Insular gigantism and dwarfism in a snake, adaptive response or spandrel to selection on gape size?

Shawn E. Vincent[1], Matthew C. Brandley[2], Takeo Kuriyama[3], Akira Mori[4], Anthony Herrel[5] & Masami Hasegawa[3]

[1]*Department of Natural, Information, and Mathematical Sciences, Indiana University Kokomo, Kokomo, IN 46902, USA,* [2] *Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520-8105 USA,* [3]*Department of Biology, Faculty of Science, Toho University, Funabashi City, Chiba, 274-8510, Japan,* [4]*Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan,* [5]*UMR 7179 C.N.R.S/M.N.H.N., Departement d'Ecologie et de Gestion de la Biodiversite, 57 rue Cuvier, Case postale 55, 75231, Paris Cedex 5, FranceDepartment of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520-8105 USA*

In biology, spandrels are phenotypic traits that evolve through their underlying developmental, genetic, and/or structural links to another trait under selection[1, 2, 3]. Despite the importance of the concept of spandrels in biology, empirical examples of spandrels are exceedingly rare at the organismal level[2, 3]. Here we test whether body size evolution in insular populations of a snake (*Elaphe quadrivirgata*) is the result of an adaptive response to differences in available prey, or the result of a non-adaptive spandrel resulting from selection on gape size. In contrast to previous hypotheses, Mantel tests show that body size does not coevolve with diet. However, gape size tightly matches diet (birds vs. lizards) across populations, even after controlling for the effects of body size, genetic, and geographic distance. Moreover, gape and body size show a strong degree of phenotypic covariation. Thus, the dramatic insular body size variation among *E. quadrivirgata* populations is at least

partially caused by its underlying phenotypic covariation with gape size rather than the result of direct selection on overall body size.

Understanding the adaptive nature of phenotypic variation in natural populations is a central goal of evolutionary biology[4, 5]. Island biota have played a prominent role in this endeavor because island-dwelling organisms often display a remarkable range of body sizes, from dwarfs to giants, across island archipelagoes. For example, carnivorous komodo dragons (*Varanus komodoensis*) have evolved remarkable differences in maximum adult body size among four islands of the Lesser Sunda region[6], which are believed to reflect differences in available prey size among islands. Case (1978) showed that many insular snake species tend toward dwarfism, which he suggested was caused by a reduction in food availability on islands compared to the mainland[7]. More contemporary reviews, however, show that island snake body sizes tend to be bimodal in their distribution[8, 9], with giants evolving on islands with larger prey sizes and dwarves on islands with smaller prey sizes.

Although numerous examples exist of body size co-varying with prey size in insular snakes[8, 9, 10], body length is at best only weakly involved in the feeding process in these highly specialized vertebrate predators. Specifically, most snakes use their mobile jaw elements to swallow prey whole. Consequently, maximum gape size primarily determines the size, shape, and type of prey that can be successfully ingested[11, 12]. One would thus expect maximum gape size in snakes to exhibit a stronger adaptive response to differences in local prey availability than body size. So why does insular gigantism and dwarfism seem to go hand-in-hand with available prey size in snakes?

We hypothesize that insular gigantism and dwarfism in snakes is a 'spandrel' of

selection on gape size. Architecturally, a spandrel is a tapering triangular space between an arch and a rectangular enclosure that arises as a natural by-product of mounting a dome on rounded arches[1, 2]. In evolutionary biology, a spandrel is a phenotypic trait that evolved through its underlying developmental, genetic, functional, and/or structural link to another trait under selection[1, 2, 3]. Although this concept has received a significant amount of attention from evolutionary biologists since its inception 30 years ago[1], empirical examples of spandrels at the whole-organismal level are exceedingly rare[2, 3]. In snakes, gape and body size often exhibit strong phenotypic and genetic covariation, both within species over ontogeny and among species[13]. Therefore, selection on gape size for foraging on different prey types may drive a non-adaptive, albeit correlated, evolutionary response in body size. Here we test this hypothesis using morphological, dietary, and genetic data for seven populations of an insular Japanese snake (*Elaphe quadrivirgata*) that exhibit replicated patterns of insular gigantism and dwarfism.

