

Genetic Assimilation and the Postcolonization Erosion of Phenotypic Plasticity in Island Tiger Snakes

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Summary

In 1942, C.H. Waddington [1] suggested that colonizing populations could initially succeed by flexibly altering their characteristics (phenotypic plasticity; [2–4]) in fitness-inducing traits, but selective forces would rapidly eliminate that plasticity to result in a canalized trait [1, 5, 6]. Waddington termed this process “genetic assimilation” [1, 7]. Despite the potential importance of genetic assimilation to evolutionary changes in founder populations [8–10], empirical evidence on this topic is rare, possibly because it happens on short timescales and is therefore difficult to detect except under unusual circumstances [11, 12]. We exploited a mosaic of snake populations isolated (or introduced) on islands from less than 30 years ago to more than 9000 years ago and exposed to selection for increased head size (i.e., ability to ingest large prey [13–16]). Here we show that a larger head size is achieved by plasticity in “young” populations and by genetic canalization in “older” populations. Island tiger snakes (*Notechis scutatus*) thus show clear empirical evidence of genetic assimilation, with the elaboration of an adaptive trait shifting from phenotypically plastic expression through to canalization within a few thousand years.

Results

Population Differences in Body and Head Sizes at Birth

Comparisons of neonates among seven populations of tiger snakes (*Notechis scutatus*) revealed significant differences in body dimensions at birth (body mass; nested analysis of variance [ANOVA] with litter nested into population, and body mass as dependent variable: population effect $F_{6, 524} = 305.95$; $p < 0.0001$; body length $F_{6, 524} = 157.69$; $p < 0.0001$; jaw length $F_{6, 523} = 243.49$; $p < 0.0001$) and in the size of the head relative to the body (jaw length relative to body length; nested analysis of covariance [ANCOVA] with jaw length as dependent variable, snout-vent length (SVL) as covariate $F_{6, 522} = 287.51$; $p < 0.0001$).

Under the hypothesis that insular habitats select for an increase in maximal ingestible prey size (because of the shift toward larger prey [13, 15–17]), we predicted that “older” (longer-established) populations would show a larger absolute head size at birth, which in turn could be achieved by increasing mean body size at birth and/or relative head size at birth (Figure 1). As predicted, the data show a trend toward longer, heavier, and larger-headed snakes in long-isolated systems (five islands and two reference [source] populations; Figures 2A–2C).

Phenotypic Plasticity in Relative Head Size

Within each of the seven populations, neonate snakes allocated to two feeding treatments (large versus small prey) did not differ significantly in mean values of any of the morphological variables that we measured ($p > 0.10$). All snakes (regardless of population or treatment group) were fed similar amounts of food over the course of the experiment (two-way ANOVA with treatment and population as factors; mean amount of food = 48.59 ± 3.04 g per snake; treatment effect $F_{1, 177} = 0.06$; $p = 0.81$; population effect $F_{6, 177} = 0.98$; $p = 0.44$; interaction term $F_{6, 177} = 0.15$; $p = 0.99$). That consistency in food intake resulted in an overall similarity in rates of growth in body length (repeated-measures [RM] ANOVA for data within each population, with treatment as a factor and body length as the repeated measure over time; all $p > 0.14$ except for New Year Island: $p < 0.002$).

Although the total mass of prey offered and consumed was similar between treatments, the size of prey items differed. “Large” mice averaged 3.16 ± 0.55 g (ranging from 0.4 to 11.2 g; prey size increased as snakes grew larger), whereas “small” mice averaged 1.69 ± 0.11 g (0.5 to 3.5 g; two-way ANOVA with population and treatment group as factors; effect of treatment $F_{1, 177} = 957.62$; $p < 0.0001$). After 242 days of growth, phenotypic differences between the “large-prey” and “small-prey” snakes emerged in some populations but not others (Table 1; see below).

Quantifying the Degree of Plasticity

Relative prey size significantly affected the rate of growth of the young snakes’ heads (skull length and jaw length, but not head width) in three insular populations (Carnac, Trefoil, and New Year islands). Most importantly, statistical analysis revealed a significant interaction between the variables population and treatment (prey size) on the rate of jaw growth over time (including body length as a covariate; RM ANCOVA interaction effect $F_{24, 704} = 3.86$; $p < 0.0001$). In order to compare phenotypic plasticity among populations, we needed an index of the magnitude of plasticity. To create such an index, we calculated the slope of the least-squares regression of log (relative jaw length) against log (snake age, i.e., days since birth) separately for each treatment group in each population (Table 2). Then we plotted the extent of phenotypic plasticity (the arithmetic difference in slopes between the two treatment groups) for each population against the length of time for which that population has been isolated. The relationship between the two variables was highly significant: phenotypic plasticity in relative head size was greatest in snakes from newly colonized areas and lower in snakes from areas that had been colonized or isolated long ago (least-squares regression $n = 5$; $R = 0.96$; $F_{1, 3} = 37.54$; $p < 0.009$; Figure 3).

