The effects of substrate and vertebral number on locomotion in the garter snake *Thamnophis elegans*

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Summary

1. Locomotor performance of limbless vertebrates depends on the substrate through which individuals move and may result in selection on vertebral number in different habitats. To evaluate the effect of push-point density on snake locomotion, the density of vegetation and other potential push-points was quantified at two sites in California (coastal and inland), where conspecific snakes differed greatly in vertebral number (230 and 256 average total vertebrae, respectively; Arnold 1988). The coastal site had significantly higher push-point densities than the inland site.

2. Five experimental push-point densities that fell within the natural range of push-point densities were employed in laboratory trials of juvenile snake locomotion. Density of push-points significantly affected both crawling speed and head-to-tail distance (HTD), an indirect measure of lateral bending. The fastest speed was achieved at an intermediate push-point density. The shortest HTD occurred when snakes moved through the lowest push-point density.

3. Sex, total number of vertebrae and total length significantly affected HTD, regardless of push-point density. Snakes with relatively more vertebrae had a shorter HTD, suggesting they were able to achieve greater lateral bending than snakes with fewer vertebrae. Coastal and inland populations did not differ in HTD during locomotion.

4. Numbers of body and tail vertebrae significantly influenced speed at different push-point densities. In general, snakes with more body vertebrae were slower than those with fewer, while snakes with more tail vertebrae were faster than those with fewer. Snakes of greater total length were faster at all densities. Coastal snakes crawled faster than inland snakes at all push-point densities.

Key-words: Crawling speed, ecomorphology, lateral bending, population variation

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Introduction

The adaptive significance of differences in snake vertebral numbers is an unsolved problem in ecological morphology. Snakes show an extraordinary radiation in vertebral numbers. Within advanced snakes of the family Colubridae, for example, the number of body vertebrae ranges from less than 100 to over 300 (Marx & Rabb 1972). Vertebral counts have been used by snake systematists since the time of Linnaeus because coefficients of variation are small within populations (typically only a few per cent), but geographical races or closely related species can differ by two or more phenotypic standard deviations. These same characteristics suggest that vertebral number is an adaptation to some feature of the environment.

Hints about selective agents come from studies of vertebral number within and among species. That selection acts on the trait is suggested by the observation that samples of adult snakes show less variation in vertebral number than samples of juveniles (Dunn 1942; Inger 1942, 1943). Furthermore, growth rate in the field, a fitness correlate, has been related to vertebral numbers in longitudinal studies (Arnold 1988; Lindell, Forsman & Merilä 1993). That selection on vertebral number is related to some aspect of the environment is indicated by studies relating trait variation among populations to habitat. In southern California, several species of snakes show parallel clines in numbers of body vertebrae: coastal populations have fewer vertebrae than inland populations (Klauber 1941). Among colubrids, species with few body vertebrae are generally aquatic or semi-fossorial, whereas species with many vertebrae are generally arboreal or terrestrial (Marx & Rabb 1972; Jayne 1982; Lindell

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1994). That selection on vertebral number is related to whole animal performance is suggested by studies relating trait variation within populations to locomotion. Garter snakes (*Thamnophis*) with relatively many (or few) body and tail vertebrae (Arnold & Bennett 1988) and with intermediate values of relative tail length (Jayne & Bennett 1989) show maximal crawling speed. To see the common thread in these results, we need an approach that links variation in vertebral number and fitness.

Studies of snake locomotion show that the vertebral column is part of a functional complex that generates propulsive forces (Gray 1946; Gans 1986; Gasc *et al.* 1989). Because individual vertebrae are coupled to the ventral scales via ribs, connective tissue and muscles, as well as to one another, they form an interactive unit that is responsible for locomotion (Mosauer 1935; Gray & Lissmann 1950; Gray 1953; Gasc 1967, 1974; Jayne 1982, 1988a,b). In the dominant mode of snake locomotion, terrestrial lateral undulation, forward movement is achieved when internally generated lateral flexion exposes the snake to external forces that act normal to the surface of its body and are equal, but opposite, to the forces of friction (Gray 1953). In this undulatory propulsion, the waves of lateral flexion are generated primarily in the anterior region of the body in association with substrate irregularities or push-points, then propagated posteriorly through the contraction of muscle units on alternating sides of the body (Gray 1953; Jayne 1988a). Thus, the snake moves forward by pushing laterally against push-points.

As a consequence of the functional relationship between vertebrae and musculature of a snake, the number of vertebrae should affect both flexibility and stiffness of a snake's vertebral column and therefore its locomotor performance. For example, given snakes of the same length, an individual with more vertebrae will have more joints permitting lateral flexion, and therefore should be more flexible (Jayne 1982, 1985). Flexibility may be particularly important when push-points are widely spaced, because a snake must form bends to find the contacts and to exert the force necessary for forward propulsion (Gray & Lissmann 1950; Gasc & Gans 1990). Flexibility may incur a cost, however, because more muscular activity is required to maintain the stiffness necessary for pushing off contact points (Blight 1977; Gans 1985; B. C. Jayne, personal communication). Given that rapid locomotion affects survival in the field (Jayne & Bennett 1990), selection may change snake vertebral number in habitats with different push-point densities. A habitat in which push-points are widely spaced should bias the compromise between flexibility and stiffness in favour of greater flexibility, and hence more vertebrae. In this paper, we explore the possibility that density of push-points is the crucial environmental feature that influences variation in snake vertebral number.

