Supplemental Data: The Rapid Mandible Strike Of A Termite Soldier

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Supplemental Figure

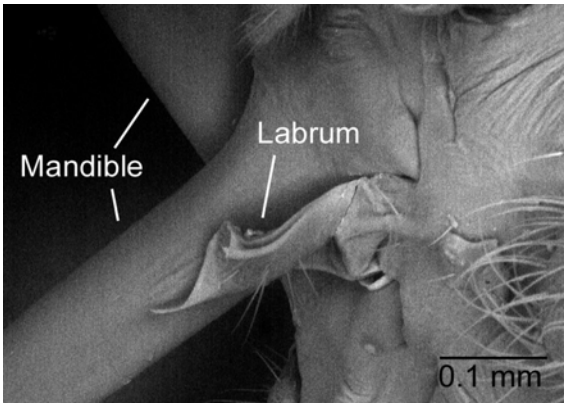

Figure S1. The labrum prevents excessive rotation of the mandibles during the strike. A scanning electron micrograph of the head of a soldier termite after it executed a strike with the labrum raised above the plane of motion of the mandibles. The mandibles are stuck in an over-rotated position and the labrum is twisted upwards.

Supplemental Experimental Procedures

Behavioural Analysis

The functional role of the mandible strike was assessed by placing *Termes panamaensis* soldiers in small, artificial, tunnels that simulate the natural tunnels, constructed of dental stone and covered with glass to enable filming and viewing. An 'invader', an individual from another termite species or an ant, was placed in the opposite side of the tunnel facing the *T. panamaensis* soldier. The species of each ant or termite invader was determined using keys [S1-3]. The interactions were rated as aggressive or non-aggressive and four major outcomes were recorded; death of *T. panamaensis* solider, death of the 'invader', retreat of the invader or a stand-off between the *T. panamaensis* solider and the invader (Table S1). A number of these interactions where also recorded using a Redlake Alliance High-Speed Digital Camera MotionPro X-3 (1000 frames per second) to allow subsequent behavioural analysis of the placement of the mandibular strike and the mechanism.

Measurements

The lengths of the mandibles, head and body of 14 *T. panamaensis* soldiers were measured using a graticule attached to a Leica MZ12.5 stereomicroscope (Table S2). The mandibles, head and body of 14 soldiers were dissected and

weighed using a Sartorius CP2P microbalance. Single mandible closer muscles (including part of the apodeme) from the heads of a further 5 soldiers were removed from the head capsule under saline, blotted dry and weighed separately [S4]. Although each closer muscle was teased away from the inside of the head capsule this may have led to an underestimate because some muscles fibres may have remained attached to the head capsule.

Scanning Electron Microscopy

Live or dried specimens were mounted on specimen holders and then examined in a Zeiss EVO 40XVP Scanning Electron Microscope.

Kinematics

T. *panamaensis* colonies were collected in Gamboa, Panama in 2007 in fallen trees and/or carton nests. Colonies were opened and soldiers removed for behavioural analysis. The kinematics of *T. panamaensis* mandibular strike was measured from 10 individuals using high speed videos recorded at either 40,000 or 50,000 frames per second, with exposure times of 2-7 μs. Videos at lower frame rates with a larger field of view were also captured to ensure angles were correctly measured. Measurements of the energy storage and resetting times, which are substantially slower than the mandible strike, were made from high speed videos recorded at 2,000 frames per second. High speed videos were captured on a Redlake Alliance High-Speed Digital Camera MotionPro X-3 attached to a Leica MZ12.5 stereomicroscope or a Nikon 105 mm macro lens. The termites were immobilized in a custom-made holder or on an adhesive surface to facilitate filming. In both cases, the mandibles extended beyond the holder/adhesive surface and were free to move. Strikes either occurred spontaneously or were elicited by touching an antenna (Video S1). Videos were analysed using Redlake MotionPro v2.02.

Calculating Force, Energy and Power

The power required to generate the rapid movements of the mandibles was obtained by modeling the mandibles as thin rods of uniform density, which rotate about a fixed axis at one end [S5-7]. Assuming the mandible is a thin rod of uniform density the moment of inertia, I, of the mandible can be calculated as:

$$I = (1/3)ML^2, \quad [1]$$

where M is the mass of the mandible and L is the length of the mandible from the axis of rotation to the tip. When a force, F, is applied perpendicularly to the long axis of the mandible,

$$F = (1/3)ML\alpha, \quad [2]$$

where α is the angular acceleration of the mandibles and was estimated to occur within 0.01 ms. This estimate is likely to be slower than the true angular acceleration, giving a conservative estimate of the force. The angular kinetic

energy, E, required to generate this force can be calculated from the moment of inertia, I, and the angular velocity, ω:

$$E = (1/2)I\omega^2, \quad [3]$$

The power, P, can be calculated from:

$$P = E/t, \quad [4]$$

where t is time. The maximum instantaneous force generated by the impact of the mandibles against an object (e.g. the head of ant or another termite) was estimated from the deceleration upon contact using equation 2 by assuming that the impact occurred at the maximum velocity.