The erosion of plasticity and assimilation of the trait jaw size is well illustrated by comparing three populations: a very young system (Trefoil Island, 30 to 40 years before present [BP]) in which jaws are small but plastic in development, a slightly older system (Carnac Island, 90 years BP) in which jaws are intermediate in size at birth but plastic in development, and an old system (Williams Island, 9100 years BP) in which jaws are large at birth but not plastic in their rates of

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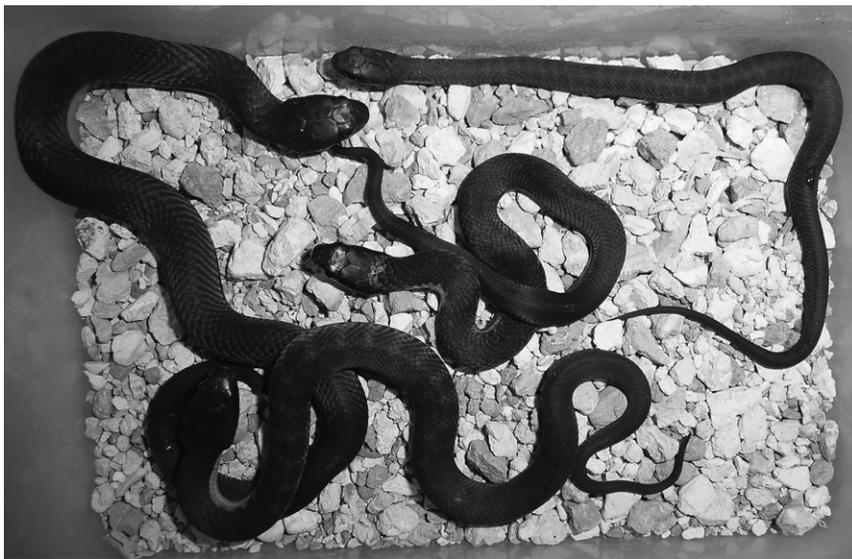


Figure 1. Variation in Body Size and Head Size at Birth among Populations of Australian Tiger Snakes

Shown are neonates from a long-isolated Williams Island population (top left), a recently isolated Carnac Island population (bottom left), and two typical mainland neonate tiger snakes from Herdsman Lake (center) and Joondalup Lake (top right). All of these neonates were born within a few days of each other and were not fed prior to being photographed.

growth (Figure 4). Thus, canalization has increasingly replaced plasticity as a function of time since colonization.

Discussion

Tiger snakes from newly colonized areas had relatively small heads at birth, but these increased rapidly in size (relative to

body length) if the young snakes were offered large prey items. In contrast, snakes from “older” (long-colonized) islands had large heads at birth, but the head size of these animals displayed little phenotypic plasticity: the relative sizes of the snakes’ heads were unaffected by the size of prey they encountered. Thus, the scenario accords well with Waddington’s hypothesis [1, 7] as well as many predictions of recent models [10]. As the animals colonized a novel type of habitat, the optimal values for major phenotypic traits (in this case, head size and body size) were different from those experienced in the ancestral (mainland) population. That challenge initially was solved by phenotypic plasticity (phenotypes were induced by local conditions, in this case prey size). Remarkably, such plasticity evolved or was selected for very quickly (in under 40 years; see above and [10, 17, 18]) from undetectable levels of plasticity (this aspect is not predicted by current evolutionary models [10]). Over time (over thousands of years), this plasticity was eroded and replaced by genes coding for canalized expression of the same phenotypic trait (a large head size) that in earlier times had been generated by plasticity.

In a scenario where plasticity is eroded, we would expect to see a progressive increase in mean body size and head size at birth, as a function of time since population isolation. That is, tiger snakes in newly colonized areas should have relatively small heads at birth, whereas conspecifics from “older” populations should have larger head sizes. In keeping with this prediction, a gradient in size at birth was evident between the “old” population on Williams Island (large neonates) and mainland snakes with no exposure to larger prey items (Joondalup Lake, small neonates). Neonate sizes were intermediate in the sites with intermediate ages since colonization (Christmas and New Year islands).

