Incline and peg spacing have interactive effects on the arboreal locomotor performance and kinematics of brown treesnakes (*Boiga irregularis*)

Bruce C. Jayne[*], Justin T. Baum and Greg Byrnes[†]

Department of Biological Sciences, University of Cincinnati, PO Box 210006, Cincinnati, OH 45221-0006, USA

*Correspondent: Bruce C. Jayne bruce.jayne@uc.edu

[†] Current Address:

Biology Department, Siena College, 515 Loudon Road, Loudonville, NY 12211

Running Head: Branch spacing affects snake locomotion

Keywords: arboreal, locomotion, performance, kinematics, snake, axial

1 Many animals move using lateral undulations, but snakes are noteworthy for using this type of

2 locomotion in an unusual diversity of environments, including trees in which both the spacing

3 and orientation of branches vary considerably. Despite branches providing discrete locations for

4 snakes to generate propulsive forces during lateral undulation, the consequences of branch

5 spacing for the locomotion of snakes are poorly understood. Hence, we determined maximal

6 speeds and kinematics of an arboreal snake (*Boiga irregularis*) crawling on horizontal and

7 vertical cylinders with pegs that simulated different spacing between secondary branches. Peg

8 spacing, perch orientation, and their two-way interaction term had widespread, significant effects

9 on both performance and kinematics. For the horizontal surfaces, maximal locomotor speed

10 occurred with intermediate peg spacing, and it was nearly twice as fast as for both the smallest

11 and largest peg spacings. By contrast, the locomotor speeds of snakes on the vertical surfaces

12 were unaffected by peg spacing, and they were uniformly slower than those for the horizontal

13 surfaces. For both perch orientations, the number of pegs touched by the snake decreased as peg

14 spacing increased, and while touching only one peg the snakes crawled with apparent ease and

15 steady speed. The snakes crawled vertically with only one peg as quickly as they did using 2-10

16 pegs. Pegs on a horizontal cylinder are probably important both for propulsion of snakes and

17 preventing long-axis rolling, whereas pegs protruding from vertical cylinders and those

18 protruding from horizontal planar surfaces are probably used almost exclusively for propulsion.

19

INTRODUCTION

20

21 Many phylogenetically diverse lineages of animals use axial bending for propulsion (Gray,

22 1968). However, compared to most other taxa that use axial bending, snakes have a remarkable

23 ability to traverse a greater variety of habitats, which is enhanced by having several different

24 modes of locomotion that use axial bending (Gray, 1946; Gans, 1974; Jayne, 1986). The most

25 common mode of snake locomotion is lateral undulation, which occurs in effectively all species

26 and is often the most common mode within a species. During lateral undulation waves of lateral

27 bending propagate posteriorly and push against objects in the environment, and the entire body

28 of the animal has sliding contact with the supporting surfaces as each point along the animal

29 follows a nearly identical path (Gray, 1946; Gans, 1974; Jayne, 1986). Despite underlying

30 qualitative similarities for this mode, the speed and waveform of snakes using lateral undulation

31 vary substantially depending on the surfaces encountered and the species of snake.

32   Terrestrial lateral undulation usually requires surfaces against which the sides of the body

33 can push to produce anteromedial propulsive forces (Gray and Lissmann, 1950; Gans, 1974).

34 Consequently, the number and spacing of such points of force application (points d'appui) are

35 key features for understanding how structural variation of the environment can affect limbless

36 locomotion. Several decades ago, a series of classic experiments with snakes moving on a

37 smooth, flat surface and with a single row of peg-like objects, established that the lateral forces

38 decrease substantially with a decreased number of points of force application until effectively all

39 of the reactive force is directed forward for movement past a single peg, but the forward-directed

40 forces remain nearly constant (Gray and Lissmann, 1950). However, the speeds of snakes

41 crawling through large arrays of evenly spaced pegs on planar surfaces appear to be maximized

42 when peg spacing is intermediate (Heckrotte, 1967; Bennet et al., 1974; Kelley et al., 1997).

43 Furthermore, some review articles on snake locomotion have variously suggested that either two

44 (Gans, 1962) or three (Gans, 1974) points of force application are required for terrestrial lateral

45 undulation.

46   Although previous work has clearly established the importance of the number and

47 spacing of points of force application for lateral undulation, several issues remain unclear. For

48 example, maximal speed occurring with intermediate peg spacing seems at odds with a

49 presumably optimal relationship between forward and lateral forces with only a single point of

50 force application. This apparent contradiction could simply reflect the limited ranges of peg

51 spacing that have been used, or perhaps larger lateral forces for a given forward force are more

52 relevant for energetic economy than for maximal speed. Previous studies that have determined

53 performance and manipulated peg spacing also have not quantified the number of pegs actually

54 touched by snakes, although snakes do seem likely to touch more pegs as the distance between

55 pegs decreases. The irregular direction of travel and irregular waveforms that often occur when

56 snakes move through arrays of pegs on a planar surface complicate measuring speed as well as

57 the angle of the body relative to the overall direction of travel, and this latter variable is related to

58 the direction of forces generated during undulatory locomotion.

59   The effects of peg spacing also have considerable ecological relevance for animals in

60 arboreal habitats because secondary branches have a peg-like shape and they create conspicuous,

61 discrete sites for force application with highly variable spacing (Mattingly and Jayne, 2004).

62 Although many species of phylogenetically diverse snakes move in trees (Lillywhite and

63 Henderson, 1993; Pizzatto et al., 2007; Hampton, 2011), the effects of branch (and peg) spacing

64 on locomotor performance are known only for a few species of arboreal limbed vertebrates

65 (Hyams et al., 2012; Jones and Jayne, 2012). For snakes, adding pegs (with constant spacing) to

66 a cylindrical surface is known to: 1) elicit lateral undulation, 2) increase the maximal speed of

67 locomotion, and 3) reduce the tendency to roll about the long axis of the supporting surface

68 (Astley and Jayne, 2009; Jayne and Herrmann, 2011). Manipulating peg spacing along a

69 cylindrical surface also has some practical advantages for clarifying the effects on waveform and

70 speed because snakes on such surfaces often move with straighter overall trajectories than when

71 they traverse wide arrays of pegs on planar surfaces.

72   We manipulated both peg spacing and the incline of the primary cylindrical surface to

73 test how both of these factors affect the locomotor performance and kinematics of a highly

74 arboreal species of snake (*Boiga irregularis*) that commonly uses lateral undulation. We used a

75 very large range of peg spacing with one extreme that transiently allowed contact with only a

76 single peg. Two alternative hypotheses seemed equally plausible for the expected effects of peg

77 spacing on performance. Previous force data suggest that speed might be greatest when

78 contacting only a single peg, whereas the results from snakes crawling through arrays of pegs

79 suggest intermediate spacing is optimal for speed. For movement on cylindrical surfaces, the

80 dual function of pegs for generating propulsive force and balancing could complicate these

81 expectations. However, since the weight of the snake on a vertical cylindrical surface does not

82   cause long axis roll, comparing locomotion on vertical and horizontal surfaces can provide a

83   useful test of whether different needs for balancing affect performance and aspects of posture

84   such as how far the crests of waves are located from the supporting surface. The need of animals

85   to lift their entire weight when climbing up a vertical surface led us to expect decreased maximal

86   speeds for snakes on the vertically inclined surfaces compared to the horizontal surfaces with

87   similar numbers of pegs, but if the prevention of long-axis rolling on horizontal surfaces is

88   overwhelmingly difficult it could be the reverse.

