

METHODS AND TECHNIQUES

Further miniaturisation of the ThermoChron iButton to create a thermal bio-logger weighing 0.3 g

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ABSTRACT

ThermoChron iButtons are commonly used by thermal biologists to continuously measure body temperature from animals. However, if unmodified, these devices are of a size that limits their use with very small animals. To allow iButtons to be used to study smaller species, methods to miniaturise them by 61% have been previously described. We present a method to reduce iButton mass by a further 71%. The modified devices have a shorter battery life, but the minimum size of vertebrates able to carry the devices is reduced from 28.9 g to 6.6 g, if the arbitrary, yet widely cited, maximum of 5% body mass for attached devices is adhered to. We demonstrate the application of our method by recording surface temperatures of captive and wild skinks and show that captive cockroaches weighing 0.8 g are also able to carry the device. We believe this to be the first time that temperature data have been recorded from an insect in this way.

KEY WORDS: Temperature datalogger, iButton modification, Thermal biology, Body temperature, Biotelemetry, Small animal

INTRODUCTION

Bio-loggers are autonomous, animal-borne electronic devices that record and store biological data from the subject or physical data relating to its immediate environment (Naito, 2004; Le Galliard, 2012). Such devices are distinguished from biotelemetry systems by the storage of data locally in the device's memory, as opposed to the transmission of data to a remote receiver (Cooke et al., 2004). Bio-loggers are able to provide continuous measurements in situations where it would not be possible to remain close enough to a target animal to continuously receive data via telemetry (Cooke et al., 2004). Bio-loggers are therefore particularly useful for taking measurements from wide-ranging animals or from animals occurring in environments that impede data transmission, e.g. underwater, provided that the animal carrying the logger can be reliably recaptured (Cooke et al., 2004; Naito, 2004). A disadvantage of bio-loggers is that they are often heavier than biotelemetry devices, as larger batteries are required to maintain the logger's memory storage (Le Galliard, 2012). Many studies adhere to the convention (in vertebrates) that animal-borne devices should weigh no more than 5% of the animal's body mass (Murray and Fuller, 2000; Horning et al., 2017). Therefore, the use of bio-loggers has been restricted to larger species.

To measure body temperatures of animals, thermal biologists commonly employ ThermoChron iButtons (Maxim Integrated, San

Jose, CA, USA) as small, relatively inexpensive and user-friendly bio-loggers (Angilletta and Krochmal, 2003; Davidson et al., 2003). These commercially available devices weighing ~2.9 g, either implanted internally or attached to the skin, are capable of recording temperatures at a resolution of 0.0625°C in real time, at user-defined intervals. ThermoChron iButtons have been used to record body temperatures in varied taxa, including marine and freshwater fish (Nowell et al., 2015; Peat et al., 2015), avian and non-avian reptiles (Kemp et al., 2017; Harlow et al., 2010), and mammals ranging in size from elephants (*Loxodonta africana*; Kinahan et al., 2007) to elephant shrews (*Elephantulus myurus*; Mzilikazi et al., 2002; Mzilikazi and Lovegrove, 2004). However, as far as we are aware, studies that have used iButtons to measure body temperature have been confined to vertebrates, as most invertebrates of interest are presumably too small to carry a device of this size.

To allow use in smaller animals, iButtons have been reduced in mass and size by removing their steel housing and other non-essential components (Robert and Thompson, 2003; Lovegrove, 2009). In a recent study, the height of modified iButtons was reduced by 46%, allowing the devices to be attached to a crevice-dwelling species (Truter et al., 2014). Modified iButtons have been produced that weigh only 1.15 g, allowing them to be carried by animals weighing as little as 29.8 g if the 5% body mass convention is adhered to (Lovegrove, 2009). Another miniaturised iButton, the iBBat (1.38 g), was developed for bats, which are of particular interest to thermal biologists because of their use of torpor. Despite these developments, there are still many species of interest that are too small to carry bio-loggers of this size; about 75% of bats, for example, have a body mass of less than 30 g (Safi et al., 2013).

