 2.

strengthen the hypothesis that chewing frequency is largely unaffected by active modulation within the gape cycle in mammals. Low variability in chewing frequency may be one mechanism to reduce tooth wear and energy expenditure during feeding (Ross et al., 2007a,b, 2010). Alternatively, foods of different properties may require gape cycles that are longer or shorter (see Fig. 1B and D) and/or more variable in duration (see Fig. 1C and D). In that case, differences between pigs and primates in the levels of between- and/or within-food variability during feeding may emphasize differences between mammalian clades in the relationship between diet, feeding morphology and the control of feeding movements. Indeed, pigs have relatively non-restrictive morphology of the dentition and temporomandibular joint (TMJ) suitable for omnivory that could allow significant range of motion during chewing if jaw-muscle motor pattern driving these movements varies from cycle to cycle.

At the same time, we expect that active modulation as well as intra-oral bolus handling alters the temporal dynamics within each gape cycle, such that the duration of intra-cycle phases (e.g. fast-closing, power stroke) is more variable, as observed in primates (Reed and Ross, 2007; Ross et al., 2010) and rabbits (Schwartz et al., 1989). This may be particularly true when comparing foods of low and high toughness. Foods of low toughness that are easily fragmented may require more manipulation by the tongue to form the fragments into a cohesive bolus. In this case, we might expect fast-opening (FO) and fast-closing (FC) phases to be longer. In comparison, the processing of tougher foods is hypothesized to require more contact between the teeth and food to allow for successful crack propagation. Therefore, we could expect an increase in occlusal phase duration and a corresponding increase in gape cycle duration (see Reed and Ross, 2010).

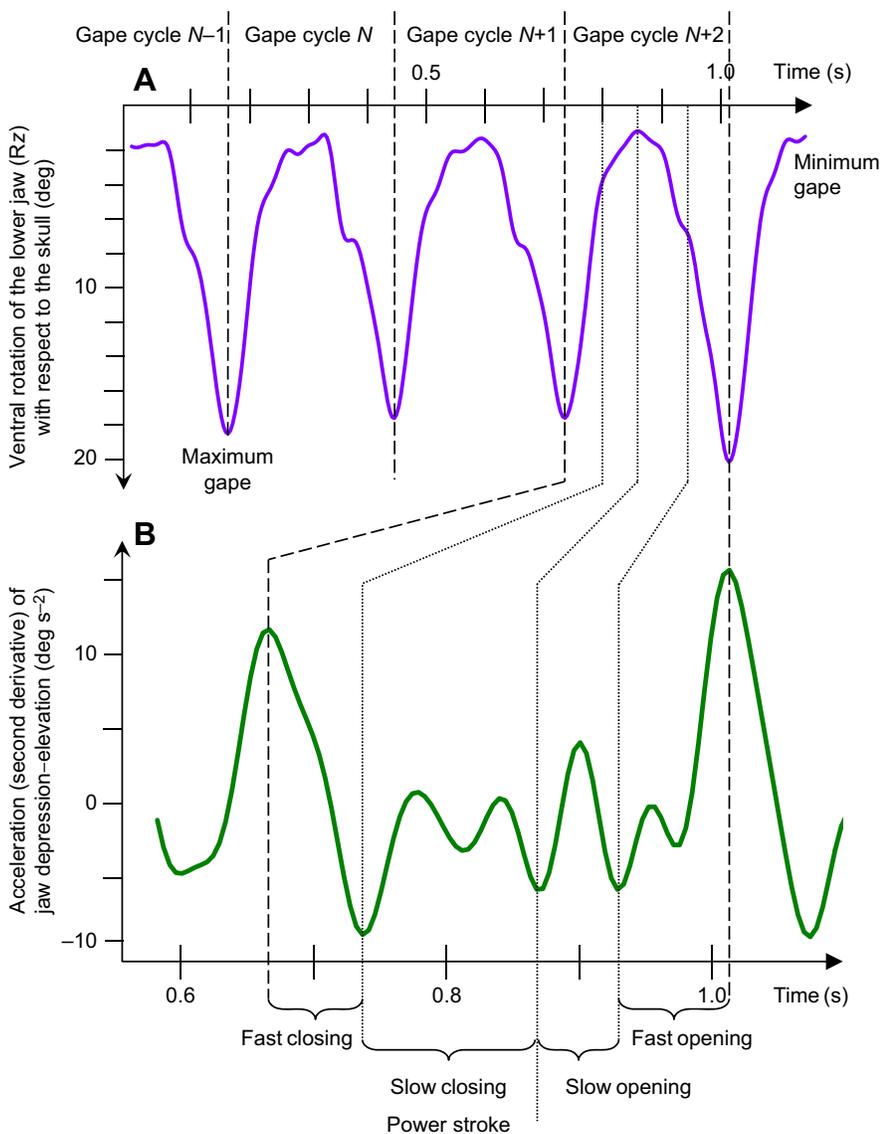
## MATERIALS AND METHODS

### Study design

The objective of our study was to investigate the effects of food toughness and stiffness independently from one another on temporal aspects of gape cycle dynamics, and their respective within-food variability, during chewing in pigs. Foods were chosen because their bivariate comparisons allow assessment of the effects of changes in one food property while maintaining the other at a comparable level. First, the effects of changes in toughness were tested by comparing jaw movements when pigs chew on two comparably low-stiffness foods varying in toughness: apples (low toughness  $56.97 \pm 17.76 \text{ J m}^{-2}$ ; low stiffness  $3.41 \pm 0.10 \text{ MPa}$ ) versus carrots (high toughness  $343.93 \pm 48.49 \text{ J m}^{-2}$ ; low stiffness  $6.86 \pm 0.46 \text{ MPa}$ ) (Williams et al., 2005). Second, the effects of changes in stiffness were tested by comparing jaw movements when chewing on two comparably high-toughness foods varying in stiffness: carrots (low stiffness  $6.86 \pm 0.46 \text{ MPa}$ ; high toughness  $343.93 \pm 48.49 \text{ J m}^{-2}$ ) versus almonds (high stiffness  $19.42 \pm 7.69 \text{ MPa}$ ; high toughness  $308.62 \pm 34.85 \text{ J m}^{-2}$ ) (Williams et al., 2005). Note that some food properties were not controlled (e.g. color, smell) and therefore may play a role in the patterns observed.

### Data collection

Temporal dynamics in jaw movements were characterized using marker-based X-ray Reconstruction Of Moving Morphology (XROMM) (Brainerd et al., 2010) in four 3-to-4-month-old female pigs (*Sus scrofa* Linnaeus 1758). Radiopaque tantalum markers (1 mm diameter, Bal-Tec, Los Angeles, CA, USA) were surgically implanted into the bones and teeth of the skull while animals were under isoflurane anesthesia. Each rigid body had a



**Fig. 2. Representative kinematic waves showing ventral rotation (Rz) of the lower jaw over the course of four consecutive gape cycles of a pig chewing on almond.** (A) Rz with respect to the skull. (B) Corresponding acceleration of jaw depression–elevation. Data are extracted from a joint coordinate system set up to quantify motion at the temporomandibular joint: Rz values of 0 illustrate the lower jaw being maximally elevated (minimum gape) whereas negative Rz values illustrate jaw depression (maximum gape). Gape cycles were determined to start at maximum gape jaw opening (maximum depression of the lower jaw or minimum ventral rotation) and were separated into four consecutive phases based on acceleration: fast closing, slow closing or power stroke, slow opening, and fast opening.

minimum of six beads. Prior to and following surgery, pigs were trained to enter and exit the restraint system and to feed comfortably in the restraint. All procedures were approved by the Ohio University Institutional Animal Care and Use Committee (IACUC protocol #12-U-009).

Fluoroscopy videos were recorded using two synchronized high-speed cameras (Oqus 310, Qualisys, Göteborg, Sweden) mounted on the output ports of two fluoroscopes (OEC-9000) (Movies 1 and 2). A Logitech webcam C210 (Lausanne, Switzerland) providing an external view of the animal was synchronized with the high-speed fluoroscopy videos in Qualisys Track Manager software (Qualisys). On average, radiation exposures were set at 80 kVp and 4.5 mA to provide sufficient contrast between markers and bones. In order to correct for distortion inherent to X-ray imaging, a perforated steel sheet with standardized hole spacing and sizes (part number 9255T641, McMaster-Carr, Robinson, NJ, USA) was imaged in each fluoroscopy view. The field of view covered by both fluoroscopes was calibrated prior to and following each feeding session by exposing a custom cube of four plastic sheets containing 64 radiopaque tantalum beads placed in a 4×4 fashion 2.5 cm apart from one another.

During feeding sessions, which occurred daily over a 2 week period, the three foods were offered in a random order. Foods were

cut to  $\sim 1 \text{ cm}^3$  pieces to nullify the effects of size differences. A bowl full of food was used to keep the subject in the field of view of the image intensifiers. The trade-off, however, is that ingestion and mastication can occur nearly continuously within a single gape cycle, making it difficult to capture individual sequences of a single piece of food being processed. This hindered our ability to quantify within-sequence variability and the effects of chew number throughout a full feeding sequence (Iriarte-Diaz et al., 2011; Reed and Ross, 2010). However, for the analysis, we were able to select chewing cycles immediately following ingestion of a single piece prior to complete trituration of the food bolus. In primates, variability between cycles within the same sequence is known to be maximal at the beginning of the sequence and decrease as the sequence progresses (Iriarte-Diaz et al., 2011; Peyron et al., 2002; Reed and Ross, 2010; Ross et al., 2016). Thus, by focusing on the earliest chewing cycles within a sequence, we likely sample the period in which the difference in mechanical properties between the foods are the greatest.