Our previous work on this snake has shown that *E. quadrivirgata* populations in the Izu archipelago, Japan exhibit pronounced differences in maximum adult body size (snout-vent length) from dwarves on Oshima (817 mm max SVL) to giants on Tadanaejima (1620 mm max SVL)[14] (Fig. 1). Furthermore, these dramatic differences in maximum body sizes tightly match the size of available prey among islands[14]. Specifically, insular gigantism occurs on two islands (Mikurajima & Tadanaejima) and in both cases snakes feed on large, nestling seabird prey and their eggs (prey mass range = 20 - 50g). By contrast, populations exhibiting insular dwarfism (e.g., Oshima) are restricted to consuming small to medium-sized lizard prey (prey mass range = 2 – 15g)[14]. Furthermore, analysis of mitochondrial DNA reveals limited genetic differentiation

among populations (0 – 1.3%; see supplemental information); therefore, body size has diverged in an extremely rapid manner among snake populations on the Izu islands. For example, snakes from the adjacent islands of Kozushima and Tadanaejima are genetically indistinguishable from one another yet exhibit considerable differences in maximum adult body size (Kozushima, mean ± 1 SE, SVL [in mm] = 1145 ± 15.6; Tadanaejima = 1405 ± 20.9), diet[14], growth trajectory and life span [15].

Morphometric analysis of snakes from lizard versus bird-eating islands show that snakes from the two bird-eating islands have significantly larger adult body ($F_{1, 256}$ = 20.1, P = 0.0001) and gape sizes ($F_{1, 256}$ = 82.2, P = 0.0001 [Fig. s 2a,b & 3]) than the five lizard-eating islands (MANOVA; prey: Wilks' Lambda = 0.756, $F_{2, 255}$ = 41.4, P = 0.0001). Moreover, differences in gape size between bird and lizard-eating islands become even more significant after adjusting for differences in overall body size (ANCOVA; $F_{1, 254}$ = 47.8, P = 0.0001 [Fig. 2c]). Hence, there has been replicated evolution of small and large gape sizes in accordance with diet in *E. quadrivirgata*.

Three-way Mantel's tests accounting for genetic and geographic distances among populations further confirm this result. Specifically, gape size is strongly and significantly correlated with diet (birds vs. lizards) across all populations (Table 1), even after controlling for genetic distance. Although the three-way Mantel's test controlling for geographic distance is not statistically significant (Table 1), it is very close to being so (P = 0.06). As the number of Izu islands this species inhabits is relatively small (n = 7), we checked the robustness of the geographic distance effect by increasing the number of permutations to 100,000. When doing so, the strength of the correlation between gape and diet after accounting for geographic distance becomes slightly stronger (Mantel's r =

0.65, $P = 0.047$), and hence statistically significant. Additionally, gape size becomes even more strongly related to diet when holding variation in body size constant across populations (Table 1). By contrast, insular body size variation is not significantly correlated with diet, genetic, or geographic distance among populations (Table 1). However, two-way Mantel's tests show that body size is significantly and strongly correlated with gape size across populations (Mantel's $r = 0.75$-0.80, $P < 0.05$ in all comparisons; Table 1). Selection for large gape to feed on massive bird prey or for small gapes to feed on small lizard prey will therefore result in concomitant changes in body size among populations of *E. quadrivirgata*. Overall, this analysis strongly suggests that the replicated evolution of insular gigantism and dwarfism in *E. quadrivirgata* is not an adaptive response to diet as previously hypothesized, but rather a spandrel of selection on gape size.

METHODS SUMMARY

We collected snakes from the seven major (Kozushima, Mikurajima, Niijima, Oshima, Shikinejima, Tadanaejima and Toshima) Izu islands, Japan this species inhabits (Fig. 1; see supplemental information for sample sizes) from late April to early May 2006-2008. We stayed between 1-8 days at each locality and collected snakes by hand by walking along roads or paths between 0800 and 1700 h. Sex was determined by either examining the external shape of the tail base or by everting the hemipenes. To avoid pseudoreplication, we gave each captured snake a unique mark to facilitate future identification.