Here, we describe a method for further miniaturisation of ThermoChron iButtons, which produces a temperature logger that is significantly lighter and smaller than those produced through previously described methods. We demonstrate the performance and utility of these devices in both captive and wild animals by logging skin temperature over 8–10 days for medium-sized, crevice-dwelling New Zealand skinks (*Oligosoma ottagense*). We also demonstrate that the device is small enough to be carried by a large insect (~0.8 g) by recording the underwing temperature of six captive American cockroaches (*Periplaneta americana*) for 6 days.

MATERIALS AND METHODS**Modifying the iButton**

Our procedure for miniaturising the DS1922L model ThermoChron iButton builds upon the methods described by Robert and Thompson (2003) and Lovegrove (2009). The terminology for internal parts of the device used in these previous works is followed here. We used a small-toothed hacksaw to make four vertical cuts in the stainless-steel casing, which was then peeled away from the inner casing using needle-nosed pliers. The circuit boards were lifted out of the plastic grommet completely, and the three terminals were scored lightly with a scalpel to facilitate the adhesion of solder

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to them. The circuit board was then cut with a small pair of wire cutters to remove three pieces of the board: 3.5 mm from the left side, 1.0 mm from the right side and 1.5 mm cut at a 45 deg angle from the upper right section (Fig. 1A; Fig. S1). The cut edges were then trimmed by holding the circuit board vertically and cutting down the edge towards the work bench with a scalpel. A tabbed, MS412FE manganese–silicon lithium rechargeable battery (Seiko Technologies, Tokyo, Japan) was then placed, negative-side down, on the circuit board so that the positive tab was positioned over the Vbat terminal and the negative tab over the Ground terminal (Fig. 1). A piece of electrical tape (100 mm in length) was then placed over the battery and circuit board, leaving the terminals exposed, such that the battery was held in position and the circuit board secured to the work bench. The battery tabs were then soldered to the underlying terminal using 0.7 mm solder (resin core, 60% tin, 40% lead) and a fine-tip soldering iron, masking each of the adjacent terminals in turn with small pieces of electrical tape to prevent cross-connections. Solder was also applied to the I/O

terminal so that the height of all three terminals remained the same. Because of their small size, MS412FE batteries can quickly exceed their recommended maximum temperature during soldering, resulting in damage to the battery (leakage or bursting) or, according to the manufacturer, fire. Therefore, the soldering iron was applied for less than 1 s, after which the device was allowed to cool. Before soldering, a steel scalpel handle was placed on a -18°C gel pack to act as a heat sink. The cooled scalpel handle was then applied to the battery between applications of the soldering iron. To insulate the devices, modified silicone conformal coating (SCC3 Conformal Coating, Electrolube, Ashby de la Zouch, UK) was applied to each side and allowed to cure for 24 h. The terminals were covered with masking tape before the coating was applied. After curing, the edge of the masking tape was scored lightly with a scalpel so that the coating did not lift when the tape was removed. If the terminals are not masked prior to coating, the devices will be fully water resistant. However, they must be programmed before the coating is applied and the coating must be carefully removed from