Comparisons between the two most recently colonized sites, Carnac Island (less than 90 years since snake introduction) and Trefoil Island (40 years), are of particular interest. Snakes from both of these “young” populations are highly plastic in rates of jaw growth, but Carnac Island neonates

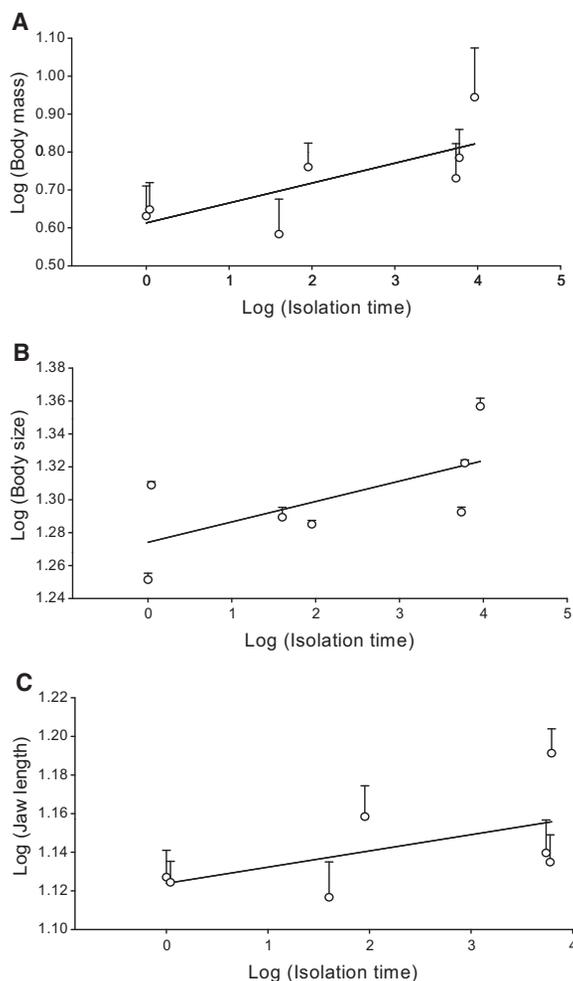


Figure 2. Body Sizes and Head Sizes of Tiger Snakes as a Function of the Time since Colonization of Novel Habitats

The graphs show mean values + standard error (SE) for body mass (A), body size (snout-vent length, B) and jaw size (jaw length, C) in neonates from five insular tiger snake populations (differing in times since colonization) and two reference (source) populations (isolation time = 0). Body mass: Spearman rank-order correlation $n = 7$; $R = 0.82$; $t = 3.19$; $p < 0.024$; body length: $n = 7$; $R = 0.75$; $t = 2.53$; $p < 0.052$; jaw length: Spearman rank-order correlation $n = 7$; $R = 0.71$; $t = 2.28$; $p < 0.071$.

Table 1. Influence of Prey Size on Head Growth

	Williams Island	Carnac Island	New Year Island	Christmas Island	Joondalup Lake	Tasmania	Trefoil Island	Across Populations
Isolation time (years BP)	9100	90	6000	6000	source	source	40	
Numbers of animals (n)	30	36	28	27	25	26	19	
Skull length (mm)	0.33	<0.002*	<0.006*	0.85	0.25	0.17	0.81	<0.032*
Jaw length (mm)	0.98	<0.0001*	<0.042*	0.14	0.11	0.31	<0.013*	<0.0001*
Head width (mm)	0.60	0.13	0.45	0.43	0.11	0.09	0.73	<0.041*

Results of statistical analyses of data on head measurements of young tiger snakes (*Notechis scutatus*) raised on either small or large food items. Isolation times for all populations are given in years before present (BP). Joondalup Lake and Tasmania are source populations. Measurements were taken every two months. Statistical analysis was performed via repeated-measure analyses of variance (ANOVAs), with treatment as factor, head measurement as the dependent variable over time, and body length as the (changing) covariate. *p < 0.05.

also are born with larger jaws than their putative mainland ancestors (Joondalup Lake), whereas the Trefoil Island snakes do not differ from their putative (Tasmanian) ancestors in mean body or head sizes at birth. Therefore, genetic assimilation may occur rapidly (over a few decades), even in relatively long-lived, late-maturing animals such as tiger snakes (2 to 3 years old at maturation [13, 18]). If such rapid change is common, then genetic assimilation will only be observed in studies specifically focused on the years immediately postcolonization. Future studies could usefully (1) assess and measure the putative costs of phenotypic plasticity in island tiger snakes and (2) explore plasticity during the postcolonization phase in other species to see whether Waddington's long-neglected concept of genetic assimilation may provide important insights into the process by which organisms adapt to novel environmental challenges.

