Developmental reaction norms vary among families of lizards in response to multivariate nest environments

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Introduction

Most organisms exhibit plastic responses to the developmental environment. These responses are adaptive if they generate phenotypes suited to conditions in spatially and/or temporally heterogeneous habitats (Lande 2009, Monaghan 2008, Arnold et al. 2019). Indeed, developmental plasticity (i.e., the capacity of a genotype to express multiple phenotypes in response to early-life environments) is one route by which organisms could overcome the challenges of environmental heterogeneity (West-Eberhard 2003, DeWitt and Scheiner 2004; Snell-Rood and Ehlman 2021). For example, some species develop defensive morphologies when predators are detected, but otherwise, do not expend energy on these traits (e.g. *Daphnia* waterfleas, Parejko and Dodson 1991; larval amphibians, Newman 1989). Such plasticity is described by a reaction norm, which is a mathematical function that describes phenotype values across different environments. Consequently, reaction norms are useful tools to calculate, visualize, and evaluate differences in plastic traits among environments, populations, and individuals (Gomulkiewicz and Kirkpatrick 1992, Brommer et al. 2005, Monaghan 2008).

Reaction norms may vary among individuals and populations, potentially due to genetic variation (Ellis and Boyce 2008; Scheiner 1993, Murren et al. 2015). This indicates an opportunity for natural selection to shape plasticity in adaptive ways (Levis and Pfenning 2016) like any trait with additive genetic variation (Hillesheim and Stearns 1991, Gavrilets and Scheiner 1993, Scheiner 2002). Environmental heterogeneity creates conditions that allow developmental plasticity to be adaptive (Gomulkiewicz and Kirkpatrick 1992, Lande 2009), as selection will favor reaction norms that match phenotypes to different environments, thereby enhancing fitness. Furthermore, adaptive plasticity should arise if the environmental cues that generate a phenotype also predict the future environment in which the phenotype is expressed and where its fitness consequences are realized (Casal et al. 2004, Beldade et al 2011).

Variation in reaction norms among individuals of a population provides an opportunity for selection to act on plasticity, whereas variation across populations may signify past selection that has shaped plasticity in response to local environments. Both theory (Gavrilets and Scheiner 1993; Lande 2009, Levis and Pfenning 2016) and empirical studies (Suzuki and Nijhout 2006) indicate that under strong selection, the magnitude (i.e., slope) of reaction norms should be maintained and become homogenized, decreasing within-population variation. Alternatively, if distinct populations experience different environmental pressures with varying levels of heterogeneity, then both the magnitude and variation of reaction norms might change (Duffy et al. 2015). Moreover, experimental evolution studies demonstrate that patterns of plasticity can vary between populations (e.g., populations with or without plasticity; van der Burg et al. 2020), and this process could be reversed with artificial or natural selection. Ultimately, a population's genetic structure may contain individuals that are more (or less) plastic to environmental conditions. Consequently, when a population is faced with a major environmental change due to natural or human-induced causes, or resulting from invasion, plasticity may be amenable to selection.

The aim of this study is to quantify variation in developmental reaction norms among individuals and within populations of a non-native lizard, the brown anole (*Anolis sagrei*). Past work on *A. sagrei* (Warner et al. 2012; Pearson & Warner 2018; Hall & Warner 2022), as well as studies on other reptiles (Mitchell et al. 2018; Noble et al. 2018; Warner et al. 2018), demonstrate strong effects of egg incubation environments on developmental rate, offspring body size, locomotor performance, behavior, physiology, and fitness; however, within- and among-population variation in reaction norms has never been examined in *A. sagrei* , but among-population variation in embryonic reaction norms of other *Anolis* species has been documented (Goodman 2008; Goodman & Heah 2010). Here, we collected lizards from two islands that differ in habitat structure and, thus, the predominant nest environments in which embryos develop (i.e., open-canopy island with warm, dry conditions versus closed-canopy with cool, moist conditions). Individuals from each island were bred in a common garden, and we incubated eggs in one of two regimes that mimic natural conditions on each island. Because each females' eggs were divided between treatments (i.e., split-clutch design), we could quantify among-individual and among-population variation in reaction norms for a range of fitness-related phenotypes.