Animals were CT-scanned multiple times to register the position of the implanted markers within the bones and to ensure that growth would not impact bone animation. Early in data collection, a first scan was performed under isoflurane anesthesia at the Ohio State University Veterinary Medical Center (Columbus, OH, USA) on a

**Table 1. Average absolute durations (in ms) of gape cycles and phases, and the associated coefficients of variation (CVs), in pigs chewing on foods varying in toughness (apple versus carrots) and stiffness (carrots versus almonds)**

	Individuals (N)									
	All (369)		5 (56)		6 (80)		9 (136)		10 (97)	
	Mean±s.e.m.	CV	Mean±s.e.m.	CV	Mean±s.e.m.	CV	Mean±s.e.m.	CV	Mean±s.e.m.	CV
Apple										
Total cycle	288.4±2.4	15.91	335.0±7.6	17.04	314.3±4.5	12.94	271.4±2.2	9.32	263.9±3.4	12.58
FC <sub>abs</sub>	83.9±1.0	23.91	97.4±2.7	20.63	92.2±2.7	26.65	79.7±1.3	18.74	75.2±1.6	20.42
SC <sub>abs</sub>	77.3±1.2	30.02	79.9±2.4	22.36	91.9±2.8	27.13	70.7±1.5	25.20	72.9±2.8	37.69
SO <sub>abs</sub>	66.2±1.2	33.72	63.5±4.1	48.62	61.2±2.6	37.39	69.8±1.8	29.24	67.0±1.8	26.10
FO <sub>abs</sub>	61.0±1.3	39.46	94.1±4.4	34.83	69.1±2.1	26.88	51.2±0.9	20.31	48.9±1.4	27.86
Carrot										
Total cycle	314.0±3.6	20.73	355.8±9.4	26.37	317.3±4.2	12.23	285.3±2.1	8.50	269.2±11.3	15.07
FC <sub>abs</sub>	94.3±1.3	25.38	100.7±3.2	31.72	102.1±2.0	18.38	86.5±1.4	18.95	76.0±3.3	15.49
SC <sub>abs</sub>	86.3±1.3	26.88	92.3±3.0	32.40	88.8±1.7	17.74	82.2±1.7	23.69	66.2±6.6	36.16
SO <sub>abs</sub>	67.7±1.4	37.78	71.6±3.4	46.17	61.4±3.0	44.42	67.8±1.4	24.44	78.5±5.2	23.96
FO <sub>abs</sub>	64.6±1.5	41.48	87.7±3.3	37.09	65.1±2.0	28.08	48.8±0.9	20.78	48.6±3.7	27.46
Almond										
Total cycle	295.9±1.9	13.25	318.3±4.2	12.32	345.5±6.0	4.91	283.7±1.7	7.68	294.3±3.5	15.70
FC <sub>abs</sub>	89.8±1.0	22.89	90.6±1.8	18.13	121.5±7.2	16.87	85.4±1.3	19.36	92.2±1.8	26.10
SC <sub>abs</sub>	90.1±0.9	19.88	97.8±2.1	19.66	93.5±4.8	14.45	91.1±1.1	15.74	85.1±1.4	22.36
SO <sub>abs</sub>	59.1±0.8	28.05	52.6±1.3	22.60	54.0±5.9	30.92	59.2±1.1	25.03	62.6±1.5	30.81
FO <sub>abs</sub>	56.9±0.9	32.89	77.2±1.8	21.86	76.5±7.8	28.70	48.1±0.9	23.14	54.5±1.3	31.68

Data are presented for all individuals pooled together and for each individual separately, along with the respective sample sizes (in parentheses).

GE Lightspeed Ultra CT scanner (General Electric, Boston, MA, USA). After all data were collected, a second scan of the frozen head was done *post mortem* at Holzer Clinic (Athens, OH, USA) on a Philips Brilliance 64 scanner (Amsterdam, The Netherlands). Scans were reconstructed in Avizo to create 3D bone models (FEI, Hillsboro, OR, USA).

### Data processing

Fluoroscopy videos were processed in the XMALab software (Knörlein et al., 2016) using the XROMM workflow (Brainerd et al., 2010). Following distortion correction, the screen position of each bead in the calibration cube was digitized in each view and matched with their respective reference *x*, *y* and *z* coordinates. Direct linear transformation was used to obtain the 3D position of each video camera, thus calibrating the field of view. Subsequently, the screen positions of the skull, jaw and teeth markers were digitized in both fluoroscopy video views. The screen coordinates of each marker were filtered using a low-pass Butterworth filter with a 25 Hz cut-off frequency. The standard deviation of the distance between markers implanted in the same bone was used to quantify measurement error (Brainerd et al., 2010). The average standard deviations of these distances were 0.14 mm (Fig 5), 0.73 mm (Fig 6), 0.47 mm (Fig 9) and 0.45 mm (Fig 10) for the skull markers, and 0.17 mm (Fig 5), 0.70 mm (Fig 6), 0.47 mm (Fig 9) and 0.38 mm (Fig 10) for the jaw markers.

By registering the digitized coordinates of the implanted markers to their respective reference coordinates extracted from the CT scans, rigid body motions of the skull and jaw were calculated using singular value decomposition. Rigid body motions were then imported into Maya (Autodesk Inc., San Rafael, CA, USA) where they were assigned to the corresponding 3D models of the skull and jaw. Each sequence was then animated frame-by-frame to allow visualization of the jaw movements.

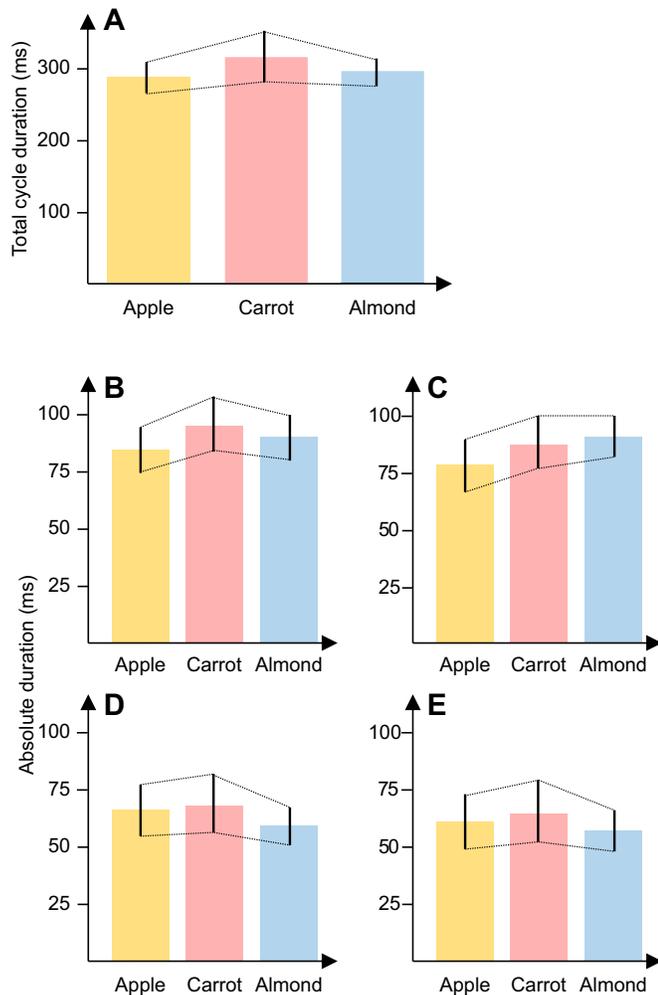
For each individual, a joint coordinate system (JCS) was created using anatomical reference systems for each skeletal element (see Brainerd et al., 2010; Menegaz et al., 2015). The JCS consisted of three perpendicular axes oriented rostrocaudally (*x*), dorsoventrally

(*y*) and mediolaterally (*z*) and positioned at the TMJ (Movie 3). The JCS was parented to the skull so that all jaw movements were measured independently of skull position and movements. This reference system allows quantifying six degrees of freedom (i.e. three rotations: Rx, Ry and Rz; and three translations: Tx, Ty and Tz) characterizing the movements of the lower jaw with respect to the skull over time (Brainerd et al., 2010; Menegaz et al., 2015). Because this study focused on the temporal characteristics of the gape cycle, only Rz (rotation about a transverse axis, i.e. pitch) was utilized (see 'Data analysis', below) for all statistical analyses.

A precision study was also conducted post mortem by imaging the frozen heads with the articulated lower jaw following the same biplanar fluoroscopy and XROMM protocol as for *in vivo* data collection. Because relative motion between bones should be zero, standard deviations from zero demonstrates digitizing noise in Rz measurements. Standard deviations for the four individuals were as follows: Fig 5, 0.10 deg; Fig 6, 0.12 deg; Fig 9, 0.32 deg; and Fig 10, 0.12 deg. These values were used to determine the precision threshold at which movements can be confidently interpreted as real motion versus noise from digitizing. The corresponding *in vivo* data were compared with the precision threshold so that only movements greater than the digitizing noise were included in the analysis. Conversely, if the magnitude of movement was lower than the precision threshold, the observation was discarded.

