# Vertebral number in adders, *Vipera berus*: direct and indirect effects on growth

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Given the importance of body size, and thereby growth rate, for many reproductive parameters in snakes, morphological traits conferring an advantage in terms of growth may be important targets of selection. Studies have demonstrated effects of vertebral number of growth rate in garter snakes. In this study effects of total number of body vertebrae and of number of abnormal body vertebrae (obtained by counting number of ventral scutes and number of abnormal scutes) on growth rate in free-ranging male and female adders, *Vipera berus* (L.), are examined by calculating directional performance gradients (estimating linear effects) and stabilizing performance gradients (estimating curvilinear effects). After controlling for body size (SVL) female adders demonstrated a significant positive directional gradient for vertebral number, and a significant interaction between body size and vertebral number, showing that females with more vertebrae have higher size-specific growth rates, and that this effect is strongest among small, fast growing individuals. Females also showed a weak stabilizing effect of abnormal vertebrae. Males, on the other hand, showed a positive directional gradient for number of abnormal vertebrae, whereas no effect of vertebral number was observed. Indirect effects of the same variables were evaluated by use of path analysis. Generally, indirect effects were weak and did not substantially increase the amount of explained variance in growth rate. Field data showed that the correlation between vertebral number and growth rate in females was stronger in years with higher overall growth rate. To evaluate whether vertebral number and food availability show an interactive effect I used captive born juvenile adders in an experiment with two different food levels. The experiment confirmed the field data. No relationship between vertebral number and growth was observed in the low food level group, whereas in the high food level group a significant positive correlation was demonstrated. Finally, the heritability of vertebral number was examined using a mother-offspring regression and a full-sib analysis. The estimated heritabilities were 0.30 and 0.39, respectively. From these results it is concluded that both vertebral number and abnormal vertebral number may significantly affect growth in adders, but that this effect may differ between sexes and among years.

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ADDITIONAL KEY WORDS: — body size – heritability – morphological variation – path analysis – performance gradients – pleomerism – snakes.

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## INTRODUCTION

In advanced snakes (families Colubridae, Elapidae and Viperidae) the number of body vertebrae shows a considerable amount of both interspecific (e.g. Boulenger, 1913; Klauber, 1956; Lindell, 1994) and intraspecific variation (e.g. Klauber, 1945; Lindell, Forsman & Merilä, 1993). Within species the range of vertebral number may be 50 vertebrae or more (e.g. FitzSimons, 1962) with a coefficient of variation typically around 2% (Klauber, 1956; Kerfoot, 1970). Only in a few cases, however, has the ecological and evolutionary significance of this large intraspecific variation been assessed. For example, Arnold (1988) showed that female *Thamnophis elegans* individuals with intermediate vertebral numbers grew faster than any of the extremes, whereas in males, number of abnormal body vertebrae negatively affected growth. Also, Arnold & Bennett (1988) suggested that number of body vertebrae in combination with number of tail vertebrae affected locomotor performance in juvenile *T. radix*. Lindell *et al.* (1993) showed that in *Vipera berus* individuals with a larger number of body vertebrae grew larger, as was also the case in juvenile *T. radix* (Arnold & Bennett, 1988) and other genera of colubrids and viperids (Klauber, 1945). The existence of an intraspecific relationship between vertebral number of growth rate or body size is of considerable evolutionary interest as body size is highly correlated with fitness in many species of snakes because of its relationship with fecundity and mating success (e.g. Shine, 1978; Fitch, 1985; Seigel & Fitch, 1985; Madsen & Shine, 1992; Madsen *et al.*, 1993) and survival (Jayne & Bennett, 1990; Forsman, 1993a). Therefore, if vertebral number is a heritable trait, it may undergo evolutionary change both as a response to selection on vertebral number *per se* and as a correlated response to selection on body size (Arnold, 1988; Lindell, 1994).

As the number of vertebrae does not change over an individual's lifetime a positive relationship between body size and vertebral number among adults can come about mainly in three ways. Individuals with more vertebrae can: (i) grow faster; (ii) survive longer; or (iii) stop growing at a larger asymptotic size than individuals with fewer vertebrae. Lindell *et al.* (1993) suggested, based on field data on adders (*Vipera berus*), that individuals with many vertebrae have higher size-specific growth rates than those with fewer vertebrae. However, we were unable to control for differences in food intake rate, nor did we analyse males and females separately. Some evidence for viability selection against low vertebral numbers in newborn snakes was found, whereas differences in asymptotic size were not examined.

In this paper I examine the effects of three morphological variables (snout-vent length, total number of body vertebrae, and number of abnormal body vertebrae) on growth rate in free-ranging adders using data from two populations obtained during 1986–1994. The number of body vertebrae was assessed by counting number of ventral scutes (Alexander & Gans, 1966). I examine whether a linear relationship may prevail between growth rate and any of the three variables, or any interaction between them, and also whether any curvilinear relationships might be found. To do this I use the method outlined by Arnold (1983) and Lande & Arnold (1983) where a number of morphological variables are used as independent variables in a multiple regression model. I also analysed the field data using path analysis to supplement the

test for direct effects using the Lande & Arnold method with a test for indirect effects (Sokal & Rohlf, 1981).

The field data indicated an interactive effect of food abundance and vertebral number on growth rate. Therefore I performed an experiment to examine whether different food levels affect the relationship between vertebral number and growth rate in young adders raised in captivity. By keeping the adders on a controlled diet at different levels two goals were achieved: (i) the effect of vertebral number *per se* could be assessed without confounding effects of differences in food intake rate, and (ii) the interactive effects of vertebral number and food intake rate could be evaluated.

