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METHODS & TECHNIQUES

Tunnel-tube and Fourier methods for measuring three-dimensional medium reaction force in burrowing animals

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ABSTRACT

Subterranean digging behaviors provide opportunities for protection, access to prey, and predator avoidance for a diverse array of vertebrates, yet studies of the biomechanics of burrowing have been limited by the technical challenges of measuring kinetics and kinematics of animals moving within a medium. We describe a new system for measuring 3D reaction forces during burrowing, called a 'tunnel-tube', which is composed of two, separately instrumented plastic tubes: an 'entry tube' with no medium, in series with a 'digging tube' filled with medium. Mean reaction forces are measured for a digging bout and Fourier analysis is used to quantify the amplitude of oscillatory digging force as a function of frequency. In sample data from pocket gophers digging in artificial and natural media, the mean ground reaction force is constant, whereas Fourier analysis resolves a reduced amplitude of oscillatory force in the artificial medium with lower compaction strength.

KEY WORDS: Digging, Rodent, X-ray, Force, Biomechanics

INTRODUCTION

Burrowing is a fundamental behavior for many species because it provides protection from environmental conditions and predators, as well as access to subterranean prey. A small number of biomechanical studies have probed the biomechanics of diverse burrowing species. Ansell and Trueman (1967, 1968) used an impedance pneumograph to measure pressure changes in wet sand caused by the burrowing patterns of freely moving aquatic invertebrates. More recently, photoelastic stress techniques (Full et al., 1995) have been used to study the biomechanics of burrowing behaviors in marine invertebrates (Che and Dorgan, 2010; Dorgan, 2015; Grill and Dorgan, 2015; Murphy and Dorgan, 2011). O'Reilly and colleagues (1997) studied the burrowing locomotion of caecilians using implanted air-filled catheters to measure pleuroperitoneal pressure together with fore–aft force measured by a vertically oriented force platform. Another study of amphisbaenid burrowing used a strain gauge contained in a sheet of plastic that the animals pushed against (Navas et al., 2004). Gambaryan and colleagues (2002) used a single plane of X-ray video combined with force measurements to study burrowing biomechanics in European moles. Their force transducer was a moveable wall connected to two springs to measure normal force exerted by the animals' forelimbs. The biomechanics of sandfish lizard subsurface locomotion have also been studied using X-ray analysis of the granular medium, which was reproduced by an instrumented robot to determine dynamics of the animal–medium interaction (Maladen et al., 2011). Here, we describe a new 'tunnel-tube' system that uses two six-axis load cells and mitigates the effect of soil mass by isolating the 'entry tube' from the soil-filled 'digging tube'. We also provide a method for extracting the amplitudes of oscillatory reaction forces exerted during scratch, chisel-tooth, head-lift or humeral rotation digging within the 'tunnel-tube'.

The dynamics of overground locomotion have been widely studied using force platforms, according to principles summarized by Heglund (1981). The basic constraints and assumptions used in force platform studies of terrestrial locomotion need to be adapted for the measurement and analysis of subterranean digging force.

First, the natural frequency of a force platform should be at least 10-fold greater than the highest frequency of force measured. By pairing lightweight top-plates with sufficiently stiff transducer elements, manufacturers typically provide natural frequencies between 100 and 400 Hz to measure human locomotor forces below 15 Hz. For studies of subterranean digging, achieving a sufficiently high natural frequency presents a challenge because the force-sensing elements must support a large enough soil mass to permit digging while remaining sensitive to small digging forces.

Second, force platform analysis assumes that terrestrial animals can only apply downward vertical force (i.e. they cannot pull upward), whereas digging animals can exert upward or downward directed force at any level of the soil column. To measure the reaction forces, the digging medium and its containing structure need to be supported by the force transducer. Separate force-instrumented sections are required to measure opposing forces – for example, the head and neck pushing upward against the roof of the tunnel while hind limbs push downward, or the forelimbs pushing forward against the medium while the hind limbs push backward against the floor of the tunnel.