Our novel study design helps fill two important knowledge gaps in studies of developmental plasticity. First, most studies of developmental plasticity consider the isolated effects of factors like incubation temperature or moisture, but such factors usually co-vary in predictable ways in the wild (e.g. warmer nests are often drier; Pruett et al. 2020). We need a better understanding of the effects of real nest environments on plasticity. For example, warmer temperatures result in greater sprint speed at hatching (Pearson & Warner 2018), but dry incubation conditions can decrease hatchling performance (Gatto & Reina 2022). Moreover, the effect of temperature on sprint speed may depend upon thermal variation, not just mean temperature (Hall & Warner 2020). Thus, we need studies that combine multiple nest conditions that represent real habitats to better understand developmental plasticity in the wild.

Second, we test the hypothesis that variation in offspring phenotypes results from the influence of the environment (i.e., open- vs closed-canopy conditions), parentally induced variation in phenotypes (i.e., family-group reaction norm intercepts), and plasticity (i.e., family-group reaction norm slopes). We address this hypothesis by quantifying the slopes of reaction norms (for morphological, physiological, and performance traits) for each family group and comparing them among families and populations. Significant variation in reaction norm slopes among family groups (indicative of genetic x environment interactions) would support this hypothesis and indicate potential for plasticity to evolve in response to future pressures. Our results have important implications for understanding how natural developmental environments generate phenotypic variation, and the capacity for populations to adapt to changing environments.

Ethics

Use of live animals was approved by Auburn University IACUC # 2019-3637. Field work was approved by the Guana Tolomato Matanzas National Estuarine Research Reserve.

Data accessibility

The data generated in this study and the code used for analysis and data visualization will be deposited in AUrora.

Declaration of AI use

We have not used AI-assisted technologies in creating this article.

Conflict of interest declaration

All authors declare no conflict of interest.

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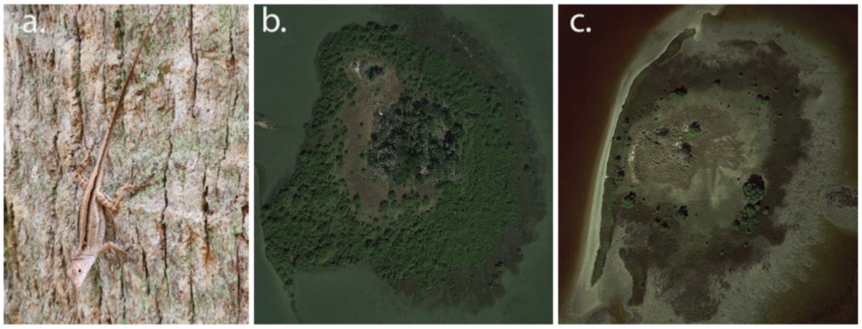
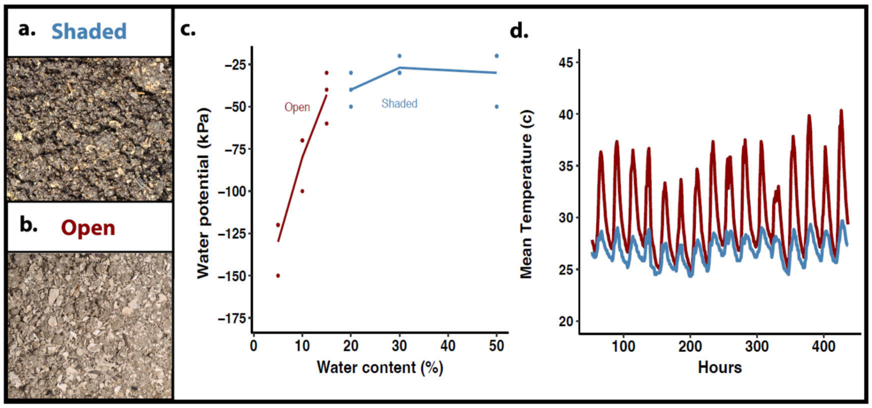


Figure 1. Study species and field sites. (a) Female brown anole (*Anolis sagrei*). (b) Shaded island with high tree density. (c) Open island with sparse tree cover.



