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RESEARCH ARTICLE

Why arboreal snakes should not be cylindrical: body shape, incline and surface roughness have interactive effects on locomotion

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ABSTRACT

Depending on animal size, shape, body plan and behaviour, variation in surface structure can affect the speed and ease of locomotion. The slope of branches and the roughness of bark both vary considerably, but their combined effects on the locomotion of arboreal animals are poorly understood. We used artificial branches with five inclines and five peg heights (≤40 mm) to test for interactive effects on the locomotion of three snake species with different body shapes. Unlike boa constrictors (*Boa constrictor*), corn snakes (*Pantherophis guttatus*) and brown tree snakes (*Boiga irregularis*) can both form ventrolateral keels, which are most pronounced in *B. irregularis*. Increasing peg height up to 10 mm elicited more of the lateral undulatory behaviour (sliding contact without gripping) rather than the concertina behaviour (periodic static gripping) and increased the speed of lateral undulation. Increased incline: (1) elicited more concertina locomotion, (2) decreased speed and (3) increased the threshold peg height that elicited lateral undulation. *Boiga irregularis* was the fastest species, and it used lateral undulation on the most surfaces, including a vertical cylinder with pegs only 1 mm high. Overall, *B. constrictor* was the slowest and used the most concertina locomotion, but this species climbed steep, smooth surfaces faster than *P. guttatus*. Our results illustrate how morphology and two different aspects of habitat structure can have interactive effects on organismal performance and behaviour. Notably, a sharper keel facilitated exploiting shorter protrusions to prevent slipping and provide propulsion, which became increasingly important as surface steepness increased.

KEY WORDS: Biomechanics, Climbing, Habitat structure, Morphology, Reptile, Scales, Slope, Tree, Tree bark, Movement ecology

INTRODUCTION

Structural variation in natural habitats can preclude animal movement altogether or affect the speed, type and ease of locomotion. For example, challenges for moving on branches in arboreal habitats include: (1) balancing on narrow cylindrical surfaces, (2) lifting the weight of the animal up inclines and (3) preventing slipping around the circumference of branches or down inclines (Cartmill, 1985). For a variety of limbed and limbless vertebrates, previous studies have quantified the effects on performance of variation in arboreal habitat structure such as surface diameter, the presence and spacing of secondary branches, and inclines (Irschick and Losos, 1999; Mattingly and Jayne, 2004; Astley and Jayne, 2009; Hyams et al.,

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2012; Jones and Jayne, 2012; Jayne et al., 2013). The roughness of tree bark also varies considerably among different species and among different branches within a single tree (Ferrenberg and Mitton, 2014), but the effects of surface roughness are poorly understood for the locomotion not only of arboreal animals but also of animals in general.

Diverse morphologies and behaviours can prevent slipping of arboreal animals. For example, phylogenetically diverse arboreal lizards and frogs have independently evolved specialized toe pads that stick to surfaces (Emerson and Diehl, 1980; Irschick et al., 1996; Autumn et al., 2002). Friction generated passively by the weight of the animal can prevent slipping on shallow inclines, but diverse animals such as chameleons (Losos et al., 1993), snakes (Byrnes and Jayne, 2014) and primates (Cartmill, 1985) use active gripping to generate sufficient friction to prevent slipping on steep surfaces. Claws are generalized structures of amniotic vertebrates that often penetrate surfaces and create interlocking shapes so that the strength of the materials rather than friction is the limiting factor that prevents slipping.

Ledge-like structures on inclines can also prevent slipping, depending on the steepness and the size and shape of the object on the incline (Fig. 1). For example, for a given height of a ledge perpendicular to an inclined surface, a square is better than a circular shape for preventing downward toppling via pivoting about the uphill edge of a ledge (Fig. 1; Movie 1). The abutment created by a ledge will also prevent a square from sliding downhill, whereas without sufficient friction a circular shape could slip as well as topple off a ledge.

Squamate reptiles have provided many insights into how variation in arboreal habitat structure and morphology affect locomotor performance, and some effects of habitat structure differ among different body plans. For example, pegs (simulating secondary branches) along the top of cylinders impede the running of limbed lizards (Jones and Jayne, 2012), whereas they facilitate generating propulsive forces as arboreal snakes push the sides of their bodies against the pegs and perform lateral undulatory locomotion (Astley and Jayne, 2009; Jayne and Herrmann, 2011). In fact, pegs can allow snakes to traverse large diameter cylinders that would otherwise be impassable (Astley and Jayne, 2009; Jayne and Herrmann, 2011).

Quantitative variation within a given body plan also may have important interactions with the effects of habitat structure on locomotor performance. For example, decreased overall animal size decreases the adverse effects of increased incline on locomotion (Taylor et al., 1972; Huey and Hertz, 1982). Quantitative differences in shape may also be important, as evidenced by different leg lengths altering the severity of the effects of branch diameter on maximal running speeds of lizards with similar overall size (Irschick and Losos, 1999).