Experimental Procedures

This study was carried out over three years under consistent laboratory conditions in a temperature-controlled room at the University of Sydney. Gravid females were captured from seven populations in January or February of 2006, 2007, and 2008. From a total of 595 neonates born in the laboratory, 191 were used for a 242-day-long common-garden experiment under the protocol described in [17]. For each population, we selected a few neonates from each litter (split-clutch design) and allocated them between two treatment groups (large versus small dead mice) as follows: Carnac Island (8 litters; 19 large-prey snakes and 17 small-prey snakes; S 32° 07' 17"; E 115° 39' 43"); Joondalup Lake (4 litters; 14 large-prey snakes and 11 small-prey snakes; S 31° 44' 52"; E 115° 45' 09"); Christmas Island (6 litters; 13 large-prey snakes and 14 small-prey snakes; S 39° 41' 13"; E 143° 49' 55"); New Year Island (3 litters; 14 large-prey snakes and

14 small-prey snakes; S 39° 40' 11"; E 143° 49' 34"); Tasmania (4 litters; 13 large-prey snakes and 13 small-prey snakes; S 41° 09' 49"; E 146° 10' 17"); Trefoil Island (3 litters; 10 large-prey snakes and 9 small-prey snakes; S 40° 37' 57"; E 144° 41' 25"); Williams Island (3 litters; 14 large-prey snakes and 16 small-prey snakes; S 35° 01' 48"; E 135° 58' 25").

Young snakes were individually housed in plastic tubs (40 cm × 25 cm × 12 cm). A heat source at one end of the tub provided basking opportunities. For each neonate, the size and the shape of the head were measured (at birth and then at two-month intervals) with digital calipers (± 0.01 mm) as follows: jaw length (from the tip of the snout to the quadrate-articular projection; see Table 1 for details); skull length (from the tip of the snout to the base of the skull); and head width (maximal width above the eyes, from the external margins of the supraoculars). Body mass was recorded on a digital scale (±0.1 g) and snout-vent length with a measuring tape (±0.1 cm). All measurements were performed by a single highly experienced person on a "blind" design via standardized procedures to minimize measurement errors.

Food was offered on a weekly basis. For all large-prey groups, prey sizes were selected based on head sizes of each snake and increased from week to week as each snake grew larger. In the frequent cases where a snake was physically unable to ingest the prey item it was offered, it was given a slightly smaller prey item the following day. Therefore, all snakes in the large-prey group were challenged with prey items so large that they were close to upper ingestible size, and that challenge remained consistent throughout the experiment as a result of our continual adjustment of prey size to snake head size.

The island systems tested in this study were selected for their similarity in prey type and availability of prey to snake populations. On islands that lack shearwater colonies, tiger snakes feed on small prey and grow to small sizes. However, on islands that contain breeding shearwaters, the chicks of those birds are the primary prey for the snakes in every case [14, 16, 17].

Estimating Island Isolation Times

Isolation times for each population were estimated from data on the timing of sea level rises compared to water depths between island and mainland by

Table 2. Growth in Jaw Length across Populations

Snake Age (Days)	Trefoil Island		Carnac Island		Christmas Island		New Year Island		Williams Island		Tasmania		Joondalup Lake		
	Large Prey	Small Prey	Large Prey	Small Prey	Large Prey	Large Prey	Large Prey	Small Prey	Small Prey	Small Prey	Small Prey	Large Prey	Small Prey	Large Prey	Small Prey
Growth in jaw length	60	13.63	13.73	14.96	14.85	13.87	13.81	13.52	13.38	14.30	14.23	15.67	15.58	13.65	13.62
Growth in jaw length	116	14.34	14.39	15.78	15.36	14.71	14.70	14.32	14.12	15.34	15.30	15.91	15.79	14.34	14.40
Growth in jaw length	186	15.37	15.25	16.85	15.97	15.98	15.62	15.16	14.96	16.34	16.19	16.81	16.97	15.30	15.28
Growth in jaw length	242	16.52	15.88	18.18	17.04	17.23	17.02	16.41	16.20	17.71	17.34	18.10	18.08	16.49	16.46
Slope		0.133	0.103	0.133	0.091	0.151	0.141	0.130	0.128	0.145	0.135	0.096	0.103	0.13	0.13

Jaw dimensions relative to snake body length (in mm) in young tiger snakes from six island populations at 60, 116, 186, and 242 days of age. The young snakes were maintained in captivity since birth on a diet of either relatively small or relatively large prey. Slopes were calculated via linear regressions of log (jaw length) against log (snake age).