89

90   **MATERIALS AND METHODS**

91   **Experimental subjects**

92   We used nine brown treesnakes (*Boiga irregularis*; Merrem, 1802) captured in Guam. All of the

93   experiments were performed in a laboratory at the University of Cincinnati. The snakes were

94   housed individually in cages with incandescent light bulbs that allowed them to behaviorally

95   thermoregulate and attain daytime body temperatures from 25-33° C. The snakes had values of

96   snout-vent length (mean$\pm$s.e.m. SVL=101$\pm$1.9 cm, range=101-118 cm), total length (mean

97   TL=140$\pm$2.4 cm, range=130-152 cm), and mass (mean=174$\pm$7.9 g, range=141-203 g) that were

98   as similar as was practical to obtain. All experiments were performed at least one week after the

99   snakes had been fed. White paint marks at 25%, 50%, 75%, and 100% SVL provided landmarks

100   for motion analysis. The care of animals and all procedures were approved by the Institutional

101   Animal Care and Use Committee at the University of Cincinnati (protocol # 07-01-08-01).

102

103   **Experimental apparatus and procedures**

104   We tested the locomotor performance of all snakes on five perches consisting of a metal cylinder

105   (diameter=2.4 cm; length=273 cm) with pegs (diameter=6 mm; length=4 cm) oriented

106   perpendicular to the long axis and arranged in a single row along the top center of the cylinder

107   (Fig. 1). We used five different distances between adjacent pegs (10, 20, 30, 40, and 80 cm).

108   The long axis of the perches had two orientations (horizontal and vertical). To encourage the

109   snakes to crawl along the perch, we attached a cylinder (diameter=7.5 cm; length=15cm) with a

110   blackened interior at the end of the perch opposite where the snakes were placed initially. To

111   provide a standardized surface with a sufficient amount of friction and texture (Astley and Jayne,

112   2007), we applied strips of duct tape (Nashua 394, Franklin, KY, USA) along the long axis of all

113 the cylindrical surfaces forming the perch. As explained in more detail elsewhere (Astley and

114 Jayne, 2007), the frictional resistance of this tape with snake skin and the texture created by the

115 rectangular array of fibers embedded in the tape seemed more likely to resemble natural branches

116 than the extraordinarily smooth surfaces (metal pipe and manufactured hardwood pegs) that the

117 tape covered. However, we also deliberately avoided using a surface for the cylinder with overly

118 large projections which could render the pegs largely irrelevant for the locomotion of the snakes.

119   Prior to an experiment, the snakes were placed in individual cloth bags within a chamber

120 that was 30-31°C. During experiments, we used a PE-1 infrared temperature gun (Pro Exotics,

121 Littleton, CO, USA) to verify that all snakes had body temperatures between 29° and 31°C,

122 which is within the range of field active body temperatures for this species (Anderson et al.,

123 2005). For each treatment, we performed three trials in rapid succession by placing a snake onto

124 the perch and gently touching the snake near the tail to encourage it to move at maximum

125 velocity. Except for one day that had three treatments per snake, all other days had two or fewer

126 treatments per snake. Whenever a snake performed more than one treatment per day, three hours

127 of rest were provided between successive treatments. To minimize confounding influences of

128 time and experience, the snakes were assigned to two groups, and each group experienced the

129 treatments in a different randomized order.

130 **Image acquisition and analysis**

131 We videotaped simultaneous dorsal and lateral views of the snakes at 30 images s^{-1} using a two-

132 camera (Basler piA640, Ahrensburg, Schleswig-Holstein, Germany) video system with MaxTraq

133 version 2.13 software (Innovision Systems, Inc., Columbiaville, MI, USA). A 30 Hz square-

134 wave voltage was used as an external trigger to synchronize the images of both cameras. The

135 cameras were positioned perpendicular to the long axis of the perches, and the midway point

136 along the perch was near the center of the field of view of both cameras. The top-center line

137 along the long axis of each perch contained the origin and the *x*-axis for our coordinate system.

138 The *y*- and *z*- axes were both perpendicular to the long axis of the perch. The *z*-axis was in the

139 plane containing the pegs, whereas the *y*-axis was perpendicular to this plane.

140   For all of the nine snakes, we quantified the average forward velocity of the landmark at

141 50% SVL for 20 cm of forward movement (*x*-direction) immediately prior to this landmark

142 passing the middle peg of each perch and another 20-cm interval immediately after this landmark

143 passed the middle peg of the perch. The locomotor performance of each snake (Max $\mathbf{v}_x$), was the

144   single greatest value of all these velocities (for 20 cm of forward movement) from the three trials

145   per treatment. We chose the largest value of peg spacing so that the snakes would have some

146   locomotion using only one peg, and depending on the snake length and how convoluted the

147   snake was, this usually occurred when the location at 50% SVL was within 20 cm of the peg at

148   the middle of the long axis of the perch. Occasionally the snakes paused during the performance

149   trials, but choosing only a 20 cm interval usually allowed us to obtain locomotion without

150   pausing. We consistently used a 20 cm interval to determine maximal speed of all of our

151   treatments because the values of maximal speed usually decrease with an increase in the distance

152   used to make this measurement.

153   For the five snakes with the greatest grand mean of Max $\mathbf{v}_x$ across all treatments, we also

154   quantified several variables describing the contact points and posture of the snake on the perch at

155   the time when the mid-body landmark was at the middle peg along the length of the perch in the

156   20-cm interval used to determine $\mathbf{v}_x$. Four variables quantified the number and nature of

157   locations where the body of the snake interacted with solid surfaces and hence where significant

158   forces were likely to occur. We counted the number of regions in which the dorsal midline of

159   the snake crossed the *x*-axis (Fig. 1, N_{cr}). Rather than merely assuming that snakes contacted

160   more pegs when the space between successive pegs was smaller, we determined the number of

161   pegs touched by the snake simultaneously at all locations (N_{peg_all}) and for locations likely

162   (N_{peg_prop}) or unlikely ($N_{peg\_nonprop}$) to be useful for propulsion (Fig. 1).

163   If we could not discern a gap between the body of the snake and a peg, then this was

164   classified as a peg that had been touched. Our video images and methods were not able to clarify

165   additional details such as how hard the snakes pressed against a peg or whether or not the body

166   wall had a curvature different from the mid-dorsal line as been described for the terrestrial

167   locomotion past pegs of some other species of snakes that are much heavier than the snakes in

168   our study (Gasc et al., 1989; Moon and Gans, 1998). Thus, whether a peg was considered a

169   "propulsive" peg was based only on its position relative to the body of the snake and the

170   direction of movement (Fig. 1). For example, if a peg was located on the trailing edge of a half

171   wave so that a normal reactive force arising from could have forward-directed component

172   parallel to the overall direction of movement (Fig. 1, green dot), then this was considered a

173   propulsive peg. By contrast, pegs that touched the leading edge of a half wave (Fig. 1, red dot)

174   or precisely at the crest of a half wave were considered non-propulsive pegs.

175   For each of the propulsive pegs, we determined α, the angle between the tangent through

176   the midline of the snake where the body of the snake touched the peg and the x-axis (Fig. 1).

177   This angle has strong implications for the orientation and proportion of forces that ultimately

178   contribute to propulsion when snakes undulate past solid objects (Gray and Lissmann, 1950). To

179   obtain an overall indicator of how convoluted the snake was, we measured the total distance

180   between the head and tail of the snake (x_{ht}) along the x-axis (Fig. 1), and we expressed this as a

181   proportion of the total length (TL) of the snake. In addition to x_{ht} clarifying the amount of

182   longitudinal space required for the snakes to move, this variable also allowed us to directly

183   compare the effect of peg spacing in our study to those observed in the most detailed previous

184   study of peg spacing and locomotor performance (Kelley et al., 1997).

185   For each half wave (0.5λ) we determined the lateral amplitude (y_{crest}), and the x-distance

186   from the anterior node to the crest of the half wave (x_{crest}) was measured and expressed as a

187   proportion of the half-wave length (Fig. 1). We determined the maximal width of the snake

188   (y_{max}) by adding the largest amplitudes of half waves to the left and the right of the snake (Fig.

