



## COMPLIMENTARY/POSTER SESSION PAPER

# Muscle Fatigue in the Latch-Mediated Spring Actuated Mandibles of Trap-Jaw Ants

Fredrick J. Larabee <sup>\*,†,¶</sup>, Josh C. Gibson<sup>†,§</sup>, Michael D. Rivera<sup>‡</sup>, Philip S. L. Anderson<sup>\*,§</sup> and Andrew V. Suarez<sup>\*,†,‡,§</sup>

\*Department of Evolution, Ecology and Behavior, University of Illinois Urbana Champaign, 515 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801, USA; †Department of Entomology, University of Illinois Urbana Champaign, 515 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801, USA; ‡Program in Ecology, Evolution and Conservation Biology, University of Illinois Urbana Champaign, 515 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801, USA; §Beckman Institute for Advanced Science and Technology, University of Illinois Urbana Champaign, 515 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801, USA; ¶Department of Entomology, National Museum of Natural History, Smithsonian Institution, 1000 Constitution Ave., Washington, DC 20560, USA

From the symposium “Best practices for bioinspired design education, research and product development” presented at the annual meeting of the Society for Integrative and Comparative Biology virtual annual meeting, January 3–February 28, 2022.

<sup>1</sup>Email: [larabeef@si.edu](mailto:larabeef@si.edu)

**Synopsis** Muscle fatigue can reduce performance potentially affecting an organism's fitness. However, some aspects of fatigue could be overcome by employing a latch-mediated spring actuated (LaMSA) system where muscle activity is decoupled from movement. We estimated the effects of muscle fatigue on different aspects of mandible performance in six species of ants, two whose mandibles are directly actuated by muscles and four that have LaMSA “trap-jaw” mandibles. We found evidence that the LaMSA system of trap-jaw ants may prevent some aspects of performance from declining with repeated use, including duration, acceleration, and peak velocity. However, inter-strike interval increased with repeated strikes suggesting that muscle fatigue still comes into play during the spring loading phase. In contrast, one species with directly actuated mandibles showed a decline in bite force over time. These results have implications for design principles aimed at minimizing the effects of fatigue on performance in spring and motor actuated systems.

## Introduction

Muscles that experience prolonged or repeated contractions eventually experience physiological fatigue, with reduced capacity to exert force and do work. Muscle fatigue can decrease whole-animal performance, which can have important effects on their ecology. Many studies have examined the cascading neuromuscular and metabolic mechanisms that cause muscle fatigue (Cairns et al. 2005; Allen et al. 2008; Enoka and Duchateau 2008) and its consequences on muscle power output and performance. Most studies focus on movements directly actuated by muscle contraction, where prolonged or repeated use of a muscle correlates with a decrease in movement performance (James et al. 1995; Maluf et al. 2005; Williams et al. 2013). In some organ-

isms, however, muscles instead load a biological spring (tendon or ligament) held in place by a latch to be released at a later time. These latch-mediated spring actuated (LaMSA) systems (Longo et al. 2019) are prevalent in biology and represent an interesting comparison with directly actuated systems. In the present study, we sought to understand the effect of muscle fatigue on the performance of a LaMSA system.

In animals that employ LaMSA mechanisms, muscle activity is temporally decoupled from movement and can be separated into phases (Gronenberg 1996a; Ilton et al. 2018; Longo et al. 2019). Starting with a latching phase, the accelerating mass (projectile, appendage, or whole body) is held in place by a latch. This enables the loading phase, where contraction of a muscle stores po-

Advance Access publication June 11, 2022

© The Author(s) 2022. Published by Oxford University Press on behalf of the Society for Integrative and Comparative Biology. All rights reserved. For permissions, please e-mail: [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

**Table 1** Collection and voucher deposition information for ants used in this study

Species	Mandible mode	Colonies	Collection location	Voucher location	Voucher number
<i>A. paripungens</i>	Spring Actuated	1	Darwin, Australia	NMNH	USNMENT01124355
<i>C. chromaiodes</i>	Directly Actuated	2	Urbana, Illinois, USA	UIUC	USNMENT01128951
<i>D. armigerum</i>	Spring Actuated	2	Nouragures, French Guiana Cayenne, French Guiana	UIUC	USNMENT01128952
<i>O. brunneus</i>	Spring Actuated	3	Venus, Florida, USA	NMNH	USNMENT01128953
<i>O. relictus</i>	Spring Actuated	2	Venus, Florida, USA	NMNH	USNMENT01128954
<i>P. californicus</i>	Directly Actuated	1	Hurricane, Utah, USA (AntsAlive)	UIUC	USNMENT01124405

tential energy in a spring. During spring actuation, the latch releases the accelerating mass (often controlled by a fast trigger mechanism) and transfers potential energy from the spring into kinetic energy of the accelerating body (Divi et al. 2020). The accelerating body transitions to ballistic motion after the spring stops exerting a force on it. Although muscle activity is limited to the beginning of this process, muscle fatigue could theoretically impact the performance of a LaMSA movement by reducing the amount of energy that is stored in the spring during the loading phase.

LaMSA mechanisms can enable greater velocity, acceleration, and power density than would be possible from direct muscle actuation. For example, salamander tongues that use LaMSA mechanisms reach velocities five times greater and power densities over 100 times greater than tongues directly actuated by muscle (Deban et al. 2020). Similarly, Sutton et al. (2019) showed that below a certain body size, insects can jump significantly higher and with greater energy density using LaMSA mechanisms. In ants, the focus of the current study, the mandibles of species that have evolved LaMSA have velocities, accelerations, and power densities orders of magnitude greater than closely related species whose mandibles are directly actuated by muscle (Booher et al. 2021). Despite the great deal of attention given to the morphology, energetics, and evolution of LaMSA movements, relatively little is known about if or how muscle fatigue plays a role in these systems.

Although most ants possess directly actuated mandibles, at least four independent clades, including more than 600 species, have convergently evolved “trap-jaws”—fast-closing mandibles powered by LaMSA mechanisms (Larabee and Suarez 2014). Each lineage uses different morphological structures to act as a latch, spring, and trigger, but they all power rapid mandible strikes that are used for predation of fast or dangerous prey (Moffett 1986; Gronenberg 1996b; De la Mora et al. 2008). In at least one genus, *Odontomachus*, mandibles are also used to power anti-predator escape jumps (Patek et al. 2006; Spagna et al.

