



SYMPOSIUM

Sources of Variance in Temporal and Spatial Aspects of Jaw Kinematics in Two Species of Primates Feeding on Foods of Different Properties

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Synopsis Chewing kinematics reflects interactions between centrally generated motor signals and peripheral sensory feedback from the constantly changing oral environment. Chewing is a strongly modulated behavior that responds to differences in material properties among different type of foods and to changes in the external physical properties of the food as the bolus gets processed. Feeding, as any complex biological behavior, presents variation at multiple hierarchical levels, from among species or higher-order levels to variation among chewing cycles within a single feeding sequence. Thus, to understand the mechanics and evolution of feeding systems requires estimation of how this variation is distributed across each of these hierarchical levels, which in turn requires large sample sizes. The development of affordable, high-resolution, three-dimensional kinematic recording systems has increased our ability to collect large amounts of data on complete or near-complete feeding sequences that can be used to shed light on the mechanisms of control in vertebrate feeding. In this study, we present data on the nature and sources of variation (from species to chewing cycle levels) in kinematics of chewing in two species of primates, *Cebus* and *Macaca*, while they feed on foods of known material properties. Variation in chewing kinematics was not evenly distributed among hierarchical levels. Most of the variation was observed among chewing cycles, most likely in response to changes in the external properties of the food bolus throughout the feeding sequence. Species differences were found in duration and vertical displacement during slow-close phase suggesting that each species exhibits different power stroke dynamics. *Cebus* exhibited more variable gape cycles than did *Macaca*, in particular when eating low-toughness foods. This increased ability to temporally and spatially modulate the gape cycle may reflect increased efficiency in processing food because *Cebus* monkeys use fewer, but longer cycles, than does *Macaca* when feeding on low-toughness foods. This is due to an increase in duration of the jaw-opening phases of the gape cycle, when the tongue repositions the food bolus in the oral cavity.

Introduction

The morphological and functional diversity of vertebrate feeding systems provides a rich array of natural experiments with which to test hypotheses regarding relationships between morphology, kinematics, and muscle-activation patterns in control of behavior. Mammalian mastication is a particular form of chewing (i.e., cyclic intra-oral food processing) in which food is extensively broken down between the postcanine teeth (Hiemae and Crompton 1985)

until the properties of the bolus are appropriate for a safe, rapid, reflexively driven swallow (Lucas et al. 2002).

Chewing kinematics reflect the interaction between centrally generated motor commands and peripheral sensory feedback about the constantly changing oral environment (Lund and Kolta 2006). The mechanisms of sensorimotor integration facilitate successful chewing and are the focus of intensive study on a number of fronts. Many studies of chewing

kinematics in humans have focused on understanding how mechanisms of control of mastication vary in response to abnormalities and pathologies of the cranio-facial system. Consequently, attention has sometimes been focused on minimizing the amount of variation between cycles within sequences, individuals or test groups in order to increase statistical power for studying disease states and syndromes (e.g., Wintergerst et al. 2004). Although minimizing natural variability has merit from a clinical and diagnostic point of view, it impedes answers to questions such as: How variable are primates' chewing kinematics? What is the relative importance of food material properties (FMPs), species-level morphological and behavioral differences, and inter-individual variability? Studies on humans (Hiimae et al. 1996; Hiimae and Palmer 1999) and nonhuman mammals (German et al. 2008; Vinyard et al. 2008; Reed and Ross 2010) focused on identifying and quantifying sources of variation in chewing kinematics and in motor control have yielded important insights into variation in chewing kinematics. In this study, we present high-resolution, three-dimensional data on the nature and sources of variance in chewing kinematics of the jaw in two species of primates, *Cebus* and *Macaca*, while they feed on foods of known material properties. These data were used to test hypotheses regarding the evolution and control of mammalian feeding.

Hypotheses

The relationship between jaw kinematics and FMPs was evaluated by testing a series of hypotheses regarding variance in the duration of gape cycles and their constituent phases, as well as variance in their vertical and lateral displacements of the jaw.

Components of variance

Variation in jaw kinematics is a product of variation in the relative orientations and/or magnitudes of the jaw muscle forces. A comprehensive study of EMG activity in eight jaw elevator muscles in primates has shown that the largest proportion of variance in the timing of peak activity in the jaw muscles is between chewing cycles within chewing sequences (Vinyard et al. 2008). This was attributed to variation in the external properties of food (i.e., bolus) and bite location within chewing sequences, as well as redundancy in the way that the jaw muscles can be activated to produce sub-maximal bite forces. Vinyard et al. also found that, for most muscles, peak EMG amplitude shows the highest variance between chewing sequences, which they attributed to differences in material properties among different types of food.

These results suggest that, if variation in jaw kinematics is caused primarily by variation in relative timing of the jaw elevator muscles, rather than by variation in their relative amplitudes, then the majority of variance in jaw kinematics should be found between chewing cycles within chewing sequences, rather than between chewing sequences on different foods. Once these inter-cycle effects are controlled, we hypothesize that variance in jaw kinematics is primarily caused by differences in type/material properties of food, predicting greater variance among sequences than among experiments, individuals, or species.

One source of variance in jaw kinematics that has been poorly studied is the species-effect (but see Hiimae and Kay 1973; Kay and Hiimae 1974). Differences in jaw muscle architecture, skeletal geometry, and dental morphology among species might be expected to produce differences in the ways in which animals modulate their feeding behavior to FMPs. We studied two species of primates that share general similarities in musculoskeletal design, but with differences in occlusal morphology: *Cebus* monkeys have flat occlusal surfaces, similar to those of humans, whereas the teeth of *Macaca* are high-crowned. External physical attributes such as the size, geometry, and adhesive properties of the surface of the food can affect jaw movements both during and prior to the power stroke (Van Der Bilt et al. 1991; Peyron et al. 1997; Lucas et al. 2002; Lucas 2004; Reed and Ross 2010). However, if differences in occlusal morphology between our subject species necessitate differences in the jaw closing phases (fast-close, when the teeth are aligned for the power stroke, and slow close, when the teeth are actually in contact with each other and with the food) then inter-specific variance in jaw kinematics might be substantial (Kay and Hiimae 1974; Hylander 1988). To identify these effects, we need to account for inter-individual differences as well. To test these hypotheses, we quantified the distribution of the magnitudes of variance using an analysis of the components of variance. We asked: are jaw kinematics more variable among species, among individuals within species, among feeding sequences (on different foods) within individuals, or among gape cycles within feeding sequences?