189   1). For each half wave that was visible in the lateral view camera, we determined the z-distance

190   between the top-center line of the perch and the most ventral portion of the half wave. For each

191   individual and treatment we determined the maximal (z_{max}) and mean (z_{crest}) value for these

192   measurements of half-wave depth. Collectively these remaining variables further determine the

193   extent to which the shape of the snake conformed to the shape and locations of the objects

194   traversed by the snake. For example, a snake could theoretically use the same large amplitude,

195   large wavelength undulations when pegs are far a part as when pegs are closer together if it

196   simply failed to touch each successive peg. Different locations of the crests of waves also have

197   different implications for stability. For example, when moving on horizontal cylindrical surfaces

198   lowering the crest of a wave beneath the cylinder is a mechanism for increasing stability by

199   lowering the center of gravity and functioning like a pendulum, whereas a large lateral excursion

200   can be disadvantageous because it could generate a torque that causes long axis rotation about

201   the supporting cylinder (Jayne and Herrmann, 2011).

202   **Data analysis**

203   Each quantity describing either performance or kinematics was used as the dependent variable in

204   a three-way mixed-model analysis of variance (ANOVA). The independent variables (factors) in

205   each ANOVA were peg spacing ($N = 5$), incline ($N = 2$) and individual, and all three of these

206 factors were fully crossed. In each ANOVA, incline and peg spacing were fixed factors, whereas

207 individual was a random factor. Data from all nine snakes were used in the ANOVA for

208 performance, whereas data from only the five fastest individuals were used in each ANOVA of a

209 kinematic variable. For dependent variables such as maximum speed and some kinematic

210 variables such as the total number of pegs touched, we had only one observation per treatment

211 per individual, whereas for each of the kinematic variables (α, y_{crest}, x_{crest}, z_{crest}, 0.5λ) with

212 multiple observations along the length of a snake within a single treatment we calculated a mean

213 value per treatment. Thus, in each ANOVA, each individual had only one value of the

214 dependent variable for each combination of incline and peg spacing. We used $P<0.05$ as the

215 criterion for statistical significance. To facilitate evaluating the effects of multiple comparisons

216 (Moran, 2003), we provide exact P-values. All mean values are reported $\pm$s.e.m.

217

218 **RESULTS**

219 For all of the trials used to quantify locomotor performance, the locomotion of the snakes

220 conformed to previous definitions of lateral undulation. Waves of bending were propagated

221 posteriorly along the body of the snake, but the waves formed a standing pattern relative to the

222 supporting surface as the pegs prevented backwards slipping (Fig. 2). All points along the snake

223 moved simultaneously with continuously sliding contact with the supporting surface. Different

224 points along the length of the snake more or less followed the same path, but this was less

225 evident for the larger distances between pegs (Fig. 2B) compared to the surfaces with smaller

226 distances between pegs (Fig. 2A).

227   A noteworthy feature of the lateral undulation that we observed, especially when snakes

228 were moving vertically, was how loosely the body was draped across the cylinder, which often

229 contributed to sizable gaps between the body and the cylinder that were evident in a dorsal view

230 (Figs 2, 3). This body posture at the crossing regions during arboreal lateral undulation differs

231 substantially from the tight wrapping and greater encirclement that has been described for the

232 arboreal concertina locomotion of snakes(Astley and Jayne, 2007; Jayne and Herrmann, 2011).

233 Surprisingly, some snakes in our study also occasionally climbed vertically with a sizeable gap

234 between their ventral scales and the cylinder at the base of a peg that they were pushing against.

235 Thus, no appreciable active gripping of the cylinder appears to occur for the type of arboreal

236 lateral undulation involving pegs that we observed.

237   A three-way ANOVA revealed that both peg spacing ($F_{4,32}$=4.2, P=0.008) and incline

238   ($F_{1,8}$=19.3, P=0.002) had significant effects on locomotor performance, but the effect of peg

239   spacing was highly dependent upon the incline (incline x spacing $F_{4,32}$=6.2, P=0.001). For the

240   horizontal surfaces, the mean value of Max $\mathbf{v}_x$ was greatest for the 40 cm peg spacing (17.8$\pm$2.4

241   cm s^{-1}) which was nearly twice as large as the values for the 10 cm (10.5$\pm$1.6 cm s^{-1}) and 80 cm

242   (10.9$\pm$1.4 cm s^{-1}) peg spacing (Fig. 4). The mean values of Max $\mathbf{v}_x$ (grand mean = 6.5 cm s^{-1}) for

243   locomotion up the vertical surface were not much more than one-half the lowest values observed

244   for snakes on the horizontal surfaces. Two-way ANOVAs (factors: peg spacing; individual)

245   performed separately for data from the two different inclines confirmed that peg spacing lacked a

246   significant effect on Max $\mathbf{v}_x$ for the vertical surfaces ($F_{4,32}$=0.4, P=0.8) but had a highly

247   significant effect for the horizontal surfaces ($F_{4,32}$=8.3, P=0.001).

248   In addition to affecting performance, peg spacing and incline commonly affected

249   kinematics. For example, the number and nature of the contact regions between the snake and

250   the supporting surfaces also varied considerably among the different treatments (Figs 3, 5; Table

251   1). The number of regions where the midline of the snake crossed the midline of the supporting

252   surface decreased significantly with increased distance between adjacent pegs, and for a given

253   peg spacing the snakes usually had fewer crossing regions when moving up the vertical surface

254   than when moving horizontally (Fig. 5A). The total number of pegs (Fig. 5B) and the number of

255   propulsive pegs (Fig. 5C) touched by the snakes also decreased significantly with increased

256   distance between pegs (Table 1), but incline did not have a significant effect on these variables

257   (Table 1). Peg spacing and incline had a significant interactive effect on $N_{\text{peg\_nonprop}}$ (Table 1) as

258   a result of snakes commonly touching one or two non-propulsive pegs while moving on the

259   horizontal surfaces with the two smallest values of peg spacing, but in most other circumstances

260   nearly all of the pegs touched by the snakes were propulsive pegs (Fig. 5D).

261   The waveforms created by the bodies of the snakes also commonly had substantial

262   variation among the different surfaces (Fig. 3). Especially on the horizontal surfaces with peg

263   spacing < 30 cm, the waves of lateral bending of the snakes closely resembled a sinusoidal

264   function, whereas with increased distance between pegs many of the waves resembled a square

265   wave with an amplitude that was small compared to the wavelength (Fig. 3). The waveforms of

266   snakes on the vertical surfaces (Fig. 3B) were often less regular than those observed for snakes

267   on the horizontal surfaces (Fig. 3A).

268   All four of the variables (y_{max}, z_{max}, y_{crest}, and z_{crest}) describing the lateral and ventral

269   measurements of the half waves varied significantly with peg spacing, but they all lacked a

270   significant peg-spacing by incline interaction effect (Table 1). For both inclines, the maximum

271   width (Fig. 6A) and mean lateral amplitude (Fig. 6C) of the snake increased from the 10 cm to

272   the 20 cm peg spacing but changed little with further increases in peg spacing. The values of

273   y_{max} and y_{crest} were also significantly greater for movement up the vertical surface than for the

274   movement on the horizontal surface (Fig. 6A,C; Table 1). For y_{max} the greatest mean value

275   (11.8$\pm$1.1 cm) occurred for locomotion on the horizontal surface with the 20 cm peg spacing.

276   Both the maximum (Fig. 6B) and mean (Fig. 6D) ventral locations of the wave crests increased

277   from the 10 to 40 cm spacing, but few differences were apparent between the two different

278   inclines. For z_{max} the greatest mean value (5.8$\pm$2.0 cm) occurred with the horizontal surface with

279   40 cm peg spacing, which indicates that the greatest amount of ventral sagging coincided with

280   the conditions that optimized speed.