2009; Larabee and Suarez 2015). As all trap-jaw ants use a large adductor muscle to load the spring, and sustain these contractions for many seconds (Gronenberg 1995a; Larabee et al. 2017), trap-jaw ants are a good case study for examining the influence of muscle fatigue on LaMSA systems.

Mandibles are critical multi-purpose tools for ants, and in arthropods generally. Therefore, factors that reduce ant performance, such as muscle fatigue, are expected to be important for shaping their evolution. We tested if performance of the LaMSA mandibles of trap-jaw ants showed signs of being influenced by muscle fatigue. Because muscles must maintain their contraction during the loading phase of a strike, we tested the hypothesis that muscle performance occurring between strikes (reflecting the time it takes to load a spring or muscle recovery) would decrease with repeated use. To accomplish this, we recorded multiple consecutive mandible strikes with high-speed videography and derived their kinematic performance for four species of trap-jaw ants. For comparison, we examined the role of muscle fatigue in two species of ants whose mandibles are actuated directly by muscles by measuring any decrease in performance during bite force experiments. Our hypothesis for directly actuated mandibles was that repeated or prolonged bites would result in decreased bite performance.

## Materials and methods

### Study organisms

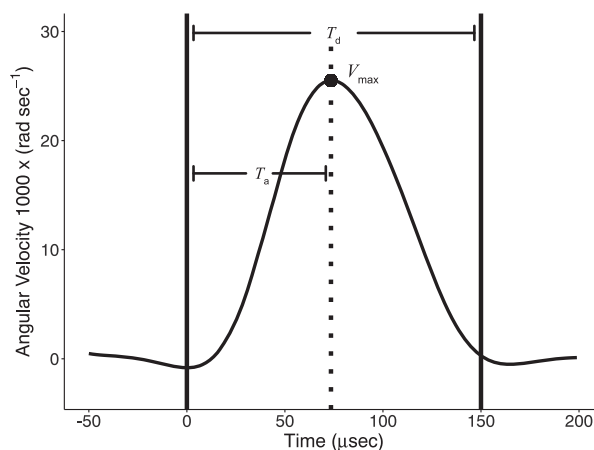
Colonies of four species of trap-jaw ants were collected live for study: *Anochetus paripungens*, *Daceton armigerum*, *Odontomachus brunneus*, and *O. relictus* (Table 1). To study directly actuated mandibles, colonies of the carpenter ant, *Camponotus chromaiodes*, were collected live and individuals of the harvester ant, *Pogonomyrmex californicus*, were purchased from a commercial vendor (AntsAlive, Hurricane, UT, USA). Laboratory nests were constructed out of plaster-filled petri dishes placed inside plastic boxes. Ants were pro-

vided with water and sugar water *ab libitum* and fed crickets, flies, or termites one to three times a week. Nests were kept at 25°C, 50% relative humidity, and a 12-h light-dark cycle.

### Trap-jaw ant performance

Trap-jaw ant mandible performance fatigue was measured using high-speed videography of repeated mandible strikes using methods similar to previous studies (Larabee et al. 2017; Gibson et al. 2018). Briefly, ants were restrained by the posterior dorsum of the head to an insect pin with utility wax (Kerr Laboratory Products, Orange, CA, USA). This pin was attached to a thin rod fitted onto a micromanipulator and ants were positioned with their mandibles perpendicular to the optical axis of a Zeiss SteREO Discovery V20 Microscope (Carl Zeiss Inc., Oberkochen, Germany). Strikes were backlit with an LED light and recorded with a Phantom V9.1 high-speed camera (Vision Research Co. Wayne, NJ, USA) at framerates of 50,000–100,000 frames per second. To account for adaptation of the mechanoreceptors that trigger the strike reflex, strikes were elicited under two different experimental protocols: (1) for *Anochetus* and *Odontomachus*, five strikes within 15 s were provoked by gentle blowing from the experimenter, (2) for *Daceton*, as many strikes as possible were elicited over the course of 2 min with air from a nasal bulb at a rate of one puff per second. Following recordings, mandible length was measured with a Leica DFC 425 digital microscope camera connected to a Leica M205 C stereo microscope (467 nm resolution) (Leica Microsystems, Wetzlar, Germany). Strikes were recorded from three workers of *A. paripungens*, 40 workers of *D. armigerum*, six workers of *O. brunneus*, and five workers of *O. relictus*. When the same individual was recorded twice, at least 5 min separated the recorded strike sequence.

Strike kinematics were estimated from digitized mandible strikes using the same method as Larabee et al. (2018). Using the  $x$ - $y$  coordinates of the mandible tip and the mandible length, the angular displacement of mandible during the strike was calculated trigonometrically. Raw displacement data were smoothed in R (ver. 4.1.2) using the *pspline* package (ver. 1.0–18), and this smoothed data was used to estimate strike kinematic traits (Fig. 1). Five traits were estimated for each strike: (1) *Strike duration*—the time from mandible rest to when they stop making forward progress. (2) *Peak angular velocity* and (3) *peak angular acceleration* were calculated from the first and second derivatives of the displacement data, respectively. (4) *Mandible acceleration duration* is an estimate of the how long the spring applies a force to the mandible and was calculated from



**Fig. 1** Stereotypical trap-jaw ant performance. Angular velocity as a function of time. Velocity was derived from the  $x$ - $y$  coordinates of mandible tips from high-speed videography. Performance traits: strike duration ( $T_d$ ), peak angular velocity ( $V_{max}$ ), peak angular acceleration (not shown—calculated from second derivative of change in displacement), acceleration duration ( $T_a$ ), and strike interval (not shown) were derived from these data.

the time of rest to peak velocity. (5) *Strike interval* was defined as the time elapsed between start times between strikes. Traits for the left and right mandible were averaged together for each strike, and estimated only for the first five recorded strikes.

