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Plastic vipers: influence of food intake on the size and shape of Gaboon vipers (*Bitis gabonica*)

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Abstract

Repeated measurements of captive-born Gaboon vipers *Bitis gabonica* from an inbred stock examined the degree to which an animal's size and shape are affected by food intake. We also used the level of asymmetry in dorsal coloration as an index for possible genetic (inbreeding?) effects. Both of these factors, and the interaction between them, affected phenotypes of the young snakes. Snakes raised with abundant food differed from their less well-fed siblings not only in size, but also in body mass relative to snout–vent length, head length relative to snout–vent length, head width relative to head length, and fang length relative to jaw length. Hence, our data show that body proportions (including the feeding apparatus) can be influenced by the environment after birth.

Key words: adaptation, *Bitis gabonica*, growth, energy allocation, phenotypic plasticity, reptiles

INTRODUCTION

The expression of many phenotypic traits is a result not only of genetic factors, but also of the local environment, and of the interaction between genes and environment (A. D. Bradshaw, 1965; Conover & Kynard, 1981; Stearns, 1989; van Noordwijk, 1989; Olsson *et al.*, 1996). Reptiles have proved to be excellent model systems for studies that seek to identify the relative roles of genetics and environment in producing the adult phenotype, because they show a high degree of ontogenetic plasticity. For example, thermal conditions during incubation can influence the body size, gender, activity levels, and locomotor performance of the hatchlings (Fox, Gordon & Fox, 1961; Bull, 1980; Gutzke & Packard, 1987; Burger & Zappalorti, 1988; Burger, 1989; Toussignant & Crews, 1995; Shine, Madsen *et al.*, 1997). Environmental factors acting later in life can also affect the reptile's phenotype. Clearly, food availability and thermoregulatory opportunities can influence overall growth rates (e.g. Madsen & Shine, 1993; O'Steen, 1998) and reproductive output (Ford & Seigel, 1989; Seigel & Ford, 1991, 1992), but it remains unclear as to whether or not such environmental cues also can modify more complex aspects of developmental trajectories. In particular, the ontogenetic lability of body shape (relative proportions of the head, trunk, tail and limbs) requires clarification. Experimental studies on snakes maintained on high *vs* low rates of food intake have revealed no plasticity in some variables (e.g. head length relative to body length: Arnold & Peterson, 1989; Forsman, 1996; Forsman & Shine, 1997; but see Queral-Regil & King, 1998), but some flexibility in others (e.g. mass relative to length: Forsman & Lindell, 1996).

The degree of environmentally induced lability in body proportions (such as relative head length or relative mass) bears directly upon current hypotheses on the evolution of life-history traits in reptiles. For example, snakes are gape-limited predators (Vezina, 1985; Cundall, 1987; Shine, 1991*a*) and hence the size and shape of the head may be under strong and spatially variable selection in relation to prey size availability (e.g. Arnold, 1993; Forsman & Lindell, 1993; Forsman, 1994). Similarly, sexual divergence in relative head sizes in snakes has been attributed to sex-specific adaptations to different ecological niches (Shine, 1991*b*; Houston & Shine, 1994). A growing body of literature interprets variations in mass relative to body length in terms of life-history adaptations to optimize relative energy allocation between the demands of maintenance, growth, storage and reproduction (Congdon, 1989; Kozlowski, 1992). These interpretations rely upon the conclusion that body proportions in snakes are 'adaptive' (and thus, must have a genetic underpinning). This inference is weakened if the traits in question shift in response to factors in the post-hatching environment (Forsman &

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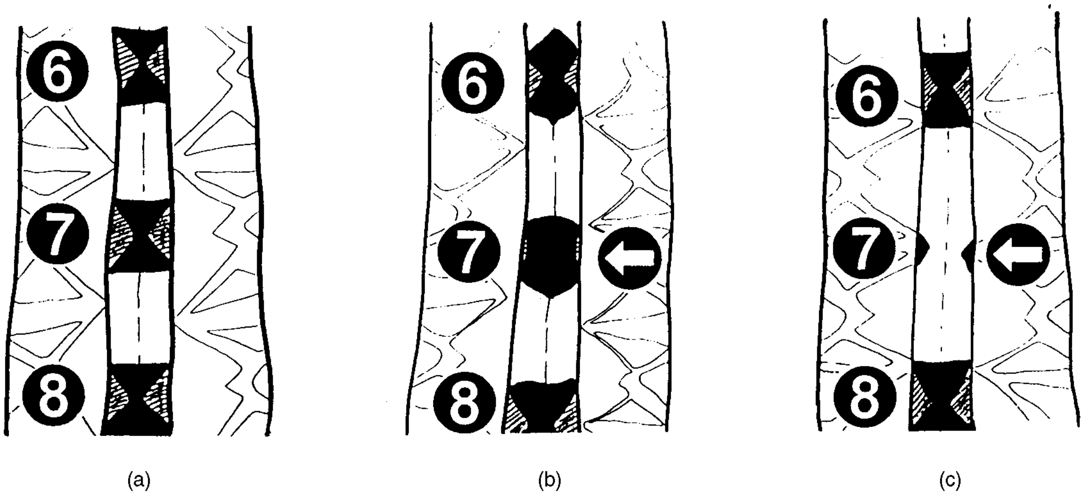

Fig. 1. Colour patterns of three Gaboon vipers *Bitis gabonica* (a, b, c). Nine to 12 'hourglass' markings (in black) occur on their dorsal surfaces. For each individual, hourglass numbers 6 to 8 (from the anterior part of the snake) are represented. The snakes display either complete longitudinal symmetry (a), or obvious asymmetry (arrow, b). Hourglass markings are sometimes absent (arrow, c). These figures were drawn from photographs.