281   The angle of the body of the snake at the point of contact with the propulsive pegs had

282   highly significant variation with peg spacing and its two-way interaction with incline (Table 1;

283   Fig. 7C). On the vertical surface α had the smallest values when peg spacing was smallest

284   (41$\pm$2.6 deg), and α increased asymptotically with increased spacing up to a maximum value of

285   nearly 90 deg (89$\pm$1.4 deg). By contrast, for the horizontal surfaces the values of α had little

286   regular variation with peg spacing, and the grand mean was 36 deg (Fig. 7C). For both inclines,

287   half wavelength had similarly small values for the smallest peg spacing (Fig. 7B). Half

288   wavelength was often greater for locomotion on the vertical surface (Fig. 7B), but incline did not

289   have a statistically significant effect on this variable (Table 1). The values of x_{crest} were usually

290   near 0.5, indicating many of the half waves were fairly symmetrical (Fig. 7A). However, for the

291   largest peg spacing and the vertical surface, the mean value of x_{crest} (0.73$\pm$0.08) indicated that the

292   crests of the waves were often shifted posteriorly and in the downhill direction (Fig. 3B). The

293   head-to-tail distance lacked variation that was clearly significant (Table 1), but x_{ht} was often

294   greatest with the smallest peg spacing and slightly smaller for the vertical compared to the

295   horizontal surfaces (Fig. 7D).

296

297   **DISCUSSION**

298   The inclines of surfaces and the spacing of branches are two features of natural arboreal

299   habitats that vary considerably (Mattingly and Jayne, 2004), and the brown treesnakes in Guam

300   that we observed and collected moved on branches with a wide range of both slopes (from

301   horizontal to vertical) and spacing between secondary branches. Both slope and branch spacing

302   had widespread effects on the locomotion of the snakes in our study. However, peg spacing had

303   more pervasive effects on the kinematics of the snakes than incline. The interactive effects of

304   peg spacing and incline on the locomotor performance (Fig. 4) and body angle (Fig. 7C) of

305   brown treesnakes were especially striking. Two unexpected findings were the apparent ease with

306   which snakes moved when touching only a single peg and the lack of a significant effect of peg

307   spacing on the locomotor performance of snakes climbing up the vertical surface.

308   Key features of environmental structure are the coarseness and regularity of how objects

309   are spatially distributed, but compared to some resources such as food, data are limited for the

310   effects of the spatial distribution of the objects involved in the locomotion of animals (Kelley et

311   al., 1997; Mattingly and Jayne, 2004; Goodman, 2009). Variation in the size, orientation, and

312   spacing of branches in arboreal habitats (Mattingly and Jayne, 2004) creates an interesting

313   amount of complexity, and all of these attributes of branches can be readily simulated. However,

314   previous experimental manipulations of branch spacing to determine effects on locomotion are

315   limited to only a few arboreal limbed vertebrates such as anole lizards (Jones and Jayne, 2012)

316   and mice (Hyams et al., 2012). For the arboreal locomotion of snakes, the presence or absence

317   of pegs has been manipulated (Astley and Jayne, 2009; Jayne and Herrmann, 2011), but our

318   study is the first to manipulate peg spacing. Consequently, most previous insights regarding the

319   effects of peg spacing on snake locomotion must be derived from experiments using variable peg

320   spacing for snakes crawling horizontally on planar surfaces (Gray and Lissmann, 1950;

321   Heckrotte, 1967; Bennet et al., 1974; Jayne, 1986; Kelley et al., 1997).

322   The effects of objects such as pegs and secondary branches on locomotion depend on the

323   body plan of the animal. For limbed animals moving horizontally, vertically oriented objects

324   such as pegs impede locomotion unless the animal has sufficient space to detour around such

325   objects without taking an overly circuitous route (Hyams et al., 2012; Jones and Jayne, 2012).

326   By contrast, peg-like objects that are perpendicular to the surface supporting the ventral surface

327   of the animal can facilitate the lateral undulation of limbless animals such as snakes by providing

328   suitably oriented surfaces for applying posterior-laterally directed forces to generate thrust (Gray

329   and Lissmann, 1950). At one extreme, pegs spaced less than a body width could also create a

330   barrier for a limbless animal, and at the other extreme, pegs more than a body length apart could

331   not be used continuously to generate propulsive forces for undulatory locomotion. However,

332   additional variation in peg spacing between these extremes can also affect snake locomotion.

333   A long-standing suggestion has been that three lateral points of force application are

334   needed for terrestrial lateral undulation (Gans, 1974). The body posture and performance of

335   snakes in this study moving past a single peg may suggest otherwise, but regions of the body

336   other than the location touching the peg may help to obtain the balance of forces necessary for

337   stable forward motion, especially when such regions touching the cylinder are flexed ventrally.

338   However, additional circumstances have been described when snakes may use lateral undulation

339   without any obvious points of lateral contact such as when boa constrictors move on very

340   slender, cylindrical, horizontal surfaces (Jayne and Herrmann, 2011) or when rat snakes move on

341   very smooth planar surfaces (Hu et al., 2009).

342   Our study and some previous studies of terrestrial snake locomotion found large effects

343   of peg spacing on performance (maximal speed). For example, juvenile garter snakes crawling

344   through arrays of pegs on a flat surface have mean speeds with a peg spacing of 8% TL that are

345   approximately 50% and 25% greater than those for peg spacings of 21% TL and 6% TL,

346   respectively (Kelley et al., 1997). For peg spacing from 5-18% TL (Jayne, 1986) rat snakes and

347   water snakes are fastest with the largest peg spacing. For another species of North American rat

348   snake, the speed of locomotion with a peg spacing of 19% TL exceeds that for a spacing of 43%

349   TL (Bennet et al., 1974). Thus, peg spacing from approximately 10-20% TL often appears well

350   suited for snakes to attain high speeds of terrestrial lateral undulation. In our study peg spacing

351   ranged from a mean value of $7\pm0.1\%$ TL to $57\pm1\%$ TL, and the maximal value of speed for

352   brown treesnakes on the horizontal surfaces, which occurred with a spacing of $28\pm0.5\%$ TL, was

353   nearly twice the values for both the smallest and largest peg spacing. Similar to some of the

354   previous studies of terrestrial lateral undulation through arrays of pegs with a limited range of

355   spacing (Bennet et al., 1974; Jayne, 1986), our results would have suggested that speed simply

356   increases with increased peg spacing rather than being optimized at an intermediate value if we

357   had not included the largest peg spacing.

358   In addition to affecting speed, peg spacing also can affect the number of waves,

359   waveform, body posture, and number of pegs touched by undulating snakes. For example, the

360   number of undulations decreases with increased distance between pegs for both garter snakes

361   (Kelley et al., 1997) and the brown treesnakes in our study. For the peg spacing that maximizes

362   speed, both garter snakes and brown treesnakes commonly have four to six half waves. Previous

363   illustrations of terrestrial locomotion show that the angle of the body of snakes (α) often

364   increases with increased distance between pegs (Gray and Lissmann, 1950, Fig. 6; Kelly et.,

365   1997, Fig. 5), and we observed this for the uphill locomotion of the brown treesnakes but not for

366   their horizontal locomotion (Figs 3, 7A). The number of pegs touched by the snakes in our study

367   decreased more rapidly with increased peg distance than the number of crossing regions (Fig. 4).

368   Consequently, even though the brown treesnakes touched an average of only two pegs for the

369   conditions that maximized speed on the horizontal surface, nearly half of the nodes between

370   undulations were not associated with a peg. Unfortunately, previous studies of locomotor

371   performance for snakes crawling though arrays of pegs have not systematically quantified the

372   number of pegs touched.