The limbless, elongate bodies of snakes superficially seem morphologically uniform, but substantial morphological variation does exist and is correlated with different habitats. For example, the
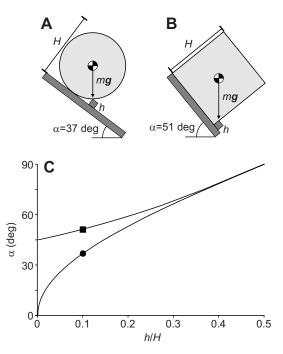
Fig. 1. Effects of ledge height, object shape and object height on the threshold angle for toppling off an inclined surface with a ledge. The circle (A) and square (B) have identical heights (H) above the inclined surface that is supporting them, and the value of h/H=0.1, where h is ledge height. Toppling occurs when the incline is slightly greater than the threshold angle α when the centre of mass of the object is directly above the uphill edge of the ledge that contacts the object. (C) Functions for α of a square [α=90−arctan(1−2h/H)] and of a circle [α=arccos(1−2h/H)]. The square will not topple until the incline exceeds 45 deg, and especially for low values of h/H, a square shape is better suited than a round shape to prevent toppling (see Movie 1).

stoutness of snakes varies considerably (Pough and Groves, 1983), and many arboreal species have convergently evolved more slender bodies than terrestrial species (Lillywhite and Henderson, 1993; Pizzatto et al., 2007; Feldman and Meiri, 2013). Many different lineages of arboreal snakes have also convergently evolved variable abilities to form a ventrolateral keel (Figs 2, 3) (Smith, 1943; Wright and Wright, 1957; Pitman, 1974), which seems well suited for catching irregularities in tree bark and preventing slipping. However, unlike the consequences of cross-sectional shape for swimming (Graham et al., 1987; Aubret and Shine, 2008) and gliding (Holden et al., 2014), the consequences of variable cross-sectional shapes for the locomotion of snakes on solid surfaces are poorly understood.

In this study, we varied incline, peg height and peg shape (Fig. 4) to test how surface features and incline might interact with each other and with the shape of snakes to affect locomotor performance and behaviour. All of our three study species climb trees, but *Boa constrictor* lacks the ability to form a ventrolateral keel (Fig. 2A). By contrast, *Boiga irregularis* (Fig. 2C) can form a rather sharp ventrolateral keel, and *Pantherophis guttatus* is somewhat intermediate (Fig. 2B). Hence, geometric considerations (Fig. 1) lead us to expect *B. irregularis* to be best and *P. guttatus* second best at using short peg heights to prevent slipping and as points of force application enabling lateral undulatory locomotion. We also expected that greater peg heights would be required by all species to prevent slipping and elicit lateral undulatory locomotion as incline increased (Fig. 1). Finally, for steeper and smoother surfaces, we expected an increased use of concertina locomotion because it uses periodic static gripping to prevent slipping.
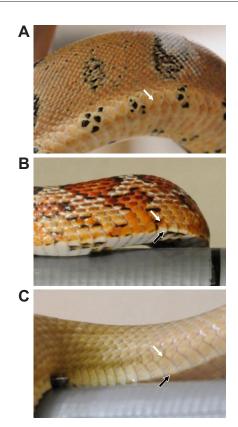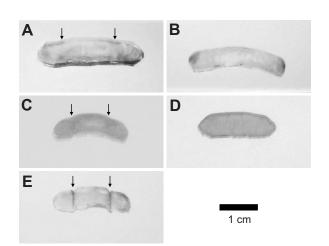

Fig. 2. Ventrolateral views of the three study species showing the variation in shape and the morphology of the ventral scales. (A) *Boa constrictor*. (B) *Pantherophis guttatus*. (C) *Boiga irregularis*. Anterior is to the left. The white arrows indicate the dorsolateral margin of the ventral scale. Unlike the other two species, the ventral scales of *B. constrictor* are not wide enough to span the width of the belly that contacts the ground, and this species does not make a sharp ventrolateral keel parallel to the long axis of the animal. *Boiga irregularis* often makes a much sharper keel (black arrow) than *P. guttatus*.
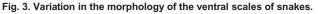
MATERIALS AND METHODS
Study species
We studied three species of phylogenetically diverse snakes with considerable variation in stoutness, cross-sectional shape and ventral scale morphology (Figs 2, 3). *Boa constrictor* Linnaeus 1758 belongs to Boidae within the superfamily Henophidia. *Boiga irregularis* (Merrem 1802) (brown tree snake) and *Pantherophis guttatus* (Linnaeus 1766) (corn snake) both belong to Colubridae within the Caenophidia, but they are not closely related (Pyron et al., 2011).

Of the three study species, *B. constrictor* was the stoutest species with the shortest tail (Table 1). *Boiga irregularis* was the most slender with the longest tail, and it was the only study species with a prehensile tail capable of supporting the entire weight of the snake by itself. *Pantherophis guttatus* had intermediate values of stoutness and tail length (Table 1). The ventral scales of *B. constrictor* only span approximately 50–60% of the width of the belly when crawling on a flat surface (B.C.J., S.J.N., personal observations of snakes crawling on glass), and the cross-sectional shape of this species was the roundest (Fig. 2A). The ventral scales of both *B. irregularis* (Fig. 2C) and *P. guttatus* (Fig. 2B) are so wide that they are readily visible in lateral view, and these species often transiently create a ventrolateral keel by bending the lateral portions of their ventral scales. Unlike *P. guttatus*, *B. irregularis* has a slight notch in the ventral scale where it creates a keel, and this probably facilitates making a sharper keel as has been described for the highly arboreal gliding snakes in the genus *Chrysopelea* (Fig. 3) (Smith, 1943).