We studied the effects of push-point density and vertebral number on locomotion in two California populations of *Thamnophis elegans* (Baird & Girard) that differ in average numbers of body and tail vertebrae (Arnold 1988). As in Klauber's (1941) study of unrelated snake species, our coastal population has fewer vertebrae than our inland population (Table 1). Vegetation is dense along the California coast, but sparse inland because of a rain shadow (Munz & Keck 1963; Hickman 1993). Our first goal was to determine whether this vegetation difference translated into a significant difference in the density of push-points encountered by *T. elegans*. Our second goal was to determine how locomotion was affected when push-point density varied over the range observed in the field. We anticipated the greatest lateral bending when push-points were sparse. We expected locomotory speed to be highest at intermediate push-point density, because propulsive forces should increase with number of push-points, but so should the lateral forces that diminish forward movement (Gray & Lissmann 1950, Fig. 7; Jayne 1988b). The few prior studies of snakes locomoting through push-point fields of different densities suggest that for snakes of a given length, there is some intermediate density at which maximal crawling speed is achieved (Gray 1946; Heckrotte 1967; Bennet, McConnell & Trubatch 1974). Our third goal was to determine whether the effect of vertebral number on locomotion was modified by push-point density. We expected snakes with more vertebrae to show greater lateral bending and to crawl faster at low push-point densities than snakes with fewer vertebrae. This result would be consistent with the hypothesis of local adaptation in vertebral number to substrate push-point densities.

Table 1. Morphology of coastal and inland *T. elegans* (mean ± SD, with range given in parentheses). Means denoted by [1] are the average of separate male and female means; all other statistics are for males and females pooled

	Coastal	Inland
Samples of newborn snakes (Arnold 1988)		
Sample size (body, tail)	874, 886	1486, 1479
Body vertebrae [1]	154 ± 3	170 ± 4
Tail vertebrae [1]	76 ± 4	86 ± 5
Subjects of performance tests		
Sample size	10	10
Body vertebrae	156 ± 4	168 ± 4
	(150–162)	(160–172)
Tail vertebrae	76 ± 5	84 ± 6
	(70–85)	(79–93)
SVL (cm)	18 ± 1·5	18 ± 1·2
	(16–20)	(16–20)
Mass (g)	2·65 ± 0·67	2·10 ± 0·57
	(1·36–3·80)	(1·30–3·10)

Methods and materials

HABITAT MEASUREMENTS

Push-point density was recorded at the coastal (mouth of the Mad River, Humboldt Co., California) and inland (southeast corner of Eagle Lake, Lassen Co., California) study sites. Push-point density was scored where *T. elegans* was seen crawling in the field. Areas frequented by snakes were patrolled until a snake was sighted. As the snake crawled, two points crossed by the snake's head 30–40 cm apart were immediately noted and marked by driving 20-cm nails into the substrate. A count was made of the number of potential push-points (plant stems and/or rock edges) contacting a 30-cm length of string stretched between the two nails a few millimetres above the substrate (Fig. 1). Twenty such push-point scores were made on 24–25 June at the coastal site, and 19 scores were made on 8–12 July at the inland site. At each site these scores represented the push-point scores for the first 19–20 snakes sighted. Each of these snakes was captured, then its snout-to-vent length (SVL) was measured to the nearest millimetre, its weight was measured to the nearest 0·1 g, and its sex was determined by noting the presence of hemipenes in males and their absence in females.

SUBJECTS FOR PERFORMANCE TESTS

Twenty individuals sampled from a group of 133 laboratory-born *T. elegans* were the subjects of locomo-

Fig. 1. Sample photographs of push-point densities at the coastal (top) and inland (bottom) study sites in California. The gnomon in the pictures is 30-cm long. To score push-point density, a string was stretched between the nails that are visible at the sides of each photograph. In the coastal and inland samples shown, 61 push-points (2·03 push-points per cm) and seven push-points (0·23 push-points per cm) contacted the string, respectively.

tion testing. These 20 snakes consisted of seven females and three males (a total of 10 individuals) from each population, representing five coastal and six inland litters. The test subjects were the offspring of wild-caught, pregnant females from the two sites. Mothers were transported from the sites, housed in individual cages in the laboratory and given food and water until their litters were born. During the day mothers had access to a thermal gradient (20–40 °C), maintained by a heating strip at one end of the cage. At night cages were kept at a spatially uniform 20–22 °C. A natural (Chicago) photoperiod was maintained.

Newborn snakes were separated from their mother, measured, and weighed on the day after birth. Neonates were housed at a constant temperature (24–26 °C) in individual plastic boxes (310 x 170 x 87 mm^3) with water dishes (Arnold 1981). No food was given to inland neonates, to avoid the effects that feeding has on performance (Garland & Arnold 1983). Inland neonates were tested on boards beginning at age 15–16 days. All yolk stores have been depleted by this age (S. J. Arnold, unpublished data). Coastal individuals were smaller at birth than inland snakes, therefore they were raised until the age of 95 days and then tested. During the additional 80 days, coastal individuals were fed freshly ground fish twice a week until 15 days before trials began and then given only water. Coastal snakes grew to approximately the same length and mass as the inland individuals at 15 days of age. The aim in standardizing size was to reduce the effects that population differences in SVL and mass might have on locomotor performance (Jayne 1986; Walton, Jayne & Bennett 1990).