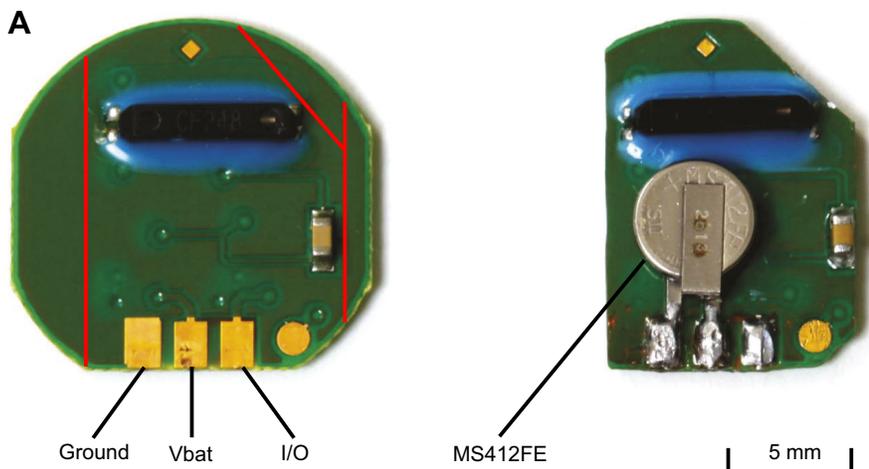


Fig. 1. Modification of the iButton and two examples of its application. (A) Left: the circuit board of a DS1922L ThermoChron iButton, showing the three terminals [the battery negative (Ground), battery positive (Vbat) and input/output (I/O) terminals]. Right: the same circuit board after removal of three sections (as shown by red lines in the left-hand figure), and the addition of a manganese–silicon lithium rechargeable battery (MS412FE). (B) A wild Otago skink (*Oligosoma otagense*, 15 g) with a miniaturised ThermoChron iButton attached to its tail with tape. (C) An American cockroach (*Periplaneta americana*) with a miniaturised ThermoChron iButton attached to its abdomen with cyanoacrylic glue. The scale bar applies only to A.



the Ground and I/O terminals when communicating with the device later (this can be done with a scalpel). Preparing the devices in this way does not noticeably affect the mass.

To communicate with the device, we constructed a receptor from a leftover plastic grommet. Lengths of wire (0.28 mm diameter, 7 core, copper, Teflon-insulated) were soldered to the ground and I/O (data) terminals on the base of a DS1402D Blue Dot™ Receptor (Maxim Integrated). The battery was then removed from a plastic grommet by cutting between the battery and battery tab with a scalpel. Machine flux was then applied to the I/O tab and ground tabs on the grommet, after which the corresponding wires were soldered to them (Fig. S2). The device was then pushed into its original place on the plastic grommet so that the terminals connected to the tabs. The positive battery tab was bent upwards towards the connector tabs to support the device, and the terminal tabs were pushed down slightly before the device was inserted to create a better connection. The devices were then interfaced with a computer via the OneWireViewer Java™ platform (Maxim Integrated) in the same way as unmodified iButtons.

The need to individually calibrate iButtons, either modified or unmodified, and the methods for doing so via a linear correction are well established (Davidson et al., 2003; Lovegrove, 2009). Our devices were calibrated by setting them to record at 10 s intervals at a resolution of 0.0625°C. They were then placed inside a vacuum-sealed plastic snap-lock bag and held first in a refrigerator for 10 min at 3°C and then in a water bath set to 20, 30 and 40°C, for 10 min each (GR120, Grant Instruments, Cambridge, UK). The temperature of the refrigerator and the water bath was verified with a reference thermometer.

Test of two bio-logging applications

The application of the device as a bio-logger was first tested by recording the skin temperature of two Otago skinks, *O. ottagensis* (McCann 1955). Although this species is relatively large (loggers weighed 0.88% and 2.25% of body mass), we had access to animals and the trials provided a useful demonstration of feasibility in this crevice-dwelling species. The devices were attached dorsally at the base of each skink's tail, with a 100 mm strip of 25 mm Leukopor tape (BSN Medical, Stockholm, Sweden). The tape was coloured black with permanent marker to match the skink's predominant tail colour. In the first trial, we attached the device to a captive adult female (38.35 g) and programmed it to record once per hour at a resolution of 0.5°C. The trial took place in the skink's usual housing: a 370 l glass terrarium containing numerous retreat sites under rock slabs, with a basking area under a 40 W incandescent bulb that was available from 09:00 h to 16:00 h for 5 days per week. A time-lapse camera placed inside the enclosure took a photograph of the skink's usual basking site every 5 min. After logging data for 190 h (7.9 days), the device was removed by cutting the surgical tape with fine scissors, and the skink was reweighed. In the second trial, the device was attached to a wild male (15 g) at Redbank Scenic Reserve in Eastern Otago in October 2017 (Fig. 1B). The device was set to record at 15 min intervals at a resolution of 0.0625°C and was active for 246.75 h (10.3 days) before the skink was recaptured and the device removed.