We used snakes with snout–vent lengths (SVLs) that were as similar as was practical to obtain, and we determined the approximate height and width of each snake at mid-body as it crossed the top-centre line of the horizontal cylinder with 10 mm pegs (Table 1). We collected all 15 *B. irregularis*

Fig. 3. Variation in the morphology of the ventral scales of snakes. (A) *Pantherophis guttatus* (snout–vent length, SVL=102 cm). (B) *Nerodia fasciata* (SVL=49 cm). (C) *Boiga irregularis* (SVL=90 cm). (D) *Boa constrictor* (SVL=104 cm). (E) *Chrysopelea ornata* (SVL=85 cm). The scales are from the shed skins of captive specimens, and they were darkened with iodine before being flattened between two microscope slides and photographed. The three species on the left are arranged from the least- to best-developed capacity to form keels (in the areas indicated by arrows). Unlike all of the other species, *N. fasciata* very rarely climbs trees. Neither *C. ornata* nor *N. fasciata* was used in our experiments, but these colubrid species are included to illustrate an extreme anatomical specialization of an arboreal specialist and a more generalized anatomy of a non-arboreal species, respectively.
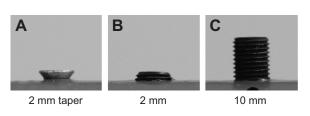
specimens in Guam during 2010 and 2011, and we brought them to a laboratory in the University of Cincinnati where all experiments were conducted during 2013. We obtained *P. guttatus* from commercial suppliers. All of the *B. constrictor* were born and raised in captivity. All of the snakes were maintained in a variety of cages with incandescent light bulbs that allowed them to regulate their daytime body temperature at 25–33°C.
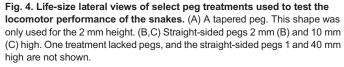
The research was conducted in compliance with the Institutional Animal Care and Use Committee of the University of Cincinnati (protocol no. 07-01-08-01), and the brown tree snakes were captured and imported under permits (MA214902-0; MA3500A-0) from the US Fish and Wildlife Service.

Experimental apparatus and procedures

All of the experiments were performed with conditions that maintained the body temperatures of the snakes between 29 and 31°C, which is within the range of field-active body temperatures of these species (Brattstrom, 1965; Montgomery and Rand, 1978; Anderson et al., 2005). The snakes were not fed for at least 7 days prior to an experiment to allow sufficient time for the completion of digestion and defecation of the last meal. To facilitate motion analysis, we painted white marks on the snakes at approximately 25%, 50% and 75% SVL.

The snakes crawled on cylinders 285 cm long and with a diameter of 5 cm. To provide a uniform texture and one that was rougher than that of the underlying material, we covered the cylinders with Nashua 394 duct tape

Fig. 4. Life-size lateral views of select peg treatments used to test the locomotor performance of the snakes. (A) A tapered peg. This shape was only used for the 2 mm height. (B,C) Straight-sided pegs 2 mm (B) and 10 mm (C) high. One treatment lacked pegs, and the straight-sided pegs 1 and 40 mm high are not shown.

(Franklin, KY, USA) in the same manner as in previous laboratory studies of arboreal snake locomotion (Astley and Jayne, 2007; Jayne and Herrmann, 2011). Lines drawn at 10 cm intervals on the cylinder provided a distance scale.

For each individual of all three species we used a total of 30 combinations of inclines and peg treatments to test the locomotor performance of the snakes. The long axis of the cylinder was 0, 5, 10, 45 or 90 deg relative to horizontal. One treatment lacked pegs, whereas others had pegs with a diameter of 8 mm and heights of 1, 2, 10 or 40 mm above the top-centre line of the cylinder and spaced at 10 cm intervals. With only one exception, the pegs were steel set screws with approximately one thread per mm (Fig. 4B,C). For the 2 mm peg height, we also used steel wood screws with a head that tapered from a diameter of 8 to 5.5 mm (Fig. 4A). We used threaded pegs to facilitate adjusting height precisely and fastening the pegs securely to the cylinder. We also conducted trials with both types of 2 mm high pegs and an incline of 120 deg (with the pegs located along the bottom-centre line of the cylinder) only for *B. irregularis* because preliminary experiments indicated that these two treatments were impassable for *P. guttatus*, and *B. constrictor* did not interact with the pegs on the lower surface of the cylinder.

After placing the snakes on the test apparatus, we lightly tapped their tails to encourage them to attain maximal speeds. We also placed a black cylinder at the end opposite the starting point to encourage the snakes to crawl. For a given treatment and individual, we usually performed three trials in rapid succession (<3 min). However, if a snake failed to make forward progress after 15 min, we assigned velocity a value of zero. Each snake experienced only one or two treatments within the same day. When two treatments were performed within a day, the snakes rested for at least 4 h between the two treatments. To reduce potential confounding effects of experience and time, we subdivided the snakes within each species into two groups, each of which experienced the treatments in a different randomized order.