PERFORMANCE TESTING

Locomotor performance was measured on square boards (123 cm^2) that were varnished to provide a smooth surface and fitted with nails at a constant density for each board. Although the varnished surface did not provide traction for the snakes, they were able to locomote by pushing against the nails. The five boards had a square central region with nails that averaged 87 cm^2. The boards spanned a range of push-point densities; in order of increasing push-point density, the nails were spaced 5·0, 4·0, 3·0, 2·0 and 1·5 cm apart, yielding, respectively, 0·2, 0·25, 0·33, 0·5 and 0·67 push-points per cm. A grid of lines 2 cm apart was drawn on the surface of each board and enabled the scoring of coordinates during snake locomotion.

For each trial, a snake was moved from the housing room to the test room 0.5 h before the trial to allow it to equilibrate to the room's temperature. The snake remained in its plastic box, but the water dish was removed. Five minutes before a trial, the snake was placed in a bottomless cylindrical container (10 cm in diameter, 14-cm tall) situated at the centre of the board. The trial was initiated by lifting the cylinder,

leaving the snake unimpeded at the centre of the board. For 30 s the snake was stimulated to move across the board by tapping lightly on its posterior with a cotton-tipped swab every few seconds. After each trial the individual was returned to its housing box and room. Ambient temperature was recorded on the surface of the test board with a thermocouple thermometer at the beginning of each trial.

Every individual was tested on a different board each day for 5 consecutive days between 1300 and 1800 hours. Each snake experienced the boards in a random order. On the day after all tests were completed, the snake was killed; then its SVL and tail length were measured to the nearest millimetre, its weight was measured to the nearest 0·01 g, and its sex was determined as described for field-caught individuals. The individual was fixed in formalin and preserved in 70% ethanol so that its ventral and subcaudal scales could be counted later (Dowling 1951). These scale counts correspond to the number of vertebrae in the body and tail, respectively (Alexander & Gans 1966).

Trials were videotaped with a Panasonic WVBD-400 camera with a Vivitar 28 mm (1:2·8) wide-angle lens located 160 cm above the trial arena and connected to a Panasonic AG-6010 Video Cassette Recorder. A time generator was used to imprint a time reading (0·01 s resolution) on each image. Analysis of videos was conducted with a 17-inch (430 mm) Panasonic monitor.

ANALYSIS OF VIDEOS

Individual trials were examined to identify the longest distance that the snake crawled on the board without changing direction or stopping. Once such a sample was identified, two performance variables were measured: (1) the distance between the head and tail of the snake (HTD), an indirect measure of lateral bending, and (2) the snake's speed (see Fig. 2). The HTD at a given time provided an estimate of its lateral bending during movement, because when the distance between the head and tail was relatively short the animal had
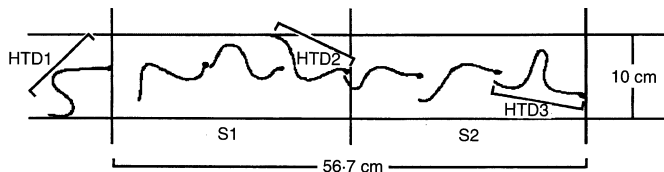
bent its body during the course of locomotion. Three measures of snake HTD were made for each trial. For speed, the linear distance travelled by the snake was calculated as the distance between the head coordinates at two successive time periods. Two speed measures were taken for each trial.

STATISTICAL ANALYSIS

All uni- and multivariate analyses were conducted using the GLM procedure of PCSAS (SAS Institute, Inc. 1989). Population differences in field densities of push-points and experimental conditions were tested with univariate analyses of variance. For the locomotion experiments, population and sex differences in the performance variables (HTD and speed) were tested using univariate repeated measures analyses of variance. For each individual, the HTD and speed values at each density were the average of three and two consecutive measurements, respectively. For all analyses, the morphological variables were $\log_{10}$-transformed to reduce their distributions' departures from normality. In the analysis of HTD, population and sex were considered main effects, the $\log_{10}$ of total vertebral number and total length (SVL plus tail length) were used as covariates, and density was treated as a repeated measure. This model was employed for HTD because we expected lateral bending to be directly related to the number of vertebrae, i.e. snakes with more vertebrae for a given length were expected to have greater lateral bending, and therefore shorter head-to-tail distance. The analysis of speed was the same, except that the covariate of the total vertebral number was replaced by body and tail vertebral numbers and their product, because these covariates have been shown to affect locomotion (Arnold & Bennett 1988). Multivariate analyses of variance of performance at each density were conducted to obtain the partial regression coefficients of the covariates specified in the models for HTD and speed. For all repeated measures analyses, the assumption of Type H covariances (Huynh & Feldt 1970) was tested by applying a sphericity test (Anderson 1958) to the orthogonal components of the data matrix using the PRINTE option of the REPEATED statement (SAS Institute, Inc. 1989). Satisfying the Huynh–Feldt condition allowed the use of unadjusted univariate statistics for the within-subject effect of density and its associated interactions.