The second application of the device was to record surface temperature of a moderately large insect species, the American cockroach, *Periplaneta americana* (Linnaeus 1758) (Fig. 1C). Adult male cockroaches ($n=6$, mean mass 0.81 g) were obtained from a captive colony where they were kept at a constant 25°C. The trial took place in a climate-controlled animal containment facility at an air temperature of 16°C. The cockroaches were housed in a 15 l

plastic container lined with paper towels. Warm and cool retreat sites, constructed from ceramic tiles, were available in opposite corners. The warm retreat site was heated by a 40 W incandescent bulb positioned 200 mm above the tiles. Each device, which was programmed to record every 10 min at a resolution of 0.0625°C, was prepared by placing a small 10 mm×10 mm piece of electrical tape on the underside. This was done to prevent glue accumulating on the device. To attach the device, each cockroach was briefly restrained to a workbench by placing a piece of Leukopor tape over its head and thorax. A small amount of cyanoacrylic glue was applied to the electrical tape attached to the device. The cockroach's wings were then lifted with fine forceps and the device was held, glue-side down, to the abdomen for 1 min with the terminals positioned anteriorly. The glue was allowed to cure for a further 5 min before the tape was carefully removed and the cockroach placed in its enclosure. The device was removed after 6 days by peeling the electrical tape away from the abdomen with forceps. Unmodified DS1921G model iButtons were placed at the warmest and coolest parts of the container programmed to record every 10 min at a resolution of 0.5°C.

RESULTS AND DISCUSSION

Our method produces a temperature logger that is significantly smaller in mass, height and length (both with and without the application of conformal silicone coating) than with previously reported modifications (Table S1). For example, the mean mass of our device (now 0.338 g with coating) is lighter by 0.81 g (71%), thinner by 4.30 mm (63%) and shorter by 3.00 mm (18%) than the miniaturisation reported by Lovegrove (2009). If the conventional guideline for vertebrates is adhered to (i.e. that an animal-borne device weighs no more than 5% of body mass), our devices can be used as thermal bio-loggers to study vertebrates with a minimum body mass of 6.6 g. This rule is, however, arbitrary; recommendations vary between taxonomic groups and authorities, and suggested limits of up to 10% of the subject's total body mass are common (Horning et al., 2017). Therefore, it is possible that our device may be tolerated by some vertebrate species weighing as little as 3.3 g.

We recorded plausible temperature profiles for the animals that carried our devices (Fig. 2). The surface temperatures recorded for skinks are broadly consistent with spot cloacal temperatures previously recorded for New Zealand skinks (Hare and Cree, 2016) and provide the first continuous temperature traces over several days for New Zealand lizards. Devices carried by cockroaches recorded temperatures that were almost always within the temperature range recorded within the enclosure (with the exception of one data point). Predictably, all but one of the six cockroaches selected mean temperatures that were within 1°C of 25°C, the temperature to which they were acclimated prior to the experiment (the other was within 2°C). All of the animals appeared to exhibit normal behaviours while the devices were attached. Time-lapse camera footage showed that both the captive and wild skinks were able to enter and exit rock crevices as normal while the device was attached. All animals exhibited normal behaviour after the devices were removed and no obvious loss of mass occurred in the time that the devices were attached. Following these trials, we are confident that these devices can be successfully used as bio-loggers for small animals in both field and laboratory studies.

Although our method reduces the mass of the device by less than 1 g when compared with the smallest previous miniaturisation, the relative reduction in mass achieved here greatly expands the number