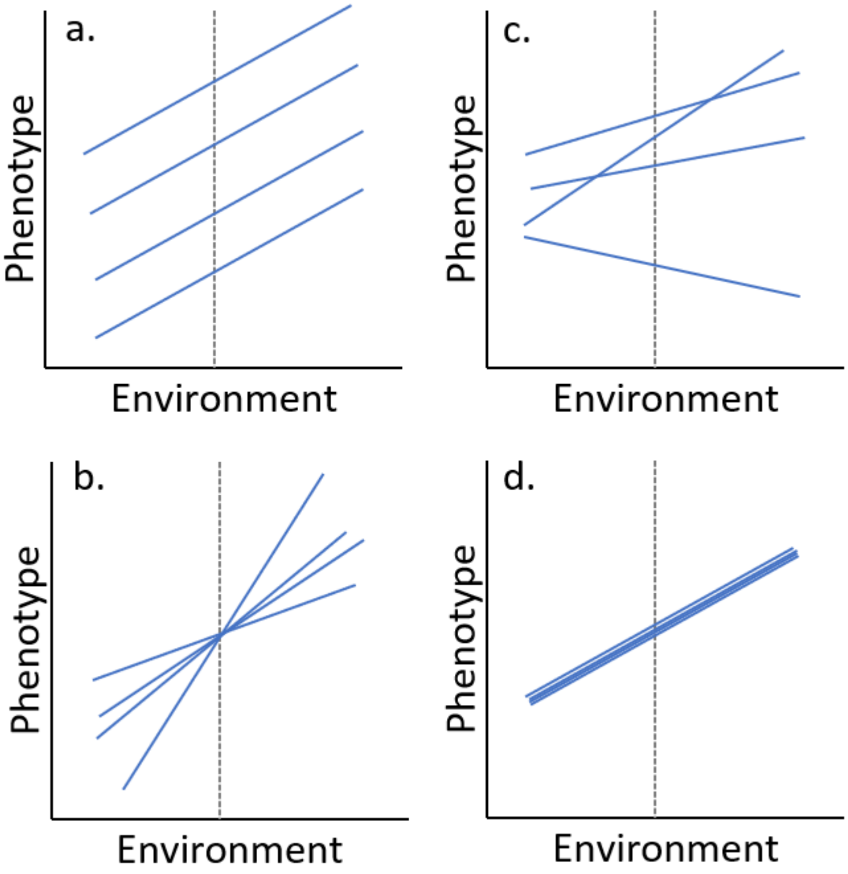


Figure 3. Possible family-level relationships between the slope and intercept of reaction norms. Each line represents a reaction norm for an individual family-group (i.e., offspring from a single female and her mate): (a) random intercept model; (b) random slope model; (c) random intercept and slope model; (d) null model with no random effect (i.e., identical slope and intercept among families).

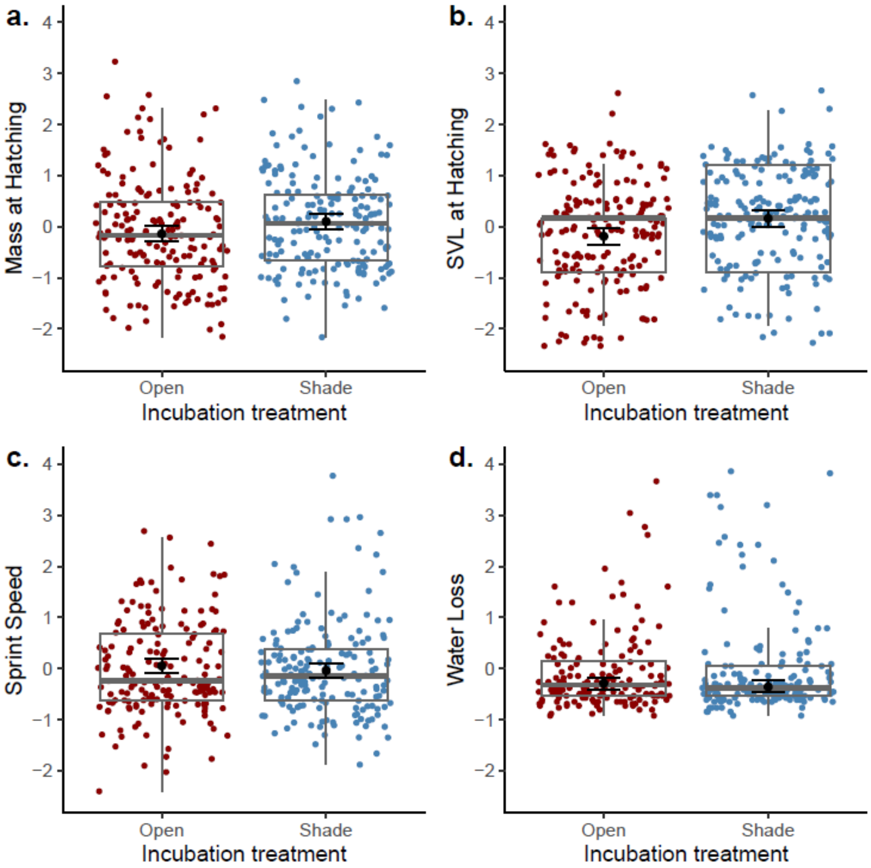


Figure 4 : Effect of incubation treatment on body size and performance of *Anolis sagrei* hatchlings. (a) Body mass at hatching; (b) Snout-vent length (SVL) at hatching; (c) Running speed; (d) Water loss during desiccation trials. All data points in the graphs are model adjusted for time of hatching, egg mass, and maternal island of origin. All points have been mean standardized as described in methods (but see supplemental figure 2 for plots of non-scaled means). Results from statistical tests are reported in Table 1.