Fig. 2. The method of measuring snake head-to-tail distance (HTD) and speed (S). A transparent template with three parallel and equidistant lines was placed on the video monitor over the path of the snake to mark three locations at which the coordinates of the head and tail would be determined. These locations correspond to 0, 28·3 and 56·7 cm from the starting location of measurements. To be chosen for analysis, the path of the snake's head had to remain within the 10-cm limits. The frame in which the head of the snake crossed each line was the one from which the coordinates were taken.

Results

HABITAT VARIATION

Push-points were more than three times denser where snakes were observed crawling at the coastal site than at the inland site ($F_{1,37} = 22\cdot14$, $P < 0\cdot0001$) (Fig. 3). Push-point density was not correlated with SVL at the coastal site ($r = 0\cdot03$, $n = 20$, $P > 0\cdot89$). In contrast,
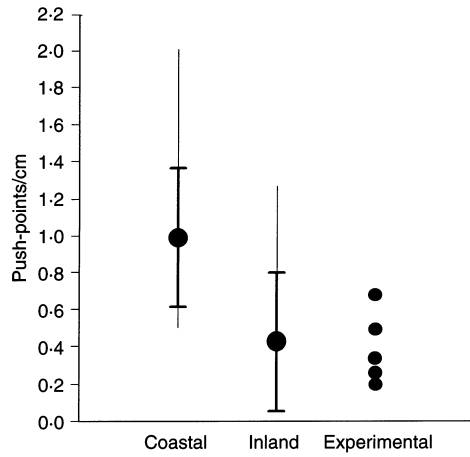
Fig. 3. The density of push-points at the two field sites and in the experimental (lab) trials. Twenty (eight males, 12 females) and 19 (11 males, eight females) individuals were the source of push-point data at the coastal and inland sites, respectively. For the two field sites the mean (●), SD (dark bars), and range (light line) are presented; for the five experimental boards, individual board values are shown (●).

push-point density was positively correlated with SVL at the inland site ($r = 0.52$, $n = 19$, $P < 0.023$), because large females tended to occur in clumps of grass, while males did not show such a tendency (females only: $r = 0.74$, $n = 8$, $P < 0.036$; males only: $r = 0.16$, $n = 11$, $P > 0.63$).

EXPERIMENTAL CONDITIONS

There was no difference in the temperature ($F_{1,18} = 4.01$, $P > 0.06$) at which trials were conducted for the two populations (coastal: 24.16 ± 0.47 °C; inland: 23.84 ± 0.19 °C). Nor was there any difference between the two populations on the trial dates in SVL ($F_{1,18} = 0.05$, $P > 0.83$), tail length ($F_{1,18} = 0.11$, $P > 0.74$) or mass ($F_{1,18} = 3.88$, $P > 0.06$) (Table 1). The populations were significantly different, however, in the number of body ($F_{1,18} = 41.63$, $P < 0.0001$) and tail ($F_{1,18} = 8.83$, $P < 0.008$) vertebrae, with coastal juveniles having fewer vertebrae on average (Table 1).

LOCOMOTORY PERFORMANCE

Both HTD (MS = 18.24, $F_{4,76} = 22.82$, $P < 0.0001$) and speed (MS = 44.61, $F_{4,76} = 12.57$, $P < 0.0001$) were significantly affected by the density of push-points through which snakes crawled (these results are for a repeated measures model in which no dependent variables other than density were included; sphericity tests: for HTD, Mauchly's Criterion = 0.4873, df = 9, $P > 0.19$; for speed, Mauchly's Criterion = 0.5527, df = 9, $P > 0.32$). A linear relationship was observed between HTD and density (Fig. 4a), while a curvilinear relationship was found between speed and density

(Fig. 4b). Tracings of a snake in motion illustrate how the pattern of movement changed with increasing density: the snake's body had fewer large bends as push-point density increased (Fig. 5). For both populations, the fastest locomotion occurred at the intermediate density of 0.5 push-points per cm (Fig. 4b).

There was no population difference in HTD when differences in total vertebral number and total length were taken into account (Table 2). There was a significant sex difference in HTD; males had a longer HTD than females at three of five densities. Total length had a significant effect on HTD, as might be expected (Table 2). All partial regression coefficients relating HTD to total length were positive and significant (for boards of increasing density: $\beta_1 = 0.041$, $\beta_2 = 0.040$,
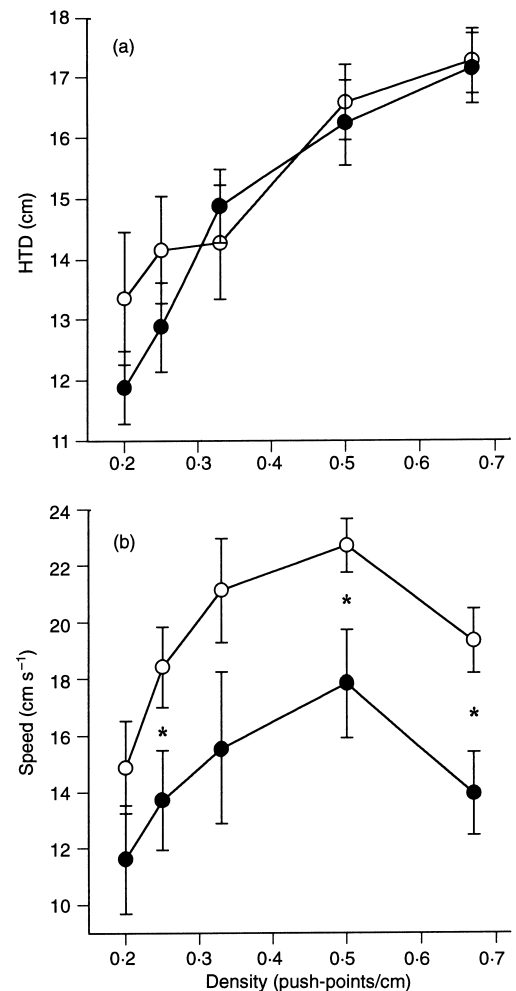