Performance fatigue was assessed for the four species of trap-jaw ant by measuring how performance traits changed over the course of five repeated strikes. To account for differences in body size (correlated with peak performance of LaMSA systems [Spagna et al. 2008]), % change in performance since the first strike was compared for these analyses. Data were analyzed separately for each species with the exception of *O. brunneus* and *O. relictus*, where these species were pooled to simplify interpretation of the results. This is justified because these taxa are closely related sister species (Larabee et al. 2016; Matos-Maraví et al. 2018; Fernandes et al. 2021), use the same trap-jaw mechanism, and are very similar in overall body size. Also, there was no difference in strike performance between the two species (two-sided  $t$ -test, mean strike duration:  $t = -0.368$ ,  $P = 0.72$ ; mean peak velocity:  $t = -1.73$ ,  $P = 0.112$ ; mean acceleration duration:  $t = -0.589$ ,  $P = 0.566$ ). Differences in strike performance were tested with a one-way repeated measures ANOVA using the *Rstatix* package (ver 0.7.0) in R. Samples that violated the assumption of sphericity had a Greenhouse-Geisser correction applied. Post-hoc pairwise  $t$ -tests with a Bonferroni correction for multiple comparisons were used to test for differences between strike 1 and strikes 2–5. In the case of strike interval, comparisons were made between interval 1 and intervals 2–4. Raw data and scripts of all analyses, in-

**Table 2** Performance summary of trap-jaw (LaMSA) ant mandibles

Species	N	Min. duration ( $\mu$ s)	Peak angular velocity (rad/s)	Peak angular acceleration (rad/s <sup>2</sup> )	Min. acceleration duration ( $\mu$ s)	Strike interval (s)
<i>A. paripungens</i> <sup>a</sup>	4	80.2 $\pm$ 11.6	43.4 $\times$ 10 <sup>3</sup> $\pm$ 2.3 $\times$ 10 <sup>3</sup>	1.33 $\times$ 10 <sup>9</sup> $\pm$ 0.151 $\times$ 10 <sup>9</sup>	51.4 $\pm$ 10.6	1.10 $\pm$ 0.22
<i>D. armigerum</i>	20	78.9 $\pm$ 23.3	24.4 $\times$ 10 <sup>3</sup> $\pm$ 8.5 $\times$ 10 <sup>3</sup>	1.09 $\times$ 10 <sup>9</sup> $\pm$ 0.994 $\times$ 10 <sup>9</sup>	64.3 $\pm$ 13.2	5.98 $\pm$ 3.34
<i>O. brunneus</i>	11	108 $\pm$ 19.5	43.5 $\times$ 10 <sup>3</sup> $\pm$ 3.3 $\times$ 10 <sup>3</sup>	1.24 $\times$ 10 <sup>9</sup> $\pm$ 0.152 $\times$ 10 <sup>9</sup>	64.6 $\pm$ 12.1	1.78 $\pm$ 0.411
<i>O. relictus</i>	7	112 $\pm$ 21.8	46.7 $\times$ 10 <sup>3</sup> $\pm$ 4.1 $\times$ 10 <sup>3</sup>	1.34 $\times$ 10 <sup>9</sup> $\pm$ 0.163 $\times$ 10 <sup>9</sup>	68.0 $\pm$ 12.3	1.76 $\pm$ 0.988

<sup>a</sup>Data originally published in Gibson et. al (2018). All values are means  $\pm$  standard deviation. Species means for strike duration, and acceleration duration are calculated from individuals minimums. Species means for peak velocity and acceleration. Species means for strike interval are based on individual means.

cluding filming conditions and kinematics calculations are available in supplementary material.

### Directly actuated mandible performance

Repeated biting performance of non-LaMSA ant mandibles was studied with bite force experiments. A total of 20 workers of *Camponotus* and 21 workers of *Pogonomyrmex* were used in bite force experiments. A Flexiforce ELF-system piezoelectric force sensor (Tekscan, South Boston, MA, USA) was trimmed, covered in parafilm, and calibrated according to the manufacturer's instructions. The sensor was sampled at 150 Hz and a gain setting ("sensitivity setting") of 18. Ant workers were restrained by the body using soft forceps and allowed to voluntarily bite the sensor. Recording was triggered when the force sensor surpassed a 0.1 N threshold and lasted for 120 or 180 s. The resulting bite force traces were cleaned with a 10 Hz low-pass Butterfield filter using the R package signal (ver. 0.7 -7).

Two methods were used to detect performance fatigue from bite force experiments. First, bite traces were divided into discrete bites by identifying local maxima using a threshold criterium. Bites were defined to start and stop whenever bite force increased above or decreased below, respectively, 10% of the experiment maximal bite force. Three performance measures were estimated from these filtered bite data: (1) *Bite duration* was calculated as the difference between bite start and stop times. (2) *Maximum bite force* was the local maximal force of each bite. (3) *Bite interval* was calculated from the time between the start of each bite. Differences in % change in bite performance for the first five bites were tested with a one-way repeated measures ANOVA similar to the analysis of trap jaw performance. The second method involved identifying individual bites from ants that were at least 30 s long. Trends in force during long bites were then tested with linear regression and a *t*-test was used to test if the mean slope was significantly different from zero.