Finally, whether or not a fitness-related trait will show evolutionary change in response to selection depends on its heritability (e.g. Falconer 1981). If there is no heritability, selection will only act on phenotypes and no change in population mean or variance will take place over generations (Lande, 1979; Endler, 1986). Therefore, heritability of vertebral numbers was also determined for adder populations.

## MATERIAL AND METHODS

### *Field data*

Adders were caught on two island groups (localities A and I) off the east coast of central Sweden (N 59° 20'; E 19° 20') in 1986–1994 (for a map see Forsman, 1991). Each snake was sexed, measured for snout-vent length (SVL) to the nearest 5 mm, weighed to the nearest gram and individually marked by branding one or two ventral scutes. Number of body vertebrae was estimated by counting number of ventral scutes, as these show a 1:1 relationship in most advanced snakes (Alexander & Gans, 1966; Voris, 1975). Sometimes abnormal scutes are present on snakes (Plummer, 1980; Schwaner, 1990; Merilä, Forsman & Lindell, 1992) with the most common type being one half scute inserted between two large ones. This usually corresponds to an abnormal vertebra with two ribs on one side and one on the other (King, 1959; Plummer, 1980). In these cases the half scute was not counted when estimating vertebral number. The number of abnormal scutes, and thus abnormal vertebrae, was noted. Growth was calculated as the increment in SVL (mm) between two capturing occations during one summer, or (more often) between a first capture in spring or summer and a second capture in early spring the next year. Growth rate was calculated by dividing growth with number of days elapsed between capturing occasions, setting the growth period to June 1–September 15.

Lande & Arnold (1983) suggested a method for calculating directional and stabilizing selection gradients using a multiple regression model. Selection gradients are equivalent to the partial regression coefficients and they represent the effect of each variable while holding all other variables constant. Relative fitness was used as the dependent variable in the Lande & Arnold model and any number of morphological variables, as well as interactions between them, are taken as independent factors. Often, however, relative fitness is difficult to measure in natural populations, or the investigator is mainly interested in the effects of a morphological variables on some component of fitness. Arnold (1983) therefore suggested that one may partition the selection gradient into one component connecting morphology

with some measure of performance (the performance gradient) and one component connecting performance with relative fitness (the fitness gradient).

In this paper I apply the method of Arnold (1983) and Lande & Arnold (1983) to calculate performance gradients for body size (SVL), vertebral number and number of abnormal vertebrae on growth rate of male and female adders. SVL and vertebral number were normally distributed in both sexes whereas number of abnormal vertebrae was Poisson distributed with a mode of zero and therefore was $\log_{10}(x + 1)$ transformed. As data were collected in two island groups during several years and mean growth rate varied among island groups and years (Forsman & Lindell, 1991; Forsman, 1993b), growth rate was standardized for both males and females separately among island groups and years to their respective overall mean. When data on growth rate of an individual were available for several years I randomly decided which year to include and each individual is therefore only used once in the analyses. As growth rate is highly dependent on body size, and because mean SVL varied among years, SVL was also standardized within year and locality to its overall mean for males and females separately. There was no significant heterogeneity of variances in any of the variables among localities and years ($F_{max}$-test, all $P > 0.05$), and there were no significant correlations among the morphological variables (Table 1). I excluded from the analyses all females that I could positively identify as gravid.

A linear multiple regression model was first used to obtain directional performance gradients ($\beta$) for the three variables. The directional performance gradients describe the linear effect of each independent variable on growth rate while holding all other variables constant. Thus, this model included the variables $z_1$ (SVL), $z_2$ (vertebral number) and $z_3$ (number of abnormal vertebrae). A quadratic multiple regression model was then used to estimate stabilizing performance gradients for the same three variables, plus the three two-way linear interactions, while controlling for their directional effects. The stabilizing performance gradients equal the partial regression coefficients calculated for the squared deviations from the mean of each variable, with a negative value indicating stabilizing selection and a positive value disruptive selection. Thus, the second model included the squared deviations from the mean of all variables divided by two, $z_1^2/2$, $z_2^2/2$, and $z_3^2/2$, as well as the unsquared values $z_1$, $z_2$, $z_3$, (representing the directional performance gradients), and all two-way interactions: $z_1 \times z_2$, $z_1 \times z_3$, $z_2 \times z_3$. Dividing by two converts the coefficients for the squared variables into measures of curvature of the performance surface (Arnold & Bennett, 1988). The coefficients for the linear variables calculated in the second

TABLE 1. Descriptive statistics of three morphological variables used in the multple regressions for estimating effects on growth rate in *Vipera berus*, with observed correlation coefficients among them. No correlations were significant

| Sex | Variable | Mean | S.D. | Range | | Correlation matrix | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | SVL | Vert. | Ab vert |
| Females | SVL (mm) | 523.2 | 105.70 | 230–670 | SVL | 1.0 | 0.111 | −0.063 |
| $n = 61$ | Vertebrae | 148.8 | 3.14 | 139–157 | Vert. | | 1.0 | −0.017 |
| | Abn. Vertebrae | 0.6 | 1.53 | 0–11 | Ab vert. | | | 1.0 |
| | | | | | | SVL | Vert | Ab vert |
| Males | SVL (mm) | 500.9 | 75.47 | 260–665 | SVL | 1.0 | 0.104 | 0.146 |
| $n = 97$ | Vertebrae | 144.9 | 2.21 | 139–150 | Vert. | | 1.0 | 0.187 |
| | Abn. Vertebrae | 0.6 | 1.03 | 0–7 | Ab vert. | | | 1.0 |

model did not coincide with the correct values obtained when linear effects were estimated alone. This arises because skewness in trait distributions will cause distortion of directional gradients when squared and product variables are included in the regression (Arnold & Bennett, 1988).