Fig. 4 Head-to-tail distance (HTD) and speed as a function of push-point density for coastal (○) and inland (●) juveniles. For the two measures, the mean (○ or ●) and 2 SE (bars) are presented. Single factor ANOVAs for each density comparing speeds of individuals in the two populations showed that on three of the five boards, the average speed of coastal individuals was significantly faster than that of inland individuals. Significant differences are indicated by * where appropriate for: board one (NS); board two ($F_{1,18} = 4.23$, $P = 0.05$); board three (NS); board four ($F_{1,18} = 5.27$, $P < 0.03$); board five ($F_{1,18} = 8.33$, $P < 0.01$).

$\beta_3 = 0.044$, $\beta_4 = 0.045$, $\beta_5 = 0.039$; for all coefficients $P < 0.05$). Importantly, there was a significant effect of total number of vertebrae on HTD (Table 2). All partial regression coefficients relating HTD to vertebral number were negative ($\beta_1 = -20.69$, $\beta_2 = -11.44$, $\beta_3 = -19.59$, $\beta_4 = -11.04$, $\beta_5 = -10.98$; for all coefficients $P > 0.05$). Thus, having more vertebrae decreased HTD, apparently by increasing some component of lateral bending. From the tracings of snake locomotion, it can be seen that the amplitude of undulatory bends increased as the density of push-points decreased (Fig. 5). It was not clear if the number of undulatory bends changed with density. There was no evidence that population, sex, vertebral number or total length had interactive effects with density (Table 2). When the effects of population, sex and the two covariates are taken into account, no difference in HTD across densities was detected (Table 2). Finally, the model with two main effects and two covariates was significant at the three most dense fields of push-points and explained a large proportion of variation in head-to-tail distance of snakes ($R_1^2 = 0.38$, $R_2^2 = 0.41$, $R_3^2 = 0.52$, $R_4^2 = 0.72$, $R_5^2 = 0.66$; for $R_3^2 - R_5^2$ $P < 0.05$).

Coastal snakes were consistently faster than inland snakes and significantly faster when locomoting through three of five densities (Fig. 4b). When population, sex and the covariates were taken into account,
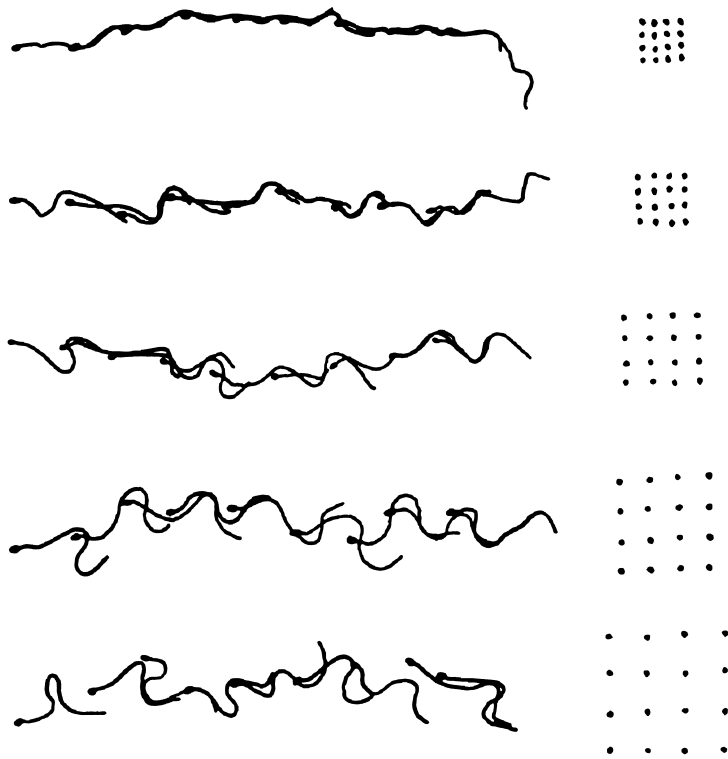
Table 2. Repeated measures analysis of variance for head-to-tail distance (HTD). Total is the $\log_{10}$ of the total number of vertebrae. Length is total snake length. Mean squares (MS) are based on Type III sums of squares. Unadjusted univariate statistics are presented, because the test for sphericity of orthogonal components was not significant (Mauchly's Criterion = 0.4814, df = 9, $P > 0.3663$)