Gape cycles

A gape cycle is the complete elevation–depression cycle of the jaw, measured in this study from minimum gape to the next minimum gape. Duration of the gape cycle has been said to be influenced by

FMPs (Peyron et al. 1997, 2002, 2004; Foster et al. 2006; Woda et al. 2006a, 2006b). In a prior study from our laboratory, we reported that *Cebus* monkeys feeding on foods of high toughness chew with relatively constant cycle duration throughout the sequence, whereas processing foods of low toughness resulted in the duration increasing throughout the sequence (Reed and Ross 2010).

The amplitudes of maximum vertical and lateral movements of the jaw during the gape cycle are expected to be affected by FMPs. Harder food items have been associated with larger maximum vertical excursions (Oron and Crompton 1985; Foster et al. 2006), maybe because harder food items are placed further distally along the tooth row to reduce the load arm about the jaw joint (Thexton et al. 1980). Similarly, some studies have shown that lateral excursion in humans increases with food hardness (Proschel and Hoffmann 1988; Takada et al. 1994; Agrawal et al. 2000; Anderson et al. 2002). In addition, Foster et al. (2006) showed that humans chew plastic foods with greater vertical and horizontal jaw displacements than they do when chewing elastic foods. Others report no consistent effect of the properties of food on the shape of the horizontal jaw-movement profile (De Guldre and De Vree 1984; Hiimeae et al. 1996; Foster et al. 2006). Reed and Ross (2010) found that tougher foods elicit greater vertical displacements whereas foods that are less tough elicit greater horizontal displacements.

Gape-cycle phases

A gape cycle consists of four gape-cycle phases (Fig. 1): slow-open (SO), fast-open (FO), fast-close (FC), and slow-close (SC) (Hiimeae 1978; Bramble and Wake 1985). Each phase is defined by key jaw kinematic events associated with transitions in sensory afferent input and are plausibly hypothesized to be key events in sensorimotor control (Lund 1991).

SC is the phase of the chewing cycle when the teeth interact with the food item, FMPs are detected and the food is broken down (Lucas 2004) and therefore, jaw movement during this phase is of particular interest. It has been argued that tough foods require greater horizontal displacements during the SC phase (Hylander 1988; Agrawal et al. 1997, 2000; Agrawal and Lucas 2002; Lucas 2004). Larger lateral excursions have been observed in humans feeding on foods of high toughness than when feeding on low-toughness foods (Agrawal et al. 2000) but this effect is not seen in *Cebus* monkeys (Reed and Ross 2010). Whether macaques resemble capuchins or humans in

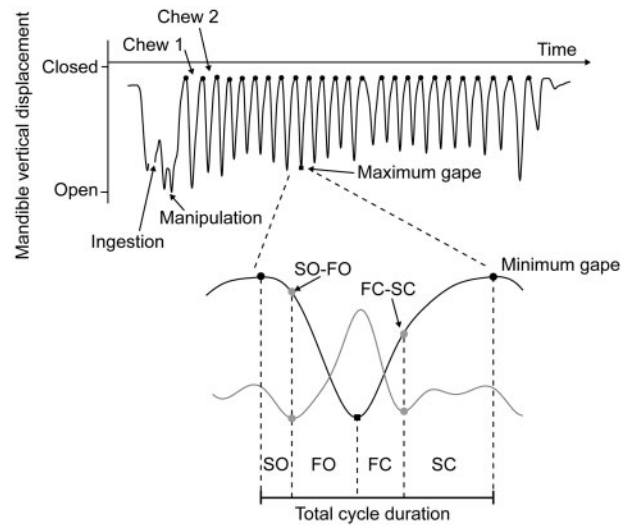


Fig. 1 Schematic of a representative feeding sequence. The black line represents the vertical position of the lower jaw. Black circles correspond to minimum gape while the black squares correspond to the maximum gape. The enlarged portion of the feeding sequence represents a single chewing cycle, divided into its four phases (following Hiimeae 1978). The gray line represents the second derivative of the vertical displacement of the mandible (Y-axes not to scale). Gray circles correspond to inflection points (i.e., the largest negative peak of the second derivative) of the vertical displacement of the lower jaw during opening and closing phases. The inflection point between minimum gape and maximum gape corresponds to the SO–FO transition and the inflection point between maximum and minimum gape corresponds to the fast-close–slow-close (FC–SC) transition. Thus, maximum and minimum gape, together with the SO–FO and FC–SC transitions define the boundaries of the four chew phases: SO, FO, FC, and SC.

the effect of food toughness on lateral mandibular displacement will be tested.

SO and FO are the phases of the chewing cycle when the tongue is brought into contact with the food item, sensory information is collected about the external properties of the bolus, and fragments are collected by the tongue and transported posteriorly toward the pharynx. We hypothesize that because low-toughness foods quickly break into many fragments, they will require larger movements of the tongue to manipulate those fragments, in turn necessitating larger vertical and lateral excursions and longer durations of the SO and FO phases. Reed and Ross (2010) reported that among *Cebus*, less tough foods elicit smaller vertical and greater horizontal excursions of the mandible than do tougher foods.

Importantly, all of these hypotheses relate variance in jaw kinematics during the phases of the gape cycle either directly or indirectly to FMPs, rather than to species-specific factors such as occlusion or facial morphology. The analyses presented here provide

Table 1 Data set used in this study

Species/individual	Sequences	Cycles	Foods
<i>Cebus apella</i>	187	4161	Almond, apricot, brazil nut, cashew, date, strawberry, gummy bear, hazelnut, peanut, pecan,
C1	76	1527	pineapple, popcorn kernel, raisin, walnut
C2	44	823	
C3	67	1811	
<i>Macaca mulatta</i>	129	3459	Almond, carrot, date, hazelnut, popcorn kernel, raisin, sweet potato
M1	55	1630	
M2	74	1829	

an opportunity to evaluate this hypothesis, and to evaluate whether there might be species-specific strategies for processing foods of different properties.

Material and methods

Chewing kinematics were recorded from three adult male capuchins (*Cebus apella*) and two adult female macaques (*Macaca mulatta*) (Table 1). Animals were housed and studied at the University of Chicago in accordance with Federal regulations and approved IACUC protocols.