MATERIALS AND METHODS

Animals

Four Botta's pocket gophers [*Thomomys bottae* (Eydoux and Gervais 1836), mean±s.d. mass=125.4±29.5 g] were live-trapped using box traps (Connior and Risch, 2009). The gophers were trapped in Sunset Park, Las Vegas, NV, USA. Animals were housed for the duration of the experiments using the simulated burrow system at the University of Nevada, Las Vegas, described by DeVries and Sikes (2009). This research was approved by the Institutional Animal Care and Use Committee at the University of Nevada, Las Vegas (protocol R0310-252).

Motion analysis

Following methods described by Brainerd et al. (2010), we recorded two axes (vertical and mediolateral views) of high-speed X-ray video from burrowing pocket gophers. Our system for 3D X-ray

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motion analysis (XMA) includes two X-ray sources (Yxlon; 15–320 kV, 0–5 mA), and two adjustable zoom image intensifiers (Medelex QXS-164; with 15, 22, 30 or 40 cm view diameters) coupled to high-speed digital video cameras (Phantom Miro 4; 12 bit, monochrome). For this study, the X-ray sources were set to 78.4 kV and 5 mA. The image intensifier was set to a 22 cm view and aluminium flashing was positioned ∼2 cm from the image intensifier surface to filter scattered (low energy) photons and improve image quality. Using X-ray video allowed us to clearly view the 3D skeletal motions and digging frequencies, but is not required in the design of a tunnel-tube system. A transparent (or windowed) tunnel-tube and a standard high-speed video camera could be used to kinematically assess digging frequency without X-ray video.

Tunnel-tube

The 'tunnel-tube' consists of two mechanically isolated plastic tubes mounted on ATI Nano-17 six-axis load cells (ATI Industrial Automation, Apex, NC, USA) and arranged in series (Fig. 1). Three-axis force transducers would serve equally well in a tunnel-tube design, albeit many biomechanics load cells are six-axis force-torque transducers. The tube through which the animal first enters (the entry tube) is unfilled and the second tube is filled with medium (the digging tube). The natural frequency of the entry tube was 165 Hz, whereas the natural frequency of the digging tube was reduced to 15 Hz by the added mass of the medium. Owing to the low natural frequency of the digging tube, only mean forces (not oscillatory forces) from the digging tube are analyzed. The entry tube, mounting hardware and platforms were designed using SolidWorks (Dassault Systemes SolidWorks Corporation, Waltham, MA, USA) and 3D printed using a Makerbot Replicator (Makerbot Industries, Brooklyn, NY, USA). Setting the layer height to 0.20 mm on our prints provided a textured surface for animals to stand on and prevents slippage while digging. In the tunnel-tube coordinate system, the *x*-axis is mediolateral, the *y*-axis is fore–aft and the *z*-axis is vertical (see Fig. 1). Given the wide availability of 3D printers, the cost of assembling a tunnel-tube system is determined by the expense of the force transducers specified, at least three channels of amplification, and an appropriate A-to-D system.

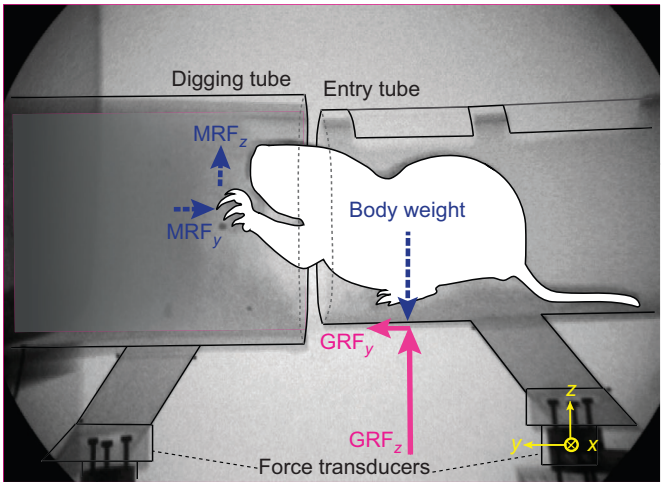

Fig. 1. Diagram of the tunnel-tube in horizontal view showing vectors representing ground reaction force (GRF) from the entry tube and medium reaction force (MRF) from the digging tube. The coordinate system defines vertical (*z*), fore–aft (*y*) and mediolateral (*x*) axes.