Effect	df	MS	F	P
Population	1	3·01	3·19	0·0945
Sex	1	7·51	7·94	0·0130
Total	1	6·30	6·67	0·0208
Length	1	50·63	53·56	0·0001
Error	15	0·95		
Density	4	0·13	0·15	0·9601
Density*Population	4	0·26	0·30	0·8764
Density*Sex	4	0·66	0·76	0·5550
Density*Total	4	0·14	0·16	0·9564
Density*Length	4	0·04	0·04	0·9965
Error (density)	60	0·87		

Table 3. Repeated measures analysis of variance for crawling speed. Body and Tail are the $\log_{10}$ of the numbers of body and tail vertebrae, respectively. Other conventions as in Table 2. Mean squares are based on Type III sums of squares. Unadjusted univariate statistics are presented, because the test for sphericity of orthogonal components was not significant (Mauchly's Criterion = 0.4986, df = 9, $P > 0.5398$)

Effect	df	MS	F	P
Population	1	21·75	0·94	0·3509
Sex	1	0·03	0·00	0·9729
Body	1	6·45	0·28	0·6070
Tail	1	6·86	0·30	0·5961
Body*Tail	1	6·63	0·29	0·6021
Length	1	76·41	3·29	0·0929
Error	13	23·23		
Density	4	8·08	3·07	0·0241
Density*Population	4	7·36	2·80	0·0353
Density*Sex	4	7·89	3·00	0·0265
Density*Body	4	7·96	3·03	0·0256
Density*Tail	4	8·14	3·09	0·0233
Density*Body*Tail	4	8·05	3·06	0·0244
Density*Length	4	11·03	4·19	0·0051
Error (Density)	52	2·63		

however, only density and its interactions were statistically significant (Table 3). The difference in speed between coastal and inland snakes was greatest at the highest densities (Fig. 4b). The effect of sex on speed changed as a function of density (Table 3), with males crawling faster than females at all but the lowest density. Additionally, the effect of vertebral numbers on speed varied with density (Table 3). The partial regression coefficients were mostly negative for the number of body vertebrae ($\beta_1 = -8.76$, $\beta_2 = -15.56$, $\beta_3 = -6.21$, $\beta_4 = -3.96$, $\beta_5 = 10.88$; for all coefficients $P > 0.05$), mostly positive for the number of tail vertebrae ($\beta_1 = -39.88$, $\beta_2 = 5.49$,

Fig. 5. Tracings of an inland female *T. elegans* locomoting through push-points of different densities, with boards one to five shown bottom to top. Nine evenly spaced tracings were made, three of which were used in the analysis of locomotion. The density of push-points on each board is shown beside the tracing. The push-point density and snake tracings are drawn to the same scale.

$\beta_3 = 34\cdot05$, $\beta_4 = 3\cdot24$, $\beta_5 = 32\cdot99$; for all coefficients $P > 0\cdot05$), and of mixed sign for their interaction ($\beta_1 = -89\cdot45$, $\beta_2 = -179\cdot1$, $\beta_3 = -367\cdot3$, $\beta_4 = 158\cdot4$, $\beta_5 = 60\cdot41$; for all coefficients $P > 0\cdot05$). These data suggest that having more body vertebrae decreased speed at all densities except the highest, while having more tail vertebrae increased speed at all densities except the lowest. A related result is that the number of body and tail vertebrae had a negative interactive effect on speed at low push-point densities and a positive interactive effect on speed at high push-point densities. Total length also had a significant interactive effect with density on speed; all its partial regression coefficients were positive ($\beta_1 = 0\cdot099$, $\beta_2 = 0\cdot032$, $\beta_3 = 0\cdot110$, $\beta_4 = 0\cdot030$, $\beta_5 = 0\cdot002$; for all coefficients $P > 0\cdot05$), suggesting that longer snakes were consistently faster. Even when the main effects and covariates were taken into account for the analysis of speed, density still had a large effect on speed. Finally, the model of main effects and covariates for speed was significant for the lowest and intermediate push-point densities and explained a fair proportion of variation in locomotory speed of snakes ($R_1^2 = 0\cdot56$, $R_2^2 = 0\cdot26$, $R_3^2 = 0\cdot52$, $R_4^2 = 0\cdot27$, $R_5^2 = 0\cdot46$; for R_1^2 and R_3^2 $P < 0\cdot05$).

Discussion

DO CONSPECIFIC POPULATIONS OF SNAKES EXPERIENCE DIFFERENT PUSH-POINT DENSITIES?

Our field measures demonstrate that a coastal population of *T. elegans* encounters substantially greater push-point densities than an inland population. Additionally, the association between push-point density and SVL of females at the inland site suggests that individuals may choose microhabitats that differ in push-point density, perhaps because of their age or physiological condition (e.g. gravidity). Furthermore, our success in measuring the push-point densities encountered by free-ranging snakes indicates that it is possible to relate experimental studies of locomotion to ecological conditions. Thus, the range of push-point densities that we used in the locomotion trials (0·2–0·67 push-points per cm) was within the total range of densities that we recorded in the field (0·13–2·03 push-points per cm). The inland mean push-point density was well within the range of experimental densities; however, the coastal mean push-point density (0·99 push-points per cm) was slightly greater than the highest experimental density. Additionally, the extreme densities at each site (highest and lowest push-point densities at the coastal and inland sites, respectively) fell outside the experimental range. In future studies of push-point effects on snake locomotion, the experimental range should be expanded. Nevertheless, ours is apparently the first study to measure push-point densities experienced by snakes in the field and the first to use in laboratory experiments a range of densities known to be experienced by snakes in nature.