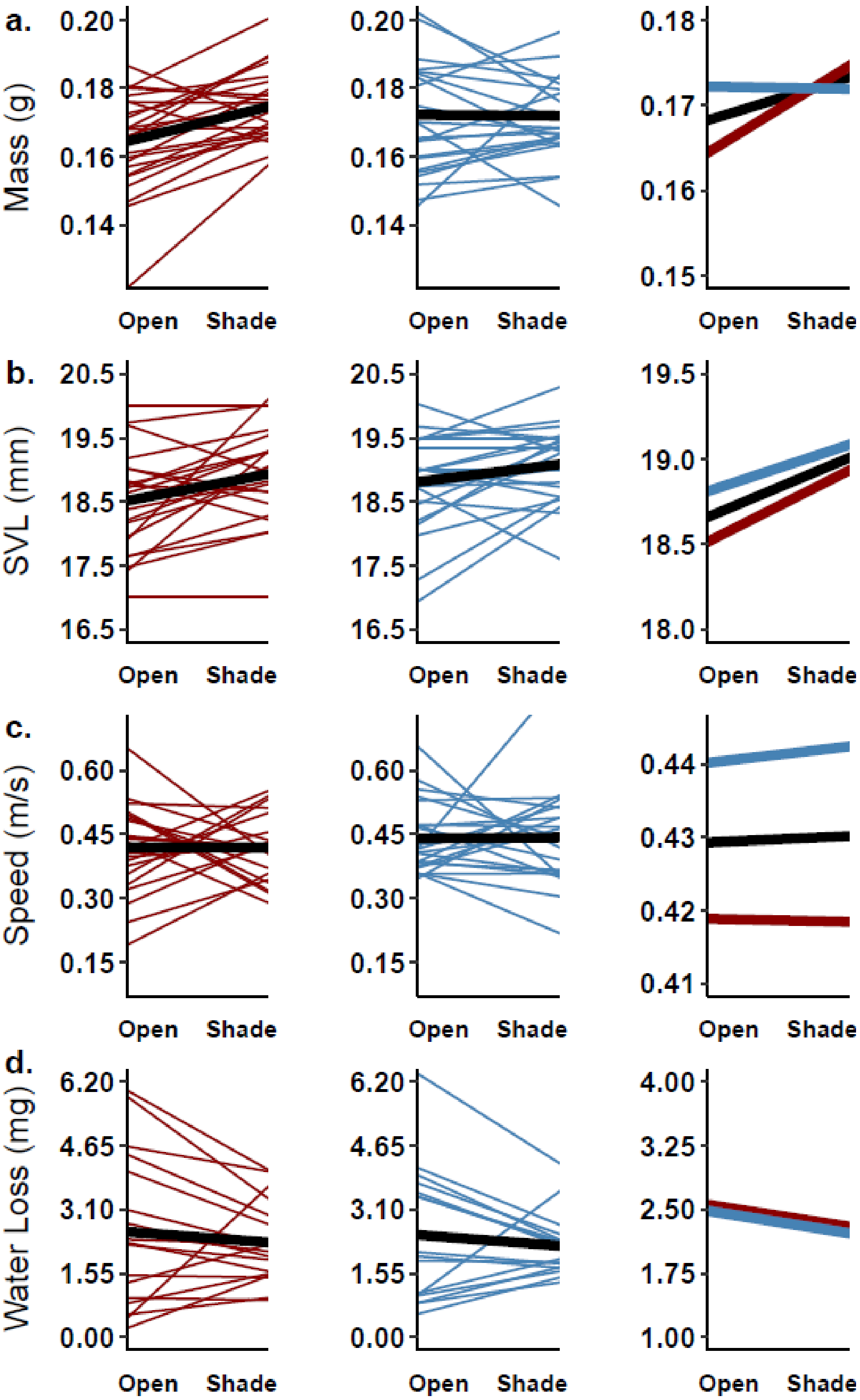


Figure 5 : Reaction norms for each family separated by source island; the first and second columns of graphs are for individuals from the open and shaded islands, respectively (i.e., reaction norms reflect the trait values for all offspring from a single mother. The third column represents the reaction norms averaged for each family group (red curve = open island, blue curve = shaded island, black curve = average of all family groups). (a) Reaction norms for body mass; (b) Reaction norms for snout-vent length; (c) Reaction

