

Phenotypic plasticity in body sizes and sexual size dimorphism in European grass snakes.

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The interrelationships between body size, sexual differences and growth rates of European grass snakes were studied. Two-factor analysis of variance and covariance were used in the analysis of the relationships. Results indicate that prey availability causes the marked differences in adult body sizes and the degree of sexual size dimorphism among populations of grass snakes without any genetic modifications involved.

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Many animal species show considerable geographic variation in average adult body sizes. Although this phenomenon offers a unique opportunity to tease apart the factors influencing body size, interpretation has proved to be difficult because several processes may contribute to the observed variation (e.g., Andrews, 1976; Case, 1978; Dunham et al., 1978). First, average body size is influenced by adult survival rates in species in which growth continues after maturity: animals may be smaller in one area than another simply because they are, on average, younger (e.g., King, 1989). Second, even if survivorships are similar among areas, so that the body-size differences are due to differences in growth patterns, there are two possible explanations for such variation in growth. Body sizes may differ either because of local genetic modifications (possibly due to adaptation) or because of a direct phenotypic effect of differing food availability on growth rates (e.g., Berry et al., 1987; Dobson and Murie, 1987; Ebenhard, 1990).

To distinguish among these alternative interpretations, we need two kinds of data on growth trajectories of animals: first from the field (to determine whether the populations differ in actual growth patterns, rather than simply survivorships) and second the response to experimental manipulation of food supply (to determine whether the observed differences in growth rates are due to genetic differences or phenotypic plasticity). This paper presents information on European grass snakes. We show that body sizes and the degree of sexual difference in body size are greatly reduced in an island population compared to the nearby mainland, that these differences are due to modified growth patterns and not just survivorship, and that the low growth rates and small asymptotic body sizes of the island snakes are a phenotypic response to local conditions (probably, low food availability).

Insular populations of snakes offer some of the most dramatic examples of geographic variation in body size (e.g., Case, 1978; Schwaner, 1985; King, 1989; Shine, 1987; Schwaner and Sarre, 1988; Hasegawa and Moriguchi, 1989; Forsman, 1991). For example, Schwaner (1985) showed that body masses of adult Australian

tigersnakes varied up to tenfold among adjacent islands. Correlational analyses suggest that predators attain larger sizes in areas where larger species of prey are available, and this may be true both for snakes (Schwaner, 1985; Hasegawa and Moriguchi, 1989) and for mammalian predators (Gittleman, 1985; Erlinge, 1987). Experimental studies on mammals have shown that geographic variation in body sizes may be due both to phenotypic plasticity (e.g., Dobson and Murie, 1987) and to local adaptation (e.g., Berry et al., 1987; Ebenhard, 1990). The only experimental study to address the determinants of such differences in snakes has been that of Barnett and Schwaner (1984), who raised juvenile tigersnakes. These authors documented rapid growth in captive snakes from a "giant" population, but obtained no comparable information on snakes from "dwarf" populations.

We studied two populations of a nonvenomous natricine colubrid species, the grass snake (*Natrix natrix*), which is abundant over much of Europe (Arnold and Burton, 1978). The mainland study area was near Maryd, 15 km south of Lund in southern Sweden (55 [degrees] 40'N, 13 [degrees] 30'E). The area contains a mixture of arable land, grazed meadow, and mixed deciduous forest. Detailed data have already been published on body sizes, sexual size dimorphism, growth rates, diets and reproductive biology of grass snakes from this area (Madsen, 1983, 1987). Those papers also describe the methods used to capture, mark and measure snakes, and to obtain prey items by forced regurgitation. The same methods were used for the study of island snakes. Our island population was on Hallands Väderö (56 [degrees] 27'N, 12 [degrees] 44'E), a small (2.6 [km.sup.2]) island approximately 3 km from the Swedish coast. One quarter of the island is forested, with the remainder consisting of meadows bordered by blackthorn, stony areas with juniper, and bare rock (Madsen and Stille, 1988). The two study areas are approximately 100 km apart. Geological evidence suggests that the two areas probably have been separated for several thousand years, since glaciation-induced reductions in sea level (Devoy, 1987). However, it is possible that the effective period of separation may have been longer than this (e.g., these oviparous snakes may not have existed in this region during glacial periods) or considerably briefer (due to fortuitous dispersal of snakes

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from one area to another).