We used Canon Vixia HFG10 cameras (Canon USA, Inc., Melville, NY, USA) to record high definition (1080×1920 pixels) video images at a rate of 30 Hz for both dorsal and lateral views of the snakes during the tests of performance. Both cameras were centred and perpendicular relative to the long axis of the cylinder. We used MaxTraq (Innovision Systems, Inc., Columbiaville, MI, USA) software to determine elapsed times and distances from the video images.

Data analysis

To determine speed, we first recorded the time (to the nearest 0.03 s) taken for the snake to cross each 10 cm interval for all three trials per individual per treatment. For the trial with the fastest speed over a consecutive 20 cm interval, we determined the average speed per cycle for all cycles of movement within the trial. Each cycle of lateral undulation was defined by the two successive times when a landmark on the snake reached maximum lateral displacement to the same side (Fig. 5A). One cycle of concertina locomotion was the time between successive initiations of static contact between a given location on the snake and the supporting surface (Fig. 5B). We used the fastest speed per cycle (V_{max}) to avoid sampling arbitrary portions of the static and moving phases that occur within each cycle of concertina locomotion (Fig. 5B).

Our main test for the effects of treatments on locomotor performance was a four-way mixed-model ANOVA in which peg height (N=5), incline (N=5) and species (N=3) were fixed and crossed factors, whereas individual was a random factor nested within species. For this analysis, the five levels of the peg height factor were 0, 1, 2, 10 and 40 mm, and we used the data only for pegs with straight sides (Fig. 4B,C). For the treatments with pegs 2 mm high, we tested the effects of different peg shapes (Fig. 4A,B) using a four-way mixed-model ANOVA in which peg shape, incline, species and individual were the factors. We used $P < 0.05$ as the criterion for statistical significance, and all mean values are reported ±s.e.m.
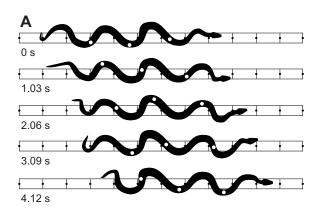
RESULTS
Locomotor behaviours

All three species performed one of two major types of locomotion: lateral undulation and concertina locomotion (Fig. 5). During

Table 1. Anatomical measurements for all of the individuals used to test the locomotor performance for each of the three study species

Measurement	Boa constrictor (N=12)	Pantherophis guttatus (N=10)	Boiga irregularis (N=15)
SVL (cm)	111±1.5 (100–116)	109±3.0 (101–134)	137±1.4 (127–145)
Tail (cm)	14±0.5 (11–16)	18±0.8 (13–22)	35±0.8 (29–40)
Mass (g)	757±35 (554–934)	420±33 (280–640)	396±10 (313–457)
Height (mm)	38±0.8 (31–41)	26±0.4 (23–28)	29±0.2 (27–30)
Width (mm)	37±0.6 (33–40)	25±0.3 (23–26)	23±0.2 (22–24)

Data are means±s.e.m., and ranges are indicated parenthetically.
SVL, snout–vent length.

lateral undulation, each point along the animal more or less followed the same path (Fig. 5A). Consequently, at a given location along the perch, snakes performing lateral undulation only required an amount of space equal to the cross-sectional area of the animal's body. Unlike lateral undulation, the concertina locomotion of *B. constrictor* required effectively the entire upper half of the surface of the cylinder because different points along the length of the snake followed different paths (Fig. 5B). Consequently, a peg could create an obstacle for this type of locomotion. However, unlike *B. constrictor*, points along the length of *P. guttatus* during concertina locomotion usually followed a similar path where the body contacted the cylinder. The concertina locomotion of
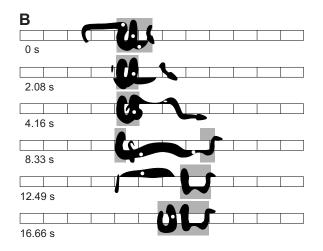

Fig. 5. Dorsal views traced from video images of a *B. constrictor* performing one cycle of each of the two different locomotor behaviours on a 45 deg incline. (A) Lateral undulation on a cylinder with 40 mm high pegs. (B) Concertina locomotion on a cylinder without pegs. The elapsed times relative to the first image are at the lower left of each illustration. The white dots indicate locations at approximately 25%, 50% and 75% SVL. The grey rectangles indicate regions of static contact where the snake was gripping the cylinder.

B. irregularis was highly variable for whether points along the body followed similar or different paths.

The mode of locomotion depended on the species of snake, peg treatment and incline (Fig. 6). Overall, *B. constrictor* was most likely to perform concertina locomotion, *B. irregularis* was most likely to perform lateral undulation, and *P. guttatus* had an intermediate use of these two behaviours (Fig. 6). When 40 mm high pegs were present, all of the snakes used only lateral undulation regardless of the incline (Fig. 6). When pegs were absent and the incline was greater than or equal to 45 deg, all of the snakes used only concertina locomotion (Fig. 6). On the 5 and 10 deg inclines without pegs, only *B. irregularis* used some lateral undulation (Fig. 6B,C).