373   The effects of peg spacing on lateral displacement (wave amplitude) differ for the

374   terrestrial lateral undulation of garter snakes compared to the arboreal undulation of brown

375   treesnakes. With increased peg distance the amplitude of lateral undulations of garter snakes

376   nearly doubles (Kelley et al., 1997, Fig. 5), whereas that of the brown treesnakes was nearly

377   constant for all but the smallest peg spacing used in our experiments (Fig. 6C). The large

378   increase in wave amplitude for garter snakes results in their head-to-tail distance decreasing from

379   approximately 70% TL to 50% TL with increased peg distance, whereas that of the brown

380   treesnakes was usually larger and nearly constant (~80% TL). Increased wave amplitude usually

381   correlates well with increased wavelength both for undulations of a snake in different

382   environments and for undulations at different longitudinal locations within the snake in a given

383   environment (Gray and Lissmann, 1950; Jayne, 1985; Jayne and Davis, 1991; Kelley et al.,

384   1997), whereas the small wave amplitudes that we observed for the arboreal locomotion of the

385   brown treesnakes were nearly independent of wavelength (Figs 3A, 6C). Such small wave

386   amplitudes could reduce the tendency for long-axis rolling during horizontal locomotion by

387   decreasing the amount of unsupported weight and decreasing the length of the lever arm that

388   contributes to the rolling torque acting on each half wave. For a given peg spacing, the brown

389   treesnakes also had undulations with very similar shape along the lengths of their bodies when

390   moving horizontally, and if the rolling torques acting on each undulation to the left and right are

391   equal, then maintaining balance should be easier.

392   Variable body orientations and numbers of contact points can have important

393   consequences for the orientation and magnitude of forces that are relevant for undulatory

394   propulsion (Gray and Lissmann, 1950). For the snakes in our study, the values of body angle, α,

395   can provide a good estimate of the orientation of the reaction forces arising from pressing against

396   the peg after making a correction for the frictional resistance between the snake and the peg (Fig.

397   8). For the surfaces that we used, the coefficient of static friction for snake skin is 0.28 (Astley

398   and Jayne, 2007), which would cause a difference of approximately 16 deg between the

399   orientation of the normal force and the resultant reaction force (Fig. 8). Sliding friction is less

400   than static friction, which would reduce the value of this correction. The grand mean of α for

401   brown treesnakes moving on the horizontal surfaces was 36 deg, which suggests that the angles

402   of the reaction forces against the pegs commonly approximated 45 deg and thus the ratio of the

403   magnitudes of the forward and lateral components of force should be approximately 1:1 (Fig. 8).

404   The only experimental data for the reaction forces of snakes performing lateral

405   undulation past a series of pegs are those of (Gray and Lissmann, 1950), who observed a snake

406   crawling on planar horizontal surfaces for five treatments with a range of one to nine contact

407   points. The spacing between successive contact points (pendulums) for these treatments with

408   two, three, and four contact points was approximately 17% TL, whereas that of the treatment

409   with nine pendulums was approximately one-half that value. From the least to most contact

410   points, the ratios of the total lateral to longitudinal components of the reaction force were 0, 0.12,

411   0.94, 1.39 and 2.27, respectively, and these values correspond to average angles of the reaction

412   force relative to the direction of forward travel of approximately 0, 7, 43, 54 and 66 deg,

413   respectively. The corresponding average values of body angle thus ranged from approximately

414   90 deg for treatment with a single pendulum to less than 30 for the largest number of contact

415   points. Thus, even when the spacing between contact points was constant, the ratio of sideways

416   to forward forces increased with increased number of contact points, and the two treatments with

417   the most contact points support the conclusion that the ratio of lateral to forward components of

418   forces also increases with decreased distance between peg-like contact points. Although forces

419   were quantified in this study (Gray and Lissmann, 1950), neither speed nor locomotor mode was

420   determined.

421   Variation in the ratio of the lateral- to forward-directed reaction forces seems unlikely to

422   account for all of the differences in performance that we observed for the brown treesnakes

423   moving on the horizontal surfaces because α was nearly constant regardless of peg spacing (Fig.

424   7C). Perhaps the greater number of changes in the direction of momentum associated with a

425   greater number of undulations contributed to decreased speed when the space between pegs was

426   small. Unlike pegs protruding from a planar surface, pegs on a cylindrical surface seem likely to

427   have the dual functions of providing sites for generating propulsive force as well as preventing

428   long-axis roll of the snake about the cylinder supporting its weight. Large values of α could

429   enhance the ratio of propulsive to lateral force, but the nearly constant body orientation of

430   approximately 45 deg at the pegs on the horizontal cylinder further suggests the primacy of

431   maintaining balance rather than generating thrust on these surfaces. Consequently, the decrease

432   in performance when moving on the surface with the largest distance between pegs may have

433   arisen primarily from the difficulties associated with maintaining balance.

434   Several factors suggest that the constraints on speed of the snakes climbing vertically

435   differ substantially from those for the snakes moving horizontally. For example, when climbing

436   vertical cylinders, the direction of the weight vectors for alternating loops of the snakes are

437   parallel to the cylinder and thus should not contribute directly to long-axis rolling. Indeed, we

438   observed very little long-axis rolling for snakes in these circumstances despite the snakes having

439   consistently larger values of lateral displacement than were observed for horizontal locomotion

440   (Fig. 6C). Despite little apparent need to counteract long-axis rolling, the maximal speeds during

441   vertical climbing were slower than those for the horizontal surface, and the maximal vertical

442   speeds lacked any clear pattern of change with peg spacing. The large values of α for snakes

443   crawling vertically while touching only one or two pegs (Figs. 3B, 7C) should reduce the

444   magnitude of laterally directed forces, and this may have increased the ease of dealing with these

445   forces and helped to prevent a decrease in performance similar to that which occurred for the

446   largest peg spacing when snakes crawled horizontally. The ventral curvature of the snake

447   interacting with the underlying cylinder provides another potential mechanism for balancing left

448   and right lateral forces in addition to modulating the orientation of the body at the pegs.

449   Some additional effects of body weight also differ for snakes climbing vertically versus

450   those moving horizontally. For example, snakes climbing vertically must apply sufficient force

451   to lift their entire weight, **W**, whereas the weight of snakes moving horizontally produces a

452 ventral frictional resistance equal to μ**W**, where μ is the coefficient of friction (Gray and

453 Lissmann, 1950). Snakes climbing vertically should have a negligible amount of ventral friction.

454 For both vertical and horizontal lateral undulation, the snakes will also encounter a lateral

455 frictional resistance where the body contacts the pegs, and this should be greater for vertical

456 movement since the snakes should have to press harder against the pegs to overcome their

457 weight rather than primarily the (lesser) ventral frictional resistance when the snake moves

458 horizontally. Consequently, the force required to move vertically should exceed that required to

459 move horizontally by more than (1-μ)**W**. Thus, generating propulsive force seems more likely to

460 limit vertical climbing speed, whereas horizontal arboreal lateral undulation may be limited more

461 by factors involving coordination and balance.

462   The different body orientations that we observed for snakes climbing vertically could

463 result from the snakes actively controlling their body posture to optimize the orientations of

464 forces, or the increased values of α (Fig. 3B) could also arise from greater amounts of passive

465 bending resulting from fewer pegs being used to generate the same total amount of propulsive

466 force. Most vertebrates with elongated body plans have acquired them via increased numbers of

467 body segments (Ward and Mehta, 2011), and if the mobility per joint is constant, then an

468 increased number of joints per unit length can enhance the flexibility of the body. Snakes are

469 certainly a noteworthy group for having both large numbers of vertebrae (Hoffstetter and Gasc,

470 1969) and an ability to bend readily, but variation in the morphology of muscles is also likely to

471 have consequences for axial flexibility and how it is controlled. For example, the numbers of

472 vertebrae spanned and lengths of tendon relative to the lengths of contractile tissue within certain

473 major axial muscles segments of specialized arboreal colubroid snakes, such as the species we

474 studied, are greater than those for nearly all groups of snakes that are not arboreal (Jayne, 1982).

475 Not only is tendon stiff, but when there are greater proportions of tendon in individual axial

476 muscle segments, a greater amount of length change in the contractile tissue will be required to

477 accommodate the same amount of axial banding as in a different species with a higher proportion

478 of contractile tissue per axial muscle segment. Hence, this morphological specialization may

479 facilitate enhancing axial stiffness, which may in turn facilitate supporting the body of arboreal

480 snakes in between branches (Hoefer and Jayne, 2013) or be well suited for moving using a

481 minimal number of contact points (Ruben, 1977). Such long tendons also might enhance the

482   ability of these snakes to cope with the large and very localized loads that occur when they climb

483   vertically using undulation past only one or two points of support.