The directional and stabilizing performance gradients describe the effect of a change in any of the morphological variables on growth rate. However, instead of expressing a rate of change in terms of the original measurement units, the standardized performance gradient $\beta'$ (i.e. standardized partial regression coefficient) will give the rate of change in standard deviation units of growth rate per one standard deviation unit of each variable. Using standardized coefficients the effects of each variable on growth rate become directly comparable (Sokal & Rohlf, 1981: 621). The standardized performance gradients were obtained by standardizing growth rate and the three independent variables to a mean of zero and a standard deviation of one before calculating the multiple regression model.

Path analysis was used to quantify indirect linear effects by the different variables (Sokal & Rohlf, 1981). In path analysis a path diagram is first constructed where the researcher decides which variables and effects (paths) to include. The strength of the relations between any two variables may then be calculated as the path coefficients ($p$) which equal the standardized partial regression coefficients ($\beta'$) as obtained by a multiple regression (e.g. Kerlinger & Pedhazur, 1973).

### Growth experiment

The mothers ($n = 5$) of the young used in the feeding experiment were collected in the field (from the same island groups as adders used in the field data) in late July 1994, brought to the laboratory in Uppsala and housed in individual terraria. They were given water in a bowl but no food. The terraria were kept in a climatic chamber with a temperature of 28° C and 45–50 % humidity. The photoperiod was 12 h light: 12 h dark, and over each terrarium a 100 W light bulb was present for additional heating. The females had access to shelter allowing them to thermoregulate to some extent. The young were born between August 7 and August 14 and subsequently placed individually in plastic containers ($18 \times 18 \times 11$ cm). The floor of the containers was covered with a paper towel and water was provided in a bowl. The young were kept at the same temperature, humidity and photoperiod as their mothers.

The young were divided into a high food level group and a low food level group such that from each litter both males and females were present in both groups. Twenty-five individuals were used over the whole experimental period. The high food level group ($n = 13$) was provided with food twice a week whereas the low food level group ($n = 12$) only once a week. Individuals in the high food level group were fed a mean of 55.5 g (SE = 0.8) of mice and the low food level group 26.7 g (SE = 0.7) over the whole experimental period. The food provided consisted of newborn mice (1.5–3.0 g), and on each feeding occasion each snake was given one live mouse. If the mouse was not eaten within 24 hours it was killed and the snake was force-fed. This feeding schedule was maintained for 16 weeks. If a snake regurgitated the mouse, it was recorded.

Over the experimental period all young were measured four times, namely before the feeding commenced, after six weeks, after eleven weeks, and then at the

termination of the experiment after 16 weeks. On each measuring occasion the young were weighed to the nearest 0.1 gram and their snout-vent length (SVL) measured to the nearest 5 mm. The young were sexed and number of body vertebrae was estimated by counting number of ventral scutes from black-and-white photographs of their ventral side. The number of abnormal scutes, and thus abnormal vertebrae, was recorded. Notes were also taken on dates of sloughing.

### Heritability estimates

The gravid females used when estimating heritabilities were collected from three island groups during four years. A total of 29 females, giving birth to 218 young, were used. Number of ventral scutes could not be counted on some of the young, and these had to be omitted from the analyses. If collected relatively early in summer (late June-mid July) the mothers were first placed in an outdoor enclosure situated in a forest area outside Uppsala until shortly before parturition when they were brought to the laboratory and put in separate terraria. Females collected in late July or August were directly placed in terraria. After parturition the young were sexed, weighed and measured, and their ventral sides photographed to allow ventral scutes to be counted. The scutes of the mothers were counted directly. In both mother and young the number of abnormal scutes was noted.

As the females were collected after the mating period and the fathers were unknown, heritability estimates ($h^2$) for vertebral number were obtained by regressing midoffspring values on mothers' values where $h^2$ = twice the slope and SE ($h^2$) = twice the SE of the slope (Falconer, 1981). Heritability estimates were also calculated by a full-sib analysis (Falconer, 1981). The same mother was only used once in each of these calculations.

Because sexual dimorphism was significant in vertebral number among the offspring (mean of males: 143.4; females 148.6; $t = 7.84$, $P < 0.0001$) and the sex ratio varied among litters, I standardized vertebral numbers of males to female equivalents before heritabilities were calculated. This was done by multiplying male measurements with a constant (1.0305) reflecting the female/male ratio observed in a larger sample ($n = 493$).

### RESULTS

### Field data

Descriptive statistics of the three morphological variables in male and female adders are presented in Table 1, and estimated directional and stabilizing performance gradients, and their interactions, for these variables in Table 2. In both sexes SVL showed a significant negative directional performance gradient (negative linear effect) for growth rate, as expected since larger individuals in most snake species, including the adder, grow more slowly than smaller ones (e.g. Andrews, 1982). In females SVL also showed a weak positive curvilinear effect, i.e. stabilizing performance gradient ($\beta' = 0.22$), reflecting that some intermediate sized females grew more slowly than expected from their body size, possibly due to pregnancy unobserved by me.