Collection of data

Kinematic data were collected using three-dimensional motion-capture methods described in detail by Reed and Ross (2010). Briefly, reflective markers were coupled to the mandible and cranium using bone screws; their positions relative to the teeth were measured with a 3D digitizer (Immersion, Microscribe G2) and their movements measured in three dimensions using either a six-camera or ten-camera Vicon system, recording at 250 frames/s. Subjects were trained to feed while restrained in a primate chair (Plas-Labs, 514-AG). The capuchins wore a jacket (Lomir Biomedical) that restrained the body but allowed the head and neck to move freely and the arms to be selectively released. The macaques' heads were restrained using a halo coupled to the cranium through chronically implanted headposts and the left arm was restrained. Both species were allowed to use at least one hand to feed themselves.

Food items were grouped into two categories following Reed and Ross (2010): (1) food items of high toughness and low stiffness [all dry foods (apricot, date, strawberry, gummy bear, pineapple, raisins), carrot, popcorn kernel, and sweet potato] and (2) food items of low toughness and high stiffness (all nuts, including almond, brazil nut, cashew, hazelnut, peanut, pecan, and walnut).

Data processing

The 3D movement of the mandibular markers was calculated in the local coordinate system of the cranium. To construct the cranial coordinate system, we fitted a plane through the occlusal surface of the upper teeth during minimum gape. The primary axis of this plane, the midline between the left and right tooth rows, was assigned the X-axis of the cranium (positive forward), with the origin at the mid-point between the right and left mandibular condyles; the secondary axis of the plane was assigned the Z-axis (positive to the right); and the Y-axis (positive vertical) was calculated as the cross-product of the X and Z-axes. Thus, the XY plane of the cranial coordinate system corresponds to the sagittal plane of the cranium. The position of the rotated and centered cranial markers during minimum gape was defined as the reference position, and at each time step, the mandibular and cranial markers were rotated and translated so that the cranial markers matched the reference position, effectively "fixing" the cranium in 3D space. The mandibular marker positions were then calculated with respect to a fixed-cranium coordinate system. The movement of a marker attached to the left anterior aspect of the mandible was selected as representative of the jaw's movement and was used in further analyses. The use of other mandibular markers produced no qualitatively different results. The data on displacement of the marker were filtered with a fourth-order, low-pass Butterworth filter with a 15-Hz cutoff frequency.

Kinematic variables

A feeding sequence can be divided into five different types of event: ingestion, manipulation, stage-1 transport, rhythmic chewing, and swallows (Thexton et al. 1980; Schwartz et al. 1989). In this study, we focused on rhythmic chewing cycles only, for which we estimated a series of temporal and spatial kinematic parameters that summarize the

mandibular movement. A chew cycle was defined by two consecutive minimum gapes. The number of chewing cycles varied among species, individuals and sequences, in some cases reaching up to 90 cycles in a sequence. Due to difficulties in defining the end of a sequence (some animals tended to clean their palates with their tongues after the last swallow), we limited our analysis to the first 40 chewing cycles.

Both temporal and spatial components were measured from each chewing cycle. Temporally, a chew cycle is defined as the time between a minimum gape and the next minimum gape. Each chew cycle was then divided into four phases: SO, FO, FC, and SC (Hiimae 1978; Bramble and Wake 1985) (Fig. 1). The duration of each phase and the timing of transitions between phases were calculated as a percentage of the duration of the chew cycle. Spatial variation was quantified by measuring the total vertical and lateral displacement of the marker during a chew cycle, as well as the vertical and lateral displacement during each phase. The vertical position of the marker with respect to the vertical position at minimum gape was calculated for maximum gape and for transitions between phases (SO–FO and FC–SC).

Statistical analyses

To identify the sources of variation in temporal and spatial variables, variance for each chew cycle was analyzed using a random, nested ANOVA model using all foods, as well as by food type (i.e., high- and low-toughness foods separately). We considered four hierarchical factors: *species*, *individuals* nested within species, *chewing sequences* nested within species and individuals, and *chewing cycles* nested within all the previous factors (cf. Vinyard et al. 2008). Variation among chewing cycles was modeled by considering chew number as a covariate (Doncaster and Davey 2007). Due to the nature of the experimental design, the ANOVA design was unbalanced (i.e., unequal numbers of individuals and sequences), preventing the estimation of an exact significant test for the random factors (Sokal and Rohlf 1995; Searle et al. 2006), and therefore we focused on the overall pattern of variation distributed among these hierarchical factors (cf. Vinyard et al. 2008). Because chew cycles cannot be replicated within a chewing sequence in this kind of experimental design, between-cycle variation is confounded with the residual error of the model. Estimation of the components of variance by ANOVA can, in some cases, yield negative estimates, despite the fact that variance cannot be negative. Negative estimates can result

from the use of an inappropriate model or if the true value of the component of variance is zero (Searle et al. 2006). Following the recommendations of Searle et al. (2006), we performed our analyses with a restricted maximum likelihood (REML) method that excludes the possibility of negative estimates of variance.

In addition, we tested specifically for differences between species and between food types by using a mixed model, with species and food type as fixed factors. Individuals and sequences were nested within species and individual, respectively, and they were considered as random factors. Because spatial and temporal parameters mostly change linearly within the first 20 chews (Reed and Ross 2010), only the first 20 chewing cycles were included in this analysis, with chew number used as a covariate. The model was factorial for the fixed factors. Data are presented as mean \pm SEM.