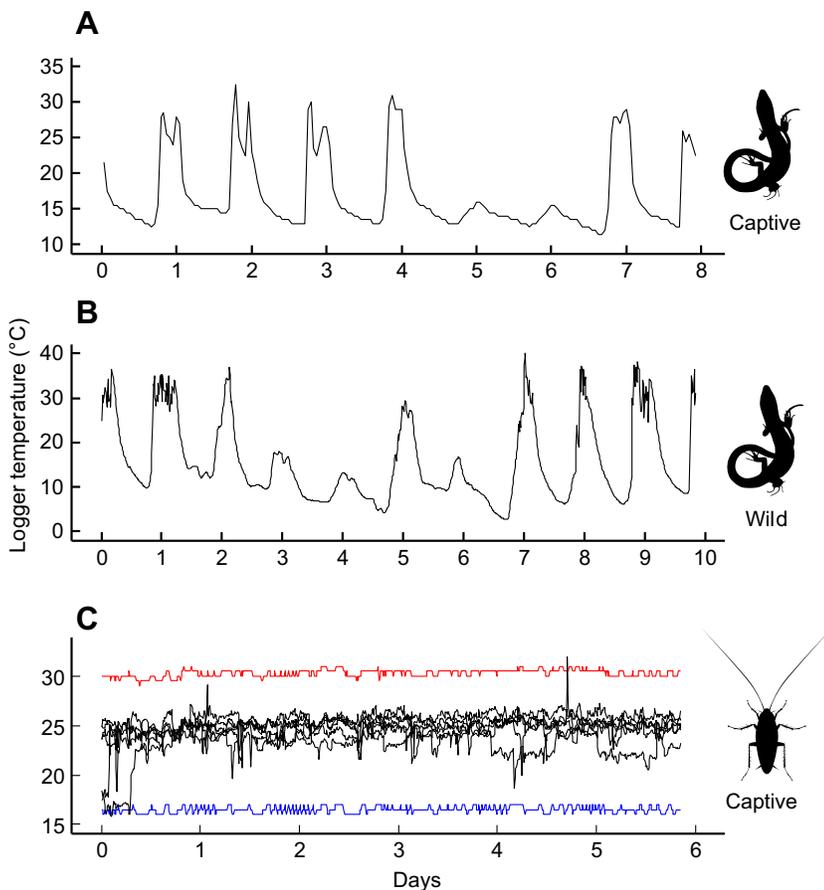


Fig. 2. Temperature profiles resulting from test applications of the modified iButton.

Profiles for a captive female (A) and wild male (B) Otago skink (*O. otagense*) and for six captive male American cockroaches (C; *P. americana*). The miniaturised Thermochron iButton bio-loggers were attached to each animal's dorsal surface, with resolutions of 0.5°C (A) and 0.0625°C (B,C). Coloured lines in C are environmental temperature profiles recorded by unmodified Thermochron iButtons (DS1921G) at a resolution of 0.5°C placed at the warmest (red) and coldest (blue) parts of the enclosure.

of potential species that can be studied. Globally, and especially for terrestrial species, body mass distributions of taxonomic groups tend to be unimodal and strongly skewed to the right (Gaston and Blackburn, 2000). In other words, smaller species are far more numerous than large species (Kozłowski and Gawelczyk, 2002). Our device will be especially useful for studying small-bodied animals in groups that have previously been of interest to thermal biologists, such as bats, lizards and birds. We estimate that our device potentially allows bio-logging studies to be conducted on an additional 56% of bat species, 37% of lizard species and 40% of bird species (Fig. 3). These estimates are based on available body mass data for these groups (Myhrvold et al., 2015), and while data for lizards are deficient (23% of all species), the data are representative of nearly all lizard families. Additionally, the body mass of most lizards is small, as the distribution of snout–vent length (SVL) of 99% of species has been shown to be strongly right skewed and SVL is strongly correlated with body mass (Meiri, 2008). Our estimates for potential use in vertebrates are made on the basis of body mass alone, and do not consider tolerance of individual species to bio-logger attachment or the potential additional mass of attachment accessories. However, the greatly reduced dimensions of our device may allow its use with vertebrate species that have previously been precluded on the basis of habitat use, regardless of mass, such as the crevice-dwelling Otago skink, as demonstrated here.

For insects, the percentage of body mass of attached devices has ranged in past studies from 2% to 100% (Kissling et al., 2014). However, in the majority of studies, devices have weighed less than one-third of insect body mass. Using this as a benchmark, our device can probably be used as a bio-logger with terrestrial insects of about 1.0 g; indeed, we have demonstrated that temperature data

can be bio-logged in a laboratory setting from cockroaches with a mean mass of 0.81 g (42% of body mass). To our knowledge, this is the first time that bio-loggers have been used to record temperature data from an invertebrate in this way.

A limitation of our modification is the small nominal capacity of the MS412FE battery (1.0 mAh). Unmodified iButtons contain CR1220 batteries (Energizer Holdings; as reported by Lovegrove, 2009) or similar, with a nominal capacity of 40.0 mAh. Although our devices have a much smaller capacity, they draw so little charge that the MS412FE is able to power the device for up to 14 days. In a test of the maximum longevity for our bio-loggers, the MS412FE powered a device programmed to record every 600 s for 344 h (~14 days) before readings became spurious (thus collecting 2065 reliable data points). Longevity can probably be extended by programming the devices to record at longer intervals than used here; however, we have not assessed this. As a caution, preliminary work showed that if the batteries discharge completely, data cannot be recovered. Therefore, establishing the discharge time of the battery at any given recording interval is necessary so that the devices can be recovered before complete discharge occurs. Another caution to note is that when the device's battery is discharged to below its minimum operational voltage, the device will record potentially spurious data. However, spurious values should be easily recognisable as spikes in readings of -41.000°C and 86.938°C . Furthermore, when the battery is nearly discharged, at low temperatures (below 10°C), readings will progressively become lower; this decline is especially obvious when the actual temperature is stable. It may also be possible to use silver epoxy, electrically conductive adhesive to connect the battery to the circuit board. This would potentially negate the risk of damaging the