Shine, 1997), although it remains possible that this plasticity is in itself adaptive (Brönmark & Miner, 1992; De Jong, 1995; Via *et al.*, 1995).

Thus, the degree of ontogenetic stability in body proportions, as well as in absolute body size, is directly relevant to the interpretation of current paradigms in the field of reptile ecology. In order to evaluate the validity and generality of the assumption of strong canalization for such traits, we need experimental studies on a wider range of species. In this paper, we report such a study. In contrast to previous work, we show that food availability during juvenile life affects not only absolute growth rate, but also the allometric relationships among morphological variables such as body length, head length, head width, and fang length.

MATERIALS AND METHODS

Study animals

Gaboon vipers *Bitis gabonica rhinoceros* are large (up to 150-cm body length, 10 kg) viviparous venomous snakes from Africa (Mehrtens, 1987; Branch, 1988). They are notable for their sluggish disposition, heavyset build, and extraordinarily long fangs (Pough & Groves, 1983; Mehrtens, 1987). Although this species is often maintained in captivity, there is little quantitative information on its biology in the field (Luiselli, Akani, Angelici *et al.*, 1998; Luiselli, Akani & Capizzi, 1998). Radiotelemetric monitoring suggests that Gaboon vipers are extremely sedentary ambush foragers, feeding almost exclusively on mammals (Bodbijl, 1994).

The data in the present paper are based on a lineage of vipers maintained in captivity for 2 generations. The grandparents (1 female and 1 male) were wild-caught animals whose exact origin is unknown (illegal traffic). The captive-bred progeny of these snakes reproduced in June, 1993, and the offspring from this litter produced their own offspring in 1997, the snakes used in this study: 18 offspring from a single litter, born in captivity on 9 October, 1997.

The progeny resulted from a mating between a brother and sister, themselves born in captivity in June 1993 from a brother–sister mating. Perhaps because of this inbreeding, 5 of the 23 neonates (22%) were stillborn (or died 1–3 days after birth), and the mother also voided 9 abortive eggs (unfertilized or died early in embryogenesis). Gaboon vipers have 9–12 'hourglass' markings on their dorsal surfaces (Fig. 1), and these usually display complete longitudinal symmetry (pers. obs.). The mother and the grandmother of the 1997 litter were symmetrical in this respect, but the father displayed 1 asymmetrical hourglass (the grandfather's colour pattern was not recorded). Among the 5 stillborn offspring in the 1997 litter, 4 (80%) showed 'asymmetrical' hourglass patterns. The same was true for 8 of the 18 living offspring (44%). The high frequency of this unusual asymmetry in the inbred litter suggests that the pattern abnormality may reflect an underlying genetic effect. Although our main goal was to examine the effect of food abundance on snake body shape, we also classified the animals into 2 additional groups (symmetric and asymmetric) based on whether or not the animal displayed any obviously non-symmetrical hourglass markings.

Our approach is based upon a single litter of animals. Previous studies have generally relied upon individuals from several litters. The only disadvantage to reliance upon a single group of full-siblings is that our litter may not be representative of mean gene frequencies in the Gaboon viper population as a whole. However, because our focus is on a simple question (phenotypic plasticity in response to food intake), if our study animals show strong phenotypic plasticity in response to the conditions under which they were reared (as indeed they did), then presumably other Gaboon vipers would respond in similar ways. By using 1 litter, we partly control for genotype, and minimize confounding effects (both genetic and non-genetic), focusing narrowly on treatment effects.

Measurements

The living neonates were measured (snout–vent length (SVL)) to the nearest 0.5 cm and weighed to the nearest 0.1 g. The initial mean SVL and mean body mass of the 18 neonates were 28.1 ± 0.8 cm (25–29 cm) and 44.0 ± 2.4 g (39.4–48.6 g), respectively. Several features of cranial morphology were also measured to the nearest 0.1 mm: head length (from the base of the skull, localized by palpation, to the tip of the snout), cranial width (across the top of the head, above the eyes), and 'jaw' length (from the quadrato-articular articulation to the tip of the snout). Our measures of head and 'jaw' lengths include the thickness of the skin overlying these structures, but such effects should be minor (and should not generate spurious patterns). Because of the great flexibility of the snake's skull, a standardized method was used to measure the length of the 'jaw'. The snake was forced to bite into a polystyrene block, and then the skull held in position against the block with the fangs still embedded. Thus, the head was immobilized during measurement. Our measures were highly repeatable. On average ($n = 10$ repeated measures per individual), the standard deviation and maximal error (in parentheses) were respectively 0.26 cm (1 cm) for SVL, 0.43 mm (1.72 mm) for head length, 0.23 mm (0.82 mm) for cranial width, 0.45 mm (1.66 mm) for 'jaw' length, and 0.33 mm (1.04 mm) for the length of the fangs (see below). In each, the standard deviation comprises < 1.8% of the mean (average 1.0%). In contrast, our experimental treatments generated differences of up to 15.6% in these variables (average 12.1%; see Results). Thus, our measurements were sufficiently precise for reliable detection of such effects. The animals were weighed at weekly intervals, and measured at approximately monthly intervals. Ten measures of both SVL and body mass (with time intervals of 33 ± 7 (SD) days during the 9-month experiment) were kept for analyses. Head dimensions were measured less frequently (because longer time intervals were necessary to detect increases in size), so that only 6 measures were available for each of the 18 juvenile snakes. The length of the fangs was estimated later in the course of the study, and only 3 repeated measures were available for this phenotypic trait. Fang length was estimated by keeping the mouth open, then erecting (90°) gently the right fang with a pen, and measuring it straight from the lip to the tip of the fang with a calliper. Hence, our measure ignores the curved shape of the fang and the exact position of its base (in fact the enlarged tip of the maxillary (that represents 20% of our 'fang' length) was included in this measure).