Digging media

Media were uniformly packed into 10 cm long sections of black ABS pipe, which could be exchanged between trials. Two digging media are tested in this report: an artificial soil made up of equal volumes of coconut fiber and walnut shell (CWM), and a natural soil collected from gopher mounds at the Sunset Park trapping location (SPM). Using a pocket penetrometer (Model E-280, Geotest Instrument Corporation, Burr Ridge, IL, USA), the compaction strength of each medium was tested. The medium compaction strength of CWM is 0.25 kg cm^{-2} and that of SPM is 0.38 kg cm^{-2}.

Data collection

The tunnel-tube was mounted inside the X-ray enclosure with separate footings for the entry and digging tubes. Simultaneous X-ray video and force data were captured using Phantom Camera Control Software (Vision Research, Wayne, NJ, USA) and LabView (National Instruments Corporation, Austin, TX, USA), respectively. Video data were collected at 500 Hz and force data were collected at 25 kHz. Synchronization was achieved by digital post-trigger (TTL) from the Phantom Miro 4 cameras to a National Instruments cDAQ™ 9188XT ethernet data acquisition chassis with four NI-9237 National Instruments bridge completion amplifier modules (two 4-channel modules were required for the six half-bridges of an ATI nano-17 transducer). The animal was placed in the entry tube and blocked from the digging tube by a thin acrylic door. Using windows cut into the top of the entry tube and a webcam, we observed the animal's behavior inside the X-ray enclosure. Once the animal was facing the digging tube, the door was raised by a pulley and the X-ray sources were turned on.

Data selection

Thirty-two uninterrupted bouts of scratch-digging from four pocket gophers were selected, split evenly between SPM and CWM. These bouts were periods when the animals were scratch-digging with the forelimbs and during which the hindlimbs did not move. From the recorded X-ray video, we determined the start and end times of digging bouts and cropped digging bouts to 0.4 s each. These 32 bouts were windowed to 20 bouts in which (1) total mean vertical reaction force across both tubes equaled body weight ±15% and (2) the magnitudes of mean fore–aft reaction forces from the entry and digging tubes were equal ±10%.

Fourier method

Fourier analysis was used to decompose the oscillatory scratch-digging force amplitude as a function of frequency (Fig. 2). Data were downsampled as described in Table S1 so that LabView's fast Fourier transform (FFT) would return amplitudes at 1 Hz intervals when processing a 0.4 s digging bout. To quantify oscillatory digging forces, mean amplitudes were determined for three frequency ranges: 3–12 Hz ('low' frequency), 13–17 Hz ('digging' frequency) and 18–28 Hz ('high' frequency). Digging frequency is defined as 13–17 Hz by the digging frequency (15.1±1.5 Hz) measured from forelimb–medium contacts counted in X-ray video. The low frequency bin of 3–12 Hz excludes frequencies below the minimum measurable frequency of 2.5 Hz for a 400 ms sample.

Statistical analysis

Using data from both the digging and entry tube in each axis of force, a one-way ANOVA was conducted to determine the effect of medium (CWM, SPM) on mean reaction force. Using data from the entry tube, a two-way mixed ANOVA was conducted to determine the effects of medium and frequency range (low, digging, high) on the amplitude of