Results

Patterns of components of variance

Variation in temporal and spatial variables is not evenly distributed among hierarchical levels (Fig. 2,

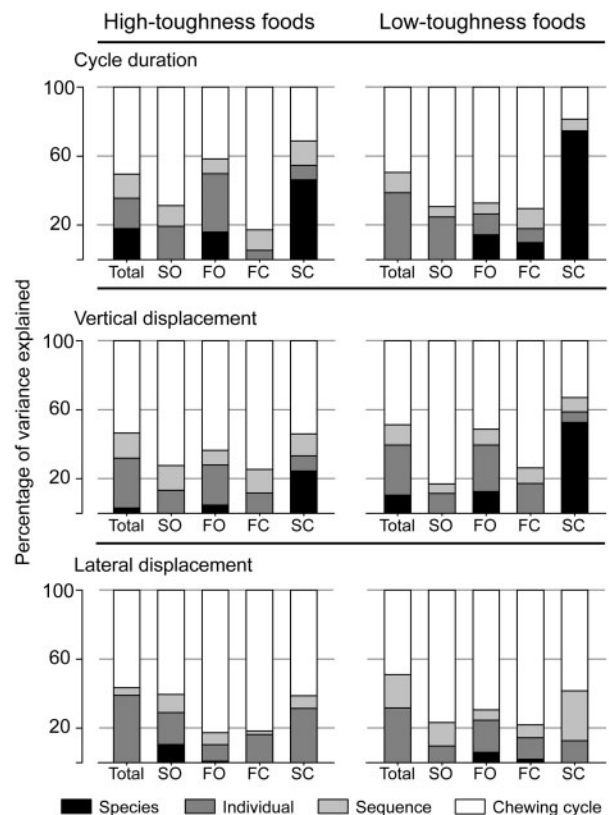


Fig. 2 The percentage of variance explained by each hierarchical factor in the nested ANOVA model for the temporal and spatial variables when animals are feeding on high-toughness and low-toughness foods, using the first 40 chewing cycles.

Supplementary Figs. S1, S2, and S3, and Supplementary Tables S1 and S2). Overall, the largest component of variance is found among chewing cycles, with small to moderate contribution of all other levels. However, a substantial species-level component of variance is found for both the duration of the SC phase and for the vertical displacement during this phase. The pattern of variance in these parameters is also affected by FMPs: larger species-level components of variance are observed for low-toughness foods than for high-toughness foods.

The largest component of variance for the duration of the chew cycle, and of the SO, FO, and FC phases is attributed to among-cycle variation (between 42% and 83%). In contrast, the largest component of variance for duration of the SC phase is at the species level, which is also affected by FMPs. For duration of the SC phase, the species component of variance is 47% for high-toughness foods and 75% for low-toughness foods. In contrast, among-cycle variation in duration of SC is greater for high-toughness foods (31%) than for low-toughness foods (19%). FMPs also affect the individual-level component of variance of total-cycle duration, with 18% for high-toughness foods and 39% for low-toughness foods.

The variation in vertical displacement of the mandible across the whole chew cycle mimics the variation observed for the duration of the total cycle and for the durations of individual phases. Among-cycle variation is the largest component of variance in vertical displacement during all phases, with the exception of the SC phase when feeding on low-toughness foods, where the variance component among species is the largest. In contrast to the

pattern observed for SC duration, the among-species variance is not as prominent for vertical displacement during the SC phase.

For the lateral displacement of the mandible, little variation can be attributed to species-level components of variance and most of the variation is due to among-cycle variation, followed by a moderate amount of variance explained by sequence and by individual factors.

Species effects

The results of ANOVAs for the effects of species, FMP, and chew number on temporal and spatial variables are summarized in Tables 2 and 3. As suggested by the patterns of distribution of variance components among hierarchical levels, we found that the duration of the SC phase in *Macaca* is 70% longer (as a percentage of the duration of the chew cycle) than in *Cebus* (37.0 ± 0.2 and $21.7 \pm 0.1\%$, respectively; Fig. 3B). This difference is also reflected in the timing of maximum gape and the FC–SC transition (Supplementary Fig. S4). For *Macaca*, the maximum gape and the FC–SC transition occur earlier in the gape cycle (42.8 ± 0.1 and $62.9 \pm 0.1\%$ of gape cycle, respectively) than for *Cebus* (56.2 ± 0.1 and $78.2 \pm 0.1\%$ of gape cycle, respectively). A similar pattern is observed when transitions between phases are analyzed in absolute time instead of as percentage of chew cycle (data not shown).

Similar to the temporal variables, we found significant differences among species in vertical displacement during the SC phase and the vertical position at the FC–SC transition (Table 3, Fig. 4 and Supplementary Fig. S5). *Macaca* shows significantly greater vertical displacements during the SC phase

Table 2 ANOVA table for temporal variables

Factor ^a	Total			SO phase			FO phase			FC phase			SC phase		
	df ^b	F	P ^c -value	df	F	P-value	df	F	P-value	df	F	P-value	df	F	P-value
Chew cycle duration															
S	3.02	1.03	0.384	3.01	0.71	0.460	3.00	2.39	0.220	2.97	3.93	0.142	2.97	46.99	0.007
ChN	3.09	26.55	0.013	2.89	4.78	0.120	3.03	1.83	0.269	2.99	1.03	0.386	3.00	6.03	0.091
S*ChN	3.09	12.85	0.035	2.89	0.01	0.936	3.03	1.91	0.260	2.99	1.01	0.389	3.00	2.14	0.240
FG	3.05	1.26	0.343	0.66	0.23	0.745	2.95	2.85	0.191	2.36	11.91	0.059	2.94	6.83	0.081
S*FG	3.05	5.17	0.106	0.66	22.63	0.220	2.95	1.34	0.332	2.36	7.40	0.094	2.94	0.48	0.538
ChN*FG	2.92	2.56	0.210	2.80	0.08	0.797	2.65	0.08	0.793	2.36	0.08	0.800	2.18	0.29	0.638
S*ChN*FG	2.92	2.85	0.192	2.80	0.31	0.621	2.65	0.31	0.624	2.36	23.52	0.029	2.18	32.73	0.024

^aFactors: S, species; ChN, chew number; FG, food group.

^bDegrees of freedom of the denominator of the F-test. The degrees of freedom of the numerator is 1 for all tests.

^cValues in bold indicate significance at $\alpha=0.05$.