HOW IS LOCOMOTOR PERFORMANCE AFFECTED BY PUSH-POINT DENSITY?

Lateral bending decreased monotonically as push-point density increased, but crawling speed was greatest at intermediate push-point densities (Fig. 4). At the lowest density, when the distance between push-points was more than a quarter of the individual's length, snakes made large bends, using the posterior part of the body and tail to reach and push away from points (Fig. 5). The lack of available push-points impeded forward progress, presumably because snakes were able to generate only a limited amount of propulsive forces. This pattern of movement was very similar to that of individuals presented with only one or two (Gray & Lissman 1950) or relatively distant push-points (Bennet *et al.* 1974; Gans & Gasc 1990). The result also is consistent with the proposal that simultaneous contact with at least three push-points is required for continuous forward progress (Gans 1970). The fastest locomotion was achieved at an intermediate density, when the distance between push-points was approximately one-ninth of the snake's total length. Apparently, the ratio of propulsive to lateral forces was optimized at this density. Additionally, it was at this density that the snake maintained a relatively constant configuration of its body while passing a push-point, a pattern that Jayne (1988a) has suggested is a distinctive feature of terrestrial lateral undulation. At the highest push-point density, when the distance between adjacent points approached one-half the individual's width, the snake made small bends and pushed against points along the entire length of its body. The reduction in speed at high push-point densities was most probably due to the increase in lateral forces in relation to propulsive forces (Gray 1953; Gray & Lissmann 1950; Jayne 1988b), especially as the distance between points restricted snakes from bending around points and approximated the conditions of moving through a tunnel. Finally, this intermediate optimum of push-point density for snakes of a given length is consistent with Heckrotte's (1967) observation that for a given push-point density, snakes of intermediate length moved the fastest.

In general, observations indicated that lateral undulation was the dominant mode employed, but snakes may have been using slide-pushing (Gans 1985; Gans & Gasc 1990) at low push-point densities (Fig. 5). Also, the small amount of lateral bending observed at higher push-point densities is strikingly similar to Jayne & Davis's (1991) results for concertina locomotion in tunnels of increasingly narrow width. We did not attempt to classify the modes of locomotion employed among different densities, because they appeared to grade into one another without obvious

changes in the two performance measures. In addition, we did not quantify the components of lateral bending, such as the maximal flexion between vertebrae or the radius of curvature over a given length of body, that contributed to changes in head-to-tail distance as a function of push-point density. For example, a decrease in head-to-tail distance could have been generated by an increase in the amplitude of the undulatory waves, as suggested, by an increase in the number of undulatory waves, or by a combination of both these factors (B. C. Jayne, personal communication). To better characterize transitions between locomotory modes and the biomechanic basis of locomotory performance at different densities, future studies should quantify the degree to which components of lateral bending contribute to changes in forward propulsion as a function of push-point density.

DOES PUSH-POINT DENSITY MODIFY THE EFFECT OF VERTEBRAL NUMBER ON LOCOMOTOR PERFORMANCE?

Two lines of evidence suggest that the number of vertebrae strongly influences locomotory performance. First, head-to-tail distance was significantly affected by the total number of vertebrae when total length was held constant (Table 2, main effects), suggesting that for a given length, having more vertebrae enables greater lateral bending. This effect was obtained regardless of density (Table 2, interaction terms). Thus, push-point density did not change the effect that vertebral number had on our measure of lateral bending. The significant effect of sex on HTD is consistent with this positive relationship, because males had more total vertebrae and were longer than females. Such a positive relationship between vertebral number and lateral bending has been suggested (Jayne 1988b); however, Jayne's proposal was for concertina locomotion through tunnels, where the force exerted is affected by the number of vertebrae that a snake may fit between two close walls. Gasc & Gans (1990) obtained a similar positive relationship between vertebral number and measures of bending in their intergeneric comparison of limbless lizards. Because our indirect measure of lateral bending did not include an estimate of the amount of flexion between adjacent vertebrae, a critical determinant of axial flexibility (Jayne 1988b), we cannot conclude that snakes with more vertebrae are more flexible, just that they showed greater lateral bending.

The second line of evidence suggesting that vertebral number affects locomotory performance comes from the analysis of snake speed. When differences between the two population test groups and the sexes were taken into account, the numbers of body and tail vertebrae had significant interactive effects on the speed of locomotion at different densities (Table 3, interaction terms). No overall effects of the covariates were found (Table 3, main effects), because the