## Results

### Trap-jaw ant performance

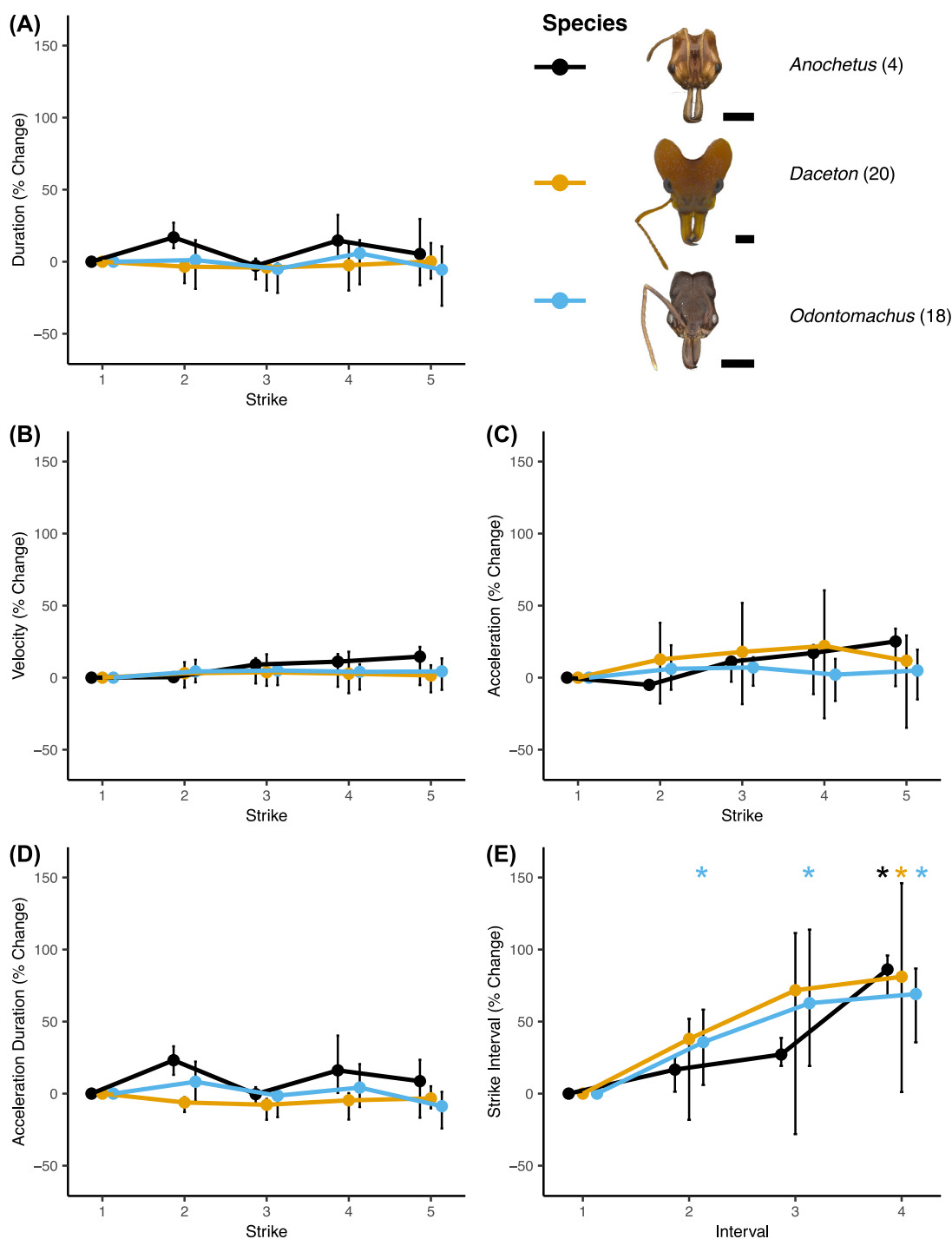
At least five strikes were elicited within the experimental trial period for 4 sequences of *Anochetus*, 20 sequences of *Daceton*, and 18 sequences of *Odontomachus*. After five successive strikes, it often became difficult to coerce ants to strike, but some individuals continued to display a willingness to use their jaws. One individual of *Daceton*, for example, released 13 strikes within 20 s. Summaries of trap-jaw strike performance are provided in Table 2.

Most trap jaw performance traits did not vary consistently across strikes (Fig. 2, Supplementary Table S1). Strike duration, peak angular velocity, peak angular acceleration, and acceleration time all displayed variation within species and individuals (coefficient of variation ranged from 9 to 85%), but the % change in performance did not vary consistently across repeated strikes (all one-way repeated measures ANOVA tests had  $P > 0.23$ , see Supplementary Table 1 for full results). The one performance trait that did show a change in all species was strike interval (one-way repeated measures ANOVA: *Anochetus*  $F_{(3,9)} = 19.2$ ,  $P < 0.001$ ; *Daceton*  $F_{(3,57)} = 3.72$ ,  $P = 0.016$ ; *Odontomachus*  $F_{(3,51)} = 10.4$ ,  $P < 0.001$ ). In *Odontomachus*, strike interval changed most quickly, increasing by  $35.7 \pm 48.8\%$  during interval 2 (post-hoc paired *t*-test:  $t = -3.10$ , adjusted  $P = 0.006$ ). By interval 4 (between strikes 4 and 5), the strike interval increased for all species, ranging from mean % change of  $69.1 \pm 73.5\%$  in *Odontomachus* to  $86.2 \pm 32.9\%$  in *Anochetus* (post-hoc paired *t*-test for all species had adjusted  $P < 0.02$ ).

### Directly actuated mandible performance

General bite behavior differed between the two non-LaMSA species (Fig. 3, Table 3), with *Camponotus* having more short bites (bite number:  $7.1 \pm 4.7$  bites, bite duration:  $6.3 \pm 4.2$  s) and *Pogonomyrmex* fewer, longer bites (bite number:  $3.6 \pm 2.4$  bites; bite du-