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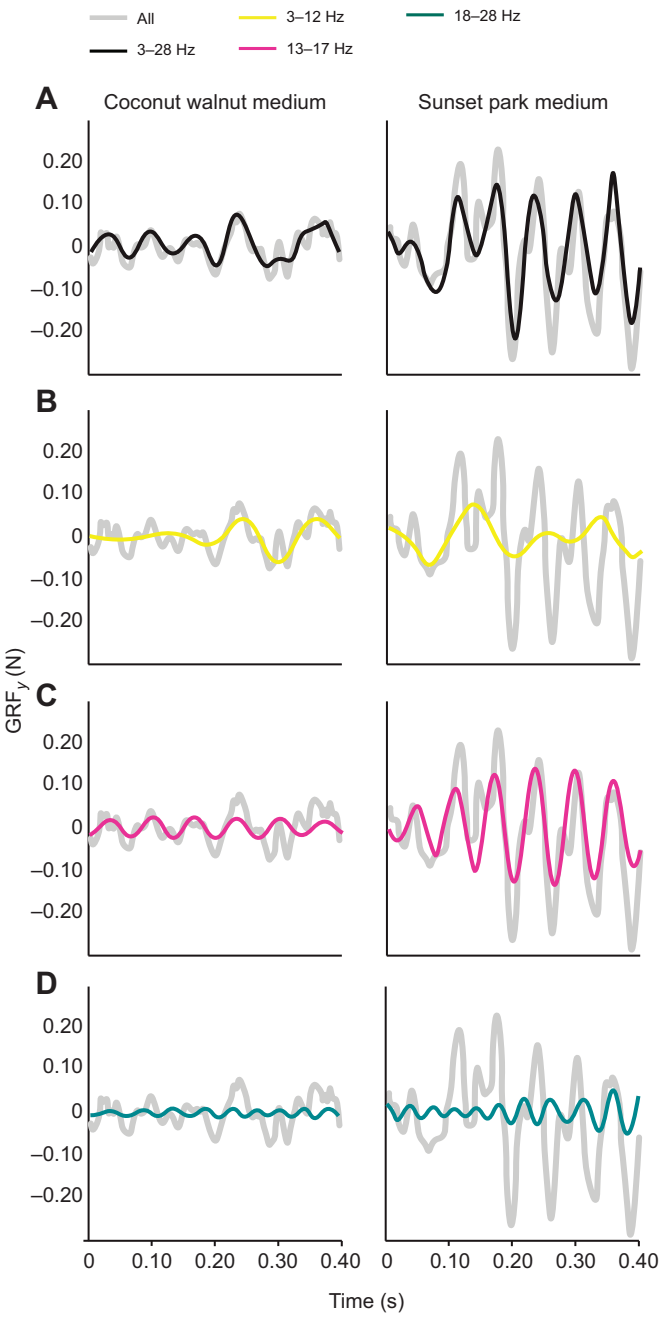

Fig. 2. Traces of fore–aft ground reaction force (GRF_y) from the entry tube, together with Fourier reconstructions using terms in the frequency ranges specified by color. (A) 3–28 Hz, (B) 3–12 Hz, (C) 13–17 Hz, (D) 18–28 Hz. The signals are zeroed by subtracting the offset as the first step in Fourier analysis.

oscillatory ground reaction force (SPSS Statistics, IBM Corporation, Armonk, NY, USA). Statistical significance was accepted at $P<0.05$ and a Bonferroni adjustment was made for multiple comparisons. Force is normalized to the body weight (BW) of the animal on the date of the trial and results are reported as means±s.d.

RESULTS AND DISCUSSION

Mean reaction force

The gophers braced themselves laterally with their hindlimbs in the entry tube while scratch-digging with their forelimbs in the medium of the digging tube (Movies 1 and 2). Mean vertical ground reaction force ($\overline{GRF_z}$) measured in the entry tube was 0.925±0.188 BW, and the vertical medium reaction force ($\overline{MRF_z}$) measured from the digging tube was 0.022±0.194 BW (Fig. 3A). Although nearly all of the body weight was supported by the hindlimbs in the entry tube, support from the forelimbs increased slightly in SPM compared with CWM, suggesting that gophers shift body weight support to their forelimbs to increase vertical digging force in more compact media (Fig. 3A). Gophers exerted mean fore–aft reaction forces (i.e. normal to the digging surface) of nearly 20% body weight to penetrate the medium ($\overline{GRF_y} = 0.183 \pm 0.076$ BW and $\overline{MRF_y} = -0.193 \pm 0.069$ BW), yet there was no significant effect of medium ($P>0.3$; Fig. 3B). Likewise, there was no significant effect of medium on the mean mediolateral reaction forces, which were generally less than 10% of body weight ($P=0.5$; Fig. 3C).

Fourier analysis of ground reaction force

Fourier analysis of oscillatory reaction forces proved to be more sensitive than mean reaction force during a digging bout – as evidenced by the ability of Fourier analysis to discriminate between digging in CWM and SPM. In this analysis, low (3–12 Hz), digging (13–17 Hz) and high (18–28 Hz) frequency oscillatory force amplitudes represent averages of all of the Fourier amplitudes within each of these ranges.