### Data analysis

For each animated feeding sequence, gape cycles were defined based on rotation of the jaw about the *z*-axis (Rz), with minimum Rz representing maximum jaw depression (i.e. maximum gape) and maximum Rz representing maximum jaw elevation (i.e. minimum gape; Fig. 2A). The total duration of each gape cycle was determined as the time difference between two consecutive, minimum Rz values. Within each gape cycle, four phases were identified based on the second derivative of changes in Rz position, which represents acceleration of the depression–elevation displacement of the lower jaw: FC, the slow closing (SC) or power stroke, slow opening (SO), and FO (Fig. 2B). The absolute (FC<sub>abs</sub>,



**Fig. 3. Flexibility in total gape cycle duration and intra-cycle phase durations, and their respective within-food variability, in response to changes in food toughness and stiffness during chewing in pigs.** (A) Gape cycle; (B) fast-closing (FC) phase; (C) slow-closing (SC) phase; (D) slow-opening (SO) phase; (E) fast-opening (FO) phase. Average absolute durations are presented with the corresponding coefficient of variation (error bars) for each food type investigated. Gape cycles are significantly longer and more variable in duration when chewing on high-toughness food (carrots) than when chewing on low-toughness food (apples) whereas they are more stereotyped in duration when chewing on high-stiffness food (almonds). All phases are significantly longer when chewing on high-toughness food (carrots) than when chewing on low-toughness food (apples). Food toughness has no effect on the stereotypy of any phase duration whereas the durations of all phases are significantly more stereotyped while chewing high-stiffness food (almonds) compared with low-stiffness foods (carrots). SC is also significantly longer when chewing on high-stiffness foods (almonds) than when chewing on low-stiffness food (carrots). This is associated with a decrease in the duration of SO and FO, revealing a trade-off in duration between SC and jaw opening when chewing on stiff foods (almonds).

$SC_{abs}$ ,  $SO_{abs}$ ,  $FO_{abs}$ ) and relative (as a % of the corresponding total cycle duration;  $FC_{rel}$ ,  $SC_{rel}$ ,  $SO_{rel}$ ,  $FO_{rel}$ ) durations of each phase were calculated.

In order to quantify between-food variability (i.e. flexibility *sensu* Wainwright et al., 2008), total cycle duration and absolute and relative phase durations were analyzed using two independent Multivariate Analyses of Variance (MANOVA) associated with univariate *F*-tests. The first MANOVA tests the effects of changes in food toughness while minimizing the effects of changes in stiffness

whereas the second MANOVA tests the effects of changes in food stiffness while minimizing the effects of changes in toughness. In both analyses, food was entered as the fixed factor and individual as the random factor. The food $\times$ individual interaction factor was also entered in the initial design. If the interaction term was not significant, it was removed from the final design. If it was significant, this indicates that individuals do not react to changes in food properties similarly, in which case differences between foods were tested within each individual separately.

In order to quantify within-food variability (i.e. stereotypy *sensu* Wainwright et al., 2008), the coefficients of variation (CVs) of each variable were calculated with all individuals pooled together as well as for each individual separately. Low CVs indicate stereotyped temporal characteristics whereas high CVs indicate variable temporal characteristics. To test whether within-food variability differs between foods, two separate MANOVAs were performed on the CVs associated with each food following the same design as previously described: food types as the fixed factor, individuals as random factor, and the food type $\times$ individual interaction term. Non-significant interaction terms were removed from the final design. If the interaction term was found to be significant, the MANOVA was performed within each individual separately.

## RESULTS

### Effects of food toughness on the temporal characteristics of the gape cycle

#### Between-food differences in cycle and phase durations

A first MANOVA compared the absolute timing parameters of the gape cycles when chewing on apple and carrot (Table 1), which differ in toughness but not stiffness (Williams et al., 2005). Significant differences between foods were found in total cycle duration ( $F_{1,693}=8.64$ ,  $P=0.003$ ; Fig. 3A) as well as in  $FC_{abs}$  ( $F_{1,693}=14.71$ ,  $P<0.001$ ; Fig. 3B) and  $FO_{abs}$  duration ( $F_{1,693}=5.90$ ,  $P=0.015$ ; Fig. 3E) without any food $\times$ individual interaction. Thus, gape cycles are longer when chewing on carrots than on apple, and they have longer  $FC_{abs}$  and  $FO_{abs}$  phases (Table 1). Significant differences between individuals were also detected in total cycle duration ( $F_{3,693}=84.09$ ,  $P<0.001$ ),  $FC_{abs}$  ( $F_{3,693}=36.17$ ,  $P<0.001$ ) and  $FO_{abs}$  ( $F_{3,693}=156.44$ ,  $P<0.001$ ).  $SO_{abs}$  was not affected by the food $\times$ individual interaction or by food effects but individual differences were significant ( $F_{3,693}=4.19$ ,  $P=0.006$ ). The food $\times$ individual interaction was significant for  $SC_{abs}$  ( $F_{3,690}=5.76$ ,  $P=0.001$ ), requiring testing food effects for each individual separately. Significant differences between foods in  $SC_{abs}$  duration in individual 5 ( $F_{1,153}=8.08$ ,  $P=0.005$ ) and individual 9 ( $F_{1,268}=25.40$ ,  $P<0.001$ ) indicate that, for these pigs, this phase is longer for carrot than for apple (see Table 1).

A second MANOVA compared the relative phase durations to investigate the effects of food toughness on phase durations independently of changes in total cycle duration. Overall, the longest phase is FC at ~30% of total cycle duration whereas FO is the shortest at ~20% of total cycle duration (Table 2). Food $\times$ individual interactions were significant for all variables except  $FO_{rel}$ .  $FO_{rel}$  is characterized by significant food differences ( $F_{1,695}=21.42$ ,  $P<0.001$ ) as well as individual differences ( $F_{1,695}=97.41$ ,  $P<0.001$ ), indicating that FO is relatively longer during apple chewing than during carrot chewing (Fig. 4).  $FC_{rel}$  and  $SC_{rel}$  each differed between foods in one individual only ( $FC_{rel}$ : individual 6,  $F_{1,163}=12.94$ ,  $P<0.001$ ;  $SC_{rel}$ : individual 9,  $F_{1,268}=14.22$ ,  $P<0.001$ ). In both cases, the phase was relatively longer when chewing carrot than when chewing apple (see Table 2).

**Table 2. Average relative durations (% of total cycle duration) of gape cycles and phases, and the associated coefficients of variation (CVs), in pigs chewing on foods varying in toughness (apple versus carrots) and stiffness (carrots versus almonds)**

	All (369)		Individuals (N)									
	Mean±s.e.m.	CV	5 (56)		6 (80)		9 (136)		10 (97)			
Apple			Mean±s.e.m.	CV	Mean±s.e.m.	CV	Mean±s.e.m.	CV	Mean±s.e.m.	CV		
FC <sub>rel</sub>	29.13±0.28	18.15	29.16±0.53	13.70	29.17±0.67	20.68	29.43±0.45	17.75	28.65±0.55	18.91		
SC <sub>rel</sub>	26.75±0.36	25.97	24.38±0.85	26.11	29.17±0.72	22.18	25.96±0.51	22.88	27.23±0.84	30.38		
SO <sub>rel</sub>	23.28±0.39	32.31	18.63±0.96	38.35	19.76±0.86	39.11	25.70±0.59	26.67	25.47±0.61	23.69		
FO <sub>rel</sub>	20.84±0.30	27.66	27.83±0.87	23.28	21.90±0.54	22.13	18.91±0.32	19.59	18.65±0.51	26.89		
Carrot	All (331)		5 (99)		6 (85)		9 (134)		10 (13)			
FC <sub>rel</sub>	30.16±0.30	18.30	28.32±0.56	19.76	32.23±0.53	15.05	30.37±0.48	18.13	28.54±1.23	15.55		
SC <sub>rel</sub>	27.59±0.32	20.96	26.16±0.61	23.01	28.08±0.51	16.61	28.65±0.50	20.27	24.21±2.03	30.22		
SO <sub>rel</sub>	21.88±0.38	31.97	20.63±0.75	36.16	19.16±0.78	37.66	23.80±0.46	22.52	29.33±1.79	21.97		
FO <sub>rel</sub>	20.37±0.33	29.71	24.89±0.68	27.30	20.52±0.57	25.72	17.18±0.30	20.28	17.91±0.96	19.24		
Almond	All (443)		5 (88)		6 (8)		9 (171)		10 (176)			
FC <sub>rel</sub>	30.24±0.24	16.54	28.54±0.46	14.97	35.16±1.97	15.82	30.03±0.37	16.13	31.08±0.39	16.65		
SC <sub>rel</sub>	30.58±0.26	17.99	30.76±0.54	16.58	27.01±1.11	11.64	32.12±0.35	14.05	29.15±0.47	21.24		
SO <sub>rel</sub>	20.10±0.26	26.88	16.58±0.36	20.61	15.59±1.62	29.36	20.90±0.38	23.65	21.29±0.44	27.47		
FO <sub>rel</sub>	19.08±0.23	25.65	24.12±0.40	15.54	22.24±2.36	30.04	16.95±0.26	19.71	18.48±0.36	25.85		

Data are presented for all individuals pooled together and for each individual separately, along with the respective sample sizes (in parentheses).