Food treatment

The snakes were allocated randomly to 2 treatments. Nine offspring (6 males and 3 females; including 4 asymmetric individuals) were each provided with 1 living mouse (27.2 ± 8.2 g) per 2 weeks ('high-food' treatment). The other 9 (6 males and 3 females; 4 asymmetric) were each given one-third this amount ('low-food' treatment: 1 living juvenile mouse, 8.1 ± 2.0 g, per 2 weeks). The time interval between feedings was the same in both treatments, all the 18 snakes being fed the same day. In the high-food group, prey size was progressively increased to 'follow' snake's growth (from 11.4 ± 1.2 g (juvenile mouse) at the beginning to 49.3 ± 21.7 g (adult mouse) at the end), whereas prey size remained constant in the low-food group (juvenile mice only). Prey sizes relative to snake sizes (mean was 21 ± 8%, range 10–46%, $n = 139$ in the high-food group; and 10 ± 3%, range 5–26%, $n = 144$ in the low-food group) were comparable to those recorded in the field (average 5–20%, extremes 1–103%; Bodbijl, 1994). Reluctant feeders were occasionally force-fed (6 occasions among 283 meals), but almost all snakes readily accepted their mice. The mass of food consumed was recorded at each feeding. The young snakes were kept separately in plastic and wooden boxes measuring 25 × 25 × 25 cm. The air temperature in the room was controlled, and ranged from 20 °C at night up to 27 °C during the day. No thermal gradient was available in the boxes. Fresh water was available *ad libitum*.

Statistics

Repeated measures analyses of variance was used with food treatment (high/low) and level of asymmetry (present/absent) as within-subjects factors. Univariate repeated measures ANOVA assumes that the changes across levels (intervals among measurements in this study) are uncorrelated across subjects. This assumption is unlikely to be valid for our data. For example, vipers that grew fast from time 1 to time 2 might then have grown less from time 2 to time 3 (because they had already reached a ceiling in their capacity for subsequent growth), or conversely, they might have grown faster (because they had already developed a more efficient machinery for processing food: e.g. Secor *et al.*, 1994). Thus, given the suspicion that the sphericity assumption for univariate ANOVA has been violated, the multivariate analysis of variance (MANOVA) was used, an approach which has gained popularity in recent years (Greenhouse & Geisser, 1959; Hulbert, 1984; O'Brien & Kaiser, 1985; Walls, 1998; see Forsman, 1996 and Forsman & Lindell, 1996 for a more complete explanation). Furthermore, the multivariate approach enables us to detect where violation of the sphericity assumption would otherwise lead to an erroneous acceptance of the interaction effects as being statistically significant. In practice, violations of the sphericity assumption were detected in all of our repeated measures tests

(Mauchley's sphericity tests: $P < 0.01$), thus the multivariate repeated-measures design was appropriate.

Another way to control statistical problems that arise in comparisons of the influences of food treatment between groups, is to restrict attention to a single sampling period ('static allometry' *sensu* Forsman, 1996). Unfortunately, such a comparison relies upon a considerable overlap in body sizes, because otherwise curvilinearity in the relationship between 2 variables can generate artifactually 'significant' differences in the slopes and intercepts of regression lines fitted to these data (Snedecor & Cochran, 1987). This assumption was valid during the first part of our experiment but not later, after the 2 groups diverged in mean body size (Fig. 2). In order to compare the effects of food treatments while maintaining overlap of body sizes between the 2 groups, static allometry analyses were conducted on the following 2 groups of snakes: the 'low-food' group at the time they attained their maximal size (9 months of age), and the 'high-food' group at the time they were similar in absolute size to these 9-month-old 'low-food' snakes. Analysis showed that the 'high-food' snakes reached this body size at 4 months of age (ANOVA with food treatment as the factor and SVL as the dependent variable; $F_{1,16} = 0.487$, $P = 0.495$). In keeping with usual practice for ANCOVA, non-significant interaction terms were deleted and intercepts effects recalculated where appropriate. One of the advantages of the 'static allometry' approach is that analyses are intuitive and thus easier to interpret.