Table 3 ANOVA table for spatial variables

Factor ^a	Chew cycle			SO			FO			FC			SC		
	df	F	P-value	df	F	P-value	df	F	P-value	df	F	P-value	df	F	P-value
Vertical displacement															
S	3.00	1.22	0.349	^b	0.06	.	3.02	1.68	0.286	2.99	0.11	0.761	3.00	19.19	0.022
ChN	3.02	34.30	0.010	.	0.01	.	2.97	57.03	0.005	2.95	0.09	0.787	3.10	22.61	0.016
S*ChN	3.02	0.24	0.657	.	0.02	.	2.97	0.71	0.462	2.95	0.69	0.468	3.10	0.10	0.768
FG	2.93	0.70	0.466	.	115.02	.	2.92	0.01	0.942	1.13	36.88	0.085	2.94	0.28	0.632
S*FG	2.93	0.06	0.823	.	121.99	.	2.92	0.21	0.676	1.13	15.65	0.135	2.94	0.27	0.638
ChN*FG	3.04	5.05	0.109	.	0.26	.	3.07	4.29	0.128	3.12	5.43	0.099	2.89	1.53	0.307
S*ChN*FG	3.04	0.52	0.521	.	1.28	.	3.07	0.02	0.898	3.12	0.06	0.823	2.89	1.51	0.310
Lateral displacement															
S	3.00	0.03	0.873	3.01	1.39	0.323	3.02	2.67	0.200	3.00	0.17	0.710	3.01	0.02	0.894
ChN	1.64	4.78	0.187	3.15	9.41	0.051	3.16	0.72	0.457	3.11	0.35	0.594	2.97	0.31	0.615
S*ChN	1.64	2.81	0.262	3.15	1.74	0.275	3.16	0.08	0.797	3.11	2.81	0.189	2.97	2.51	0.212
FG	3.20	43.88	0.006	3.09	0.17	0.707	3.03	0.01	0.914	2.88	10.34	0.052	3.30	22.67	0.014
S*FG	3.20	0.04	0.856	3.09	3.53	0.154	3.03	0.58	0.502	2.88	1.95	0.261	3.30	0.36	0.585
ChN*FG	2.87	2.64	0.207	3.04	0.00	0.965	3.13	0.65	0.478	3.03	0.12	0.752	0.54	8.69	0.350
S*ChN*FG	2.87	8.35	0.067	3.04	0.12	0.757	3.13	0.00	0.977	3.03	3.50	0.157	0.54	14.85	0.304

^aSee Table 2 for explanation.

^bDots indicate that the no satisfactory convergence was achieved for the test and that the degrees of freedoms could not be estimated.

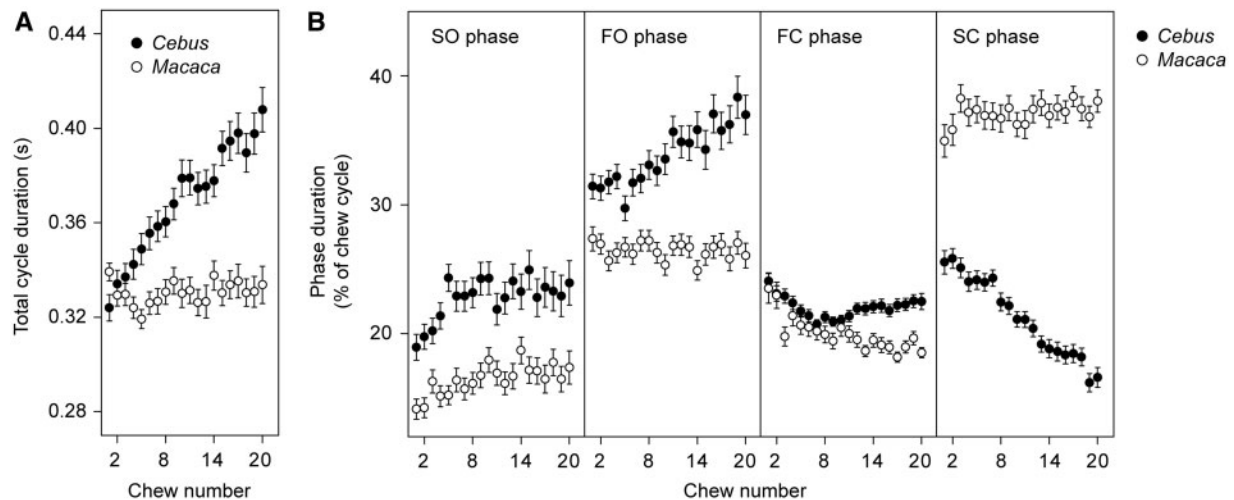


Fig. 3 Total duration of the chew cycle (**A**) and the duration of each phase of the chew cycle (**B**) during the first 20 chewing cycles for *Cebus* (closed circles) and *Macaca* (open circles). Data represent the mean \pm SEM for all individuals of each species feeding on both high- and low-toughness foods.

than does *Cebus* (5.16 ± 0.05 and 2.83 ± 0.03 mm, respectively). Vertical position at the FC–SC transition in *Macaca* is significantly lower than in *Cebus* (-5.18 ± 0.05 and -2.93 ± 0.03 mm, respectively).

Effect of FMPs

The only significant differences between high- and low-toughness foods were in the lateral displacement of the mandible (Table 3 and Fig. 5). Low-toughness

foods elicit larger lateral displacements than do high-toughness foods, both across the whole cycle (3.32 ± 0.02 and 2.70 ± 0.02 mm, for low- and high-toughness foods, respectively) and during the SC phase (1.78 ± 0.02 and 1.17 ± 0.02 mm, for low- and high-toughness foods, respectively). The absence of a FMP–species interaction term means that this applies equally to both species: low-toughness foods elicit more lateral displacement than do high-toughness foods.