484   Many aspects of the shape of the brown treesnakes did change with peg spacing, but

485   some details of waveform and body shape did not conform strictly to either peg spacing or some

486   other features of the supporting surfaces. For example, with the smallest peg spacing the snakes

487   commonly did not contact each successive peg as they often used a larger waveform than would

488   be possible if they did this (Fig. 3). Similarly, the ventral curvature of the snakes was usually far

489   less than the tight conformity to the circumference of a cylindrical surface that is probably

490   required to generate an effective frictional grip, as has been described for arboreal concertina

491   locomotion (Astley and Jayne, 2007; Jayne and Herrmann, 2011). Nonetheless, the amount of

492   ventral curvature and total dorso-ventral displacement that we observed for arboreal lateral

493   undulation does seem likely to exceed that which occurs during lateral undulation on natural

494   terrestrial surfaces even though many of them can deviate substantially from a simple horizontal

495   plane.

496   The extent to which the ventral curvature of snakes on arboreal surfaces is caused

497   actively or passively poses an interesting area for future work. Ventral curvature when the

498   snakes climbed vertical surfaces seems quite likely to be caused by active ventral flexion of the

499   snake, but for snakes moving horizontally on a cylindrical surface ventral curvature could be

500   caused solely by the weight of the snake. In this latter case activity of the most dorsal muscles

501   might occur bilaterally to prevent or modulate the amount of sagging in a fashion similar to how

502   these muscles arch the back of snakes during sidewinding locomotion with a motor pattern that

503   deviates from that of terrestrial lateral undulation (Jayne, 1988). Modulating the depth and width

504   of the waves of snakes while on cylindrical surfaces could also serve as an important mechanism

505   for enhancing the stability arboreal snake locomotion. Hence, all of these factors could cause

506   arboreal lateral undulation to deviate substantially from some of the major features of terrestrial

507   lateral undulation while retaining many of the kinematic similarities including a posteriorly

508   propagated wave, all points following a similar path and continuous sliding contact.

509   Many studies have quantified maximum speeds of diverse species of animals (Irschick

510   and Garland, 2001). However, much remains to be learned regarding what factors generally

511   limit speed and the extent to which the constraints on speed vary with physiology, animal body

512   plan, environmental structure, and interactions between these factors. For example, under some

513   conditions the limbs of some small lizards have stride frequencies close to those predicted based

514   on the time course of force development and relaxation of a single muscle twitch (Marsh and

515   Bennett, 1985), but the frequencies of undulation of snakes observed in this and other studies are

516   so slow that this factor is irrelevant to their maximal locomotor speeds. Our results also provide

517   a striking example of how different aspects of environmental structure can have interactive

518   effects on locomotor performance and can create some circumstances where force production

519   may limit speed and other circumstances where balance and coordination may be the primary

520   constraints on performance. Although the need for balance may seem most obvious for animals

521   moving on narrow branches, investigating the rich complexity of additional natural habitats

522   (Tucker and McBrayer, 2012) seems likely to hold much additional promise for identifying

523   factors besides muscle force production (Higham et al., 2011) that may limit locomotor

524   performance.

525

526

536

REFERENCES

Anderson, N. L., Hetherington, T. E., Coupe, B., Perry, G., Williams, J. B. and Lehman, J. (2005). Thermoregulation in a nocturnal, tropical, arboreal snake. *J. Herpetol.* **39**, 82-90.

Astley, H. C. and Jayne, B. C. (2007). Effects of perch diameter and incline on the kinematics, performance and modes of arboreal locomotion of corn snakes (*Elaphe guttata*). *J. Exp. Biol.* **210**, 3862-3872.

Astley, H. C. and Jayne, B. C. (2009). Arboreal habitat structure affects the performance and modes of locomotion of corn snakes (*Elaphe guttata*). *J. Exp. Zool.* **311A**, 207-216.

Bennet, S., McConnel.T and Trubatch, S. L. (1974). Quantitative-analysis of speed of snakes as a function of peg spacing. *J. Exp. Biol.* **60**, 161-165.

Gans, C. (1962). Terrestrial locomotion without limbs. *Am. Zool.* **2**, 167-182.

Gans, C. (1974). *Biomechanics. An Approach to Vertebrate Biology*. Ann Arbor: University of Michigan Press.

Gasc, J. P., Cattaert, D., Chaserat, C. and Clarac, F. (1989). Propulsive action of a snake pushing against a single site: its combined analysis. *J. Morphol.* **201**, 315-329.

Goodman, B. A. (2009). Nowhere to run: the role of habitat openness and refuge use in defining patterns of morphological and performance evolution in tropical lizards. *J. Evol. Biol.* **22**, 1535-1544.

Gray, J. (1946). The mechanism of locomotion in snakes. *J. Exp. Biol.* **23**, 101-120.

Gray, J. (1968). *Animal Locomotion*. London: Weidenfield and Nicolson.

Gray, J. and Lissmann, H. W. (1950). The kinetics of locomotion of the grass snake. *J. Exp. Biol.* **94**, 15-42.

Hampton, P. M. (2011). Ventral and sub-caudal scale counts are associated with macrohabitat use and tail specialization in viperid snakes. *Evol. Ecol.* **25**, 531-546.

Heckrotte, C. (1967). Relations of body temperature, size, and crawling speed of the common garter snake, *Thamnophis s. sirtalis*. *Copeia* **1967**, 759-763.

Higham, T. E., Korchari, P. G. and McBrayer, L. D. (2011). How muscles define maximum running performance in lizards: an analysis using swing- and stance-phase muscles. *J. Exp. Biol.* **214**, 1685-1691.

Hoefer, K. M. and Jayne, B. C. (2013). Three-dimensional locations of destinations have species-dependent effects on the choice of paths and the gap-bridging performance of arboreal snakes. *J. Exp. Zool. A* **319**, 124-137.

Hoffstetter, R. and Gasc, J.-P. (1969). Vertebrae and ribs of modern reptiles. In *Biology of the Reptilia. Vol. 1.*, (ed. C. Gans, T. S. Parsons and A. A. Bellairs), pp. 201-310. New York: Academic Press.

Hu, D. L., Nirody, J., Scott, T. and Shelley, M. J. (2009). The mechanics of slithering locomotion. *Proc. Natl. Acad. Sci. USA* **106**, 10081-10085.

Hyams, S. E., Jayne, B. C. and Cameron, G. N. (2012). Arboreal habitat structure affects locomotor speed and perch choice of white-footed mice (*Peromyscus leucopus*). *J. Exp. Zool. A* **317A**, 540-551.

Irschick, D. J. and Garland, T. J. (2001). Integrating function and ecology in studies of adaptation: Investigations of locomotor capacity as a model system. *Ann. Rev. Ecol. Syst.* **32**, 367-396.

Jayne, B. C. (1982). Comparative morphology of the semispinalis-spinalis muscle of snakes and correlations with locomotion and constriction. *J. Morphol.* **172**, 83-96.