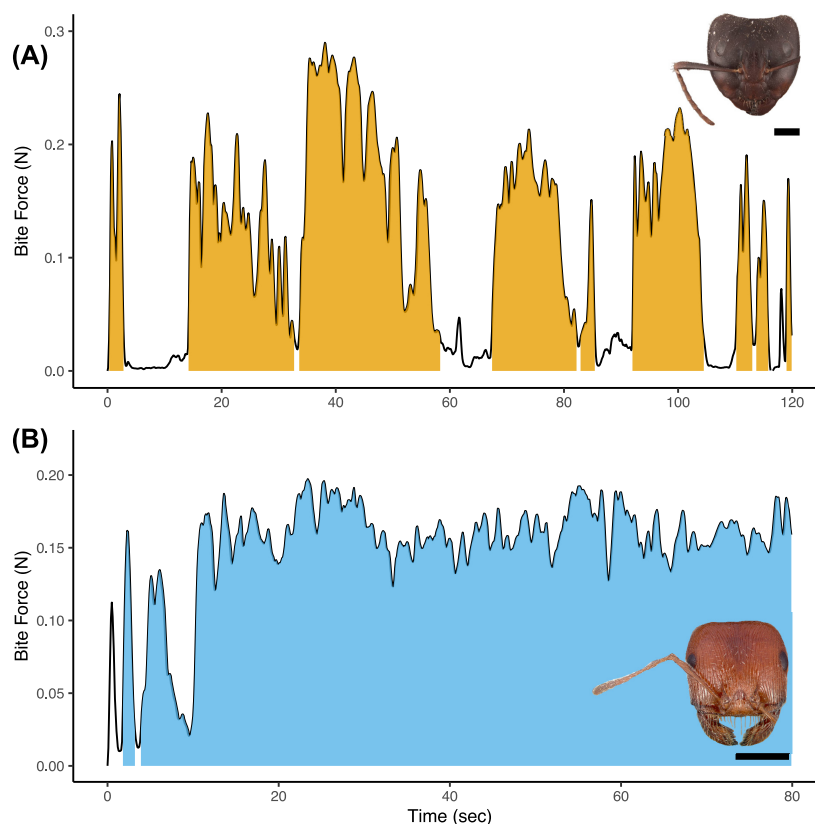




**Fig. 2** Most performance traits of trap-jaw strikes do not change over repeated strikes. Percent change in performance since strike 1 is shown for each performance trait **(A)** strike duration, **(B)** peak angular velocity, **(C)** peak angular acceleration, **(D)** acceleration duration, and **(E)** strike interval. All values are means  $\pm$  interquartile range. Strikes that have a significant change in performance since the first strike are labeled with asterisks. Species denoted by line color: *Anochetus* (black), *Daceton* (yellow), and *Odontomachus* (blue). Images for *A. paripungens* (CASENT0217510) and *O. brunneus* (CASENT0270601) were downloaded and edited from AntWeb. Version 8.73. California Academy of Science, online at <http://antweb.org>. Accessed March 14, 2022. Image of *D. armigerum* (JCG2017065) is from J.C. Gibson. Scale bars = 1 mm.

ration:  $30 \pm 27$  s). Sixteen *Camponotus* workers and seven workers of *Pogonomyrmex* had at least five bites for a repeated measures test. Bite force decreased significantly over the course of five bites only in *Cam-*

*ponotus* (one-way repeated measures ANOVA: *Camponotus*  $F_{(2.75, 41.3)} = 3.56$ ,  $P < 0.025$ ; *Pogonomyrmex*  $F_{(1.8, 10.8)} = 2.5$ ,  $P = 0.131$ ). By bites four and five, peak bite force decreased by 29.5 and 25.9%, re-



**Fig. 3** Stereotypical bite force plot from a directly actuated mandible. Bite force (N) plotted over time (s) after lowpass Butterfield filter was applied to data. Bites were defined (shaded regions) after force increased over 10% of maximal bite force and lasted longer than 0.1 s. Data shown for **(A)** *Camponotus* and **(B)** *Pogonomyrmex*. Images for *C. chromaiodes* (FMNHINS0000045774) and *P. californicus* (CASENT0005710) were downloaded and edited from AntWeb. Version 8.73. California Academy of Science, online at <http://antweb.org>. Accessed March 14, 2022. Scale bars = 1 mm.

**Table 3** Performance summary of directly actuated (non-LaMSA) ant mandibles

Species	N	Number of bites	Peak bite force (N)	Bite with peak force	Mean bite duration (s)	Mean bite interval (s)
<i>C. chromaiodes</i>	20	7.13 ± 4.73	0.335 ± 0.298	2.67 ± 2.63	6.32 ± 4.20	9.34 ± 3.52
<i>P. californicus</i>	18	3.61 ± 2.45	0.233 ± 0.061	2.8 ± 2.59	30.0 ± 27.2	13.6 ± 12.2

All values are means ± standard deviation. Species means for bite duration and interval are calculated from individual means.

spectively (Fig. 4). Bite duration and interval did not change over repeated bites for either species (Table 4).

A total of 4 individuals of *Camponotus* and 13 individuals of *Pogonomyrmex* had individual bites longer than 30 s that were used to analyze the change in bite performance in a single bite (Supplementary Figure 1). The three bites that started with the highest force, all declined by over 50% over the course of their duration. However on average, the slope of force during these long bites was not significantly different from zero (one sample *t*-test:  $t = -1.3711$ ,  $df = 16$ ,  $P = 0.189$ ). A total of 11 bites out of 17 bites had a negative slope, with force decreasing over time, but this was not significantly dif-

ferent from a random distribution (Chi-square analysis of independence:  $X^2 = 2.25$ ,  $df = 1$ ,  $P = 0.134$ ).

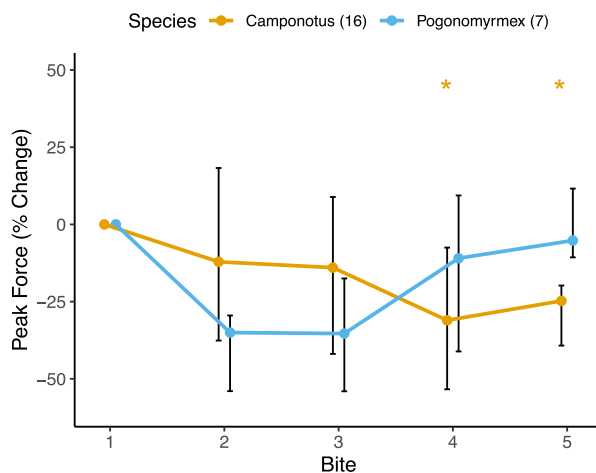
## Discussion

We sought to determine how muscle fatigue affects LaMSA systems by measuring the change in performance of repeated mandible strikes in trap-jaw ants. We found evidence for fatigue in only one performance trait, strike interval, which increased over repeated mandible strikes by 69–86%. Because strike interval is when the adductor muscle is loading the spring and recovering from any fatigue, these results are consistent with our hypothesis that muscle fatigue would most af-

**Table 4** One-way repeated measures ANOVA of % change in bite performance

Species	N	Trait	df	F	P	Bite 2	Bite 3	Bite 4	Bite 5
<i>Camponotus</i>	18	Duration*	2.11, 31.6	2.14	0.132	-	-	-	-
		Peak bite force*	2.75, 41.3	3.56	0.025	0.904	0.976	<b>0.003</b>	<b>0.006</b>
		Bite interval	2, 30	1.48	0.244	-	-	-	-
<i>Pogonomyrmex</i>	20	Duration*	1.78, 10.7	1.30	0.307	-	-	-	-
		Peak bite force*	1.8, 10.8	2.50	0.131	-	-	-	-
		Bite interval*	1.06, 6.36	0.808	0.409	-	-	-	-

Samples that violated the assumption of sphericity had a Greenhouse-Geisser correction applied to reduce the degrees of freedom (asterisks). For peak bite force, the only trait with significant differences between bite, post-hoc pairwise t-tests between bite 1 and the four following bites were performed. *P*-values had a Bonferroni correction applied for multiple comparisons, and tests with a *P*-value < 0.05 are in **bold**.