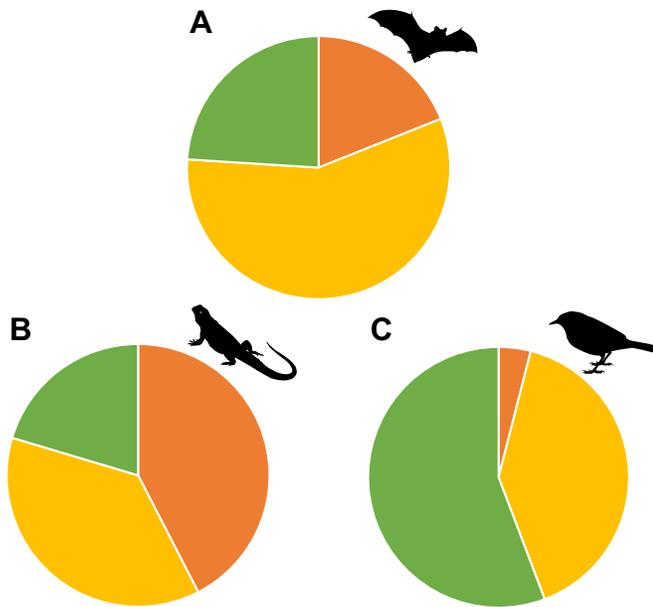


Fig. 3. New possibilities for iButtons as bio-loggers in vertebrates.

The percentage of bats (A), lizards (B) and birds (C) in three size classes, defined as: 29.8 g and above, or species that could carry the smallest previous configuration of the ThermoChron iButton, assuming devices weigh less than 5% of the subject's body mass (green); 29.7–6.6 g, or species that were too small to carry previous iButton configurations but can carry iButtons miniaturised through our methods (yellow); and less than 6.6 g, or species that are too small to carry any existing iButton configuration including ours (orange). Body mass data were obtained from the Amniote Life History Database (Myhrvold et al., 2015), representing 75.43% of bat species (<http://bat1k.ucd.ie>), 22.88% of lizard species (<http://www.reptile-database.org>) and 73.24% of bird species (BirdLife International, 2017).

battery during soldering; however, this has not been attempted here. Other common problems that we have encountered during interfacing of miniaturised iButtons, and their potential solutions, are provided in the supplementary material (Table S2).

Although the MS412FE is reportedly rechargeable, we have had limited success recharging these batteries to the manufacturer's specifications. We were able to charge the cells back to their nominal voltage after use, but capacity was diminished following each charge. This reduction is probably due to over-discharge of the battery below 2.0 V. Although the devices do not have a function that prevents them from discharging below 2.0 V, short-term use that does not over-discharge the battery may allow the devices to be used and recharged for many cycles. Additionally, if the battery has discharged to the point where the device can no longer be interfaced with normally, then running a charge through the battery while interfacing the device with a computer has, in our experience, allowed data recovery in some cases.

To our knowledge, our method produces the smallest thermal bio-logger currently available. It opens up the possibility of recording temperature profiles from many animals that were previously too small to be studied with existing thermal bio-loggers. Additionally, while emerging technologies are often costly, our method is relatively inexpensive (batteries and iButtons cost ~3USD and ~70USD, respectively). Our device is by no means the *ne plus ultra* and smaller devices are likely to be possible as battery sizes decrease in the future. However, our method does not require advanced technical skills to implement and is accessible to anyone with basic soldering experience and a steady hand.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.V., A.C.; Methodology: J.V.; Investigation: J.V.; Writing - original draft: J.V.; Writing - review & editing: A.C.; Supervision: A.C.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.176354.supplemental>

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