TABLE 2.Performance gradients in *Vipera berus*. For each sex a linear multiple regression model was first used to calculate the directional performance gradients (linear effects) for all three morphological variables on growth rate. Then another multiple regression was performed where the interactive effects and the stabilizing performance variables (curvilinear effects) for the same three variables were calculated, while also controlling for their linear effects. (For more methodological details, see Material and Methods.) For each variable and interaction term the $F$-value and significance are given, together with the calculated performance gradient ($\beta$) and standardized performance gradient ($\beta'$), with their respective standard errors (SE and SE'). All multiple regression models performed were highly significant ($P<0.0001$)

| Sex | Test | Variable | $F$ | $P$ | $\beta$ | SE | $\beta'$ | SE' |
|---|---|---|---|---|---|---|---|---|
| Females | Directional | SVL | 96.2 | 0.0001 | −0.0026 | 0.0003 | −0.7830 | 0.0798 |
| $n = 61$ | performance | Vertebrae | 10.5 | 0.002 | 0.0278 | 0.0086 | 0.2583 | 0.0797 |
| | gradients | Abn. Vertebrae | 0.5 | 0.50 | 0.0841 | 0.1241 | 0.0538 | 0.0793 |
| | Interactions | SVL * Vertebrae | 12.1 | 0.001 | −0.0003 | 0.0001 | −0.3090 | 0.0890 |
| | | SVL * Abn. Vertebrae | 1.3 | 0.26 | −0.0018 | 0.0016 | −0.1159 | 0.1018 |
| | | Vert. * Abn. Vertebrae | 3.2 | 0.08 | −0.0670 | 0.0377 | −0.1344 | 0.0755 |
| | Stabilizing | SVL | 5.1 | 0.03 | 0.000004 | 0.000002 | 0.2211 | 0.0979 |
| | performance | Vertebrae | 1.1 | 0.29 | 0.0017 | 0.0016 | 0.0884 | 0.0833 |
| | gradients | Abn. Vertebrae | 4.8 | 0.03 | −0.8745 | 0.3987 | −0.3083 | 0.1406 |
| Males | Directional | SVL | 119.0 | 0.0001 | −0.0023 | 0.0002 | −0.7435 | 0.0681 |
| $n = 97$ | performance | Vertebrae | 0.00002 | 0.99 | −0.00003 | 0.0066 | −0.0003 | 0.0693 |
| | gradients | Abn. Vertebrae | 6.1 | 0.02 | 0.1784 | 0.0720 | 0.1709 | 0.0689 |
| | Interactions | SVL * Vertebrae | 0.1 | 0.82 | 0.00002 | 0.0001 | 0.0172 | 0.0759 |
| | | SVL * Abn. Vertebrae | 0.1 | 0.71 | −0.0005 | 0.0013 | −0.0310 | 0.0838 |
| | | Vert. * Abn. Vertebrae | 2.7 | 0.10 | 0.0632 | 0.0384 | 0.1338 | 0.0813 |
| | Stabilizing | SVL | 0.1 | 0.76 | 0.000001 | 0.000004 | 0.0476 | 0.1538 |
| | performance | Vertebrae | 0.5 | 0.49 | −0.0035 | 0.0050 | −0.1017 | 0.1477 |
| | gradients | Abn. Vertebrae | 0.1 | 0.79 | 0.1610 | 0.6136 | 0.0526 | 0.2004 |

The effects of vertebral number and number of abnormal vertebrae differed between sexes. Males showed a weak, but significant, positive directional performance gradient for number of abnormal vertebrae, but no effect of vertebral number, and there were no interactive or curvilinear effects. The standardized directional performance gradient for number of abnormal vertebrae ($\beta' = 0.17$) shows that, in a male of a mean SVL (501 mm; Table 1), an increase in number of abnormal vertebrae with one SD (0.59 vertebrae) will increase growth rate with 0.17 SD (0.04 mm day$^{-1}$). In contrast, females showed no directional gradient of abnormal vertebrae on growth rate but a significant negative stabilizing one ($\beta' = -0.31$) showing that females with intermediate numbers of abnormal vertebrae had a slight growth advantage over the extremes. Females also showed a significant positive directional performance gradient for vertebral number when controlling for SVL. The corresponding standardized performance gradient ($\beta' = 0.26$) shows that for a female of a mean size (c. 523 mm, Table 1) an increase in vertebral number with one SD (3.14 vertebrae) will increase growth rate with 0.26 SD (0.09 mm day$^{-1}$). In addition, there was a significant interaction between SVL and vertebral number with a corresponding negative performance gradient ($\beta' = 0.31$), showing that the positive effect of increased vertebral number decreases with SVL. This is illustrated in Figure 1 where growth rate is plotted as a function of SVL and vertebral number and the curvature of the plane illustrates the interactive term. Note that the positive effect of vertebral number is strongest among small individuals.

Because the three morphological traits used show some covariation (Table 1) they may show both direct and indirect effects on growth rate. One way to estimate these indirect effects is by path analysis. For both sexes a number of path diagrams depicting possible interactive effects were constructed. However, these models did not explain a great deal more than a model including only direct effects (i.e. a multiple regression model), indicating that indirect effects were generally small. In fact, the coefficient of determination ($r^2$) when including indirect effects only
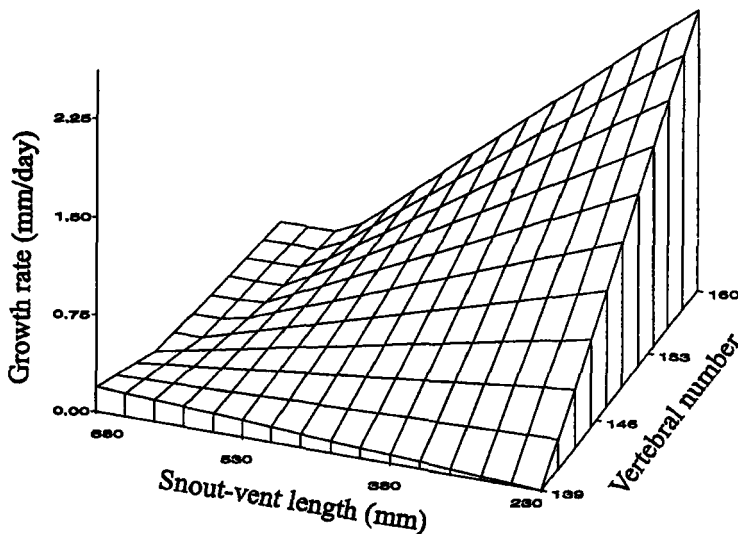