direction of the vertebral number effects on speed changed with density. Although none of the partial regression coefficients of vertebral numbers was significant for speed at different densities, the following interpretations are suggested by their directions. At all except the highest density, more body vertebrae decreased speed, whereas at all except the lowest density, more tail vertebrae increased speed. The directions of these relationships between vertebral number and speed are the same as those found for the burst speed of juvenile *T. radix* crawling on an artificial grass substrate (Arnold & Bennett 1988). The first relationship is also consistent with the results of two other studies. Gasc & Gans (1990) showed that a limbless lizard (*Anguis*) with more body vertebrae was slower than one (*Ophisaurus*) with fewer body vertebrae. Jayne (1988a,b) found that a snake with more vertebrae (*Elaphe*) was better at concertina locomotion through a tunnel (similar to the highest push-point density), whereas a snake with fewer vertebrae (*Nerodia*) was faster in lateral undulation through moderately spaced push-points. Additionally, we found that the interaction of body and tail vertebral number differed with density. At the three lowest densities the effect was negative, while at the highest two densities the effect was positive. Interestingly, only the latter relationship is consistent with the one obtained by Arnold & Bennett (1988), i.e. the joint effect of body and tail vertebral number on locomotion of juvenile snakes moving on artificial grass was most similar to that of snakes locomoting through relatively dense push-points. Perhaps crawling on artificial grass is equivalent to crawling through many push-points, without the restriction of lateral movement that tall push-points such as nails or stems provide. Also, we found that longer snakes were faster at all densities, a result consistent with previous studies (Bennet *et al.* 1974; Arnold & Bennett 1988; but see Heckrotte 1967), and that males were typically faster than females.

DO COASTAL SNAKES PERFORM BETTER AT HIGHER PUSH-POINT DENSITIES?

In contrast to our prediction of adaptation to local conditions, coastal snakes were faster than inland snakes at all push-point densities, and thus snakes with more vertebrae did not perform better when locomoting through sparse push-points. Thus, our results reject the hypothesis of local adaptation to push-point density by juvenile *T. elegans*. This rejection could arise for at least three reasons. First, maladaptation of inland juveniles may be real, but at later ages and larger sizes inland snakes might outperform coastal snakes at low push-point densities. This possibility is suggested by the fact that *T. elegans* at our inland site are characteristically larger than *T. elegans* at our coastal site (Arnold 1988). Second, inland juveniles may have performed less well than coastal juveniles because they

were younger. In order to control for the effect that size might have on locomotion, coastal snakes were substantially older (95 days) than inland snakes (15 days) at the time of testing. Previous studies have demonstrated that crawling speed is influenced by size (Jayne 1986; Arnold & Bennett 1988; Walton *et al.* 1990); however, speed also might increase with age (a maturation effect), independent of size. Both of these interpretations of the superiority of coastal snakes could be tested by evaluating locomotor performance over a range of push-point densities for a sequence of ages. A third reason for the contradiction between the prediction and results is that our simple model of flexibility and stiffness as a function of vertebral number might not be correct. In fact, Jayne (1988a) has proposed that increased flexibility, and thus more vertebrae, are required at high push-point densities, while Gans (1970) has suggested that greater stiffness, and thus fewer vertebrae, are required at low push-point densities. The problem is that few studies have taken an explicitly functional approach to the study of vertebral variation and there are few unambiguous predictions for the effect that variable vertebral number will have on locomotory performance.

Two alternative hypotheses can be identified for the difference in vertebral numbers between the coastal and inland populations. First, selection may act on vertebral number in relation to push-point density, not for its effect on flexibility, but for its effect on body size. For example, at the inland site where push-points are sparse an individual that is larger, because of having more vertebrae, may be more able to reach and efficiently use the few push-points that are available. This hypothesis is consistent with evidence of selection for large body size and many vertebrae (Lindsey 1975; Jayne & Bennett 1990; Lindell 1994) and would be supported by partial regression coefficients for length on speed that were positive and larger at lower push-point densities (L. E. Lindell, personal communication). We did find significant positive coefficients for length on speed at all densities, but found no change in the magnitude of the effect with decreased push-point density. Thus, the data provide partial support for the hypothesis of selection on vertebral number for its effect on body size. Second, selection may act on vertebral number in relation to different locomotory modes employed at the two sites. Perhaps the density of push-points at the coastal site is so great that snakes are locomoting like burrowers, a mode that is associated with having relatively few vertebrae (Jayne 1982). Additional data on the microhabitat choice of snakes of different vertebral numbers and measures of snake locomotory performance in the field may provide crucial tests for these alternative explanations.

While descriptive and interspecific comparisons of vertebral variation and locomotory performance have laid the foundation for studies such as ours, we strongly encourage the development of biomechanical models that explicitly evaluate the functional consequences of variation in vertebral number on snake locomotion through push-points of different densities. A similar functional approach has been productive in the mechanistic analysis of undulatory swimming in fishes (e.g. Westneat *et al.* 1993; McHenry, Pell & Long 1995). One issue that might benefit from a mechanistic analysis is the apparent difference in effects of body and tail vertebrae on speed of locomotion. Very little work has focused on the propulsive action of the tail compared with that of the body of snakes (Cundall 1987), even though there are noticeable differences among species in relative tail length and these differences appear to affect locomotion (Jayne 1988b; Gasc & Gans 1990). Modelling how undulatory waves are generated anteriorly and propagated posteriorly, especially in the context of differing push-point densities, might reveal a biomechanic basis for the effects of body and tail vertebrae independently and their interactive effect on snake crawling speed. For example, is there a change in where or how propulsive forces are generated along the snake's length as push-point density decreases? Does the number of vertebrae for a given length change how propulsive forces are generated? Biomechanical models of lateral undulation could provide more explicit predictions for vertebral effects on locomotion and would enable stronger tests of the hypothesis that selection acts to change vertebral number through its effect on locomotory performance at different push-point densities.

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