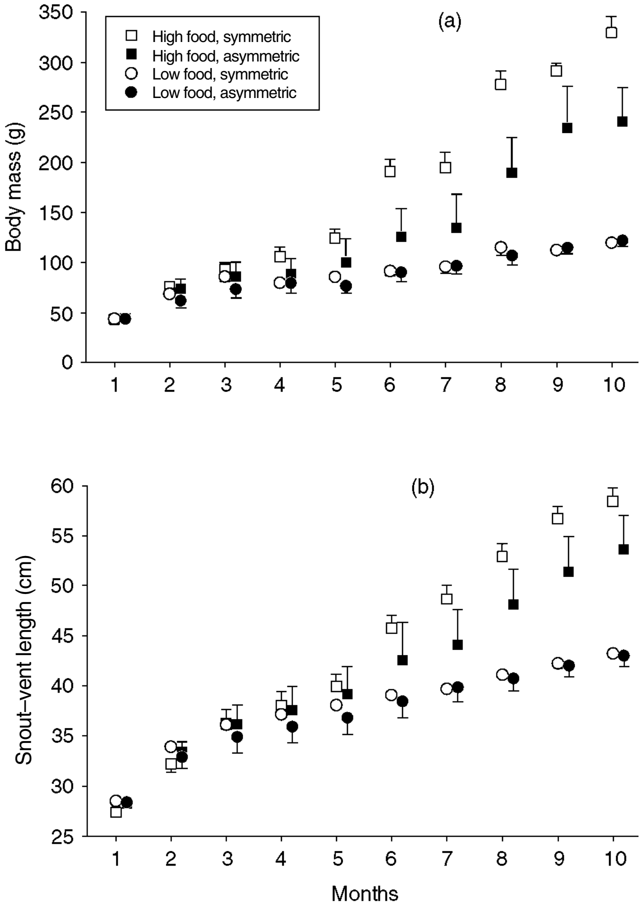

Fig. 2. Growth trajectories of juvenile Gaboon vipers *Bitis gabonica* as a function of food availability ('high-food' *vs* 'low-food' treatments), and symmetry level (symmetrical *vs* asymmetrical snakes) during a 9-month experiment. Increases over time in: (a) body mass; (b) snout–vent length. Each symbol represents the mean value (± SE) calculated for each snake group. Sample sizes are five snakes for both symmetric groups, and four snakes for both asymmetric groups (total = 18 snakes). See text for statistics.

These 2 alternative analytical approaches (MANOVA for repeated measures *vs* ANCOVA restricted to a single data point per individual) yielded very similar results, and conclusions from the 2 procedures were essentially identical (see Results). Occasionally, conventional significance was reached with only 1 of the 2 procedures, but no substantive conflict arose between them. Both sets of tests are reported for 2 reasons. First, our results will be directly comparable to those reported in previous studies (e.g. Forsman, 1996). Second, differences in the results obtained using the 2 techniques may identify cases in which a divergence between the 2 treatment groups in relative size of some body component is an indirect consequence of differences in age/size relationships rather than a direct effect of food intake. For example, if the head grows less rapidly than the body, the 'static allometry' analysis on animals of the same overall size but different ages (ANCOVA on 4- *vs* 9-month-old snakes) would detect significant effects of food treatment on relative head size. However, the MANOVA approach (repeated-measures tests, comparing snakes of similar age classes over time) would show that this effect was the result of age rather than diet *per se* (O'Brien & Kaiser, 1985). Such age effects (e.g. at the same body size, younger snakes have relatively larger heads) are 1 plausible mechanism that could generate shifts in body proportions in response to different levels of food intake (and thus, growth rate).

RESULTS

Measurements of the neonates confirmed that our random allocation of animals to treatment groups had resulted in a lack of significant differences between the two groups in any of the variables measured at the beginning (all $P > 0.10$; Fig. 2). Similarly, no significant differences were detected between symmetric and asymmetric snakes at birth (all $P > 0.30$). All 18 snakes survived to the end of the experiment (9 months of age). Our preliminary analyses of the overall data set revealed no significant differences in absolute body sizes or body proportions between the two sexes, or interactions between sex and other factors. Thus, we have pooled males and females for the results reported below. Females and males were distributed equally across food treatments, and any undetected sex effect should not alter our conclusions.

Table 1. Effect of food treatment (high-food *vs* low-food group) and symmetry level on size (snout–vent length, body mass and condition) and shape (relative head size, head shape, and relative fang length) of Gaboon vipers *Bitis gabonica*. Static measures were performed using two factor ANOVAs (with food treatment and symmetry levels as the factors) or ANCOVAs (with SVL[a], head length[b], or jaw length[c] as the covariates). Body condition represents the body mass scaled by size. Repeated measures over time were performed using MANOVAs. To characterize body shape, mean-square residual values from the linear regression between the traits of interest were used (i.e. residuals from the regression of log body mass against log SVL were used as our indices of body condition; or residuals from the regression of head width *vs* head length were used as our measure of relative head shape). Bold numbers indicate significant effects