Oscillatory vertical force amplitude was not significantly influenced by medium type ($P=0.540$), although greater amplitudes were measured in SPM (0.0254±0.0071 BW) than in CWM (0.0213±0.0071 BW) at the digging frequency (Fig. 3D). The two-way interaction between medium type and frequency was also not statistically significant ($P=0.229$). Greater vertical force amplitude in SPM is consistent with the greater compaction strength, and likely greater resistance to shear, suggesting that greater oscillatory forces in all three axes are needed to fracture this medium.

Oscillatory fore–aft force amplitude was significantly affected by both frequency ($P<0.0005$) and medium ($P=0.014$), and the two-way interaction between medium type and frequency was not statistically significant ($P=0.685$). The amplitude of oscillatory fore–aft force was greater in SPM (0.0160±0.0092 BW) than in CWM (0.0089±0.0053 BW; Fig. 3E), suggesting that gophers either exert greater force in proportion with the greater compaction strength of SPM or that digging force is limited by the lower compaction strength of CWM.

Oscillatory mediolateral force was significantly affected by medium ($P=0.006$) but not by frequency range ($P=0.693$), and the two-way interaction between medium type and frequency was not statistically significant ($P=0.429$). There was a substantial mediolateral component of force when scratch-digging in SPM (0.0157±0.0105 BW) but a significantly lower amplitude in CWM (0.0061±0.0015 BW; Fig. 3F), suggesting that the shear strength of CWM limits its ability to resist mediolateral scratch-digging force.

Design considerations

Designing a transducer system to measure reaction forces during burrowing requires high sensitivity of force-sensing elements (potentially having lower stiffness) together with a greater unsprung mass owing to the volume of digging medium required. Here, the use of a separate, unfilled entry tube provides a solution with a natural frequency safely 10-fold greater than the scratch-digging behavior measured. Achieving a sufficiently high natural frequency in the tunnel tube is a much greater challenge because its unsprung mass includes the digging medium. In the current design, we could approximately double the natural frequency of the digging tube by using a tunnel-tube one-fourth the length – i.e. in proportion

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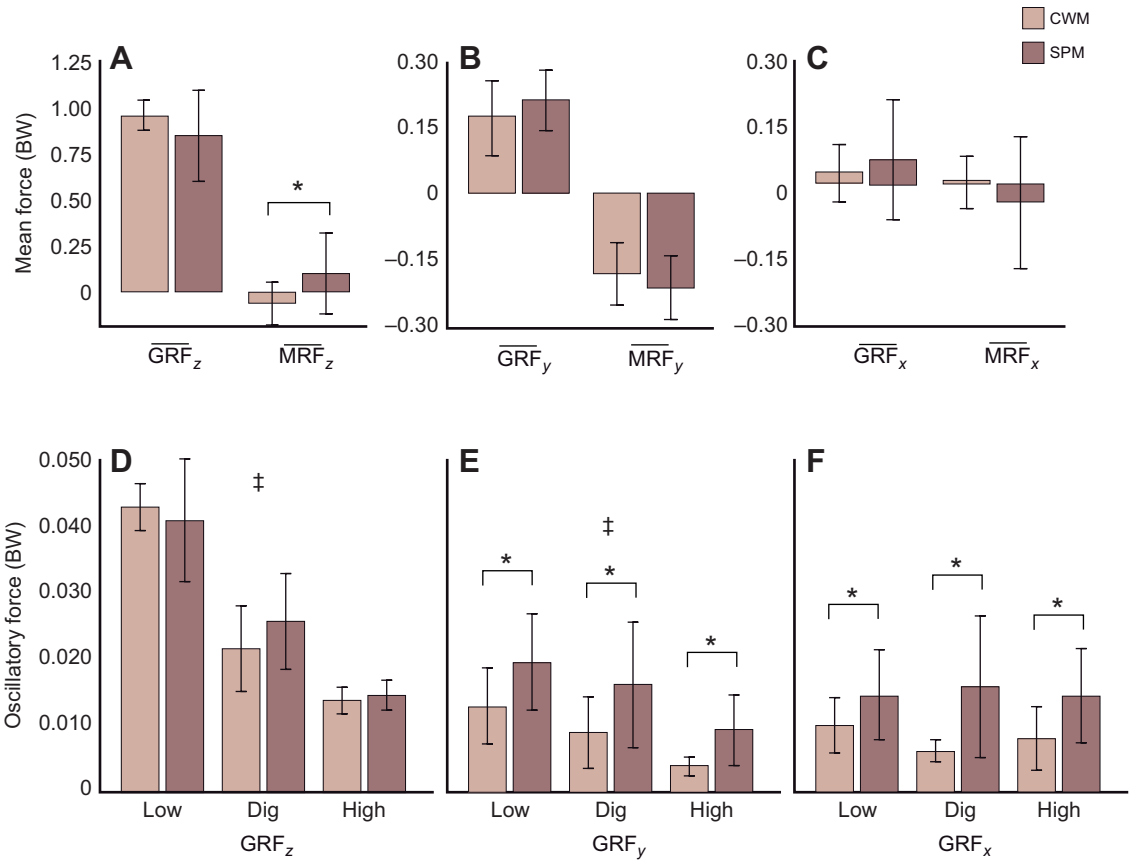