As incline increased, the threshold peg height required to elicit lateral undulation increased within each of the three species, but for all inclines this threshold peg height was least for *B. irregularis* (Fig. 6). Some *B. irregularis* also climbed the vertical cylinder using only lateral undulation (Fig. 6E) by pushing against pegs that were only 1 mm high. By contrast, the body of *B. constrictor* commonly appeared to slide passively over pegs that were only 1 or 2 mm high. Unexpectedly, some of the *B. constrictor* actively lifted their body up and over the 10 mm pegs during concertina locomotion on the 90 deg incline (Fig. 6E). On the three shallowest inclines, *B. constrictor* performed more lateral undulation with the tapered compared with the straight-sided pegs (Fig. 6A–C). When either straight-sided or tapered 2 mm pegs were present, *B. irregularis* also performed lateral undulation by pushing against the pegs on the underside of the cylinder that was inclined 30 deg beyond vertical (Fig. 7; Movie 2).

Locomotor performance

In the four-way ANOVA of V_{max}, the main effects of peg height ($F_{4,136}$=95.8), incline ($F_{4,136}$=87.7) and species ($F_{2,34}$=81.8) were all highly significant (all $P<10^{-13}$). Overall, V_{max} increased with increased peg height, with mean values (pooled across all inclines and species) of 1.9±0.1, 4.2±0.3, 5.2±0.4, 7.7±0.4 and 8.4± 0.4% SVL s^{-1} for the peg heights of 0, 1, 2, 10 and 40 mm, respectively. Overall, V_{max} decreased with increased incline, with mean values (pooled across all peg heights and species) of 6.9±0.4, 6.8±0.4, 6.5±0.4, 4.8±0.4 and 2.3±0.2% SVL s^{-1} for the inclines of 0, 5, 10, 45 and 90 deg, respectively. Overall, *B. irregularis* was the fastest and *B. constrictor* was the slowest, with mean values of V_{max} (pooled across all peg heights and inclines) of 8.6±0.3, 4.1±0.3 and 2.6±0.1% SVL s^{-1} for *B. irregularis*, *P. guttatus* and *B. constrictor*, respectively.

For every combination of peg height and incline, *B. irregularis* had mean values of V_{max} that were greater than or statistically indistinguishable from those of the other species for the same treatment (Fig. 6). Whether *B. constrictor* was slower than *P. guttatus* depended on the combination of incline and peg height. The two steepest inclines without pegs and the vertical cylinder with 1 mm pegs were all impassable for *P. guttatus* but
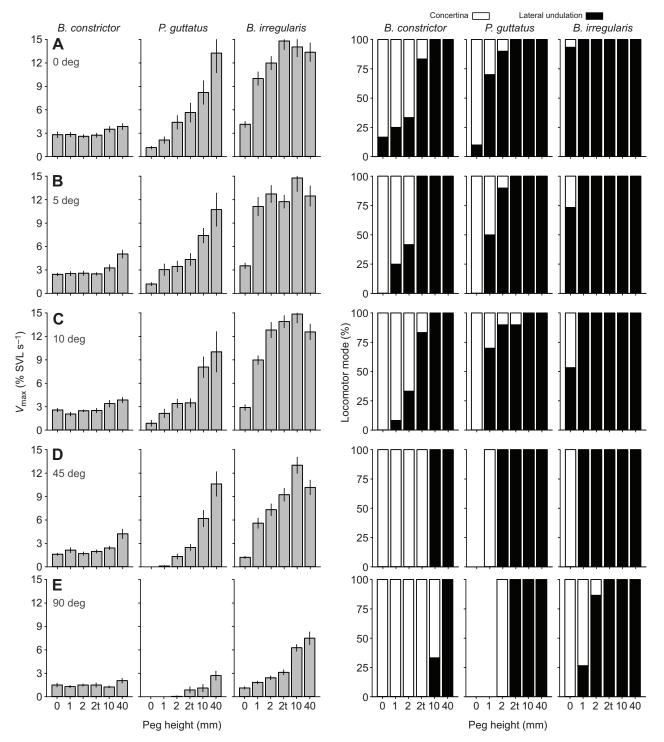
Fig. 6. Effects of peg height and species on the locomotor performance (left) and behaviour (right) of snakes on cylinders with different inclines. Cylinders were inclined 0 deg (A), 5 deg (B), 10 deg (C), 45 deg (D) and 90 deg (E) relative to horizontal. For the 2 mm peg height, t indicates the tapered head of the wood screw (see Fig. 4A). The mean values of performance (fastest speed per cycle, V_{max}) are shown ±s.e.m.; N=12, 10 and 15 individuals for *B. constrictor*, *P. guttatus* and *B. irregularis*, respectively. In the mixed-model four-way ANOVA of performance, the main effects of species, incline and peg height were all highly significant ($P<10^{-13}$). The occurrence of different locomotor behaviours is given as the percentage of individuals making forward progress that performed a particular mode of locomotion during their fastest cycle of movement. The absence of some bars indicating the type of locomotion for *P. guttatus* on the two steepest inclines is because none of the snakes made forward progress.

not *B. constrictor* (Fig. 6D,E). For the surfaces with pegs 40 and 10 mm high, *P. guttatus* was usually much faster than *B. constrictor* on all inclines, but increased incline decreased the differences in speed between these two species (Fig. 6). For

P. guttatus, many of the increases in speed associated with taller pegs were correlated with the increased use of lateral undulation (Fig. 6A–C), whereas this trend was not so apparent for the other species. Increasing peg height from 10 to 40 mm enhanced the

Fig. 7. Lateral view of a *Boiga irregularis* climbing up a surface with an incline of 120 deg and with 2 mm tapered pegs. The snake was performing lateral undulation as indicated by all points along the body simultaneously having sliding contact and moving smoothly and continuously as waves of lateral bending pushed against the tapered pegs (see Fig. 4A) on the underside of the cylinder to propel the animal. The arrows indicate where the body of the snake was pushing against the pegs. See also Movie 2.

speed of *P. guttatus* on all inclines but had no consistent effect on *B. irregularis* (Fig. 6).