583 **Jayne, B. C.** (1985). Swimming in constricting (*Elaphe g. guttata*) and nonconstricting (*Nerodia*
584   *fasciata pictiventris*) colubrid snakes. *Copeia* **1985**, 195-208.
585 **Jayne, B. C.** (1986). Kinematics of terrestrial snake locomotion. *Copeia* **1986**, 195-208.
586 **Jayne, B. C.** (1988). Muscular mechanisms of snake locomotion: an electromyographic study of
587   the sidewinding and concertina modes of *Crotalus cerastes*, *Nerodia fasciata* and *Elaphe*
588   *obsoleta*. *J. Exp. Biol.* **140**, 1-33.
589 **Jayne, B. C. and Davis, J. D.** (1991). Kinematics and performance capacity for the concertina
590   locomotion of a snake (*Coluber constrictor*). *J. Exp. Biol.* **156**, 539-556.
591 **Jayne, B. C. and Herrmann, M. P.** (2011). Perch size and structure have species-dependent
592   effects on the arboreal locomotion of rat snakes and boa constrictors. *J. Exp. Biol.* **214**,
593   2189-2201.
594 **Jones, Z. M. and Jayne, B. C.** (2012). Perch diameter and secondary branching have interactive
595   effects on the locomotion of anole lizards. *J. Exp. Biol.* **215**, 2128-2134.
596 **Kelley, K. C., Arnold, S. J. and Glatstone, J.** (1997). The effects of substrate and vertebral
597   number on locomotion in the garter snake *Thamnophis elegans*. *Func. Ecol.* **11**, 189-198.
598 **Lillywhite, H. B. and Henderson, R. W.** (1993). Behavioral and functional ecology of arboreal
599   snakes. In *Snakes - Ecology and Behavior*, (ed. R. A. Seigel and J. T. Collins), pp. 1-48.
600   New York: McGraw Hill Inc.
601 **Marsh, R. L. and Bennett, A. F.** (1985). Thermal dependence of isotonic contractile properties
602   of skeletal muscle and sprint performance of the lizard *Dipsosaurus dorsalis*. *J. Comp.*
603   *Physiol. (B)* **155**, 541-551.
604 **Mattingly, W. B. and Jayne, B. C.** (2004). Resource use in arboreal habitats: Structure affects
605   locomotion of four ecomorphs of *Anolis* lizards. *Ecology* **85**, 1111-1124.
606 **Moon, B. R. and Gans, C.** (1998). Kinematics, muscular activity and propulsion in gopher
607   snakes. *J. Exp. Biol.* **201**, 2669-2684.
608 **Moran, M. D.** (2003). Arguments for rejecting the sequential Bonferroni ecological studies.
609   *Oikos* **100**, 403-405.
610 **Pizzatto, L., Almeida-Santos, S. M. and Shine, R.** (2007). Life-history adaptations to
611   arboreality in snakes. *Ecology* **88**, 359-366.
612 **Ruben, J. A.** (1977). Morphological correlates of predatory modes in the coachwhip
613   (*Masticophis flagellum*) and rosy boa (*Lichanura roseofusca*). *Herpetologica* **33**, 1-6.
614 **Tucker, D. B. and McBrayer, L. D.** (2012). Overcoming obstacles: the effect of obstacles on
615   locomotor performance and behaviour. *Biol. J. Linn. Soc.* **107**, 813-823.
616 **Ward, A. B. and Mehta, R. S.** (2011). Axial elongation in fishes: using morphological
617   approaches to elucidate developmental mechanisms in studying body shape. *Integr.*
618   *Comp. Biol* **50**, 1106-1119.
619
620

621

622 **FIGURE LEGENDS**

623 Fig. 1. Schematic illustration of methods used for analyzing kinematics. The gray rectangle

624 represents the primary cylindrical surface of the perch. Black, green, and red circles indicate

625 pegs that were not touched by the snakes, pegs that were touched and useful for propulsion, or

626 pegs that were touched but not likely to be useful for propulsion, respectively. In this example,

627 the number of crossing regions (N_{cr}) was 5, and the numbers of propulsive (N_{peg_prop}) and non-

628 propulsive pegs ($N_{peg\_nonprop}$) touched were 3 and 1, respectively. Each white wedge indicates

629 the angle (α) between the tangent of the body of the snake at a propulsive peg and the x-axis.

630 The overall length of the snake from head to tail along the x-axis is x_{ht}. For each half wave, the

631 length along the x-axis (0.5λ) and the lateral amplitude (y_{crest}) were recorded. The maximum

632 width of the snake (y_{max}) was the sum of the greatest magnitudes of y_{crest} for half waves to the

633 left and right. Values of x_{crest} were expressed as a proportion of 0.5λ.

634

635 Fig. 2. Tracings from dorsal-view video images of a single snake performing lateral undulation

636 on surfaces with different inclines and peg spacing. (A). A horizontal surface with pegs every

637 10 cm. (B) Locomotion up a vertical surface with pegs every 80 cm. The times between

638 successive images in A and B were 0.3 and 1.2 s, respectively. This snake had SVL and total

639 length of 108 and 137 cm, respectively. The white marks on the snake indicate locations of

640 approximately 25, 50, 75 and 100% SVL.

641

642 Fig. 3. Tracings from dorsal-view video images showing the body posture of a single snake

643 while crawling on all ten combinations of incline and peg spacing that were used to test

644 performance. The small white circles represent the pegs. The number of waves increased with

645 increased numbers of pegs, and the waveforms on the horizontal surfaces are often more

646 regular and symmetric than those when the snake was climbing the vertical surface.

647

648 Fig. 4. The effects of peg spacing and incline on the mean values of locomotor performance. N

649 = 9 individuals. The black circles and red triangles indicate mean values for the horizontal and

650 uphill vertical treatment, respectively. For the horizontal surface an intermediate peg spacing

651 maximized performance, whereas peg spacing lacked a significant effect on performance for

652 snakes crawling up the vertical surface.

653

654 Fig. 5. The effects of peg spacing and incline on the mean values involving contact points

655 between the snake and the surfaces. $N = 5$ individuals. The black circles and red triangles

656 indicate mean values for the horizontal and uphill vertical treatment, respectively. (A) The

657 number of crossing regions. (B) The total number of pegs touched by the snake. (C) The

658 number of propulsive pegs touched. (D) The number of non-propulsive pegs touched. When

659 the distance between successive pegs was large, the snakes were more likely to cross the

660 primary supporting surface without touching a peg ($N_{cr} > N_{peg\_all}$). When snakes crawled up the

661 vertical surface, the pegs touched by snakes were almost always located posterior to the body

662 of the snake, whereas snakes crawling on horizontal surfaces with the smallest distance

663 between pegs often touched one or two pegs anterior to the body.

664

665

666 Fig. 6. The effects of peg spacing and incline on the mean values of lateral and vertical location

667 of wave crests. $N = 5$ individuals. The black circles and red triangles indicate mean values for

668 the horizontal and uphill vertical treatment, respectively. (A) The maximum width of the snake.

669 Snakes climbing up vertical surfaces often used more lateral space. (B) The maximum depth of

670 a wave crest. (C) The lateral amplitude of half waves was greater for vertical locomotion. (D)

671 The depth of half waves. The depth of half waves of snakes crawling up the vertical surface

672 was greater than that of the horizontal surface for the largest distance between pegs, and for

673 horizontal locomotion the greatest mean value coincided with the conditions that maximized

674 performance.

675

676 Fig. 7. The effects of peg spacing and incline on the mean values of waveform and overall

677 posture. $N = 5$ individuals. The black circles and red triangles indicate mean values for the

678 horizontal and uphill vertical treatment, respectively. (A) Wave crest location. Asymmetric half

679 waves were most common for snakes crawling up the vertical surface with the largest distance

680 between pegs. (B) Half wave length. (C) The angle (α) of the body of the snake at the point of

681 contact with the pegs. For snakes climbing up the vertical surface, α increased asymptotically

682 to a value of 90 deg as peg spacing increased, whereas peg spacing for the horizontal surface

683 had little effect on α. (D) The head-to-tail distance of the snakes.

684

685 Fig. 8. A schematic diagram of the orientation of forces acting on a peg. The light gray

686 rectangle represents the body of the snake oriented relative to the peg (dark gray circle) with the

687 value of the grand mean of α observed for all peg spacings for the horizontal surface. The red

688 arrows show how a given normal force applied by the snake against a peg generates a frictional

689 force that opposes the movement past the peg, and the resultant sum of these two vectors is

690   oriented posterio-medially (Gray and Lissmann, 1950). The black arrows indicate the

691   corresponding reaction forces with a resultant that is oriented anterio-medially, and black

692   dashed arrows indicate the magnitudes of the forward (propulsive) and sideways components of

693   the reaction force in this example are approximately equal. Note that frictional resistance

694   against the peg causes the resultant reaction force to be oriented closer to the overall direction

695   of travel (~ 16 deg) compared to the orientation of the normal reaction force.