Trait	Source	Static measures		Repeated measures		
		F(df)	P	Wilks' λ	*F*(df)	P
Body mass	Food	84.5 (1, 14)	**0.001**	0.012	41.3 (5, 10)	**0.001**
	Symmetry	5.9 (1, 14)	**0.03**	0.12	3.7 (5, 10)	0.08
	Interaction	6.5 (1, 14)	**0.03**	0.06	8.4 (5, 10)	**0.01**
Snout–vent length	Food	54.6 (1, 14)	**0.001**	0.06	8.5 (5, 10)	**0.01**
	Symmetry	2.1 (1, 14)	0.17	0.40	0.8 (5, 10)	0.67
	Interaction	1.0 (1, 14)	0.21	0.13	3.2 (5, 10)	0.10
Body condition	Food	16.7 (1, 13)	**0.002**[a]	0.12	3.7 (5, 10	0.08
	Symmetry	14.0 (1, 13)	**0.003**[a]	0.29	1.3 (5, 10)	0.42
	Interaction	20.3 (1, 13)	**0.001**[a]	0.19	2.1 (5, 10)	0.22
Relative head size	Food	5.6 (1, 13)	**0.05**[a]	0.26	4.2 (6, 9)	**0.03**
	Symmetry	3.0 (1, 13)	0.11[a]	0.42	2.1 (6, 9)	0.15
	Interaction	0.9 (1, 13)	0.37[a]	0.72	0.6 (6, 9)	0.73
Head shape	Food	4.8 (1, 13)	**0.05**[b]	0.11	13.4 (6, 9)	**0.001**
	Symmetry	0.0 (1, 13)	0.89[b]	0.52	1.4 (6, 9)	0.33
	Interaction	0.4 (1, 13)	0.54[b]	0.78	0.42 (6, 9)	0.85
Relative fang size	Food	12.3 (1, 13)	**0.005**[c]	0.44	5.14 (3, 12)	**0.02**
	Symmetry	13.9 (1, 13)	**0.003**[c]	0.94	0.3 (3, 12)	0.84
	Interaction	14.0 (1, 13)	**0.003**[c]	0.76	1.8 (3, 12)	0.33

Absolute body size and body mass

Static measures

The difference in food treatment translated into a massive difference in absolute body size over the course of the experiment (Fig. 2, Table 1). By the time the snakes were 9 months old, the high-food animals weighed about twice as much as did their siblings raised in the low-food treatment (means of 288 *vs* 119 g, 56.2 *vs* 43.0 cm SVL; see Fig. 2). A two-factor ANOVA on the data for body mass when the snakes were 9 months of age (i.e. at the final weighing), with food treatment and asymmetry level as the factors, confirms a statistically significant effect of treatment and asymmetry level as well as a significant interaction between these two factors (symmetrical and well-fed snakes were heavier; see Fig. 2, Table 1). In contrast, the same analysis on SVL detects only the treatment effect (well-fed snakes were longer), with asymmetry level and the interaction term failing to explain significant variance in body length (Table 1).

Repeated measures over time

A MANOVA for repeated measures design confirms that rates of growth in mass and length were significantly influenced by food treatment (Table 1). Symmetry levels alone did not influence these two traits. The interaction between food treatment and symmetry level was significant for body mass but not for SVL (Table 1).

Overall, both MANOVA for repeated measurements and ANOVA performed at the end of the experiment led to similar conclusions. Mass and length of the juvenile snakes were significantly affected by the food treatment. Inspection of the data shows that this interaction results from the fact that the level of asymmetry had little effect on growth rate in the low-food animals, but substantially affected the mass and length of the high-food vipers (Fig. 2).

Body mass relative to snout–vent length

There was no difference in the mass/SVL relationship (body condition index) among the four groups at the beginning of the experiment ($P > 0.18$ for all factors in all comparisons).

Static measures

We used a two-factor ANCOVA, the covariate was SVL, the dependent variable was mass, and the factors were food treatment and level of asymmetry (Fig. 3, Table 1).