All of the effects on V_{max} from the two-way interaction terms involving peg height, incline and species were also highly significant (all $P<10^{-8}$). The species×incline effect ($F_{8,136}=12.8$) was evident from incline having little effect on V_{max} of *B. constrictor* but substantially decreasing V_{max} of the other species as incline increased from 10 to 90 deg (Fig. 8A). Similarly, the species×peg height interaction ($F_{8,136}=23.0$) reflected a minimal effect of peg height on *B. constrictor* compared with the other species (Fig. 8B). The incline×peg height interaction ($F_{16,544}=5.0$) resulted from a modest effect of peg height on V_{max} for the vertical surface compared with much larger effects of peg height (from 0 to 10 mm) for the three shallowest inclines (Fig. 8C).

The main effect of peg shape (Fig. 4A,B) on V_{max} in the four-way ANOVA for the treatments with 2 mm high pegs was marginally significant ($F_{1,34}=4.75$, $P=0.036$) as the mean value of V_{max} for the tapered shape ($5.93\pm0.39\%$ SVL s^{-1}) was slightly greater than that of the straight-sided pegs ($5.16\pm0.36\%$ SVL s^{-1}). Neither of the two-way interaction terms involving peg shape was significant (peg shape×incline $F_{4,136}=1.73$, $P=0.15$; peg shape×species $F_{2,34}=1.20$, $P=0.31$). For *B. irregularis* on the 120 deg incline, the tapered pegs increased values of V_{max} compared with the straight-sided pegs (3.2 ± 0.3 versus $2.3\pm0.4\%$ SVL s^{-1}; paired $t_{14}=2.65$, two-tail $P=0.019$). Furthermore, the greater mean values of V_{max} for both *P. guttatus* and *B. irregularis* when pushing against tapered versus straight-sided 2 mm high pegs (Fig. 6D,E) suggest that a wedge shape may convey benefits for increased speed on very steep surfaces for those species that can form a more pronounced ventrolateral keel.

DISCUSSION

In natural vegetation many aspects of the size and structure of surfaces are correlated to each other (Mattingly and Jayne, 2004). Consequently, experimental manipulations are very useful for

decoupling these traits and isolating which factors are causally related to variation in performance and behaviour. This is especially true for tree bark, for which surface roughness (rugosity) often increases with increased branch diameter (Sheridan et al., 2013). Hence, not only do tree trunks have large diameters but they also commonly have rougher surfaces and very steep inclines compared with other parts within a single tree.

Surface roughness increases as the disparities between the heights of the high and low points of a surface increase. Consequently, our surfaces with greater peg heights could be considered rougher than treatments with lower pegs or no pegs. However, many other features of surface texture probably affect animal locomotion even though they remain largely unstudied. For example, the long axes of crevices and ridges with equal roughness can be either parallel or perpendicular to the long axis of a branch. If length-wise crevices are wide enough, snakes could push laterally to wedge themselves between the walls of a large crevice and perform the tunnel variant
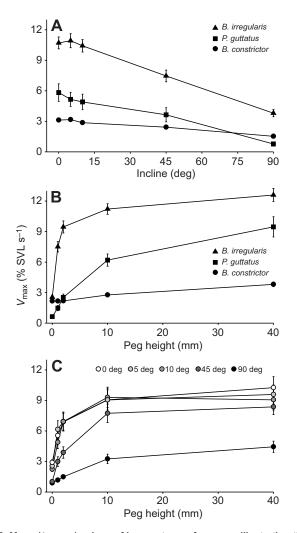

Fig. 8. Mean (±s.e.m.) values of locomotor performance illustrating the significant two-way interaction effects. (A) Species×incline interaction (mean of values pooled across peg height). (B) Species×peg height interaction (mean of values pooled across inclines). (C) Incline×peg height interaction (mean of values pooled across species). $N=60$, 50 and 75, respectively, for each mean value of *B. constrictor*, *P. guttatus* and *B. irregularis* in A and B, and $N=37$ for each point in C. All three of these two-way interaction terms between fixed effects in the mixed-model four-way ANOVA were highly significant ($P<10^{-8}$).

of concertina locomotion (Astley and Jayne, 2009). By contrast, concertina locomotion on a relatively smooth cylinder requires exerting forces medially and ventrally to grip and prevent slipping (Byrnes and Jayne, 2014). Any protrusion with a surface perpendicular to the long axis of a branch may help prevent slipping downhill for limbless and limbed animals, but it also may create an obstacle depending on how the animal moves.