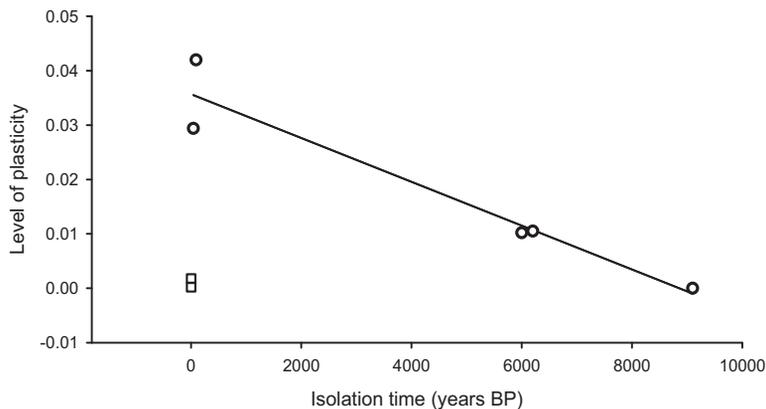


Figure 3. Magnitude of Plasticity as a Function of Isolation Time

The magnitude of phenotypic plasticity (change in jaw growth rate induced by an experimentally imposed shift in prey size) declines with the time since island colonization (isolation time) in tiger snake populations (least-squares regression $n = 5$; $R = 0.96$; $F_{1, 3} = 37.54$; $p < 0.009$). ○, island populations; □, source populations.

using nautical charts and previous detailed studies [19–22]. Carnac Island tiger snakes were introduced from the mainland around 1920 [15, 23]. Williams Island was isolated 9100 years ago [22]. Christmas Island and New Year Island were both isolated around 6000 years ago [24]. Trefoil Island tiger snakes were introduced from mainland Tasmania between 30 and 40 years ago [25, 26]. Despite their wide and fragmented distribution, tiger snake populations across Australia are genetically homogeneous, with a maximum overall genetic distance of only 1.4% between western and eastern Australian populations; island populations are most closely related to conspecific populations from the adjacent mainland, confirming that colonizations are phylogenetically independent events [16, 27].

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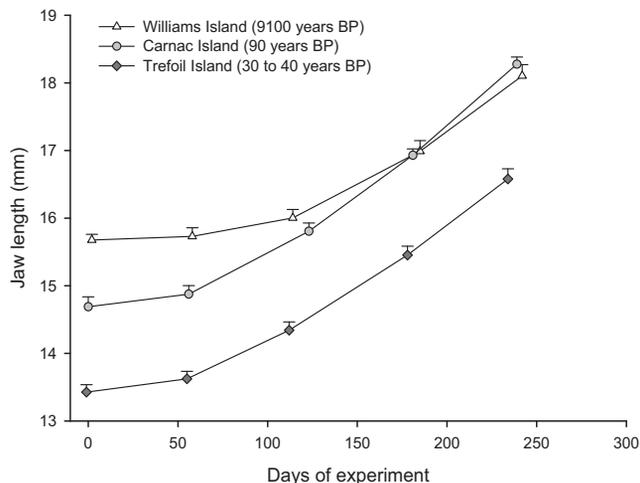


Figure 4. Growth in Jaw Size in Young Tiger Snakes

Rates of ontogenetic increase in jaw size (and hence maximum ingestible prey size) in young tiger snakes from three islands (Williams, Carnac, and Trefoil islands) that were colonized by the snakes either very recently (30 to 40 years ago), recently (90 years ago), or long ago (9100 years ago). From birth, these snakes were fed large prey items (mimicking the sizes of prey available on islands but not available to the mainland [source] populations) for 242 days, and their jaw sizes were measured at regular intervals. Mean values + SE are plotted. Snakes from a very recently colonized island had small jaws at birth, but these increased considerably if the animals were given large prey items. Snakes from “older” colonization events had larger jaws at birth and exhibited less plasticity in this trait.

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