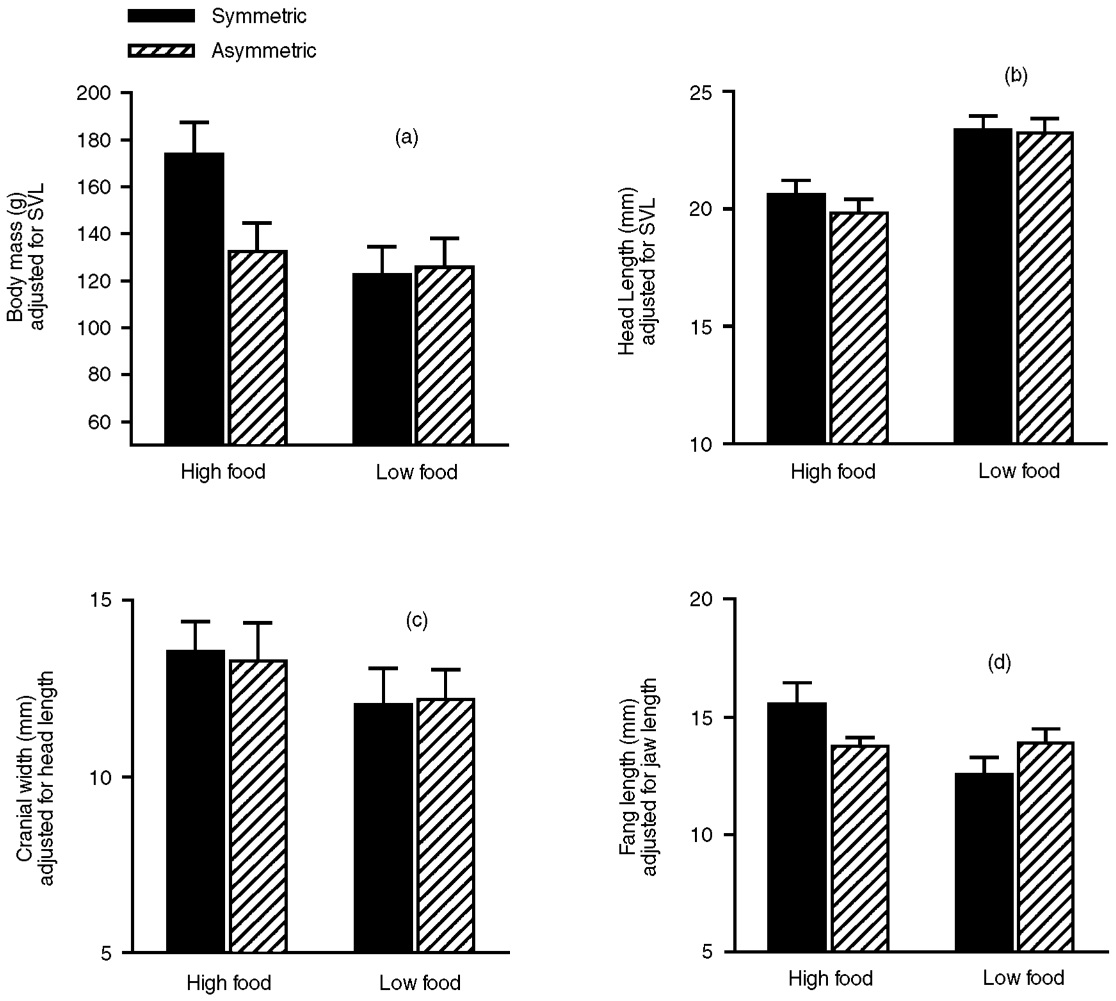

Fig. 3. Effects of food intake and level of asymmetry on body proportions of juvenile Gaboon vipers *Bitis gabonica*. Although statistical tests in the text are based on static allometric (ANCOVA) and repeated measures design (MANOVA), for simplicity adjusted (least-squares) means and associated SDs are shown. (a) Body mass relative to snout–vent length; (b) head length relative to snout–vent length; (c) head width relative to head length; (d) fang length relative to head length. SVL, snout–vent length.

Body shape was affected by food availability (high-food siblings were heavier-bodied at the same length) and differed between symmetric and non-symmetric individuals with a highly significant interaction between these two factors (the 'positive' effect of symmetry on body condition was detected only in well-fed snakes).

Repeated measures over time

A MANOVA for repeated measures design suggests that changes in mean body condition over time were not affected by food treatment or symmetry level, nor by an interaction between these two factors (Table1). However, a significant effect was obvious when the power of the analysis was enhanced by restricting computations to the influence of food treatment (Wilks' lambda = 0.13, $F_{10,\,7} = 4.44$, $P = 0.\ 03$).

Thus, static and repeated measures analyses led to slightly different conclusions, reflecting an ontogenetic change in body condition (Wilks' lambda = 0.19, $F_{9,8} = 3.80$, $P = 0.037$). As the snakes grew, they tended to become thinner as well as longer; hence, average body condition index decreased through time (Fig. 4). Faster-growing (high-food) snakes were heavier-bodied than their slower-growing (low-food) siblings at the same mean body length. However, this effect was primarily because of the age difference between the two groups (at the same mean body length, the faster-growing snakes were younger) rather than effects of food intake *per se*.

Head length relative to snout–vent length

Static measures

Using the same ANCOVA design as above (but with head length as the dependent variable), we found that animals on the high-food treatment had significantly

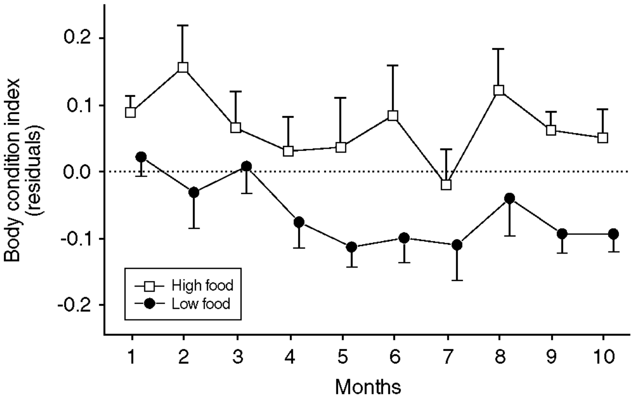

Fig. 4. Average body condition of juvenile Gaboon vipers *Bitis gabonica* as a function of food availability ('high-food' *vs* 'low-food' treatments) during a 9-month experiment. Body condition was calculated as the residual scores from the regression between ln body mass (g) against ln snout–vent length (cm). Each symbol represents the mean value (± SE) calculated for each snake group. Sample size was nine in both groups. See text for statistics.

shorter heads, relative to their SVL, than did their low-food siblings (Fig. 3, Table 1). Relative head length was not affected by asymmetry level, nor by the interaction between food availability and asymmetry (Fig. 3).

Repeated measures over time

Although relative head size was affected by ontogeny (time effect alone: Wilks' lambda = 0.08, $F_{5,10} = 23.87$, $P < 0.0001$), this effect did not modify the above static results (interaction among time, food treatment and symmetry level: Wilks' lambda = 0.80, $F_{5,10} = 0.49$, $P = 0.78$). High food treatment resulted in a relatively shorter head, with no effect of symmetry, and no interaction (Table 1). Further analyses (e.g. testing specific effects to conserve degrees of freedom) failed to provide additional information.