For each captured snake, we recorded snout-vent length (SVL), maximum head width with the quadrate bones flared laterally at the quadrato-supratemporal joint (Fig. 3), and length of the lower jaw. We used these two head measurements to compute a gape index that represents the cross-sectional area as the area of an ellipse with major and minor axes equal to jaw length and head width, respectively[16]. Snout-vent length and the computed gape index were used in all statistical comparisons among populations. Populations were categorized according to their main dietary item based upon long-term mark-recapture data published elsewhere[14].

We obtained tissue samples for a total of 261 individuals of *Elaphe quadrivirgata* from these seven localites. To capture the genetic diversity within each population, we sampled a large number of individuals (range = 16 – 57, median = 35). We amplified the entire 1117 base pair cytochrome b gene in two overlapping fragments using the primers, H14910: 5'– GAC CTG YGA TMT GAA AAA CCA CYG TT –3'[17], EQintR: 5'- AAG TGG AGG GCR AAG AAT CGA GTT AAG GT -3', EQ-mH2: 5'- AGC TTT GTC TTA CAA GGA CAA CGC -3', and EQ-mL2: 5'- CCA TGA GGA CAA ATA TCA TTC TGA G -3' using standard PCR techniques. PCR products were cleaned using ExoSap-IT (USB Corp.). Purified templates were dye-labeled using BigDye™ (ABI) and sequenced on an ABI 3077™ automated DNA sequencer using the same primers. Nucleotide sequences were examined and aligned by eye and an open reading frame for this gene was verified using MacClade version 4.08[18]. The mean uncorrected genetic distances between each of the nine populations was calculated using the "Mean distance between groups" function in MEGA version 4[19].

We tested for adaptive relationships between morphology and diet using Mantel tests with 9999 permutations of pairwise difference matrices among all localities[20]. The morphological matrices represent the Euclidean distance between a pair of localities, calculated using the mean population value for either SVL or gape index. For diet (birds or lizards), the difference matrix contained a "0" if diet between two populations was the same, and a "1" if they were different. We controlled for mean genetic and/or geographic distance using three-way Mantel tests (see Table 1).

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Authors' Contributions statement: S.E. Vincent contributed to data collection, carried out all statistical analyses and contributed to the writing process; M. Brandley and T. Kuriyama obtained field and laboratory data, conduced genetic analyses, and contributed to the writing process; M. Hasegawa, A. Herrel, & A. Mori obtained data and contributed to the writing process. All the authors equally contributed to research progress, discussion of the results and production of the paper.

Correspondence and requests for materials should be addressed to S. E. Vincent (shvincen@iuk.edu).

Figure 1 Map of the main island of Japan (Honshu) and the Izu islands indicating the seven localities sampled in this study. Populations are coloured according to diet: Blue = lizard-eating, Red = bird-eating.
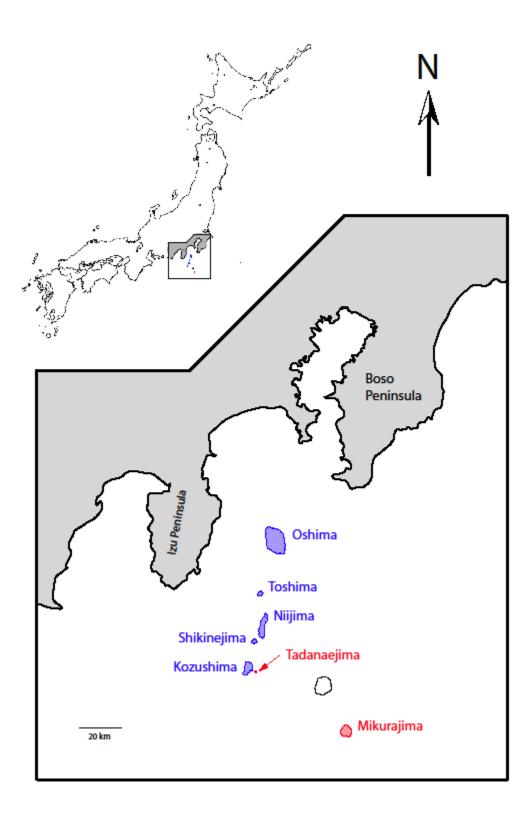
Figure 2 (**a**) Bar-graphs showing mean ± 1 SE snout-vent length (mm), (**b**) gape index (mm^2), and (**c**) body size-adjusted gape index for adult snakes collected from bird vs. lizard-eating islands. Body size-adjusted gape index was calculated using the residuals from an ordinary least-squares regression of log SVL (*x*-axis) versus log gape index (*y*-axis). Snakes from bird-eating islands have significantly larger adult body ($F_{1, 256} = 20.1$, $P = 0.0001$) and gape sizes ($F_{1, 256} = 82.2$, $P = 0.0001$) than snakes from lizard-eating islands.