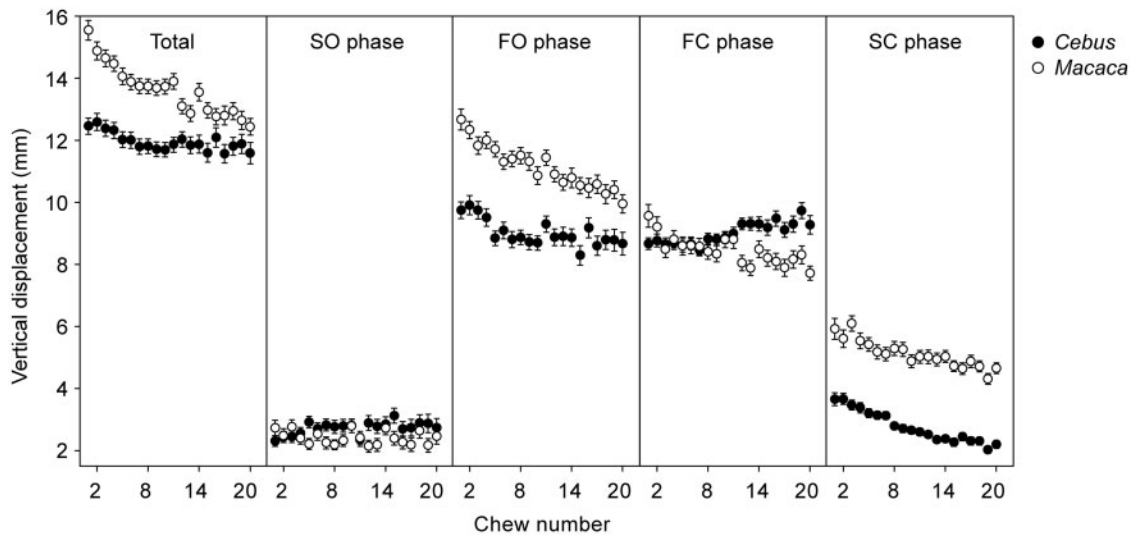


Fig. 4 Vertical displacement of the jaw during the complete chew cycle and during each phase of the chew cycle during the first 20 chewing cycles for *Cebus* (closed circles) and *Macaca* (open circles). Data represent the mean \pm SEM for all individuals of each species feeding on both high- and low-toughness foods.

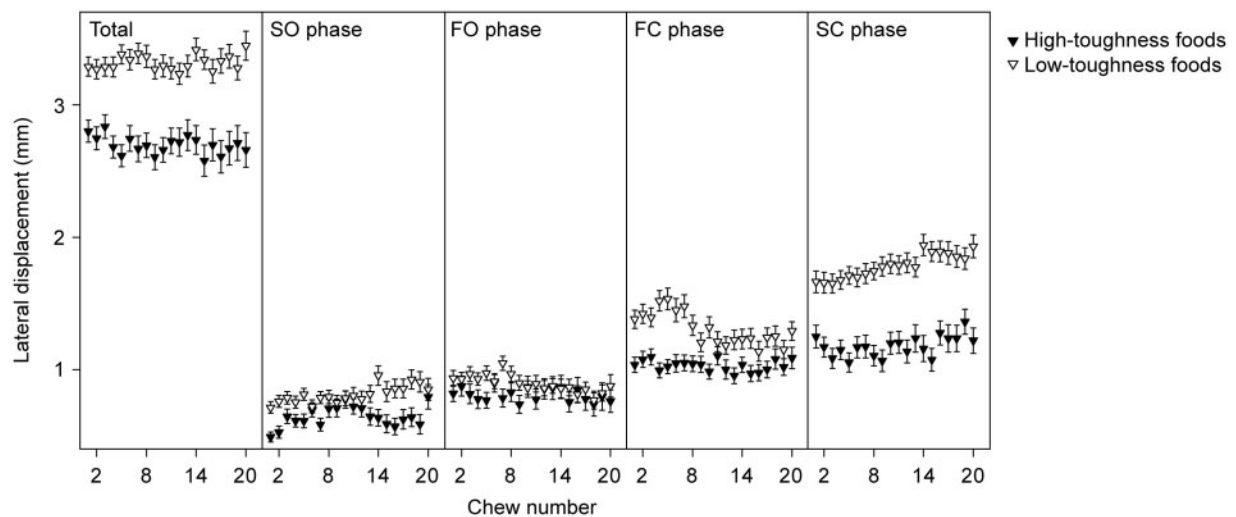


Fig. 5 Lateral displacement of the jaw during a complete cycle and during each phase of the chewing cycle during the first 20 chewing cycles when feeding on high-toughness (closed circles) and low-toughness foods (open circles). Data represent the mean \pm SEM for all individuals. Because no species effect was found, data from *Cebus* and *Macaca* were pooled.

Effect of chew number

We found significant linear relationships between chew number and the duration of the total cycle and the timing of maximum gape (Table 2). Total-cycle duration increases throughout a feeding sequence; however, the significant interaction between the species factor and the chew number suggests that the significance of chew number could be driven by the strong chew-number effect seen in *Cebus*. When the same analysis is performed separately for each species by food type, the chew-number effect only remains significant for *Cebus*

feeding on low-toughness foods ($F_{1,2.01} = 20.22$, $P = 0.045$); it is not significant for *Cebus* feeding on high-toughness foods ($F_{1,1.81} = 5.71$, $P = 0.153$) nor is it for *Macaca* feeding on either food group ($P > 0.05$ for both test).

Although we did not find a significant chew-number effect (or interaction) for the duration of the FO phase, its duration tends to increase in *Cebus*. When we performed an ANOVA for each species separated by food type we found that the duration of FO increases significantly with chew number in *Cebus* feeding on low-toughness foods

($F_{1,2.06} = 19.96$, $P = 0.044$) but not when feeding on high-toughness foods ($F_{1,1.72} = 8.25$, $P = 0.122$).

The duration of the SC phase shows a marginally significant effect of chew number; however, because there is a significant interaction effect between chew number, species and food type, we performed the analysis separately for each species and food type. We found a small decrease in duration of the SC phase through the feeding sequence only in *Cebus* feeding on high-toughness foods ($F_{1,2.22} = 28.69$, $P = 0.026$). No effect of chew number is found for either *Cebus* feeding on low-toughness foods, or for *Macaca* feeding on either type of food ($P > 0.18$ for all).

As chew number increases, total displacement and displacement during the FO and SC phases decrease, whereas no significant change is observed during the SO and FC phases (Table 3 and Fig. 4). Although we were unable to get reliable significance for the SO phase because the ANOVA could not converge on a solution, the pattern observed in Fig. 4 does not seem to change with chew number. The vertical position at transitions between phases decreases significantly for maximum gape and for the FC–SC transition but not for the SO–FO transition (Table 3 and Supplementary Fig. S5).

No systematic changes in lateral displacement of the mandible were observed with chew number (Table 3 and Fig. 5).

Discussion

The high-resolution, 3D kinematic data presented here were used to explore the sources of variation in jaw kinematics in two species of primates while they chewed foods of known material properties. We focused on the overall patterns of variance across hierarchical levels, from species to chew cycles, as well as for species-specific differences in jaw kinematics and the influence of FMPs. This article complements a previous study on the feeding kinematics of *Cebus* (Reed and Ross 2010) and increases the limited number of high-resolution kinematic studies in human and non-human mammals (Thexton et al. 1980; Hiiemae et al. 1995, 1996; Foster et al. 2006).