Head shape: cranial width relative to head length

Static measures

The two-factor ANCOVA with head length as the covariate and cranial width as the dependent variable, showed that the low-food vipers had narrower heads than did the high-food snakes at the same head lengths (Fig. 3). However, relative head widths were not affected by asymmetry level or by an interaction between asymmetry and food supply (Table 1).

Repeated measures over time

Head shape fluctuated through time (time effect alone: Wilks' lambda = 0.11, $F_{5,10} = 16.70$, $P < 0.001$), but again, this effect did not modify the static results (interaction among time, food treatment and symmetry level: Wilks' lambda = 0.78, $F_{5,10} = 0.56$, $P = 0.73$). Low food snakes had narrower heads with no effect of symmetry, and no interaction (Table 1).

Fang length relative to jaw length

Static measures

Because we did not attempt to quantify fang lengths until the young snakes were 7 months old, the strongest comparison is between low-food snakes at 9 months of age *vs* high-food snakes at 7 months of age. The two-factor ANCOVA for these animals shows that snakes in the high-food group had longer fangs relative to jaw length than did their siblings from the low-food treatment (Fig. 3), and that symmetric snakes had longer fangs than asymmetric snakes, with a significant interaction between these two factors (Table 1, the effect being visible only in the high-food group).

Repeated measures over time

Ontogenetic effects (time effect alone: Wilks' lambda = 0.67, $F_{2,13} = 3.25$, $P < 0.07$) were non-significant, perhaps because of the short period of sampling (3 months). Low-food treatment resulted in relatively shorter fangs, but with no effect of symmetry, and no interaction (Table 1). Visual inspection of the data suggests that the symmetry effect disappeared in the MANOVA procedure as a result of ontogenetic shifts, but this interpretation remains speculative because of our small sample size (only three time periods).

DISCUSSION

Our experimental design confounds two factors (mean prey size and total mass of food consumed), either (or both) of which might plausibly affect developmental trajectories. However, our goal was to determine whether or not differences in diet induced shifts in morphology, not to tease apart the factor (or combination of factors) responsible for such an effect. Overall, our results reinforce the idea that snakes display extraordinary flexibility in a wide range of phenotypic traits (e.g. Ford & Seigel, 1989, 1994). In particular, our experiment suggests that food availability (prey size?) can modify developmental trajectories. The size and shape of the young vipers was also affected by some unmeasured factor, for which our asymmetry score provided an index. This factor probably involves genetics (especially, inbreeding effects), but it remains possible that it is another environmental (maternal) effect, e.g. perhaps these asymmetric neonates developed in a particular region of the maternal oviduct where placental function was less efficient (Vom Saal, 1981).

Some of our results are not surprising. For example, previous studies have provided abundant evidence that food supply influences growth rates in snakes (as in many other kinds of animals: Reznick & Yang, 1993; Smith & Palmer, 1994) and that geographic variation in mean body sizes within a single species may result largely from such direct effects rather than from life-history adaptation (e.g. Madsen & Shine, 1993). There are also both experimental studies and field data to support a causal link between feeding rates and body condition in snakes (Forsman & Lindell, 1996; Shine & Madsen, 1997). However, the ontogenetic shifts in mass/length relationships (body reserves) that were observed obscure the picture. Body condition indices responded rapidly to food intake, and this extreme lability requires further experiments to understand how juvenile snakes are shaped in the course of growth. Temporal lability in experimentally induced phenotypic plasticity has been observed in other vertebrates (Meyer, 1987).

Other results from our study are more surprising: in particular, the discovery of significant differences in head dimensions as a consequence of feeding regimes. Previous experiments on American gartersnakes *Thamnophis sirtalis* (Arnold & Peterson, 1989) and European adders *Vipera berus* (Forsman, 1996) have concluded that snakes raised under different thermal or nutritional regimes none the less follow the same allometric relationship between body length and head length. This canalization of reaction norms has been used to support adaptationist interpretations of divergence in relative head size among populations (Forsman, 1994; Forsman & Shine, 1997) and between the sexes within single populations (Shine, 1991*b*; Houston & Shine, 1994; Forsman & Shine, 1997; Shine, Branch *et al.*, 1998). Our data suggest that caution is needed before extrapolating these results to other species. Cranial structures of our Gaboon vipers responded directly to food treatments. Other studies suggest that even low frequencies of dynamic loads (as are imposed on cranial structures during feeding) can produce remodelling of bone structure (Rubin & Lanyon, 1984). In the Gaboon vipers, these skeletal structures responded over a longer time interval than did body condition (static measures: differences in body condition were detected only 1 month after the start of the experiment, but 5 months were necessary to observe any detectable effect on relative head length). Experiments run over shorter time periods (as all have been) may detect only body-reserve fluctuations, and not experimentally induced skeletal modifications.

Conceivably, bones from the feeding apparatus may respond to different treatments only after long time intervals and/or large enough differences in the dynamic loads applied on them (Rubin & Lanyon, 1984; Meyer, 1987). Upon completion of the present experiment, the cumulative increases in body mass in the high-food and low-food groups were respectively 555% and 119% *vs* 209% and 88% for the previous study, which did not detect phenotypic response to diet (102% and 51% *vs* 52% and 32% for SVL) (Forsman, 1996). Importantly, natural populations of snakes may well be confronted with even more divergent levels of prey abundance than those we imposed on our juvenile snakes (e.g. Schwaner & Sarre, 1988).