The shapes of crevices and ridges in tree bark also commonly differ. We only examined the effects of two shapes of protrusions (Fig. 4A,B), both of which seemed well suited to prevent slipping (Fig. 1). However, a taper from the base to the top of a protrusion could greatly diminish the effectiveness for preventing slipping. The wedge-like peg shape (Fig. 4A) lacked an obvious effect on the speed of *B. constrictor*, but it often slightly enhanced the speed of the other two species, especially on steep inclines (Fig. 6). Perhaps this resulted from the wedge shape (Fig. 3A) enhancing the interlocking capability of the ventrolateral keel of the snakes, somewhat similar to one-half of a dovetail joint in furniture (Fig. 2C; Movies 1, 2). Whether similar shapes in the crevices in bark could also enhance the performance of limbed animal species with claws would also be interesting to examine. Although claw shape varies substantially among species, its consequences for interacting with different surface shapes and roughness are not well understood (Tulli et al., 2009).

The results of our laboratory experiments on the effects of surface roughness on the locomotor performance of arboreal snakes are supported by some field observations and may have significant implications for biological control. For example, the rat snake, *Pantherophis obsoletus*, is a formidable predator on the nests of birds, but nests located in trees with smoother bark are less likely to be preyed upon by this species (Mullin and Cooper, 2002). Furthermore, shaving a smooth band in the bark around the circumference of a tree trunk creates an effective barrier to prevent *P. obsoletus* climbing tree trunks and gaining access to bird nests (Saenz et al., 1999). In Guam, *B. irregularis* is an invasive predatory species that nearly exterminated the local species of birds and small mammals (Rodda et al., 1999). Given that sizable individuals of *B. irregularis* can climb vertical surfaces using protrusions only 1 mm high, designs for effective barriers for this species probably need very smooth surfaces.

Whether surface roughness and surface diameter interact to affect the locomotion of snakes has not been tested previously. However, for pegs taller than snakes and for a large range of cylinder diameters (5–21 cm), the presence of pegs largely negates the detrimental effects of increased cylinder diameter on the locomotor performance of brown tree snakes (Jayne and Byrnes, 2015) compared with when pegs are absent. Furthermore, when tall pegs are present on vertical cylinders, rather than wrapping closely around the cylinder, brown tree snakes commonly have gaps between their ventral surface and the cylinder, suggesting that the diameter of the cylinder may be minimally important as long as the surface is very rough (simulated by tall pegs). However, given the extremely large diameters of many tree trunks and the highly variable roughness of bark, experiments directly testing the issues above are desirable.

Long pegs nicely simulate key attributes of secondary branches in natural vegetation. Furthermore, many previous studies have used pegs to control and manipulate the spacing and number of points contacted by snakes to clarify the effects of surface structure on locomotor performance, kinematics and force transmission during the terrestrial and arboreal lateral undulation of snakes (Gray and Lissmann, 1950; Bennet et al., 1974; Kelley et al., 1997; Jayne et al.,

2013). However, all of these previous studies of snakes and additional studies of limbed arboreal animals (Hyams et al., 2012; Jones and Jayne, 2012) used pegs taller than the height of the animal.

A key question we sought to address is at what height does an object protruding from a surface begin to affect the locomotion of animals? For the snakes in our study, the threshold height for affecting locomotion depended considerably on the shape (ability to form a keel) and species of snake as well as the incline of the surface. Of our three study species, *B. irregularis* was able to make the sharpest ventrolateral keel. Not only was *B. irregularis* faster than the other two species but it also used the most lateral undulation, partly as a result of having the lowest threshold peg height for eliciting this behaviour.

Although speed is the most commonly used metric for assessing locomotor performance, differences in the energetic economy probably exist for the two modes that we observed. The energetic cost has not been determined for any type of arboreal snake locomotion. However, the energetic cost of concertina locomotion in horizontal, flat-bottomed, parallel-sided tunnels is approximately seven times greater than that of the terrestrial lateral undulation of snakes (Walton et al., 1990). Compared with lateral undulation, where the snake has continuous sliding contact, concertina has large changes in momentum from periodically stopping and starting, and static friction resistance is greater than sliding friction (Walton et al., 1990). Furthermore, during arboreal concertina locomotion, snakes apply sizable normal forces that contribute to active gripping but lack a component of force that can contribute directly to propulsion (Byrnes and Jayne, 2014). All of these factors also seem likely to make arboreal concertina locomotion energetically more expensive than arboreal lateral undulation. Hence, even though the speeds of boa constrictors and corn snakes are similar for some surfaces with short pegs (Fig. 6), the ventrolateral keel may provide a benefit for the energetic economy of the corn snakes by allowing them to use lateral undulation rather than concertina locomotion.

For limbed animals, peg-like objects as high as the animal create obstacles that impede running (Hyams et al., 2012; Jones and Jayne, 2012). However, because the limbs of animals elevate their bodies above surfaces, certain heights of irregularities in the surface could be of little consequence. For arboreal limbed animals, such as anole lizards and mice running parallel to the long axis of a cylinder, decreased cylinder diameter decreases the clearance between the surface and the belly for a given limb posture, but unlike snakes, limbed animals can modulate their limb posture to increase clearance between their belly and the surface (Spezzano and Jayne, 2004; Hyams et al., 2012; Jones and Jayne, 2012). Of the snakes we studied, *B. constrictor* was the only species that occasionally actively used vertebral flexion to clear pegs as high as 10 mm (during concertina locomotion). Compared with other aspects of habitat structure, the effects of surface roughness and clearance remain minimally studied. Consequently, a productive area for future study could be determining how protrusions from surfaces, such as irregularities in bark, affect locomotor performance and perhaps habitat preference of a wide variety of animals. For example, given that their overall size is rather small and their belly clearance is rather modest, do anole lizards run faster on smooth surfaces and also prefer species of trees and branches with smoother texture?