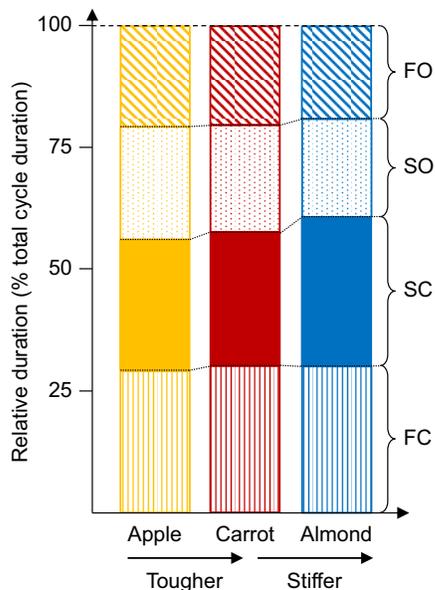
Finally, SO<sub>rel</sub> differed between foods in two individuals (individual 9,  $F_{1,268}=6.46$ ,  $P=0.012$  and individual 10,  $F_{1,108}=4.61$ ,  $P=0.034$ ). However, each individual altered SO<sub>rel</sub> differently: shorter during carrot chewing than during apple chewing in individual 9 but longer in individual 10 (see Table 2).

#### Between-food differences in CV

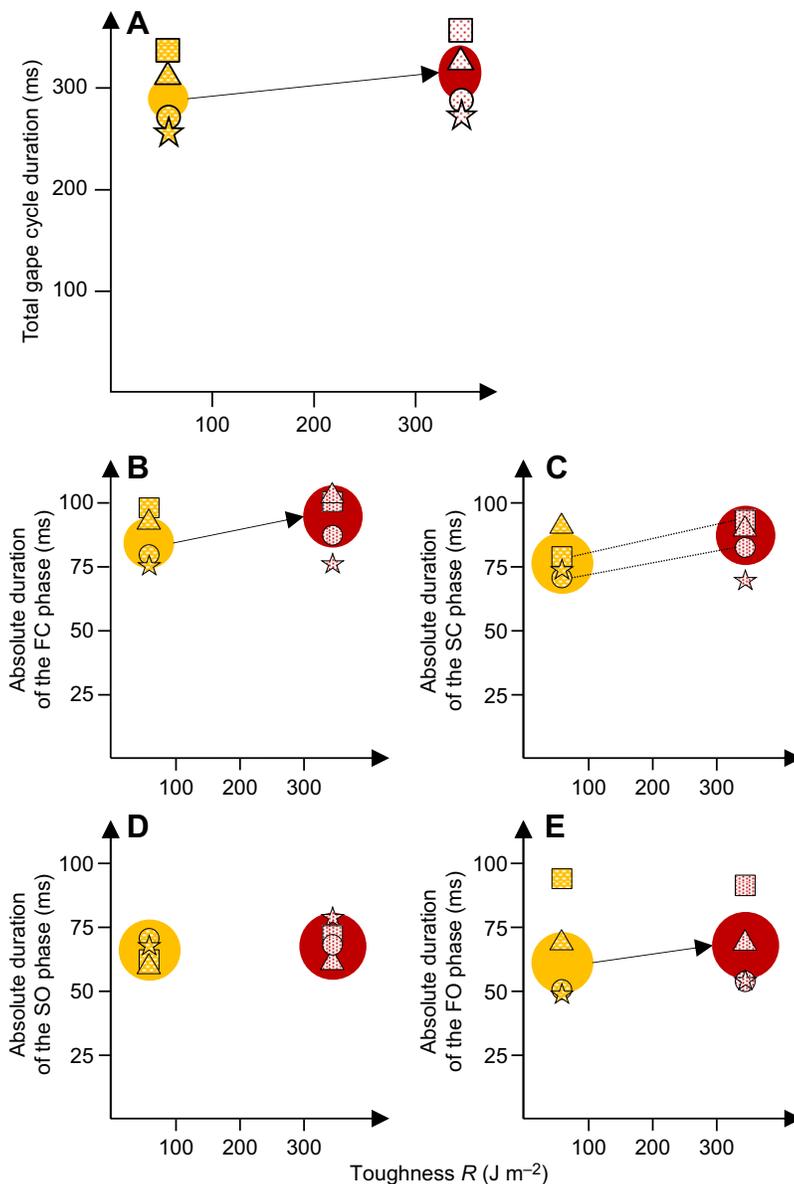
Based on the CVs for each phase, representing within-food variability, FO<sub>abs</sub> is the most variable whereas FC<sub>abs</sub> is the least variable phase (Table 1). To test whether variability in temporal

parameters differs between foods, a MANOVA was performed on the CV for each timing variable. The CVs of SO<sub>abs</sub> and FO<sub>abs</sub> did not differ between foods (see Table 1; Fig. 3D and E) and were not affected by food×individual interaction, although individual differences were significant ( $F_{3,693}=21.20$ ,  $P<0.001$  and  $F_{3,693}=11.10$ ,  $P<0.001$ , respectively). In contrast, the food×individual interaction was significant for the CV of total cycle duration ( $F_{3,690}=8.61$ ,  $P<0.001$ ) as well as for FC<sub>abs</sub> and SC<sub>abs</sub> ( $F_{3,690}=7.36$ ,  $P<0.001$  and  $F_{3,690}=6.85$ ,  $P<0.001$ , respectively). Significant differences between foods in the CV of total cycle duration were only detected in individual 5 ( $F_{1,153}=17.33$ ,  $P<0.001$ ), indicating greater within-food variability during carrot chewing than during apple chewing (see Table 1). The CVs of FC<sub>abs</sub> and SC<sub>abs</sub> differed significantly between foods in the same two individuals (FC<sub>abs</sub>: individual 5,  $F_{1,153}=12.25$ ,  $P<0.001$  and individual 6,  $F_{1,163}=5.01$ ,  $P=0.027$ ; SC<sub>abs</sub>: individual 5,  $F_{1,153}=14.46$ ,  $P<0.001$  and individual 6,  $F_{1,163}=4.69$ ,  $P=0.032$ ). However, these individuals differed from one another as absolute phase durations are more variable in individual 5 chewing on carrot versus apple whereas in individual 6 they are less variable (see Table 1).

No significant food×individual interactions were found for the CVs of SC<sub>rel</sub>, SO<sub>rel</sub> and FO<sub>rel</sub>. Among these three variables, significant differences between foods were only detected in the CV of SC<sub>rel</sub> ( $F_{1,695}=5.40$ ,  $P=0.020$ ), revealing that this phase is significantly more variable for apple than for carrot chewing (Table 2). Food effects were not significant for the CVs of SO<sub>rel</sub> and FO<sub>rel</sub>. Individual differences were significant for the CVs of SC<sub>rel</sub> ( $F_{3,695}=8.17$ ,  $P<0.001$ ), SO<sub>rel</sub> ( $F_{3,695}=16.23$ ,  $P<0.001$ ) and FO<sub>rel</sub> ( $F_{3,695}=5.71$ ,  $P=0.001$ ). Finally, the food×individual interaction was significant for the CV of FC<sub>rel</sub> ( $F_{3,692}=4.32$ ,  $P=0.005$ ). For this variable, differences between foods were only significant for two of the four individuals: individual 5 ( $F_{1,153}=7.66$ ,  $P=0.006$ ) and individual 6 ( $F_{1,163}=4.69$ ,  $P=0.032$ ). The effects of food toughness on within-food variability in FC<sub>rel</sub> differed between these individuals. In individual 5, FC<sub>rel</sub> is more variable when chewing on carrots than on apple whereas the reverse is true for individual 6 (see Table 2).



**Fig. 4. Flexibility in relative intra-cycle phase durations in response to changes in food toughness and stiffness during chewing in pigs.** Relative durations of each phase are presented as a percentage of the total gape cycle duration for each food. The relative duration of SC increases significantly as food toughness and stiffness increase whereas that of SO decreases. This demonstrates a trade-off in duration between SC and SO in response to food properties.



**Fig. 5. Summary of the effects of food toughness on the absolute durations of jaw movements during chewing in pigs.** (A) Total cycle duration; (B) FC phase; (C) SC phase; (D) SO phase; (E) FO phase. Food types are illustrated by colors: apple in yellow and carrots in red. Full solid circles represent all data (i.e. mean across all individuals) whereas symbols represent the four different individuals: square for individual #5, triangle for individual #6, circle for individual #9, and star for individual #10. Solid arrows indicate significant differences between foods and dotted lines indicate significant differences between foods limited to a specific individual.