Male snakes from the island were similar to the mainland animals in mean SVL (Fig. 1; $N = 22, 41$; means = 52.2 versus 53.0 cm, $t = 0.40$, $df = 61$, $P = 0.69$) but females were much smaller on the island ($N = 28, 44$; means 59.7 versus 69.9 cm, $t = 4.14$, $df = 70$, $P < 0.001$). Indeed, some male snakes from the mainland actually attained larger sizes than did any of the females from the island population, although the mean values were lower (Fig. 1). Two-factor analysis of variance of these data (with gender and locality as the factors) revealed a significant interaction term ($F = 8.85$, $df = 1, 131$, $P < 0.004$), indicating that the degree of sexual size dimorphism was greater in the mainland snakes than in the island population (Fig. 1). Analyses using body mass rather than length show the same patterns, although the difference between island and mainland females is greatly increased (means of 87.0 versus 137.4 g). The relationship between mass and length did not differ significantly between snakes of the two areas (analysis of covariance of log-transformed mass versus SVL: slopes $F = 1.01$, $df = 1, 81$, $P > 0.30$; intercepts $F = 0.02$, $df = 1, 82$, $P > 0.88$).

Why are Snakes Smaller

on the Island?

Information on prey availability and consumption suggests that the small body size of the island females may be a consequence of the lack of large prey items, as has been previously inferred for other dwarfed island populations (Schwaner, 1985; Hasegawa and Moriguchi, 1989). The mainland population of grass snakes has access to a diverse spectrum of prey species, and analysis of prey items via forced regurgitation from 73 snakes in this area showed that adult females fed mostly on large toads (*Bufo bufo*) whereas males ate frogs (*Rana* spp.) and juvenile toads (Madsen, 1983). Neither of these prey types are available on the island of Hallands Vadero, where the only amphibians are newts, *Triturus vulgaris* (Andren and Nilson, 1979). We palpated 12 prey items from island snakes (1 from a male, 11 from females), and all were newts. The newts are much smaller (maximum mass 2 g) than are the toads (maximum mass 40 g) consumed by mainland snakes.

Do Growth Patterns Differ between

the Two Populations?

Differences in body size between the sexes, or among populations, could result simply from survivorship differences in taxa that show continued growth after

maturity (e.g., Howard, 1981; Halliday and Verrell, 1986; but see Shine, 1990). Hence, it is important to determine whether the difference in mean body sizes between island and mainland snakes is simply a result of different survival rates, or an actual difference in growth trajectories. Recaptures of marked island snakes show that these animals cease growing at small sizes (Fig. 2). All 15 measured growth rates from the island snakes were lower than the mean rates observed in Madsen's (1983) mark-recapture study of Maryd grass snakes, enabling strong rejection of the null hypothesis that growth rates are as high in Hallands Vadero as in Maryd (using a sign test: females, $N = 5$, $x = 0$, $P < 0.04$; males, $N = 10$, $x = 0$, $P = 0.001$; Siegel, 1956). Hence, we conclude that the island snakes were smaller because they grew more slowly, and ceased growing at smaller sizes, than did the mainland animals.

Is the Growth Difference

Genetically Determined?

Many authors have attributed geographic variation in growth rates, adult body sizes and the degree of sexual size dimorphism to intraspecific genetic differentiation, and especially to local adaptation (e.g., Schwaner and Sarre, 1988; Hasegawa and Moriguchi, 1989; Forsman, 1991). Nonetheless, it remains plausible that such differences are entirely due to phenotypic plasticity (e.g., Dunham et al., 1978; Stearns, 1983; Shine, 1987). To evaluate this possibility, we captured five ovigerous female grass snakes from each population in summer 1983, and maintained them in the laboratory until oviposition. Two hatchlings (one male, one female) from each clutch were kept, and raised in captivity at the University of Lund. The young snakes were kept individually in cages measuring 10 x 20 x 10 cm, being transferred to larger enclosures (50 x 45 x 45 cm) as they grew larger. Incandescent bulbs provided heat to each cage, and toads and frogs were provided as food. As far as was possible, food was provided ad libitum. The snakes were measured at the same time each year for six years, by which time they had approached asymptotic body size (Fig. 3).