**Fig. 4** Directly actuated mandibles display some decrease in performance over repeated use. Percent change in bite force over repeated bites. All values are means  $\pm$  interquartile range. Bites that have a significant change in performance since the first bite are labeled with asterisks. Species are denoted by line color: *Camponotus* (yellow), *Pogonomyrmex* (blue).

fect performance traits related to the loading phase of the LaMSA mechanism. During a loading phase sometimes lasting seconds, the adductor muscle is likely experiencing tetany and an increased interval reflects the muscle's need to replenish ATP or  $\text{Ca}^{2+}$  or a longer time required to load the spring.

Performance traits associated with other phases of the LaMSA mechanism displayed no signs of fatigue in our experiments. Acceleration time, reflecting the spring actuation phase (Longo et al. 2019), did not change significantly across all strikes and species. This suggests that ants compressed their springs to a consistent strain over repeated strikes. Similarly, peak velocity and acceleration did not decrease over repeated strikes. Strike velocity and acceleration occur during the ballistic phase of the strike and are the result of the integrated dynamics of all the components of the LaMSA system (latch, motor, spring, and unlatching motor) and how

they interact during the preceding phases of the LaMSA mechanism (Ilton et al. 2018).

The repeatability of trap-jaw mandible strike performance is consistent with the idea that once a LaMSA system has loaded their spring to some threshold compression, performance is fixed with little room for modulation. Once a given spring, whose stiffness and mechanical performance is fixed, is loaded, performance would be determined by trigger dynamics (Divi et al. 2020). In other LaMSA-powered systems, such as mantis shrimp, performance can be modulated, with strike speed and energy depending on behavioral context or medium (water vs. air) (Kagaya and Patek 2016; Green et al. 2019; Feller et al. 2020). Behavioral motivation in a given context likely plays an important role in determining how much the spring is compressed, but little is known about the sensory or control mechanisms that influence spring compression.

Increased strike intervals were similar across all three genera of trap-jaw ants under study, despite them using different LaMSA mechanisms. Ants in the genus *Daceton* use their labrum as a latch to block their mandibles from closing like a doorstop, and a fast trigger muscle pulls the labrum out of the way to release a strike (Gronenberg 1996b). In contrast, *Anochetus* and *Odontomachus* use a modification of the mandible joint to latch their jaws open (Gronenberg et al. 1993; Gronenberg 1995a, 1995b; Gronenberg and Ehmer 1996). A fast trigger muscle derived from the mandible adductor muscle unlatches the jaws and allows them to snap shut. The trend of increased strike interval was consistent even across slight differences in experimental protocol, with *Daceton* allowed to strike repeatedly over a much longer period (2 min) than *Anochetus* and *Odontomachus* (15 s). However, the longer trial time for *Daceton* may explain the increased variability in their strike interval (Fig. 2) whether due to more variation in behavioral motivation or more time for the adductor muscle to recover from fatigue. In either case, these results are consistent with our hypothesis that fatigue in a

LaMSA system is related to muscle activity during the loading phase.

An alternative interpretation of the increased strike interval of LaMSA mandibles is that mechanosensory neurons adapted to the stimulus that elicited mandible strikes. We attempted to mitigate this possibility by using two different stimuli (continual puffs of air from the experimenter compared with timed puffs of air from a nasal bulb), which resulted in similar strike interval increases. Our experimental protocol cannot identify the exact mechanism responsible, but both sensory adaptation and muscle fatigue are steps in a cascade of mechanisms that regulate physiological fatigue (Cairns et al. 2005; Enoka and Duchateau 2008; Tuthill and Wilson 2016). Future studies will require intracellular or electrophysiological recordings of the adductor muscle and relevant mechanoreceptors involved to disentangle these two potential factors.

While we found some evidence of performance fatigue in non-LaMSA ant mandibles, our protocol likely limited the conclusions we can draw from those experiments. We found a decrease in peak bite force in *Camponotus* over repeated bites, but measuring bite force under voluntary conditions can be influenced by many factors that were not controlled for in our experiments. Animal motivation, for example, can increase the inter-individual measurement variation for traits like bite force and locomotion in vertebrates (Losos et al. 2002; Anderson et al. 2008). Controlling for this behavioral by measuring muscle activity directly (*in vivo* or in isolated muscle) (Syme and Tonks 2004; Wilson and James 2004), or even monitoring the metabolic signs of fatigue (such as intracellular concentrations of lactate or ATP) (Williams et al. 2013; Kirkton and Yazdani 2021) would provide clearer understanding of the role fatigue plays in ant muscle. Combined with data on muscle geometry and fiber composition (Paul and Gronenberg 1999; Paul 2001; Püffel et al. 2021), this kind of fatigue data would provide a robust model of the determinates of bite force and performance in both trap-jaw ants and biting ants.

Contrasting results from our fatigue experiments for LaMSA versus non-LaMSA mandibles reinforce the conclusion that LaMSA mechanisms overcome some of the performance consequences of muscle fatigue. Assuming mandible adductor muscle fatigue is responsible for the reduced bite force performance of directly actuated mandibles and increased strike interval of LaMSA mandibles (see above for caveats), only directly actuated muscles experience a decrease in mandible performance from repeated use. Trap-jaw mandible performance that is relevant to their ecology (duration, velocity, acceleration, and likely mandible impact force) was unaffected. We suggest this is because unlinking muscle activity from appendage motion also unlinks

the reduced performance of a fatigued muscle from mandible performance.