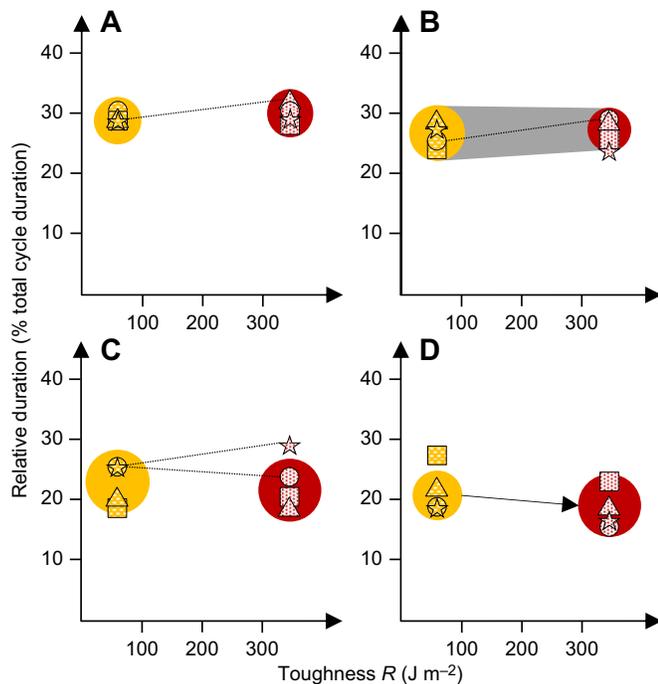
### Effects of food stiffness on the temporal characteristics of the gape cycle

#### Between-food differences in cycle and phase durations

A MANOVA compared the timing parameters of the gape cycles when chewing on carrot and almond, which differ in stiffness but have similar toughness (Williams et al., 2005). Significant differences between these foods were detected in  $SC_{abs}$  ( $F_{1,767}=25.90$ ,  $P<0.001$ ) and  $SO_{abs}$  ( $F_{1,767}=52.12$ ,  $P<0.001$ ). There was no food $\times$ individual interaction in either variable, although individual differences were detected ( $F_{3,767}=16.80$ ,  $P<0.001$  and  $F_{3,767}=7.01$ ,  $P<0.001$ , respectively). Thus, gape cycles during chewing on stiffer foods (almonds) are characterized by a longer SC and shorter SO (Table 1; Fig. 3C,D). MANOVA results also detected a significant food $\times$ individual interaction in the three remaining absolute timing variables: total cycle duration ( $F_{3,764}=8.80$ ,  $P<0.001$ ),  $FC_{abs}$  ( $F_{3,764}=7.58$ ,  $P<0.001$ ), and  $FO_{abs}$  ( $F_{3,764}=4.95$ ,  $P<0.001$ ). Total cycle duration was significantly different between foods in two of the four individuals (individual 5,  $F_{1,185}=12.21$ ,  $P=0.001$  and individual 6,  $F_{1,91}=4.11$ ,  $P=0.046$ ). However, food effects differed between these

two individuals: carrot gape cycles are longer than almond gape cycles in individual 5 whereas the reverse is true for individual 6 (see Table 1).  $FC_{abs}$  differed between foods in three of the four individuals: individual 5 ( $F_{1,185}=7.11$ ,  $P=0.008$ ), individual 6 ( $F_{1,91}=7.64$ ,  $P=0.007$ ), and individual 10 ( $F_{1,187}=5.77$ ,  $P=0.017$ ).  $FC_{abs}$  is longer for almond chewing than for carrot chewing in individuals 6 and 10 but shorter in individual 5 (see Table 1). Finally,  $FO_{abs}$  decreased significantly in response to an increase in food stiffness in one individual (individual 5,  $F_{1,185}=7.45$ ,  $P=0.007$ ; see Table 1).

Among relative phase durations, FO is the shortest at  $\sim 20\%$  of total cycle duration whereas SC and FC are the longest for both foods (Table 2; Fig. 4). Significant differences between foods were only detected for  $SC_{rel}$  ( $F_{3,769}=65.05$ ,  $P<0.001$ ) with no food $\times$ individual interaction but with significant individual differences ( $F_{3,769}=12.91$ ,  $P<0.001$ ). Thus,  $SC_{rel}$  increases when chewing on almond (see Table 2; Fig. 4). Neither  $FC_{rel}$  nor  $FO_{rel}$  were affected by significant food $\times$ individual interaction or food effects but there were significant individual differences ( $F_{3,769}=15.13$ ,  $P<0.001$  and  $F_{3,769}=104.72$ ,  $P<0.001$ , respectively).



**Fig. 6. Summary of the effects of food toughness on the absolute durations of jaw movements during chewing in pigs.** (A) FC phase; (B) SC phase; (C) SO phase; (D) FO phase. Food types are illustrated by colors: apple in yellow and carrots in red. Full solid circles represent all data (i.e. mean across all individuals) whereas symbols represent the four different individuals: square for individual #5, triangle for individual #6, circle for individual #9, and star for individual #10. Solid arrows indicate significant differences between foods, dotted lines indicate significant differences between foods limited to a specific individual, and the shaded area represents significant differences in the CV.

In contrast, a significant food $\times$ individual interaction was found for  $SO_{rel}$  ( $F_{3,766}=2.82$ ,  $P=0.038$ ).  $SO_{rel}$  was significantly shorter for almond than for carrot in three of the four individuals: individual 5 ( $F_{1,185}=21.82$ ,  $P<0.001$ ), individual 9 ( $F_{1,303}=24.00$ ,  $P<0.001$ ), and individual 10 ( $F_{1,187}=22.57$ ,  $P<0.001$ ) (see Table 2).

#### Between-food differences in CV

Based on the average CVs of all individuals, SO and FO are the most variable phases (Tables 1 and 2; Fig. 3). Food $\times$ individual interactions were significant for the comparisons of CVs of all absolute durations. The CV of total cycle duration differed significantly between foods in only one individual (individual 5,  $F_{1,183}=57.27$ ,  $P<0.001$ ), revealing that it is significantly more variable for carrot than for almond (Table 1). In this same individual, the CVs of all four phases were also more variable for carrot ( $FC_{abs}$ :  $F_{1,183}=28.58$ ,  $P<0.001$ ;  $SC_{abs}$ :  $F_{1,183}=27.10$ ,  $P<0.001$ ;  $SO_{abs}$ :  $F_{1,183}=44.58$ ,  $P<0.001$ ; and  $FO_{abs}$ :  $F_{1,183}=8.19$ ,  $P=0.005$ ). Thus, in individual 5, increasing stiffness consistently reduces variability within each food in temporal parameters (Table 1). Finally, differences between foods in the CV of  $SC_{abs}$  were also significant in individual 9 ( $F_{1,303}=13.17$ ,  $P<0.001$ ) and individual 10 ( $F_{1,187}=14.704$ ,  $P<0.001$ ), indicating a similar response to increased stiffness (Table 1).

Finally, significant differences between foods were found for the CV of  $FC_{rel}$  and  $SC_{rel}$  ( $F_{1,769}=8.00$ ,  $P=0.005$  and  $F_{1,769}=25.18$ ,  $P<0.001$ , respectively) with no food $\times$ individual interaction. This indicates that both  $FC_{rel}$  and  $SC_{rel}$  are more variable for carrot than

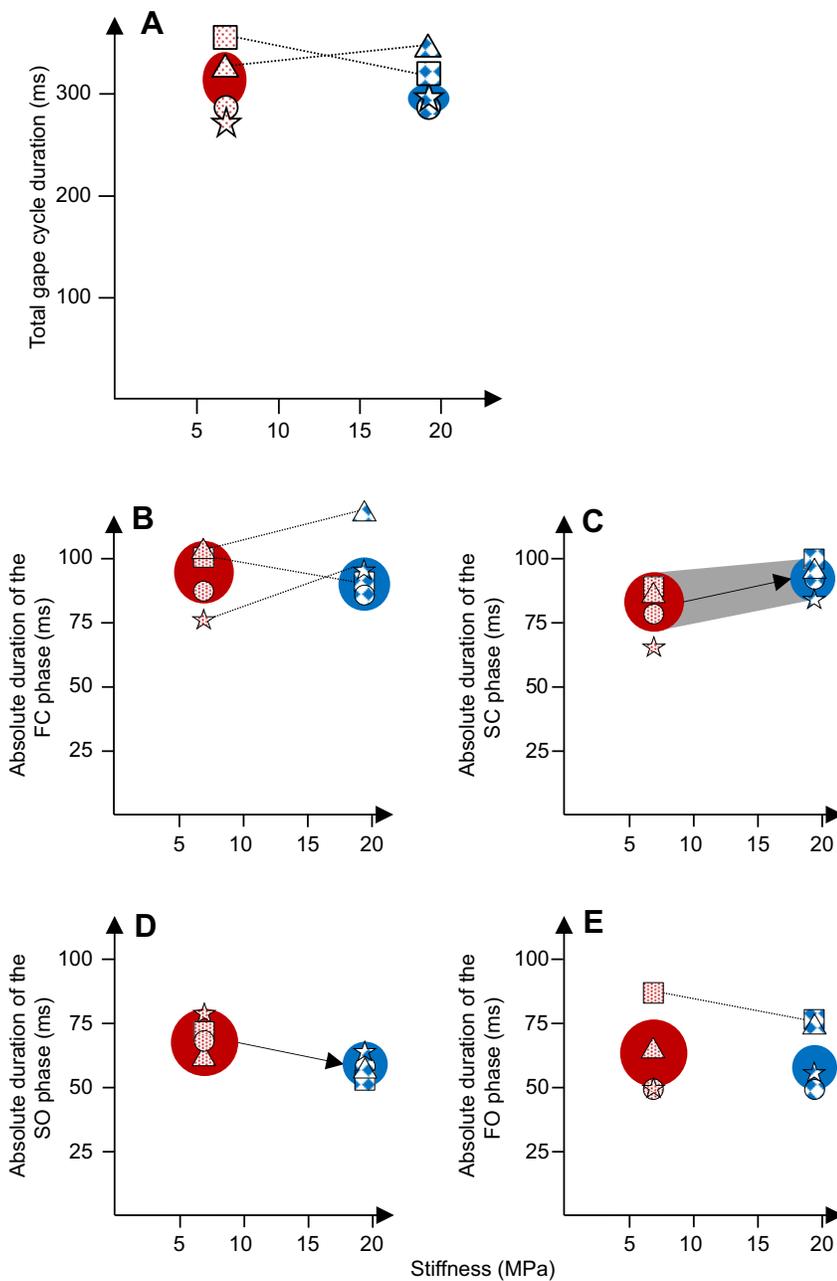
for almond (Table 2). Individual differences were significant for the CV of  $SC_{rel}$  ( $F_{1,769}=13.21$ ,  $P<0.001$ ) but not for that of  $FC_{rel}$ . Significant food $\times$ individual interactions were found for the CV of  $SO_{rel}$  and  $FO_{rel}$  ( $F_{3,766}=5.00$ ,  $P=0.002$  and  $F_{3,766}=4.29$ ,  $P=0.005$ , respectively). Significant differences between foods were only detected in the CV of these phases for individual 5 ( $SO_{rel}$ :  $F_{1,185}=21.38$ ,  $P<0.001$ ;  $FO_{rel}$ :  $F_{1,185}=13.41$ ,  $P<0.001$ ). Thus, the relative duration of these phases is significantly less variable as stiffness increases (see Table 2).