Fig. 3. Digging in artificial 'coconut–walnut' medium (CWM; light brown) and natural 'Sunset Park' medium (SPM; dark brown). (A–C) Normalized mean ground reaction force ($\overline{GRF}$) in the entry tube and mean medium reaction force ($\overline{MRF}$) in the digging tube in (A) vertical, (B) fore–aft and (C) mediolateral directions. (D–F) Normalized oscillatory ground reaction force determined by Fourier amplitudes summed across low, digging or high frequency bins in the (D) vertical, (E) fore–aft and (F) mediolateral directions. A statistically significant (P<0.05) pairwise difference between medium conditions is indicated by *. A statistically significant (P<0.05) main effect of frequency bin is indicated by ‡.

to the square-root of the inverse of mass. Because modes of oscillation are influenced by rotational inertia, decreasing the distance of the soil column from transducer by 4-fold would increase the natural frequency by 4-fold (i.e. in proportion to the square-root of the inverse of rotational inertia). Hence, future designs should minimize soil mass and the distance of the soil column from the transducer. Tunnel-tubes can be printed at any size to emulate the burrow characteristics of moles or rodents, although transducer capacity or stiffness might become more limiting for more massive diggers. We did not observe any hindlimb slippage but recommend that future iterations use rubberized tape or coating on the inside of the entry tube to reduce the chance of slippage. It would also be of interest to test the effects of different tunnel-tube surface properties on digging force and kinematics in order to assess whether animals modulate their behavior to accommodate surface conditions. Future tunnel-tubes may include a split-tube design with additional transducers to separately measure reaction forces dorsally and ventrally (and/or on left and right sides) in a given section of tube. Such a concept would be most easily applied in the entry tube section, where measurements would not be confounded by dynamic soil compaction forces.

Conclusions

We describe an innovative tunnel-tube system with separate entry and digging tubes used to measure reaction forces during scratch-digging and other burrowing behaviors. Previous burrowing studies have measured peak or mean digging forces; however, the tunnel-tube allows the amplitude of oscillatory forces to be measured by Fourier analysis. In our sample data from scratch-digging of gophers, oscillatory force amplitudes were significantly greater in the digging medium with the greatest compaction strength, whereas mean forces were not significantly different across digging media. The tunnel-tube approach is enhanced by incorporating X-ray motion analysis to measure 3D musculoskeletal biomechanics, albeit standard high-speed video is sufficient for kinematic determination of digging frequency.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.M., C.J.B., D.V.L.; Methodology: L.M., C.J.B., D.V.L.; Software: L.M., D.V.L.; Validation: L.M., C.J.B., D.V.L.; Formal analysis: L.M.; Investigation: L.M.; Resources: L.M., D.V.L.; Data curation: L.M.; Writing - original draft: L.M.; Writing - review & editing: L.M., D.V.L.; Visualization: L.M.; Supervision: D.V.L.; Project administration: L.M., D.V.L.; Funding acquisition: D.V.L.

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Supplementary information

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