Muscle fatigue could, therefore, have very different effects on the ecology of trap-jaw mandibles compared to directly actuated mandibles. Most trap-jaw ants are solitary foragers, individually capturing prey items and bringing them back to the nest (Fowler 1980; Ehmer and Holldobler 1995). Consequently, a factor like fatigue, that reduces the performance of their mandibles (strike speed or force) could theoretically reduce a colony's ability to acquire nutrition. In contrast, non-trap-jaw ants often recruit nestmates for foraging and cooperatively capture and transport prey or other resources. The cooperative chains formed by *Leptogenys* foragers to capture large millipede prey offer a dramatic example of this behavior (Peeters and Greef 2015). Cooperative behavior minimizes the impact of performance reduction on any single individual and allows for functional redundancy. For example, older workers of leaf-cutting ants whose mandibles are worn down and less efficient at cutting leaves switch to other tasks like leaf-carrying (Schofield et al. 2010). Future work could attempt to quantify the burden muscle fatigue might have on foraging efficiency in trap-jaw ants.

By decoupling muscle activity and motion, LaMSA systems overcome many of the constraints inherent to muscle performance. Most commonly noted, trade-offs between muscle velocity and force can generally limit how quickly a motion can occur, especially in small animals (Sutton et al. 2019). LaMSA systems are also more robust to the effects of temperature than direct muscle action, as has been demonstrated in vertebrates with ballistic tongue projection, such as frogs, chameleons, and salamanders (Anderson and Deban 2010; Deban and Lappin 2011; Anderson et al. 2014). Our data suggest the performance of LaMSA mechanisms are also more resistant to muscle fatigue. This feature of LaMSA movements could serve as another source for bio-inspired design that seek to optimize limiting fatigue in motor driven movements.

## Supplementary data

Supplementary Data available at [ICB](#) online.

## Acknowledgments

The authors would like to thank the organizers of the complementary session for the symposium Best Practices for Bioinspired Design Education, Research, and Product Development: Marianne Alleyne, Will Barley, and Aimy Wissa. The authors are also grateful to Alan Dejean and Corrie Moreau for helping collect colonies of *Daceton*. Assistance with filming the ants was provided by Daniel Garza, Mateusz Grobelny, Brendan



Jameson, Rachel Siller, Alex Varado, Dajia Ye, and Vicky Zhou. Dajia Ye also assisted manually digitizing *Daceton* strikes. All high-speed videography took place in the Imaging Technology Group at the Beckman Institute for Advanced Science and Technology at the University of Illinois, Urbana-Champaign. The authors would also like to thank members of the Anderson Lab for discussion and comments on early versions of this project. The authors are grateful to the comments of three anonymous reviewers, which substantially improved this manuscript.

## Funding

This work was supported by the National Science Foundation [grant numbers IOS 17–55336 to P.S.L.A. and A.V.S., DBI 20–22129 to A.V.S., DEB 1407279 to F.J.L., IGERT 1069157 to M.D.R., GFRP 2015196745 to M.D.R.].

## References

- Allen DG, Lamb GD, Westerblad H. 2008. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88:287–332.
- Anderson CV, Deban SM. 2010. Ballistic tongue projection in chameleons maintains high performance at low temperature. *Proc Natl Acad Sci* 107:5495–9.
- Anderson CV, Larghi NP, Deban SM. 2014. Thermal effects on the performance, motor control and muscle dynamics of ballistic feeding in the salamander *Eurycea guttolineata*. *J Exp Biol* 217:3146–58.
- Anderson RA, Mcbrayer LD, Herrel A. 2008. Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biol J Linn Soc* 93:709–20.
- Booher DB, Gibson JC, Liu C, Longino JT, Fisher BL, Janda M, Narula N, Toulkeridou E, Mikheyev AS, Suarez AV, et al.. 2021. Functional innovation promotes diversification of form in the evolution of an ultrafast trap-jaw mechanism in ants. *PLoS Biol* 19:e3001031.
- Cairns SP, Knicker AJ, Thompson MW, Sjøgaard G. 2005. Evaluation of models used to study neuromuscular fatigue. *Exerc Sport Sci Rev* 33:9–16.
- De la Mora A, Perez-Lachaud G, Lachaud J. 2008. Mandible strike: the lethal weapon of *Odontomachus opaciventris* against small prey. *Behav Process* 78:64–75.
- Deban SM, Lappin AK. 2011. Thermal effects on the dynamics and motor control of ballistic prey capture in toads: maintaining high performance at low temperature. *J Exp Biol* 214:1333–46.
- Deban SM, Scales JA, Bloom SV, Easterling CM, O'Donnell MK, Olberding JP. 2020. Evolution of a high-performance and functionally robust musculoskeletal system in salamanders. *Proc Natl Acad Sci* 117:10445–54.
- Divi S, Ma X, Ilton M, RSt Pierre, Eslami B, Patek SN, Bergbreiter S. 2020. Latch-based control of energy output in spring actuated systems. *J R Soc Interface* 17:20200070.
- Ehmer B, Holldobler B. 1995. Foraging behavior of *Odontomachus bauri* on Barro Colorado Island. *Psyche J Entomol* 102:215–24.
- Enoka RM, Duchateau J. 2008. Muscle fatigue: what, why and how it influences muscle function. *J Physiol* 586:11–23.
- Feller KD, Sutton GP, Gonzalez-Bellido PT. 2020. Medium compensation in a spring-actuated system. *J Exp Biol* 223:jeb208678.
- Fernandes IO, Larabee FJ, Oliveira ML, Delabie JHC, Schultz TR. 2021. A global phylogenetic analysis of trap-jaw ants, *Anochetus* Mayr and *Odontomachus* Latreille (Hymenoptera: Formicidae: Ponerinae). *Syst Entomol* 46:685–703.
- Fowler H. 1980. Populations, prey capture and sharing, and foraging of the Paraguayan Ponerine *Odontomachus chelifer* Latreille. *J Nat Hist* 14:79–84.
- Gibson JC, Larabee FJ, Touchard A, Orivel J, Suarez AV. 2018. Mandible strike kinematics of the trap-jaw ant genus *Anochetus* Mayr (Hymenoptera: Formicidae). *J Zool* 306:119–28.
- Green PA, McHenry MJ, Patek SN. 2019. Context-dependent scaling of kinematics and energetics during contests and feeding in mantis shrimp. *J Exp Biol* 222:jeb198085.
- Gronenberg W, Ehmer B. 1996. The mandible mechanism of the ant genus *Anochetus* (Hymenoptera, Formicidae) and the possible evolution of trap-jaws. *Zoology* 99:153–62.
- Gronenberg W, Tautz J, Holldobler B. 1993. Fast trap jaws and giant neurons in the Ant *Odontomachus*. *Science* 262:561–3.
- Gronenberg W. 1995. The fast mandible strike in the trap-jaw ant *Odontomachus* I. Temporal properties and morphological characteristics. *J Comp Physiol A* 176:391–8.
- Gronenberg W. 1995. The fast mandible strike in the trap-jaw ant *Odontomachus* II. *J Comp Physiol A* 176:399–408.
- Gronenberg W. 1996. Fast actions in small animals: springs and click mechanisms. *J Comp Physiol A* 178:727–34.
- Gronenberg W. 1996. The trap-jaw mechanism in the dacetine ants *Daceton armigerum* and *Strumigenys* sp. *J Exp Biol* 199:2021–33.
- Ilton M, Bhamla MS, Ma X, Cox SM, Fitchett LL, Kim Y, Koh J, Krishnamurthy D, Kuo C-Y, Temel FZ, et al.. 2018. The principles of cascading power limits in small, fast biological and engineered systems. *Science* 360:eao1082–13.
- James C, Sacco P, Jones DA. 1995. Loss of power during fatigue of human leg muscles. *J Physiol* 484:237–46.
- Kagaya K, Patek SN. 2016. Feed-forward motor control of ultrafast, ballistic movements. *J Exp Biol* 219:319–33.
- Kirkton SD, Yazdani AA. 2021. Chronic electrical stimulation reduces reliance on anaerobic metabolism in locust jumping muscle. *Comp Biochem Physiol A Mol Integr Physiol* 257:110954.
- Larabee FJ, Fisher BL, Schmidt CA, Matos-Maravi P, Janda M, Suarez AV. 2016. Molecular phylogenetics and diversification of trap-jaw ants in the genera *Anochetus* and *Odontomachus* (Hymenoptera: Formicidae). *Mol Phylogenet Evol* 103:143–54.
- Larabee FJ, Gronenberg W, Suarez AV. 2017. Performance, morphology and control of power-amplified mandibles in the trap-jaw ant *Myrmoteras* (Hymenoptera: Formicidae). *J Exp Biol* 220:3062–71.
- Larabee FJ, Suarez AV. 2014. The evolution and functional morphology of trap-jaw ants (Hymenoptera: Formicidae). *Myrmecol News* 20:25–36.
- Larabee FJ, Suarez AV. 2015. Mandible-powered escape jumps in trap-jaw ants increase survival rates during predator-prey encounters. *PLoS One* 10:e0124871.