We do not suggest that all instances of divergence in bodily proportions (especially, head length relative to body length) among and within snake populations should be reinterpreted as phenotypic plasticity rather than adaptation. For example, a genetic (and hence, plausibly, adaptive) basis for sexual divergence in relative head size is supported by observations that head sizes are sexually dimorphic at birth in some species (Shine, 1991*b*), and that this trait is controlled by sex-steroid levels in at least one of these taxa (Shine & Crews, 1988). None the less, our data suggest that at least in *Bitis gabonica*, body proportions respond directly to environmental factors. A recent experimental study on American watersnakes *Nerodia sipedon* by Queral-Regil & King (1998) also suggested that relative head proportions might be modified by prey size, suggesting that *Bitis* may not be unique in this respect.

Given the complex inter-relationships among different body parts, it is difficult to be sure that all of the divergences that we have documented are genuinely independent effects. For example, one might attribute the simultaneous diet-induced shifts in: (1) mass relative to body length; (2) head length relative to body length, as both reflecting the modification of a single trait (body length) rather than as independent effects. However, a closer analysis refutes this interpretation, because food intake strongly influences the relationship between these two dependent variables (two-factor ANCOVA with mass as covariate, head length as dependent variable; treatment effect: $P < 0.001$). The same conclusion holds true for most other comparisons among the variables measured (e.g. cranial width *vs* head length; fang length *vs* jaw length). Thus, our data document substantial shifting of body proportions in response to food supply.

Why did our vipers change their morphology in response to feeding level? Several possibilities seem plausible:

(1) *Mass relative to body length*. The heavier build of well fed vipers may reflect an adaptive response to prey availability (relative allocation of nutrients to storage *vs* growth), as advocated for adders by Forsman & Lindell (1996). However, a simpler possibility, also consistent with both data sets, is that building additional skeletal and muscular tissues to increase in size takes time, and hence that the well-fed animals store surplus energy in the form of fat reserves. Such physiological responses may not represent an adaptation (Gould & Lewontin, 1979; Bradshaw, 1997).

(2) *Head length relative to body length*. As for the trait above, the shorter head of relatively well fed snakes could be interpreted in terms of adaptation (i.e. they derive no benefit from increasing maximal ingestible prey size; Wimberger, 1991). However, it is also possible

that constraints on maximal growth rate differ between different parts of the body, so that head growth cannot keep up with the rate of body growth in these animals. The observation that male sand lizards *Lacerta agilis* continue to increase in head size, after attaining maximal body length (Olsson & Shine, 1996) supports the notion of some kind of decoupling between growth rates of these two components of the phenotype. More generally, modifications of feeding structures as a direct response to diet have been documented in a variety of invertebrates (e.g. Colonna Cuffaro, 1978; Bernays, 1986) and vertebrates (e.g. Holbrook, 1982; Swennen *et al.*, 1983; Meyer, 1987; Walls, Belanger & Blaustein, 1993).

(3) *Head width relative to head length.* The biomechanics of feeding are complex, and dynamic loads imposed on skeleto-muscular structures to swallow large prey in the 'high-food' group may well produce complex plastic responses (Rubin & Lanyon, 1984; Govind & Pearce, 1986; Garland & Huey, 1987). However, larger prey also provide more energy, and thus our protocol does not allow us to tease apart the respective contributions of energetic *vs* mechanical effects.

(4) *Fang length relative to jaw length.* For most venomous snakes, longer fangs presumably function to penetrate the prey better and to deliver venom to kill the animal and begin to digest it (Thomas & Pough, 1979). However, the fangs of *B. gabonica* are much larger than those of any other snake (Pough & Groves, 1983; Mehrtens, 1987), suggesting that other selective forces may have been involved in their evolution. Two observations are of interest in this respect. First, our vipers clearly use their fangs to manipulate prey items while swallowing (pers. obs.), and longer and more powerful 'fangs' (as measured in our study, this includes the maxillary bone as well as the actual fang) may expedite this process. Second, Gaboon vipers differ from many other viperid snakes in that they often retain their grip on the prey after the initial strike. Thus, they may be under strong selection pressure to kill the prey rapidly (before it can retaliate), and the physical damage from their massive fangs may function in this way.

In conclusion, our study reinforces the idea that snakes offer logistically feasible model systems with which to investigate the interplay between genetic and environmental factors as determinants of size and shape. Our data indicate that this flexibility extends even further than has been suggested from previous experimental work, and that a range of biologically significant traits (such as energy allocation to storage *vs* growth, and the relative size and shape of the head and fangs) can be modified by differences in the conditions experienced by juveniles. This plasticity could be adaptive, for example by increasing the swallowing capacities of the snakes in relation to their main type of prey (Forsman & Lindell, 1993; Walls *et al.*, 1993; Sheen & Whiteman, 1998). The contrasts between our results and those of workers dealing with other snake species (Arnold & Peterson, 1989; Forsman, 1996) suggest that interspecific diversity in the level of trait canalization may offer opportunities to further unravel the evolutionary significance of phenotypic plasticity.

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