Components of variance

Variation in temporal and spatial chewing kinematics of the two species of primates studied was not evenly distributed across hierarchical levels. Overall, the largest component of variance in both temporal and spatial kinematic parameters was found between chewing cycles within chewing sequences. (SC phase

is an exception; see discussion below). This agrees with the findings of Vinyard et al. (2008) that most of the variance in the timing of peak EMG activity is found among cycles within a feeding sequence and suggests that variation in jaw kinematics within a feeding sequence dominates the variance in the primate jaw kinematics of primates. Vinyard et al. (2008) suggested that this variation could be attributed to changes in the physical properties of the bolus and/or to variation in bite location throughout the feeding sequence. Our data corroborate this hypothesis: inter-cycle, intra-sequence variation in jaw kinematics likely reflects variation in external physical attributes of the bolus, including adhesive and cohesive properties, size and shape, which vary through the chewing sequence (Supplementary Fig. S4). In contrast, *intrinsic material properties* do not vary within a sequence and can only impact components of variance between sequences on different pieces of food. The magnitude of variance explained at the sequence level (5–20%) is not as large as that attributed to among-cycle variation. Intermediate levels of variance were observed at the individual level (0.2–40% for temporal variables and 6–38% for spatial variables).

The largest species effect is seen in the duration of the SC phase, vertical displacement during SC, and the related timing and vertical position of the FC–SC transition. The duration of the SC phase is significantly longer and with a larger vertical displacement in *Macaca* compared to that of *Cebus*. Possible explanations for this effect are explored below.

Variation within the chewing sequence

Previous studies have shown that chewing kinematics varies throughout a feeding sequence due to changes in the physical properties of the food bolus as it gets processed (Plesh et al. 1986; Foster et al. 2006; Woda et al. 2006a, 2006b). Our data show, in agreement with Reed and Ross (2010), that total duration of cycles increases through the sequences of *Cebus* feeding on low-toughness foods but not on high-toughness foods. This increase was driven by an increase in the duration of the FO phase (Fig. 3), which was also reflected in the timing of maximum gape and the FC–SC transition later in the chew cycle as the feeding sequence progresses (Supplementary Fig. S4). In contrast, we found no change in duration of cycle in *Macaca*, regardless of food type.

Changes in cycle duration are mirrored in the vertical displacement of the mandible. In both species, total vertical displacement during a gape cycle decreases through the feeding sequence. This is a

result of decreases in vertical displacement during the FO and SC phases in both species and decreases in vertical displacement during FC in *Macaca*. Neither species showed effects of cycle number on vertical displacement during SO. Variation in vertical displacement through the feeding cycle was also reflected in position of the mandible at maximum gape and at the FC–SC transition. The vertical position of the mandible at the FC–SC transition decreases as chew number increases, and correspondingly the vertical displacement during the SC phase. These changes plausibly are related to the processing and progressive decrease in size of the food bolus.

Effect of FMPs on the chewing kinematics

As noted previously, we found an increase in duration of cycle through the feeding sequence but only in *Cebus* feeding on low-toughness foods, suggesting an interaction between FMPs and chew number, as reported previously (Reed and Ross 2010). Reed and Ross hypothesized that longer durations of cycle are needed when chewing on low-toughness foods because such foods fragment faster, requiring more complex tongue movements to manipulate the larger number of fragments. Accordingly, this would require longer phases in which the tongue interacts with the food bolus, namely the SO and FO phases. We found an increase in duration of FO with chew number in *Cebus* feeding on low-toughness foods but not in duration of the SO phase. However, duration of SO tends to increase in the first ten chews, after which the duration remains constant. This suggests that duration of SO does not increase linearly throughout the 20 chew cycles analyzed and it could explain why no significant effect of chew number was found by modeling chew number as a covariate in the ANOVA. Alternate nonlinear models may be required to study this effect.

High-toughness foods have been argued to require larger lateral mandibular displacements, in particular during the SC phase (Hylander 1988; Agrawal et al. 1997, 2000; Agrawal and Lucas 2002), especially in animals with flat occlusal teeth morphology, such as found in *Cebus* and humans. However, we found that both *Macaca* and *Cebus* showed larger lateral displacements when eating low-toughness foods than they did when eating high-toughness foods (Fig. 5), in agreement with previous observations on *Cebus* (Reed and Ross 2010) but in direct contradiction to findings on humans. Differences in occlusal morphology cannot explain the differences between these results, because *Cebus* and humans share relatively flat occlusal surfaces, in contrast to

the more high-crowned occlusal surface of macaques. An alternative explanation is that the chewing mechanics of humans are substantially different from those of other primates, a view partially supported by EMG data (from chewing muscles) that show more variable patterns of activity in humans compared to nonhuman primates (Vinyard et al. 2008). Also, properties of the food other than stiffness and toughness could potentially explain the observed differences. Foster et al. (2006) reported that, in humans, plastic foods elicited larger lateral displacements of the jaw than elastic foods; it is possible that differences in these rheological properties explain our results, but these properties were not measured in our study.

Species' differences in kinematics

Based on the similarities in musculoskeletal design between the study species, we expected little inter-specific variance in overall jaw kinematics. Surprisingly, consistent kinematic differences between *Cebus* and *Macaca* were found in the SC phase of the gape cycle in both temporal and spatial variables. Macaques presented proportionally longer SC phases with larger vertical excursions compared to capuchins, regardless of FMPs (Figs 3 and 4). As previously mentioned, the SC phase is of particular interest because its kinematics are driven by the interaction between the food bolus and the teeth, so differences in this phase might indicate important differences between the species in the mechanics of food processing. Although jaw length of *Macaca* (84.87 mm; Ross et al. 2009) is almost 50% longer than that of *Cebus* (57.53 mm; Ross et al. 2009), meaning that the marker on which these data are based was positioned further from the jaw joint, it is unlikely that differences in size can completely explain the differences in the SC phase alone, because we would expect to find larger vertical displacements across all phases. Moreover, the fact that the duration of SC is also longer in macaques than in capuchins suggests real differences in chewing kinematics between species. Because subjects were allowed to feed themselves, differences in bite size might explain the longer durations of SC and the larger vertical excursions in macaques. However, if this were the case, we would expect that the vertical position at the FC–SC transition or the vertical displacement during SC of both species to converge as the feeding sequence progresses and food particles are gradually reduced. Both vertical displacement and position reached a “plateau” after chew 20 but no convergence was observed between macaques and

capuchins. Another possibility is that differences in biting position along the tooth row among species could explain the observed pattern. By positioning the food bolus more posteriorly on the tooth row, larger gape angles, a lower vertical position of the mandible at the FC–SC transition, and longer duration of SC would be expected. Unfortunately, no information is available at this time about species-specific differences in biting position along the tooth row. Finally, the more high-crowned teeth of macaques compared to those of capuchins, might also explain the differences in vertical displacement between these species. In comparison to the relatively flat occlusal surface of capuchins, the high-crowned molar of macaques might be expected to contact the food earlier in the chewing cycle, resulting in a larger vertical displacement during the SC phase.