- Longo SJ, Cox SM, Azizi E, Ilton M, Olberding JP, Pierre RS, Patek SN. 2019. Beyond power amplification: latch-mediated spring actuation is an emerging framework for the study of diverse elastic systems. *J Exp Biol* 222:jeb197889.
- Losos JB, Creer DA, Schulte JA. 2002. Cautionary comments on the measurement of maximum locomotor capabilities. *J Zool* 258:57–61.
- Maluf KS, Shinohara M, Stephenson JL, Enoka RM. 2005. Muscle activation and time to task failure differ with load type and contraction intensity for a human hand muscle. *Exp Brain Res* 167:165–77.
- Matos-Maraví P, Matzke NJ, Larabee FJ, Clouse RM, Wheeler WC, Sorger DM, Suarez AV, Janda M. 2018. Taxon cycle predictions supported by model-based inference in Indo-Pacific trap-jaw ants (Hymenoptera: Formicidae: *Odontomachus*). *Mol Ecol* 27:4090–107.
- Moffett MW. 1986. Trap-jaw predation and other observations on two species of *Myrmoterias* (Hymenoptera: Formicidae). *Insectes Soc* 33:85–99.
- Patek SN, Baio JE, Fisher BL, Suarez AV. 2006. Multifunctionality and mechanical origins: ballistic jaw propulsion in trap-jaw ants. *Proc Natl Acad Sci* 103:12787–92.
- Paul J, Gronenberg W. 1999. Optimizing force and velocity: mandible muscle fibre attachments in ants. *J Exp Biol* 202:797–808.
- Paul J. 2001. Mandible movements in ants. *Comp Biochem Physiol A Mol Integr Physiol* 131:7–20.
- Peeters C, Greef SD. 2015. Predation on large millipedes and self-assembling chains in *Leptogenys* ants from Cambodia. *Insectes Soc* 62:471–7.
- Püffel F, Pouget A, Liu X, Zuber M, van de Kamp T, Roces F, Labonte D. 2021. Morphological determinants of bite force capacity in insects: a biomechanical analysis of polymorphic leaf-cutter ants. *J R Soc Interface* 18:20210424.
- Schofield RMS, Emmett KD, Niedbala JC, Nesson MH. 2011. Leaf-cutter ants with worn mandibles cut half as fast, spend twice the energy, and tend to carry instead of cut. *Behav Ecol Sociobiol* 65:969–82.
- Spagna JC, Schelkopf A, Carrillo T, Suarez AV. 2009. Evidence of behavioral co-option from context-dependent variation in mandible use in trap-jaw ants (*Odontomachus* spp.). *Naturwissenschaften* 96:243–50.
- Spagna JC, Vakis AI, Schmidt CA, Patek SN, Zhang X, Tsutsui ND, Suarez AV. 2008. Phylogeny, scaling, and the generation of extreme forces in trap-jaw ants. *J Exp Biol* 211:2358–68.
- Sutton GP, Mendoza E, Azizi E, Longo SJ, Olberding JP, Ilton M, Patek SN. 2019. Why do large animals never actuate their jumps with latch-mediated springs? Because they can jump higher without them. *Integr Comp Biol* 59:1609–18.
- Syme DA, Tonks DM. 2004. Fatigue and recovery of dynamic and steady-state performance in frog skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 286:R916–26.
- Tuthill JC, Wilson RI. 2016. Mechanosensation and adaptive motor control in insects. *Curr Biol* 26:R1022–38.
- Williams JH, Batts TW, Lees S. 2013. Reduced muscle glycogen differentially affects exercise performance and muscle fatigue. *ISRN Physiol* 2013:1–8.
- Wilson RS, James RS. 2004. Constraints on muscular performance: tradeoffs between power output and fatigue resistance. *Proc R Soc Lond B Biol Sci* 271:S222–25.