696

697   Table 1. Effects of peg spacing and incline on kinematics

Dependent variable	ANOVA effect		
	Spacing (d.f. = 4, 16)	Incline (d.f. = 1, 4)	Spacing x Incline (d.f. = 4, 16)
N_{cr}	8.9 (0.001)	35.8 (0.004)	1.6 (0.225)
N_{peg_all}	97.6 (<0.001)	4 (0.115)	2.2 (0.11)
N_{peg_prop}	126.2 (<0.001)	1.4 (0.306)	1 (0.455)
$N_{peg\_nonprop}$	4.3 (0.015)	8.7 (0.042)	3.7 (0.025)
y_{crest}	3.8 (0.024)	77.8 (0.001)	0.2 (0.948)
z_{crest}	7.9 (0.001)	10 (0.034)	1.6 (0.223)
y_{max}	5.9 (0.004)	48.3 (0.002)	0.1 (0.991)
z_{max}	5.8 (0.004)	0.1 (0.803)	1 (0.448)
0.5λ	3.1 (0.044)	6.3 (0.066)	0.9 (0.508)
x_{crest}	2.4 (0.098)	11.3 (0.028)	2.9 (0.055)
α	49.4 (<0.001)	473.5 (<0.001)	9.9 (<0.001)
x_{ht}	3.2 (0.043)	7.2 (0.055)	0.2 (0.926)

Values are F (P). Abbreviations: N_{cr}, number of crossing regions. N_{peg_all}, total number of pegs touched by the snake. N_{peg_prop}, number of propulsive pegs touched by a snake. $N_{peg\_nonprop}$, number of non-propulsive pegs touched by a snake. y_{crest}, lateral amplitude of a half wave. z_{crest}, depth of a half wave. y_{max}, maximum width of a snake. z_{max}, maximum depth of half waves. 0.5λ, half wave length. x_{crest}, location of wave crest. α, angle of snake body at point of peg contact. x_{ht}, head-to-tail distance.
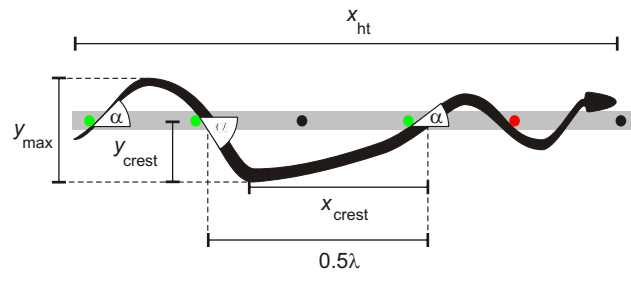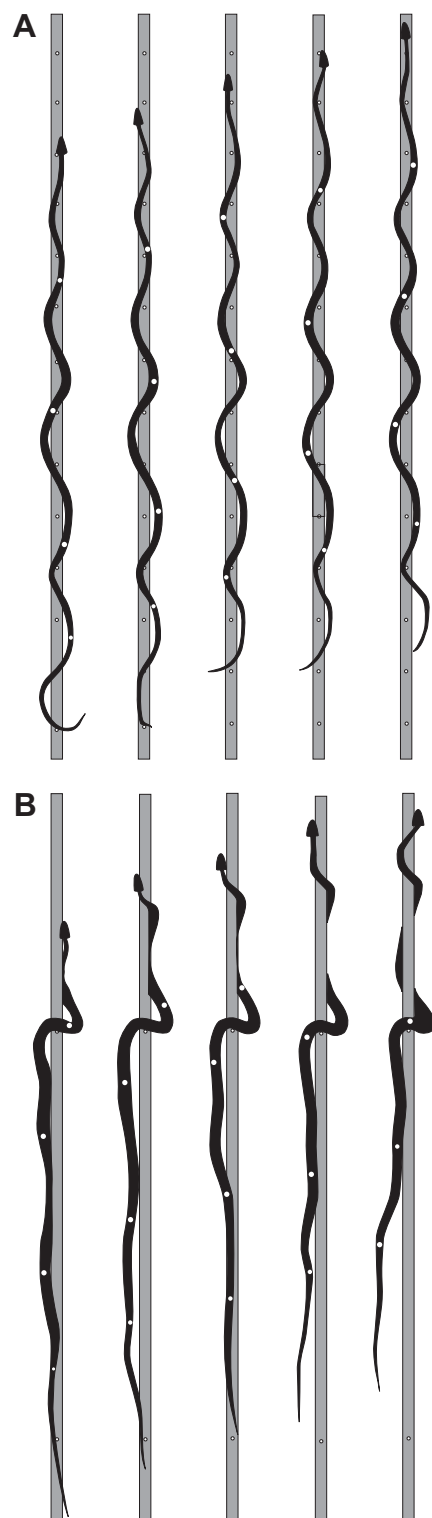
698

699

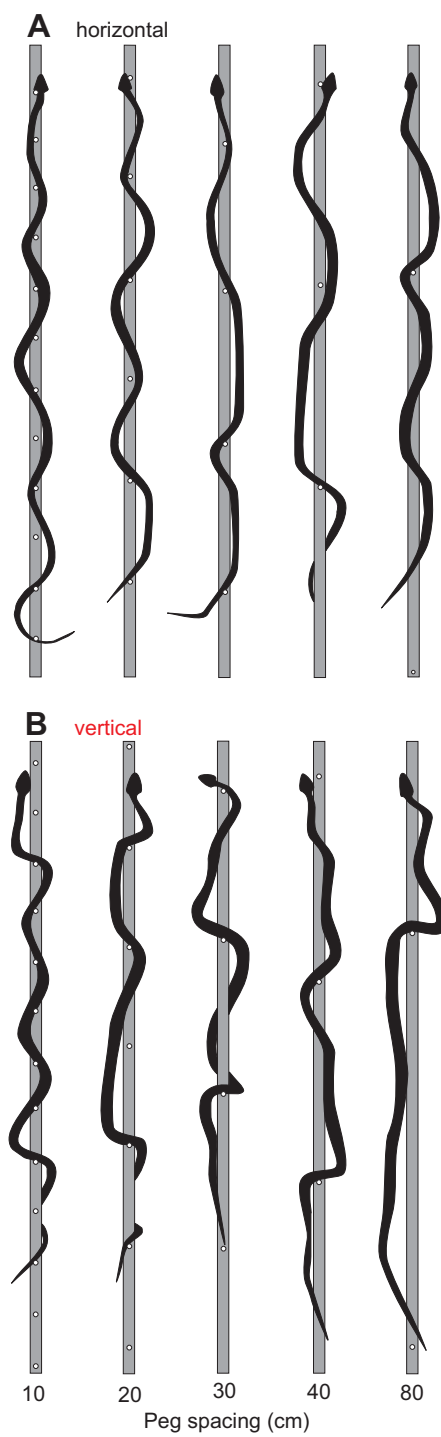700

Fig. 1

Fig. 2

Fig. 3

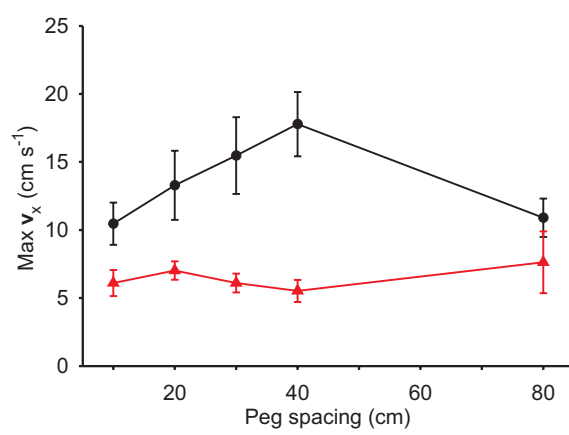**A** horizontal

B vertical

10   20   30   40   80

Peg spacing (cm)

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8