Figure 3 Photographs of an adult Kozushima lizard-eating (left), and a Tadanaejima bird-eating (right) snake taken before and after compression of the quadrate bones.

Table 1 | Mantel test results (α = 0.05). Matrix A represents the pairwise dietary difference matrix between populations (0 = diets are the same; 1 = diets are different) or the distance matrices for mean body size or mean gape size among populations. Matrix B represents the distance matrices for mean body size, mean gape size, mean genetic or geographic distance among populations. Matrix B was used to test for significant correlations between the variables included in matrices B and A. Matrix C was used to take into account the potentially confounding influence of another correlated variable when testing for correlations between matrices A and B. See Methods Summary for details on how the difference matrices were constructed. Significant results are indicated in bold.

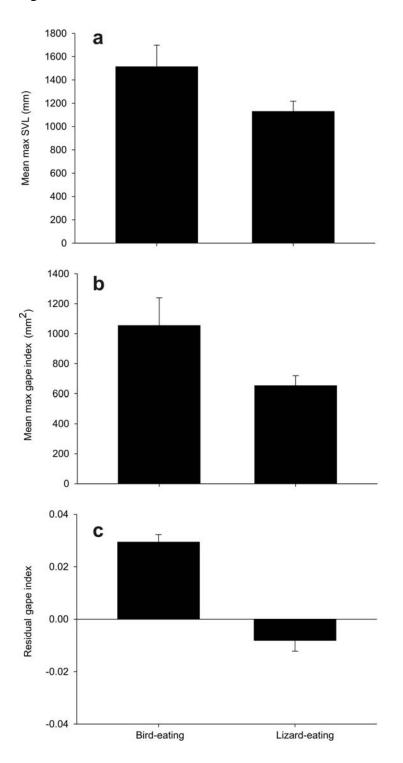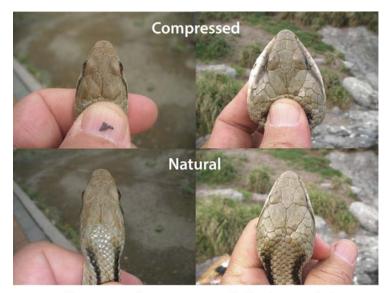| Matrix A	Matrix B	Matrix C	Mantel r-value	P-value
Diet	Body size	-	0.29	0.38
Diet	Body size	Genetic	0.54	0.11
Diet	Body size	Geographic	0.21	0.54
Diet	Body size	Gape size	-0.39	0.24
Diet	**Gape size**	**-**	**0.65**	**0.05**
Diet	**Gape size**	**Body size**	**0.68**	**0.04**
Diet	**Gape size**	**Genetic**	**0.73**	**0.03**
Diet	Gape size	Geographic	0.63	0.06
Diet	Geographic	-	0.26	0.44
Diet	Geographic	Genetic	0.44	0.20
Diet	Genetic	-	-0.17	0.61
Diet	Genetic	Geographic	-0.41	0.22
Body size	Geographic	-	0.42	0.22
Body size	Geographic	Genetic	0.10	0.73
Body size	Genetic	-	0.64	0.07
Body size	Genetic	Geographic	0.53	0.12
Body size	**Gape size**	**-**	**0.75**	**0.04**
Body size	**Gape size**	**Genetic**	**0.80**	**0.04**
Body size	**Gape size**	**Geographic**	**0.75**	**0.03**
Gape size	Geographic	-	0.22	0.50
Gape size	Geographic	Genetic	0.10	0.73
Gape size	Genetic	-	0.24	0.46
Gape size	Genetic	Geographic	0.15	0.61

Figure 1

Figure 2

Figure 3

Compressed

Natural

Lizard-eating population   Bird-eating population