Figure 1. Response surface showing the effect of snout-vent length (mm) and number of vertebrae on growth rate (mm day$^{-1}$) in female *Vipera berus*.

increased from 68.27% to, at the most, 68.80% in females and from 58.200% to 58.204% in males. However, some of the indirect effects were rather large and these path diagrams may still serve to illustrate how the three variables may affect growth rate.

Figure 2 shows path diagrams for male and female adders separately. The diagrams shown are those that I consider contain the most plausible indirect effects. It can be seen that in males the direct effect of vertebral number on growth rate is very close to zero ($p = -0.0003$, see also Table 2) and that the indirect effect of vertebral number via SVL is also negative and amounts to $p = -0.076$ ($0.1019 \times -0.7435$). However, there is also a weak positive indirect effect of vertebral number via number of abnormal vertebrae of $p = 0.032$. Thus, the total negative effect of vertebral number is somewhat larger (but still very small) than was suggested by the multiple regression model (Table 2). Also, as can be seen, the indirect effect of number of abnormal vertebrae through SVL amounts to $p = -0.011$, thus counteracting its positive direct effect. It should be noted that this model yields a $r^2$-value that is lower than a model containing only direct effects. A somewhat better model is achieved by dropping the path connecting number of abnormal vertebrae and SVL. However, the model is successful in reproducing the observed correlation matrix (Kerlinger & Pedhazur, 1973).

In females the path diagram indicates an indirect effect of vertebral number on
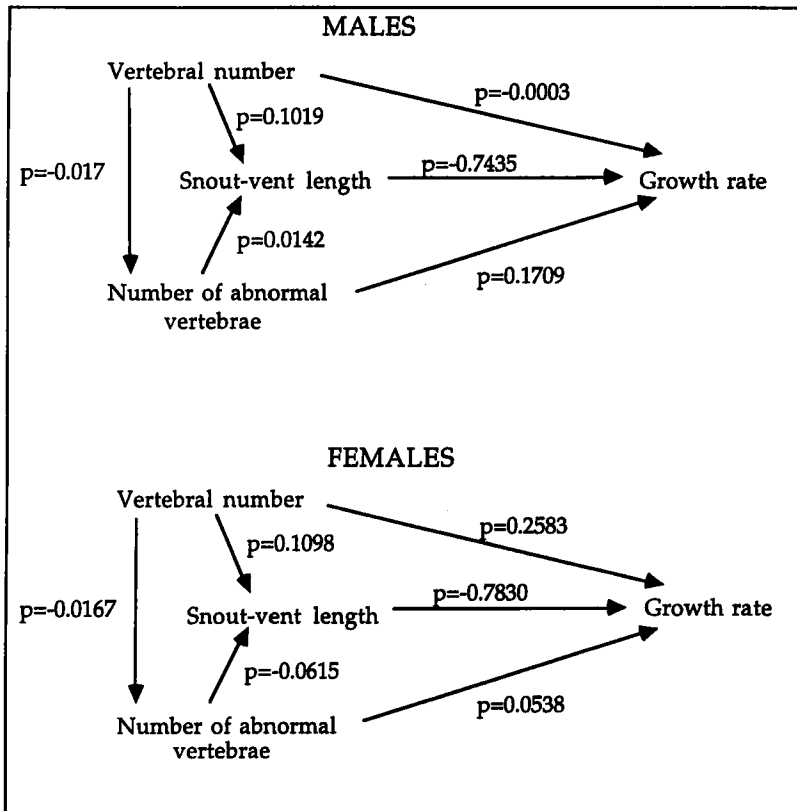


Figure 2. Path diagrams for male and female *Vipera berus* depicting direct and indirect effects of snout-vent length (SVL), vertebral number and number of abnormal vertebrae on growth rate.

growth rate via SVL of $p = -0.086$, showing that the total positive effect of vertebral number is lower than suggested by the multiple regression model (Table 2). The indirect effect via abnormal vertebrae is also negative but very close to zero. This may then explain why the direct effect of vertebral number is 0.26 but the correlation coefficient between vertebral number and growth rate is only 0.17. The multiple regression model also suggests a weak, non-significant positive effect of abnormal vertebrae. The path analysis, in addition, shows that this effect is boosted by a positive indirect effect via SVL ($p = 0.048$). Again, this model is not the one giving the highest $r^2$-value, which instead is achieved by omitting the path connecting vertebral number and SVL, but reproduces the correlation matrix well.

Finally, when separating the female data by year and locality, a significant positive relationship was observed between the overall growth rate (calculated as the least square means adjusted for SVL) and the correlation coefficient between vertebral number and relative growth rate (Figure 3). This suggests that the effect of vertebral number on growth rate in females is stronger in years when they are growing faster.