Labrum Function

The role of the labrum in resetting the strike mechanism was assessed either by holding it out of the plane of the mandible movements during a strike (n=4) or removing it entirely before a strike (n=3). In both cases, the mandibles were temporarily immobilized using a custom made plastic restraint. A strike was initiated after the restraint was removed either spontaneously or by touching the tip of the antennae.

Reconstruction of the Head Capsule

Termites were anesthetized in ice-cold fixative (2% paraformaldehyde, 6% glutaraldehyde, 0.2 M cacodylate buffer, pH 7.4) and head capsules were removed from the body. Severing the head from the body produced an opening in the head capsule through which fixative could enter. The head capsules were then submerged in the fixative for at least two hours and post-fixed with 1.5% OsO4 in 0.1 M cacodylate buffer (pH 7.4) for an additional 2 hours. The capsules were then dehydrated in 2,2-Dimethoxiproan and 100% acetone in preparation for epon infiltration and embedding. The capsules were incubated in epon/acetone solution for 3 hours followed by two 100% epon incubations for 3 hours and then placed in beem capsules in 100% epon and cured overnight.

Embedded head capsules were serial-sectioned using a Diatome diamond histo-knife at a thickness of 1 μm on a Leica EM UC6 Ultra microtome. Serial sections were placed on glass slides, photographed and viewed using a Nikon Coolpix 8700 attached to a Nikon E600 microscope. Serial images were aligned digitally using Reconstruct software [S8] and the magnification was determined using a calibration slide at resolution of 0.01 mm. Volumetric measurements and three-dimensional reconstructions were made by individually tracing the head capsule, muscular system and neuronal and glandular structures on individual sections using Reconstruct software [S8].

Statistical Analysis

The outcomes of behavioural trials in tunnels were analysed to determine whether all species were equally susceptible to the *T. panamaensis* mandible

strike. To determine whether members of the soldier caste of various invaders were less susceptible to the mandible strike were used the G-test of Independence with William's correction [S9]. Fisher's Exact Test [S9] was used to determine whether a particular species was less susceptible to the mandible strike than the average ant or termite species.

Supplemental References

S1. Bolton, B. (1994). *Identification Guide to the Ant Genera of the World*. (Cambridge, MA: Harvard University Press).

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S3. Nickle, D.A. and Collins, M.S. The termites of Panama (Isoptera). In Quintero, D. and Aiello, A. Eds. (1992). *Insects of Panama and Mesoamerica: Selected Studies*. (Oxford: OUP).

S4. Wehner, R., Fukushi, T. and Isler, K. (2007). On being small: brain allometry in ants. Brain Behav. Evol. *69*, 220-228.

S5. Halliday, D., Resnick, R. and Walker, J. (2007). *The Fundamentals of Physics*. (NJ: John Wiley & Sons)

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S7. Alexander, R.M. (1983). Animal Mechanics. 2nd edition. (Oxford: Blackwell Scientific Publications)..

S8. Fiala J.C. (2005). Reconstruct: a free editor for serial section microscopy. J. Microscopy *218*, 52-61.

S9. Sokal, R.R. and Rohlf, F.J. (1995). Biometry: the principles and practice of statistics in biological research. 3rd edition. (New York: W.H. Freeman and Co.).

Table S1. The outcomes of encounters between *T. panamaensis* soldiers and invaders in artificial tunnels.

Species	Trials	Deaths (%)	Retreats (%)	Stand-offs (%)	*T. panamaensis* deaths (%)
Termites					
Microcerotermes sp.1 Worker	10	80	20	0	0
Soldier	5	20	0	60	20
Microcerotermes sp.2 Worker	10	90	10	0	0
Soldier	5	80	20	0	0
Nasutitermes corniger Worker	10	80	10	0	10*
Soldier	5	100	0	0	0
Embiratermes chagresi Worker	10	90	10	0	0
Ants					
Pheidole sp. 1 Worker	10	80	0	20	0
Soldier	5	60	40	0	0
Pheidole sp. 2 Worker	10	70	0	30	0
Soldier	5	0	20	80	0
Azteca sp. 1	10	90	0	0	10
Azteca sp. 2	10	100	0	0	0
Dolichoderus sp.	10	80	0	20	0
Gnamptogenys sp.	10	10	90	0	0
Camponotus sp.	10	60	0	20	20
Solenopsis sp.	10	80	20	0	0
Crematogaster sp.	10	70	0	20	10

*Worker termites managed occasionally to manoeuvre around the mandibles and bite the head of the *T. panamaensis* soldier.

Table S2. Length and mass measurements of *T. panamaensis* soldiers.

Measurement	Number of Individuals	Mean	Standard Deviation
Body mass (mg)	14	1.76	0.146
Head mass (mg)	14	0.69	0.094
Mandible mass (mg)	14	0.03	0.006
Closer muscle mass (mg)	5	0.15	0.077
Body length (mm)	14	5.0	0.21
Head length (mm)	14	1.4	0.04
Mandible length (mm)	14	1.5	0.07

Video S1 A video of a *Termes panamaensis* soldier termite making a spontaneous strike taken at 2000 frames per second using a Redlake Alliance High-Speed Digital Camera MotionPro X-3 attached to a Leica MZ12.5 stereomicroscope. The antennae are deflected laterally before the strike in executed.