#### DISCUSSION

Both toughness and stiffness affect the temporal characteristic of the gape cycles in pigs, albeit differently. Results indicate that changes in food properties not only alter the duration of the gape cycle and that of its constituent phases but also their respective variability within each food. Thus, pigs are characterized by masticatory movements that are flexible in timing and timing variability in response to changes in food toughness and stiffness. The implications of this flexibility in the timing parameters of jaw movements during the chewing cycle are interesting in light of recent work on the evolution of rhythmic chewing in mammals (e.g. Ross et al., 2007a,b, 2010) and in the context of occlusal dynamics during the SC and SO phases.

Compared with low-toughness food (i.e. apple), gape cycles during chewing on high-toughness foods (i.e. carrots) are longer with longer constituent phases, indicating that temporal parameters of chewing in pigs are flexible in response to changes in food toughness (Figs 5 and 6). Importantly, SC, the period when contact is made with the food, exhibits flexibility in absolute and relative durations of SC (Figs 5C and 6B, respectively) but also in its within-food variability in relative duration (Fig. 6B), which decreases with increasing toughness (in a pattern similar to Fig. 1D). In contrast, FO is only flexible in response to changes in food toughness in its duration (Figs 5E and 6D) but this flexibility is associated with a constant level of variability between foods (i.e. inflexibility in stereotypy; in a pattern similar to Fig. 1B). When chewing tougher foods, pigs increase the duration of FO while maintaining within-food variability but decrease within-food variability in the duration of tooth–food–tooth contact. Finally, note that SO is inflexible in both its absolute duration and its within-food variability in response to changes in food toughness (Figs 5D and 6C; in a pattern similar to Fig. 1A).

The temporal characteristics of the gape cycle are also flexible in response to changes in food stiffness (Figs 7 and 8) but the effects of stiffness are different than those of toughness. Compared with low-stiffness food (i.e. carrot), the gape cycles used to chew on high-stiffness food (i.e. almond) are characterized by a longer and more stereotyped SC (Figs 7C and 8B; in a pattern similar to Fig. 1D). Thus, the duration of tooth–food–tooth contact is adjusted in its amplitude and in its CV depending on the stiffness of the food item. This result is interesting because it suggests that compliance is detected by periodontal mechanoreceptors, and this information is utilized to adjust the power stroke within each chew. This result is in line with work in humans, which demonstrates that stiffness is perceived as resistance to jaw movement once the food is loaded between the teeth (Agrawal et al., 1997). A longer SC when chewing stiff foods could protect the teeth and TMJ if it enables more controlled food fracture. Moreover, in humans, there is a significant positive correlation between stiffness and the breakage function of food, a measure of the change in specific surface area that occurs with fragmentation, and thus we would expect that to be the case for pigs as well. In contrast,  $FC_{rel}$  remains constant between



**Fig. 7. Summary of the effects of food stiffness on the absolute durations of jaw movements during chewing in pigs.** (A) Total cycle duration; (B) FC phase; (C) SC phase; (D) SO phase; (E) FO phase. Food types are illustrated by colors: carrots in red and almonds in blue. Full solid circles represent all data (i.e. mean across all individuals) whereas symbols represent the four different individuals: square for individual #5, triangle for individual #6, circle for individual #9, and star for individual #10. Solid arrows indicate significant differences between foods, dotted lines indicate significant differences between foods limited to a specific individual, and the shaded area represents significant differences in the CV.

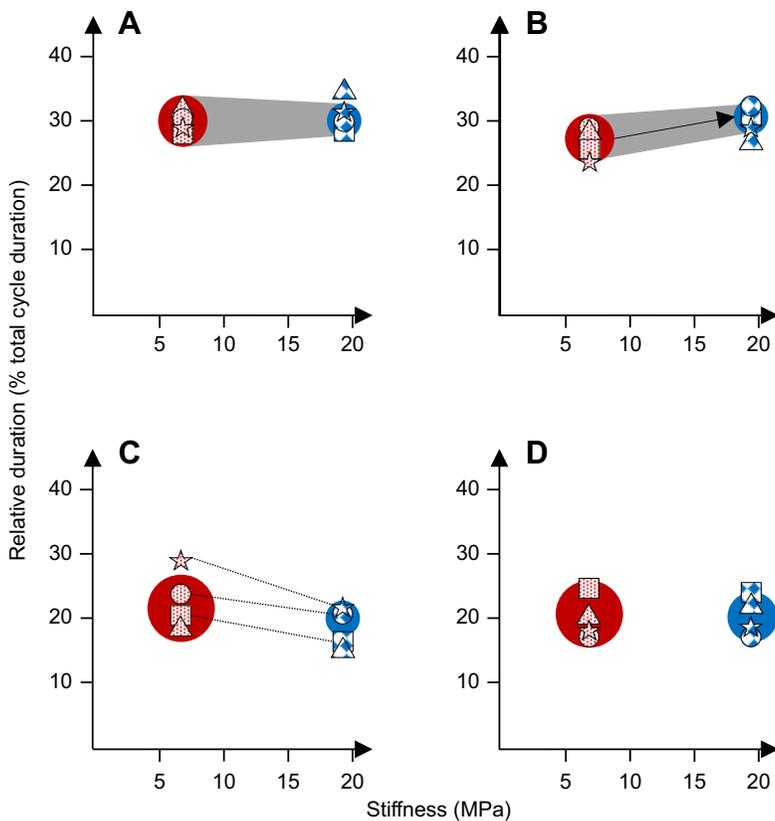
foods (i.e. is inflexible in duration), yet is flexible in within-food variability (Fig. 8A; in a pattern similar to Fig. 1C).

#### Flexibility in gape and phase temporal dynamics

Compared with apple, the processing of carrot in pigs produces longer gape cycles (Fig. 5A). At first, this result may appear to conflict with the data from primates demonstrating no effect of increased toughness on cycle duration (see table 4 in Reed and Ross, 2010 and table 2 in Iriarte-Diaz et al., 2011). However, this is the case for the average chewing cycle duration for complete chewing sequences only. In primates, the effects of increased toughness are apparent during the first 15 chews of a sequence but are mollified as the feeding sequence progresses, which suggest that their cycle dynamics are flexible (Reed and Ross, 2010). Our results based on the earliest gape cycles during chewing also demonstrate that temporal dynamics in pigs are flexible. These earliest cycles may be

the most flexible because this is when the widest range of material (including shape) and mechanical properties are encountered. This may also be when the food requires significant manipulation by the tongue to maintain placement between the teeth before trituration and mixing with saliva to form a more consistent bolus. In contrast, the cycles occurring at the end of a chewing sequence may be more similar because bolus properties are more homogeneous.