*Growth experiment*

The large difference in feeding regimes was reflected in a large difference in growth rate between the two groups (Figure 4). In addition, the high food level group sloughed their skin on average 3.4 times versus only 2.8 for the low food level group ($t = 2.86$, $P = 0.009$). At the beginning of the experiment, i.e. about one week after birth, there was a highly significant positive correlation between vertebral number and SVL in both males and females (males: $r = 0.85$, $P < 0.001$; females: $r = 0.76$, $P < 0.001$). This positive relationship remained throughout the experiment in both sexes.

To test for possible effects of vertebral number on growth rate I only consider the
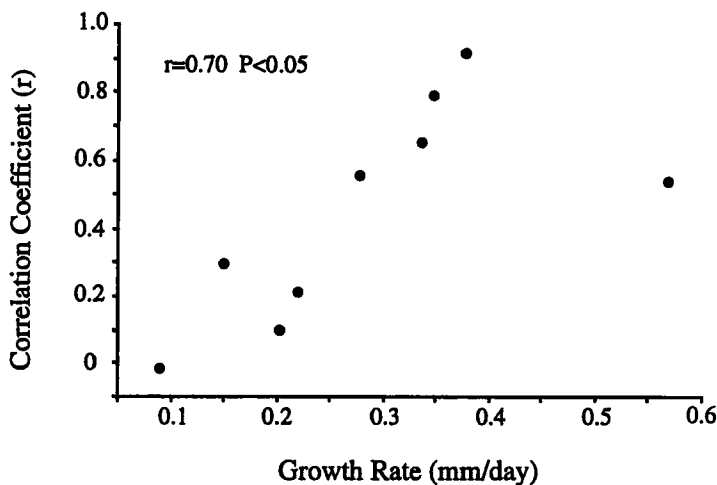


Figure 3. Scattergram of the correlation coefficient between individual vertebral number and growth rate of female *Vipera berus* versus their overall growth rates. Points represent data separated by locality and year. Overall growth rate on the x-axis was calculated as the least square means of individual growth rate (mm day$^{-1}$) adjusted for SVL (mm), using analysis of covariance.
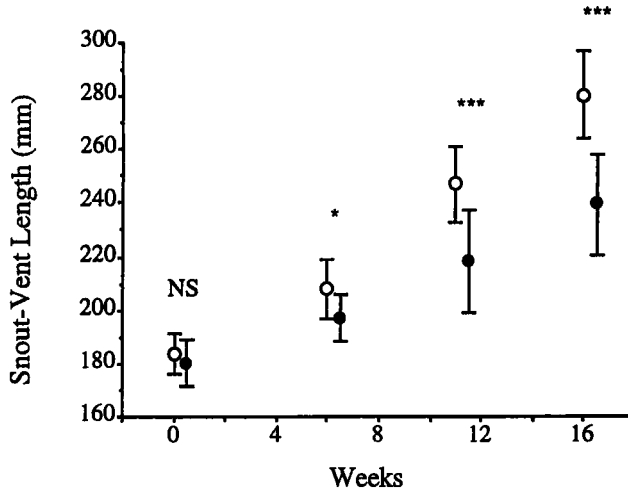
Figure 4. Increase in snout-vent length in two groups of juvenile *Vipera berus* receiving different amounts of food. Open circles represent the high food level group, closed circles the low food level group. Graph shows mean values ± one SD on four measuring occasions over a period of 16 weeks, with tests of differences in means between groups (unpaired *t*-test: NC indicate $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ and ***$p < 0.001$).

whole experimental period, and therefore growth rate refers to the increase in SVL between the start of the experiment and its termination after 16 weeks. Sexes did not differ in growth during the experiment after controlling for food level ($F_{1,17} = 0.90$, $P = 0.36$), but since males and females differ in vertebral number (see above and Lindell *et al.*, 1993), I standardized male numbers to female equivalents (see above) to be able to pool the sexes. There was no effect of litter on growth rate in either of the two groups when controlling for food level ($F_{4,17} = 0.49$, $P = 0.75$), and all litters were therefore pooled. In addition, there was no effect of initial body size on subsequent growth rate ($F_{1,17} = 1.95$, $P = 0.18$), and therefore body size was not included as in independent variable in the analyses.

The effect of vertebral number on growth in the two groups is illustrated in Figure 5. One female in the high food level group had an extremely low number of vertebrae (124) and might be considered an outlier. In the low food level group no relationship was observed between vertebral number and growth ($r = -0.16$, $P = 0.62$), whereas in the high food level group, omitting the outlier, there was a non-significant tendency to higher growth in individuals with more vertebrae ($r = 0.44$, $P = 0.15$). When the outlier was included, the correlation became significantly positive ($r = 0.62$, $P = 0.02$). The correlation coefficients for the two groups ($r = -0.16$ and $r = 0.62$) were close to significantly different (z-transformed r-values, $t = 1.94$, $P = 0.052$).

### Heritability estimates

The mother-midoffspring regression yielded a slope of b = 0.149 giving a heritability estimate of $h^2 = 0.30$ (SE = 0.29, $n = 29$), but this slope was not significantly different from zero ($P = 0.31$). The full-sib analysis yielded a higher

heritability estimate, which was significantly different from zero ($h^2$ = 0.39, SE = 0.14, $P$ < 0.0001, $n$ = 29 litters and 213 young).