Because we are comparing only two populations, our analysis of these experimental data is technically for a "location effect" rather than a treatment effect (Hurlbert, 1984). Under some circumstances, a lack of replication at the populational level can preclude the use of inferential statistics, because of biases due to pseudoreplication. Such statistical difficulties are not a problem with our study, however, because our interest lies in the comparison between these two specific populations rather than in the more general question of whether geographic

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differences in adult body size throughout the range of *Natrix* are explicable by phenotypic plasticity. Nonetheless, we have avoided statistical comparisons of the body sizes of captive-raised snakes with those of their free-ranging relatives, because such comparisons assume no temporal shifts in mean adult body sizes in the wild populations (e.g., Hurlbert, 1984).

Two-factor analysis of variance showed no difference in mean body lengths of the hatchling snakes with respect either to sex ($[F_{sub.1,16}] = 0.13$, $P > 0.70$) or to geographic origin ($F = 1.13$, $df = 1, 16$, $P > 0.30$) or any interaction between these two factors ($F = 0.13$, $df = 1, 16$, $P > 0.70$). A Kruskal-Wallis nonparametric test similarly showed no significant influence of gender or geographic origin on hatchling body length (using four treatments for this test, depending on whether the hatchling was male or female and had a parent from the mainland or island population: $H = 1.14$, $df = 3$, $P = 0.77$). As previously described for field populations (Madsen, 1983), males grew more slowly than females, and ceased growing at much smaller sizes (Fig. 3). However, there was remarkably little variation in growth rates among individuals of the same sex, regardless of whether they came from the island or mainland populations. All of the snakes attained sizes comparable to those of mainland adults, and far larger than observed on the island of Hallands Vadero (Fig. 3). The pattern of sexual dimorphism was thus also similar to that observed in mainland populations (i.e., very marked), rather than observed on the island (i.e., relatively monomorphic). Two-factor analysis of covariance of the relationship between log-transformed age and body size (with locality and gender as the factors) confirmed that the growth pattern did not differ between mainland and island snakes raised in captivity (no significant main effects or interaction terms involving locality, $P > 0.05$). Two-factor ANOVA (with gender and locality as the factors) showed no significant differences between the six-year-old island and mainland snakes raised in captivity, either in absolute sizes of each sex, or in the degree of dimorphism ($F = 0.09$, $df = 1, 16$, $P = 0.76$; interaction between gender and locality $F = 0.51$, $df = 1, 16$, $P = 0.49$).

These analyses strongly suggest that the growth rates and asymptotic body sizes of our island grass snakes may be reduced because of low food availability, whereas our mainland snakes apparently were able to obtain enough prey to grow at much higher rates and attain much larger body sizes. Females seemed to be much more drastically affected than were males in terms of the asymptotic sizes obtained in the laboratory (reductions of approximately 35% versus less than 10%), presumably because they are larger and must invest more energy into reproduction

(Madsen, 1983). Male natricine snakes are typically smaller than females (e.g., Shine, 1978; Fitch, 1981) and tend to eat less and grow more slowly than do conspecific females (Feaver, 1977; Crews et al., 1985; Madsen, 1983). Thus, food scarcity is likely to have less impact on males than on females.

In summary, our data suggest that the marked differences in adult body sizes and the degree of sexual size dimorphism between our two populations of grass snakes result from direct influences of prey availability, without any genetic modification of the populations. Such genetic modifications may well arise between populations that are separated for longer periods of time or are subject to intense selection, but our results document at least one case in which significant geographic differences in body size in snakes have arisen without any detectable evolutionary change. The similarity in growth trajectories of the captive-reared snakes from island and mainland populations (Fig. 3) suggest that the two populations share a single "norm of reaction" (Stearns, 1983) for the response of growth rates to food availability. Recent experimental studies have also revealed extensive phenotypic plasticity in reproductive traits in snakes (Ford and Seigel, 1989). Hence, many geographically variable characteristics among snakes (as in other vertebrates - e.g., Berven, 1982; James, 1983; Stearns, 1983; Dobson and Murie, 1987) may be attributed more parsimoniously to phenotypic plasticity than to microevolutionary changes.

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