Most snakes have at least two ways in which they can modulate their shape to conform to surfaces. First, the elongate overall shape and large numbers of vertebrae in snakes (Hoffstetter and Gasc, 1969) form a body plan that readily bends and allows the long axis

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of the animal to conform to a wide variety of surface shapes. Second, highly mobile ribs and the ability to move the skin relative to the body wall also allow many species of snakes to modulate the cross-sectional shape in the pre-cloacal region of their body, but these capacities vary among different species. The variable presence of ventrolateral keels within individual snakes suggests that this is accomplished via muscle activity. The ventral and ventrolateral regions of the skin of snakes have several intrinsic cutaneous muscles as well as costocutaneous muscles that extend from the tips of the ribs to the skin (Buffa, 1904), and modulating the activity of some of these may be an important underlying mechanism for how the shape of the keel can be modulated.

The ease of bending the skin into a keel could be enhanced from decreased stiffness of the tissue, but reptilian scales are thicker and stiffer than the skin in the surrounding hinge regions. Highly arboreal snakes in the closely related genera *Chrysopelea* and *Dendrelaphis* have a conspicuous thinning along notches on both sides of each ventral scale (Tweedie, 1983) precisely where the scale folds and forms a keel. Although they are not as large as in *Chrysopelea*, notches in the trailing edges of the ventral scales occur where the keel forms in *B. irregularis*, but *P. guttatus* lacks this specialized morphology (Figs 2, 3). Hence, some specialized ventral scale morphology long recognized and commonly used in taxonomic keys (Smith, 1943; Tweedie, 1983) correlates well with keel formation and function. This seemingly subtle morphological difference may have contributed substantially to the much greater ability of *B. irregularis* to use small protrusions while climbing compared with *P. guttatus*. However, the ability of all arboreal snake species to make a ventrolateral keel could be compromised by the circumferential distension of skin that occurs after a large meal or with pregnancy, and the arboreal locomotor performance of *B. irregularis* does decrease after large meals (Crotty and Jayne, 2015).

Some aspects of morphology besides the prominence of the ventrolateral keel varied among our study species. To minimize the effects of overall size, we gave primacy to selecting snakes with a SVL as similar as was practical to obtain because the length of snakes relative to peg spacing can affect locomotor performance (Jayne et al., 2013). Furthermore, a sizable fraction of tail length may not affect the crawling speed of some species (Jayne and Bennett, 1989). Although the ranges in mass, height and width overlapped for corn snakes and brown tree snakes, the boa constrictors were uniformly more massive with a larger diameter (Table 1). A larger diameter by itself can decrease the ability to catch on ledges of a particular height, especially with increased steepness (Fig. 1). However, the brown tree snakes readily performed lateral undulation on surfaces with 1 mm pegs, which were 3.4% mean mid-body height, whereas the boa constrictors used exclusively concertina locomotion for the 2 mm peg height, which was 5.4% mean mid-body height (Fig. 6). Thus, relative peg height was not sufficient to account for many of the differences that we observed between the species. Potentially, increased weight could also be detrimental for the climbing performance of heavier species. However, in our study species, much of the increased weight is from increased bulk of axial musculature, and the interspecific differences in the cross-sectional areas of the major axial muscles may be adequate to generate enough force to nearly compensate for increased total weight (Hoefer and Jayne, 2013).

The extent to which the size and shape of animals conform to the size and shape of structures in their environment intuitively seems likely to affect the capacities to move and to prevent slipping (Fig. 1), and human rock climbers regularly must deal with the combined effects of ledge height, ledge shape and incline on performance. Overall, the different abilities of our study species to form a sharp keel correlated well with their performance and behaviour, and this correlation conformed well with the theoretical expectation that objects with sharper edges are better suited than more circular shapes for catching small ledges (Fig. 1). Although several previous studies have quantified the effects of overall animal size and simple linear dimensions of the animal or the surface, such as limb length and cylinder diameter (Huey and Hertz, 1982; Irschick and Losos, 1999; Astley and Jayne, 2007; Hyams et al., 2012; Sathe and Husak, 2015), comparative data on the effects of variation in the shape of anatomical structures that contact with the branches remain quite sparse. Nevertheless, our results provide a vivid example of how body shape, surface structure and incline can have profound interactive effects on locomotion, and in several instances, quantitative variation in surface features had threshold and non-linear effects rather than simple linear effects (Figs 6, 8). Despite the logistical challenge of performing fully crossed experimental designs with more than one factor, a significant benefit is the ability to explicitly test for interactive effects. For our study, not only did body shape (variable ventrolateral keel) have interactive effects with two surface features but also two features of surfaces (roughness and incline) had interactive effects with each other on both behaviour and whole-animal performance.

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