## DISCUSSION

The analyses of the field data revealed that both vertebral number and number of abnormal vertebrae can affect growth rate in adders, but their effect may differ between sexes. There was a significant positive directional performance gradient for vertebral number on growth rate in female adders after controlling for SVL, showing that females with more vertebrae have an advantage in terms of growth. Calculations show that this advantage can be quite significant. For example, during a summer period (107 days) a female of mean size (523 mm) with 157 vertebrae will grow more
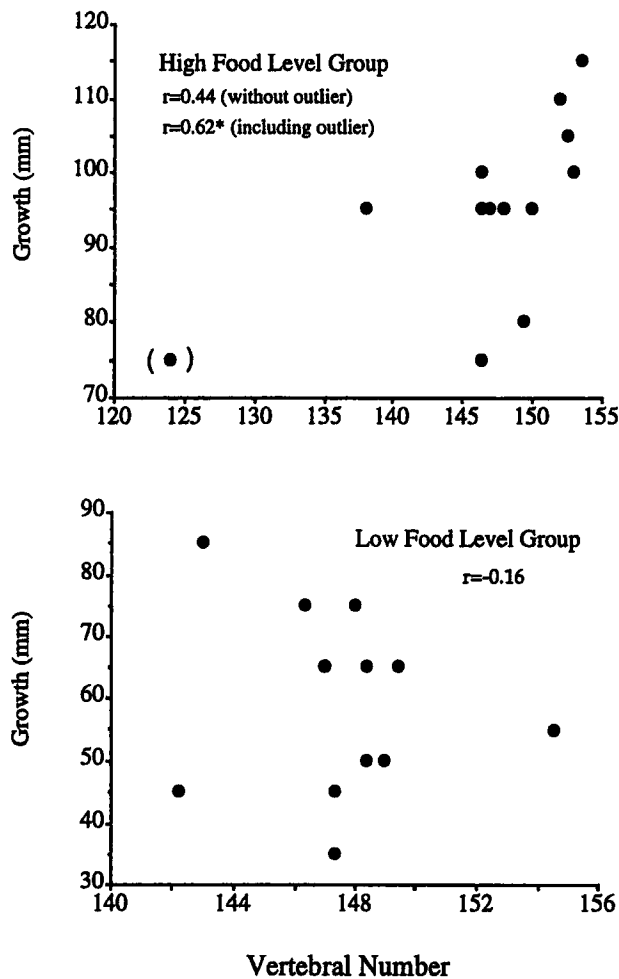


Figure 5. Relationship between vertebral number and growth (mm) over an experimental period of 16 weeks in two groups of juvenile *Vipera berus* receiving different amounts of food. Male vertebral numbers were standardized to female equivalents. Note different scales on the x-axes. Correlation coefficient denoted by * is significant (*P* < 0.05).

than 30 mm more than an equally sized female with only 147 vertebrae, a size advantage that may be translated into a fitness advantage considering that an increase in snout-vent length of 30 mm would increase litter size by approximately 0.8 young (Lindell, unpublished). Females also showed a significant interaction between vertebral number and SVL demonstrating that the positive effect of vertebral number is largest among small individuals (Table 2, Fig. 1). Females also showed a weak stabilizing effect of number of abnormal vertebrae. In addition, the positive correlation between vertebral number and growth rate in females was stronger in years of higher overall growth rate (Fig. 3). No effect of vertebral number was present in the males but they, strangely showed a positive directional performance gradient for abnormal vertebrae. The path analysis showed that indirect effects on growth rate were generally small.

The observed positive linear effect of vertebral number on growth rate in females is in contrast to findings of Arnold (1988), who showed that in female *Thamnophis elegans* individuals with intermediate vertebral number grew faster than any of the extremes. Why females of *T. elegans* and adders should respond differently to increased vertebral number is puzzling, but one possibility might be that *T. elegans* is a semi-aquatic, more actively foraging species than the terrestrial adder which uses ambush as its main prey-capturing tactic. Perhaps individual *T. elegans* with intermediate vertebral number have an advantage in terms of swimming and general locomotion and thereby achieve a higher foraging success than individuals with either many or few. Alternatively, genetic correlations with other traits influencing growth may explain the observed difference between the two species. Arnold (1988) further observed that in male *T. elegans* the number of abnormal body vertebrae negatively affected growth, indicating that individuals with more abnormal vertebrae are in generally worse condition. I, on the other hand, found a positive effect in male adders, and no effect in females. Why males with more abnormal vertebrae should grow faster, rather than slower, is strange. Inspection of the data shows that the observed positive effect is mainly produced by three individuals with many abnormal vertebrae who also have especially high growth rates; removing these three also removes the significant effect of number of abnormal vertebrae.

It is interesting that for *T. elegans* an effect of vertebral number on growth was also evident only in females, which, like in *V. berus*, grow faster and to a larger size than males (e.g. Carpenter, 1952). This suggests either that the effect of vertebral number on growth is sex-linked, or that vertebral number mainly affects growth in the fastest growing sex. The latter alternative could occur as vertebral number becoming a limiting factor at high growth rates. This hypothesis is supported by the field observations that the relationship between vertebral number and growth rate in female adders was strongest in years with high food availability and growth rate (Fig. 3), and by the fact that the positive effect of vertebral number in females was strongest among the smallest and fastest growing individuals (Fig. 1). My experiment also supports this hypothesis as it failed to find any relationship between vertebral number and growth among individuals in the low food level group, whereas there was a significant positive effect in the high food level group (Fig. 5).