The inter-specific differences reported here can be summarized by comparing the average vertical position of the jaw at the phase transitions of chew 1 and chew 20, by species and food type (Fig. 6). The most striking difference is the lengthening of the duration of the gape cycle through the feeding sequence in capuchins feeding on low-toughness foods (Fig. 6C). This lengthening is the result of an increase in the duration of the SO and FO phases. The slope for each of the phases measures average vertical velocity of the jaw, and the shallow slope for *Cebus* in chew 20 compared to chew 1 suggests a decrease in velocity at which the jaw opens through the sequence. Decrease in jaw-opening velocity is also observed in capuchins feeding on high-toughness foods but without an increase in total duration of the cycle (Fig. 6A). No clear change in vertical velocity of the jaw is observed in the closing phases. Reed and Ross (2010) attributed the increase in the durations of the opening phases in capuchins to the more complex tongue movements necessary for handling the larger number of food fragments produced by low-toughness foods. Why then is the same pattern not observed in macaques?

We propose at least three, not mutually exclusive, explanations. First, the relatively flat occlusal surface of capuchins' teeth might be less effective than the more high-crowned teeth of macaques in concentrating food fragments in the oral cavity. Second, cheek pouches in macaques may be used to collect and store fragments so that the food item need not be processed completely at once. Third, there could be inherent differences between the species in the ability to modulate the chew cycle and facilitate food handling. Figure 6 suggests smaller individual variability and a more stereotypic gape cycle in macaques compared to capuchins. Increased variance in the

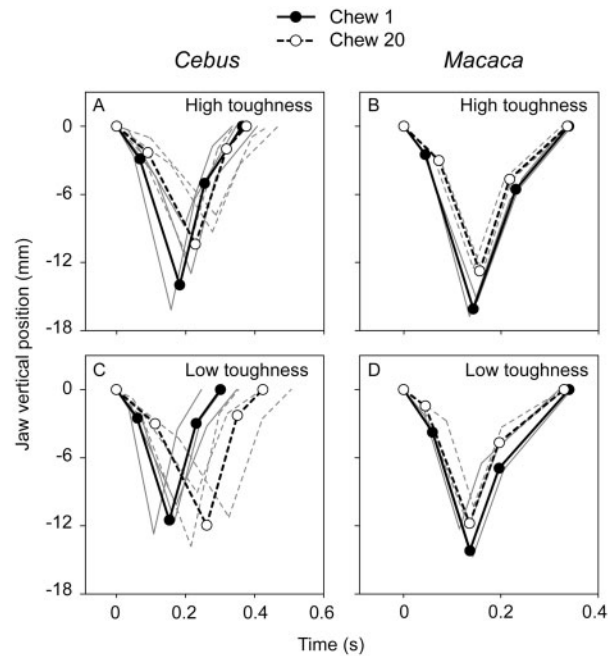


Fig. 6 Comparison of profiles of the average vertical position of the jaw in the gape cycle between the first chew and chew 20, for *Cebus* and *Macaca* feeding on high- and low-toughness foods. Symbols represent the average position of all individuals at transitions between phases (minimum gape, SO–FO transition, maximum gape, FC–SC transition, and minimum gape, respectively). Closed symbols and solid lines represent the vertical position during chew 1 and open symbols and dashed lines represent the jaw vertical position during chew 20. Gray lines represent the profile of vertical position of the jaw for each individual of each species to show the amount of inter-individual variability.

kinematics of the gape cycle in capuchins is associated with an increase in the efficiency with which food is processed, as measured by a lower number of chewing cycles per sequence. Our data show that macaques use more chewing cycles than do capuchins when feeding on low-toughness foods (inter-quartile range: 30–57 versus 16–33 chew cycles for macaques and capuchins, respectively). This suggests that capuchins may use more variable, but fewer, chewing cycles than do macaques when feeding on low-toughness foods that quickly fragment and consequently require more complex movements of the tongue to manipulate the food bolus. The relative importance of inter-specific differences in tongue morphology, tongue-jaw coordination, and composition of saliva (for example) in explaining these differences remains to be explored.

Concluding remarks

The advent of high-speed, high-resolution 3D-kinematic systems has allowed detailed investigation of

variability in chewing kinematics in species other than humans. The ability to collect and share large datasets of complete and near-complete feeding sequences through the FEED database will significantly improve our understanding of the mechanics and evolution of feeding systems in mammals. In this study we show that, like the relative timing of jaw-elevator EMG activity (Vinyard et al. 2008), most of the variability in temporal and spatial kinematics occurs *among* chewing cycles *within* sequences. Part of this among-cycle variation is most likely the result of changes in the external properties of the food bolus (i.e., size, geometry, and adhesive properties of the bolus' surface) through the feeding sequence. Significant differences between *Cebus* and *Macaca* were observed, in particular during the SC phase of the gape cycle, when the teeth contact the food and the power stroke occurs. One explanation for such differences might be differences in occlusal morphology between the species, which in turn might affect the mechanics and dynamics of food processing. Interestingly, our data also suggest that macaques have a more stereotypic gape cycle than do capuchins, and are less variable in response to variation in intrinsic FMPs. Whether these differences are related to features of the musculoskeletal periphery or represent higher-order differences in motor control remains to be evaluated. Answers to these kinds of questions depend on collection of kinematic data, as well as EMG data, from other species. A centralized and efficient repository of data on different aspects of feeding in mammals would help to generate and test hypotheses about the mechanics and evolution of feeding in mammals.

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Supplementary Data

Supplementary Data available at *ICB* online.

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