Cycle duration tends to decrease when processing stiffer foods but the effects of stiffness are masked by significant inter-individual differences (Fig. 7A), which make this observation preliminary and not conclusive. It is unclear whether the initial compliance of a food is a property that directly impacts temporal aspects of total chewing cycle duration in pigs, at least for foods of low to moderate stiffness. It may be that increasing food stiffness even more would result in a clearer signal for total cycle duration. For example, Menegaz et al. (2015) recently demonstrated that following the initial fracture of



**Fig. 8. Summary of the effects of food stiffness on the relative durations of jaw movements during chewing in pigs.** (A) FC phase; (B) SC phase; (C) SO phase; (D) FO phase. Food types are illustrated by colors: carrots in red and almonds in blue. Full solid circles represent all data (i.e. mean across all individuals) whereas symbols represent the four different individuals: square for individual #5, triangle for individual #6, circle for individual #9, and star for individual #10. Solid arrows indicate significant differences between foods, dotted lines indicate significant differences between foods limited to a specific individual, and the shaded area represents significant differences in the CV.

brazil nuts within their shells, the kinematics of the gape cycle of pigs converges on the movements that occur during chewing of pellets. As nut shells are typically much stiffer than the internal seed by several orders of magnitude (e.g. Jennings and Macmillan, 1986; Lucas, 2004; Lucas et al., 2009), it may be that offering foods with increased stiffness may induce a more consistent response across individuals at the level of the gape cycle, particularly if altering temporal dynamics from chew to chew is a mechanism to protect the teeth and TMJs (Ross et al., 2010).

The increase in gape cycle duration when processing tougher foods results from an increase in phase durations (Fig. 5), especially that of FC, SC and FO (Fig. 5B,C,E, respectively). When processing stiff foods, SC is also longer (Fig. 7C) but the duration of the subsequent SO and FO phases decreases (Fig. 7D and E, respectively). This illustrates a trade-off between SC and jaw opening to accommodate increased food stiffness, and suggests that the breakdown of stiff foods necessitates longer tooth–food–tooth contact prior to fracture. Note, however, that whereas increased food toughness is met with concomitant changes in the temporal characteristics of the chewing cycle (i.e. increases in phase durations induce an increase in total cycle duration), increased food stiffness does not alter total cycle duration (Fig. 7A), and thus it is the temporal contribution of each phase that is adjusted and flexible.

#### Flexibility in the stereotypy of temporal dynamics

Our study demonstrates that within-food variability of the timing of chewing movements in pigs differs significantly between foods of different mechanical properties (Tables 1 and 2; Figs 6B, 7C and 8A,B). We argue that this flexibility in the magnitude of stereotypy contributes to the overall functional flexibility necessary during feeding in omnivores such as pigs. Moreover, if the evolution of

functional flexibility requires significant changes in dentoskeletal morphology as well as in the neuromotor systems driving feeding movements, this may relate to why, among mammals, transitions to omnivory from more specialized diets are relatively rare (Price et al., 2012; Price and Hopkins, 2015). However, the fact that mammalian omnivores exhibit higher extinction rates suggests that functional flexibility is not sufficient to mitigate other environmental or ecological pressures (Colles et al., 2009). Of course, additional data from other omnivores as well as comparisons with species that are specialized for a particular diet are necessary to fully test this hypothesis.

For a subset of the individuals, total cycle duration is more variable when chewing on tough and elastic food such as carrots whereas it is more stereotyped when processing low-toughness food like apple or stiff foods like almonds (Table 1, Fig. 3). In contrast, an increase in food stiffness results in more stereotyped gape cycle durations and thus chewing frequency (Fig. 3A). Because variability in gape cycle duration could relate to variability in chewing frequency, these results suggest that changes in different food properties may affect chewing rhythmicity differently. Because of the energetic demands of endothermy in mammals that require increased food consumption, more rhythmic chewing is hypothesized to reduce energy expenditure during feeding (Ross et al., 2007a,b). The processing of tough but elastic foods such as carrots is characterized by a series of chewing cycles that are highly variable in total duration. If increased variability in cycle duration reduces chewing frequency, longer chewing bouts are required to process the same bolus. Moreover, a reduction in chewing frequency may also reduce rhythmicity thereby increasing muscle fatigue. The combined effect of these changes would negatively impact the metabolic cost of chewing (Ross et al., 2007a,b).

Nevertheless, the proximate role of the feed-forward and feed-back mechanisms within the sensory systems of the feeding apparatus ensure that chewing is controlled and adjusted in accordance to ever-changing food properties prior to the swallow. If bolus properties do not change throughout a chewing sequence, the expectation is that temporal dynamics of the gape cycle do not need to be adjusted, i.e. they can be stereotyped. In comparison, if the bolus requires significant alteration in its material properties, ongoing adjustments and modifications from one cycle to the next may occur within a sequence. Based on our results, tough but elastic foods such as carrots may undergo the most changes in material properties throughout the chewing sequence, leading to significant changes in the mechanical demands driving overall chewing performance.

Changes in food toughness and stiffness also alter the level of within-food variability in SC. The duration of tooth–food–tooth contact becomes more stereotyped with an increase in food toughness and stiffness (Figs 6B, 7C and 8B). The fact that the duration of SC is more variable during the processing of low-toughness/low-stiffness foods such as apple (Fig. 7B) may be linked to the fact that it is more rapidly broken down into smaller fragments with each chew and the position of these fragments within the oral cavity may be highly variable from one cycle to the next. In contrast, cracks do not propagate as easily in tougher and stiffer foods such as carrots or almonds, so their fragmentation may require altered occlusal forces and/or kinematics. This is supported by research on the primate *Cebus apella* showing that increased toughness requires fewer total chews within a sequence (Reed and Ross, 2010), suggesting that either or both of these mechanisms may be at play.

## Conclusions

In pigs, flexibility in the timing of chewing movements in response to changes in toughness and stiffness manifests differently. Changes in food toughness affect the chewing cycle as a whole whereas changes in food stiffness alter the relative contribution and duration of the constituent phases of the chewing cycle, while total cycle duration remains more or less constant. These differences may have consequences at different levels. Increased food toughness induces an increase in the absolute duration of intra-cycle phases, especially SC during which contact with the food occurs, which results in an increase in the total cycle duration. On the one hand, this implies a lengthening of the power stroke to promote crack propagation through tougher foods. On the other hand, increasing total cycle duration can be hypothesized to affect the neuromotor control of the muscles generating jaw movements, and thus impact energy expenditure necessary to complete chewing bouts. In contrast, changes in food stiffness result in a trade-off between SC and the subsequent jaw-opening phases. In other words, flexibility in the timing of chewing movements in pigs occurs: (i) at the level of the chewing cycle as a whole in response to change in food toughness, and (ii) within the temporal architecture of the chewing cycle in response to changes in food stiffness. These results suggest that the temporal response to increased toughness directly impacts food fracture whereas the temporal response to increased stiffness may be more protective in nature.

Within-food variability in jaw movements during chewing in pigs is also flexible demonstrating differences in how stereotyped the response is to different food properties. In one individual (pig 5), the duration of jaw movement during processing of low-toughness foods is more stereotyped whereas it is more variable during processing tough but elastic foods. These results suggest that chewing rhythmicity is affected differently by different food properties. In addition, within each cycle, the duration of SC is

variable during the processing of low-toughness foods whereas it is stereotyped in tough or stiff foods. This is hypothesized to be linked to food fragmentation and the swallowability of the particles generated by crack propagation. Therefore, our study emphasizes the fact that flexibility in biological motion in response to changes in the environmental conditions may not only be observed in amplitude but also in their variability within each condition.

Finally, the focus of this study is on the temporal parameters underlying rhythmic chewing in pigs but the phases of the mammalian chewing cycle are characterized by specific movements (i.e. rotations and translations) of the lower jaw with respect to the skull. Flexibility of chewing movements in their duration, frequency and rhythmicity in response to food properties may be associated with flexibility in the amplitude of these movements themselves. Indeed, as the present study reports that phase durations are modified in response to food toughness and stiffness, the next question is to test whether such changes in timing are associated with changes in other kinematic parameters, such as changes in the amplitude of rotation and translation of the mandibular condyles as well as specific occlusal interactions.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: S.J.M., S.H.W.; Methodology: S.J.M., R.O., S.H.W.; Validation: S.J.M., R.O., H.C., J.S., S.H.W.; Formal analysis: S.J.M., S.H.W.; Investigation: S.J.M., R.O., S.H.W.; Resources: S.J.M., R.O., H.C., J.S., S.H.W.; Data curation: S.J.M., R.O., H.C., J.S., S.H.W.; Writing – original draft: S.J.M.; Writing – review & editing: S.J.M., R.O., H.C., J.S., S.H.W.; Visualization: S.J.M., S.H.W.; Supervision: S.H.W.; Project administration: S.H.W.; Funding acquisition: S.H.W.

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## Data availability

All data used for this study, including metadata, CT scan data and the original unprocessed X-ray movies, are available from the X-ray Motion Analysis Portal (<http://xmaportal.org/webportal/>).

## Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.168088.supplemental>

## References

- Agrawal, K. R., Lucas, P. W., Prinz, J. F. and Bruce, I. C. (1997). Mechanical properties of foods responsible for resisting food breakdown in the human mouth. *Arch. Oral Biol.* **42**, 1–9.
- Agrawal, K. R., Lucas, P. W., Bruce, I. C. and Prinz, J. F. (1998). Food properties that influence neuromuscular activity during human mastication. *J. Dent. Res.* **77**, 1931–1938.
- Anderson, K., Throckmorton, G. S., Buschang, P. H. and Hayasaki, H. (2002). The effects of bolus hardness on masticatory kinematics. *J. Oral Rehabil.* **29**, 689–696.
- Brainerd, E. L., Baier, D. B., Gatesy, S. M., Hedrick, T. L., Metzger, K. A., Gilbert, S. L. and Crisco, J. J. (2010). X-ray reconstruction of moving morphology (XROMM): precision, accuracy and applications in comparative biomechanics research. *J. Exp. Zool. A Ecol. Genet. Physiol.* **313A**, 262–279.