I found a significant positive correlation between SVL and number of vertebrae at birth in both male and female adders. This is in agreement with earlier observations in the adder (Lindell *et al.*, 1993), as well as in a few other snake species (Klauber, 1945; Arnold & Bennett, 1988; but see Voris, 1975). The reason for this positive relationship could be that: (i) some individuals develop more vertebrae, and

consequently grow faster in their mothers' oviducts, than those with fewer because many vertebrae *per se* promotes higher growth rate; or (ii) some eggs produce more yolk at vitellogenesis such that some developing young receive more energy than their siblings and therefore end up with both more vertebrae and faster growth rates than those from smaller eggs. That the amount of yolk affects the size of the young has been shown experimentally in lizards (Sinervo, 1990), but it was not reported whether this also affected the number of vertebrae. However, both the field data and the experimental evidence indicate that a larger number of vertebrae is associated with higher growth rates after birth when the amount of yolk in the egg is no longer of importance, and it is therefore likely that the same mechanism operates in the embryos as later in life.

The positive effect of vertebral number on growth may explain the observed positive relationship between body size and vertebral number in adult adders (Lindell *et al.*, 1993). Even though there is a positive correlation between vertebral number and SVL at birth, this is not enough to explain the corresponding pattern among adults as the difference in SVL between individuals with few vertebrae and those with many is much larger in the adults. One possible proximate mechanism behind this pattern was suggested by Lindell *et al.* (1993). If each 'body segment' (vertebra with its attached tissues and ventral scute) of an individual grows independently but, at times of high prey densities and rates of food ingestion, at a certain fixed maximum rate and to a fixed maximum size, an individual with many vertebrae will automatically achieve a higher overall growth rate and attain a larger age-specific body size than one possessing fewer vertebrae. At times of low prey availability, however, each body segment may grow at various, lower, rates. In this situation there will be no relationship between vertebral number and growth rate. This may also explain the observation that a positive correlation between vertebral number and growth was evident only at high overall growth rates (Figs 3 & 5).

Given a relationship between vertebral number and growth, and a connection between body size and fitness, vertebral number is likely to be under selective pressure and to undergo evolutionary change if there is heritability for vertebral number. However, the observed heritability estimates were low. The full-sib analysis yielded $h^2 = 0.39$, which was significantly different from zero, whereas the mother-offspring regression gave a lower estimate (0.30), and was not significant. A higher estimate using a full-sib analysis is commonly observed, but both values are in the range of estimates commonly found for morphological traits (Mousseau & Roff, 1987). Unfortunately, these estimates are likely to be a slight underestimation as there is reason to believe that in some litters litter mates are only half-sibs (Stille, Madsen & Niklasson, 1986; Stille & Niklasson, 1987; Höggren & Tegelström, 1995). Other studies have also reported heritability estimates of vertebral number in snakes. Thus, in *Thamnophis elegans* it was calculated that $h^2$ range between 0.52 and 0.96 among populations with a pooled estimate of 0.79 using mother-offspring regressions (Arnold, 1988), in *T. sirtalis* $h^2$ equals 0.32 and 0.51 for full-sib analysis and mother-offspring regression, respectively (Dohm & Garland, 1993), and in *Natrix sipedon* $h^2 = 0.75$ using a full-sib analysis (Beatson, 1976). In view of this usually quite high heritability of vertebral number, and the connection between vertebral number and fitness correlated traits such as growth rate and body size demonstrated here, one may predict that vertebral number may undergo evolutionary change over time. That this has occurred has been established across a broader taxonomical range and on an evolutionary time scale (Lindell, 1994). It was demonstrated that the evolution

of larger body size has been accompanied by a parallel increase in vertebral number in both Australian elapids and Old World viperids after phylogenetic conservatism was accounted for. In addition, that study showed that vertebral number has responded to selective pressures other than body size. Type of habitat used, body shape and whether or not a species uses constriction to subdue prey are factors selecting for a change in vertebral number beyond what is expected from the species body size.

### Conclusions and future research

In *Vipera berus*, vertebral number is likely to be under selective pressure and to undergo evolutionary change; it is positively related to growth rate and thus body size (at high prey availability), which in turn is a fitness correlated trait, and it shows some heritability. Whether evolutionary change will in fact take place in response to selection on either body size (and thus indirectly on vertebral number) or vertebral number *per se*, may, however, depend on local conditions. For example, if prey availability is consistently low, and thus growth rate (Lindell, unpublished), the connection between growth rate and vertebral number will be weakened, and selection on growth rate or body size will not greatly affect vertebral number. In contrast, if prey availability is stable and high and there is selection for larger body size, selection for many vertebrae should be correspondingly strong. Similarly, in environments where microclimate may cause gravid females to experience suboptimal conditions the environmentally induced variance in offspring vertebral number may result in reduced heritabilities and thus weaker response to selection.

As for further research in this area it would be valuable to learn what factors affect the relationship between number of vertebrae and abnormal vertebrae and growth rates. For example, is the effect of vertebral number on growth rate often dependent on food availability? If so, I would predict that vertebral number and body size would to a higher degree show parallel patterns among populations of snakes living in areas with consistently high prey availability compared to those in areas with low. Also, the presented results, as well as those of Arnold (1988) on *T. elegans*, suggest that vertebral number may influence growth rate differently in the two sexes, with a stronger effect in females, alternatively, the fastest growing sex. It would therefore be valuable to see more data on effects of vertebral number on growth in males and females of other species; especially in taxa where males are larger than females, such as, for example, rattlesnakes. Finally, it would be interesting to see more heritability estimates of vertebral number in various taxa of snakes. Especially estimates from oviparous species would be welcomed to compare with those of viviparous species such as *N. sipedon*, *T. elegans*, *T. sirtalis* and *V. berus* discussed here, as variation induced by environmental conditions during incubation and gestation may vary between these two groups of snakes.

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