- Clauss, M., Nunn, C., Fritz, J. and Hummel, J.** (2009). Evidence for a tradeoff between retention time and chewing efficiency in large mammalian herbivores. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **154**, 376-382.
- Colles, A., Liow, L. H. and Prinzing, A.** (2009). Are specialists at risk under environmental change? Neoeological, paleoecological and phylogenetic approaches. *Ecol. Lett.* **12**, 849-863.
- Davis, J.** (2014). Functional morphology of mastication in musteloid carnivorans. PhD thesis, Department of Biological Sciences, Ohio University, 234 pp.
- De Gueldre, G. and de Vree, F.** (1984). Movements of the mandibles and tongue during mastication and swallowing in *Pteropus giganteus* (megachiroptera): a cineradiographical study. *J. Morphol.* **179**, 95-114.
- De Gueldre, G. and De Vree, F.** (1988). Quantitative electromyography of the masticatory muscles of *Pteropus giganteus* (Megachiroptera). *J. Morphol.* **196**, 73-106.
- De Vree, F. and Gans, C.** (1976). Mastication in pygmy goats (*Capra hircus*). *Annales de la Societe Royale Zoologique de Belgique* **105**, 255-306.
- Dotsch, C.** (1986). Mastication in the musk shrew, *Suncus murinus* (Mammalia, Soricidae). *J. Morphol.* **189**, 25-43.
- Dotsch, C. and Dantuma, R.** (1989). Electromyography and masticatory behavior in shrews (Insectivora). *Prog. Zool.* **35**, 146-147.
- Fritz, J., Hummel, J., Kienzle, E., Arnold, C., Nunn, C. and Clauss, M.** (2009). Comparative chewing efficiency in mammalian herbivores. *Oikos* **118**, 1623-1632.
- Futuyama, D. J. and Moreno, G.** (1988). The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* **19**, 207-233.
- Gorniak, G. C. and Gans, C.** (1980). Quantitative assay of electromyograms during mastication in domestic cats (*Felis catus*). *J. Morphol.* **163**, 253-281.
- Hylander, W. L., Vinyard, C. J., Ravosa, M. J., Ross, C. F., Wall, C. E. and Johnson, K. R.** (2004). Jaw adductor force and symphyseal fusion. In *Shaping Primate Evolution: Papers in Honor of Charles Oxnard* (ed. F. Anapol, R. Z. German and N. G. Jablonski), pp. 229-257. Cambridge: Cambridge University Press.
- Iriarte-Diaz, J., Reed, D. A. and Ross, C. F.** (2011). Sources of variance in temporal and spatial aspects of jaw kinematics in two species of primates feeding on foods of different properties. *Integr. Comp. Biol.* **51**, 307-319.
- Jennings, J. S. and Macmillan, N. H.** (1986). A tough nut to crack. *J. Mater. Sci.* **21**, 1517-1524.
- Karsov, W. H., Petrossian, E., Rosenberg, L. and Diamond, J. M.** (1986). How do food passage rate and assimilation differ between herbivorous lizards and nonruminant mammals? *J. Comp. Physiol. B* **156**, 599-609.
- Knörlein, B. J., Baier, D. B., Gatesy, S. M., Laurence-Chasen, J. D. and Brainerd, E. L.** (2016). Validation of XMALab software for marker-based XROMM. *J. Exp. Biol.* **219**, 3701-3711.
- Koyama, K., Mioche, L. and Martin, F.** (2002). Chewing patterns of various texture foods studied by electromyography in young and elderly populations. *J. Texture Stud.* **33**, 269-283.
- Lucas, P. W.** (2004). *Dental Functional Morphology: How Teeth Work*. Cambridge: Cambridge University Press.
- Lucas, P. W., Constantino, P. J., Chalk, J., Ziscovici, C., Wright, B. W., Fragaszy, D. M., Hill, D. A., Lee, J. J.-W., Chai, H., Darvell, B. W. et al.** (2009). Indentation as a technique to assess the mechanical properties of fallback foods. *Am. J. Phys. Anthropol.* **140**, 643-652.
- Menegaz, R. A., Baier, D. B., Metzger, K. A., Herring, S. W., Brainerd, E. L.** (2015). XROMM analysis of tooth occlusion and temporomandibular joint kinematics during feeding in juvenile miniature pigs. *J. Exp. Biol.* **218**, 2573-2584.
- Peyron, A., Lassauzay, C. and Woda, A.** (2002). Effects of increased hardness on jaw movement and muscle activity during chewing of visco-elastic model foods. *Exp. Brain Res.* **142**, 41-51.
- Price, S. A. and Hopkins, S. S. B.** (2015). The macroevolutionary relationship between diet and body mass across mammals. *Biol. J. Linn. Soc.* **115**, 173-184.
- Price, S. A., Hopkins, S. S. B., Smith, K. K. and Roth, V. L.** (2012). Tempo of trophic evolution and its impact on mammalian diversification. *Proc. Natl. Acad. Sci. USA* **109**, 7008-7012.
- Reed, D. A. and Ross, C. F.** (2007). Quantifying the modulation of mastication with high-resolution, three-dimensional kinematics. *J. Morphol.* **268**, 1123.
- Reed, D. A. and Ross, C. F.** (2010). The influence of food material properties on jaw kinematics in the primate, *Cebus*. *Arch. Oral Biol.* **55**, 946-962.
- Ross, C. F., Dharia, R., Herring, S. W., Hylander, W. L., Liu, Z.-J., Rafferty, K. L., Ravosa, M. J. and Williams, S. H.** (2007a). Modulation of mandibular loading and bite force in mammals during mastication. *J. Exp. Biol.* **210**, 1046-1063.
- Ross, C. F., Eckhardt, A., Herrel, A., Hylander, W. L., Metzger, K. A., Schaeerlaeken, V., Washington, R. L. and Williams, S. H.** (2007b). Modulation of intra-oral processing in mammals and lepidosaurs. *Integr. Comp. Biol.* **47**, 118-136.
- Ross, C. F., Baden, A. L., Georgi, J., Herrel, A., Metzger, K. A., Reed, D. A., Schaeerlaeken, V. and Wolff, M. S.** (2010). Chewing variation in lepidosaurs and primates. *J. Exp. Biol.* **213**, 572-584.
- Ross, C. F., Iriarte-Diaz, J., Reed, D. A., Stewart, T. A. and Taylor, A. B.** (2016). In vivo bone strain in the mandibular corpus of *Sapajus* during a range of oral food processing behaviors. *J. Hum. Evol.* **98**, 36-65.
- Schwartz, G., Enomoto, S., Valiquette, C. and Lund, J. P.** (1989). Mastication in the rabbit: a description of movement and muscle activity. *J. Neurophysiol.* **62**, 273-287.
- Thexton, A. J. and Crompton, A. W.** (1989). Effect of sensory input from the tongue on jaw movement in normal feeding in the opossum. *J. Exp. Zool.* **250**, 233-243.
- Thexton, A. and Hiemae, K. M.** (1997). The effect of food consistency upon jaw movement in the macaque: a cineradiographic study. *J. Dent. Res.* **76**, 552-560.
- Trulsson, M.** (2006). Sensory-motor function of human periodontal mechanoreceptors. *J. Oral Rehabil.* **33**, 262-273.
- Trulsson, M.** (2007). Force encoding by human periodontal mechanoreceptors during mastication. *Arch. Oral Biol.* **52**, 357-360.
- Trulsson, M. and Johansson, R. S.** (2002). Orofacial mechanoreceptors in humans: encoding characteristics and responses during natural orofacial behaviors. *Behav. Brain Res.* **135**, 27-33.
- Vinyard, C. J., Wall, C. E., Williams, S. H. and Hylander, W. L.** (2008). Patterns of variation across primates in jaw-muscle electromyography during mastication. *Integr. Comp. Biol.* **48**, 294-311.
- Wainwright, P. C., Mehta, R. S. and Higham, T. E.** (2008). Stereotypy, flexibility and coordination: key concepts in behavioral functional morphology. *J. Exp. Biol.* **211**, 3523-3528.
- Weijs, W. A. and de Jongh, H. J.** (1977). Strain in mandibular alveolar bone during mastication in the rabbit. *Arch. Oral Biol.* **22**, 667-675.
- Weijs, W. A., Brugman, P. and Grimbergen, C. A.** (1989). Jaw movements and muscle activity during mastication in growing rabbits. *Anat. Rec.* **224**, 407-416.
- Williams, S. H., Wright, B. W., Truong, V. D., Daubert, C. R. and Vinyard, C. J.** (2005). Mechanical properties of foods used in experimental studies of primate masticatory function. *Am. J. Primatol.* **67**, 329-346.
- Woda, A., Foster, K., Mishellany, A. and Peyron, M. A.** (2006). Adaptation of healthy mastication to factors pertaining to the individual or to the food. *Physiol. Behav.* **89**, 28-35.
- Yamada, Y. and Yamamura, K.** (1996). Possible factors which may affect phase durations in the natural chewing rhythm. *Brain Res.* **706**, 237-242.