norms for spring speed; (d) Reaction norms for water loss. All reaction norm slopes reported are measured directionally from the open to the shaded incubation treatment. That is, a positive slope conveys a larger trait value in the shaded treatment and a negative slope conveys a larger trait value in the open treatment. The y -axis has been changed for the plots showing the average reaction norms to better see the patterns.

Table 1. Effect of incubation treatment, island, and covariates on hatchling phenotypes. Statistically significant interaction terms were retained in the final models. Descriptive statistics (means and standard deviations) for each island and incubation treatment are reported in supplemental Table 2). Estimates for treatment and source island effects were calculated with the shaded incubation treatment and shaded island as references. In the random effects columns σ^2 = the residual variance, τ_{00} = the random intercept variance (maternal ID was the random effect in models for mass and SVL), ICC = interclass correlation.

	Estimates	*95% CI*	*d.f.*	*f value*	*P*
Hatchling Mass (N=358, conditional r^2=0.425, marginal r^2=0.254)					
Treatment (Shade)	0.2391	0.09 – 0.39	292.73	9.3538	**0.00**
Island (Shade)	-0.0049	-0.26 – 0.25	43.94	0.0015	0.969
Hatch Day	0.0898	-0.17 – -0.01	329.19	4.7965	**0.02**
Egg mass	0.4568	0.37 – 0.55	340.63	98.7034	**<0.0**
Random effect (σ^2 = 0.53, τ_{00} = 0.16, *ICC = 0.23, N = 63)*					
Hatchling SVL (N=358, conditional r^2=0.274, marginal r^2=0.101)					
Treatment (Shade)	0.3579	0.18 – 0.54	301.64	15.5641	**<0.0**
Island (Shade)	0.1349	-0.14 – 0.41	50.37	0.9292	0.339
Hatch Day	-0.0781	-0.17 – 0.02	335.5	2.7053	0.100
Egg mass	0.3659	0.22 – 0.51	329.13	23.5409	**<0.0**
Treatment:Eggmass	-0.2220	-0.40 – -0.04	316.5	507535	**0.01**
Random effect (σ^2 = 0.72, τ_{00} = 0.17, *ICC = 0.19, N = 63)*					
Sprint Speed (N=341, r^2= *0.223)*					
Treatment (Shade)	-0.094	-0.29 – 0.10	335	0.1801	0.383
Island (Shade)	0.02	-0.17 – 0.21	335	0.0346	0.908
Snout-vent Length	0.19	0.09 – 0.29	335	27.2059	**0.00**
Trial Temperature	0.08	-0.02 – 0.18	335	4.2103	0.107
Number of Stops	-0.39	-0.48 – -0.29	335	65.001	**>0.0**
Desiccation (N=328, r^2=0.94)					
Treatment (Shade)	-0.0643	-0.22 – 0.09	328	0.6041	0.438
Island (Shade)	0.0325	-0.12 – 0.19	328	0.2039	0.692
Relative humidity (%)	0.0545	-0.02 – 0.13	328	0.2175	0.183
Starting Mass	0.2330	0.15 – 0.31	328	31.2057	**>0.0**

Table 2: Models to test for difference in variance of reaction norms. Results of Fishers F-test for variance and Flinger – Killeen test for homogeneity of group variance. The null hypothesis for both tests is that population variances are equal. The +/- slope count records the number of positive or negative slopes in the group.

not-yet-known not-yet-known not-yet-known unknown

	Slope Mean	Slope Range	Slope variance	F-test of variance	Fligner-Killeen	+/- S
Body mass (mg)	4.585	-23.58 – 34.65	167.453	F1,23= 0.684, p = 0.373	x2=0.191, p = 0.661	31/16
Open Island	9.230	-15.15 – 32.81	120.505			18/6
Shaded Island	-0.260	-23.58 – 34.65	176.062			13/10
SVL (mm)	0.315	-1.00 – 2.00	0.392	F1,23=1.212, p = 0.653	x2=0.002, p = 0.961	6/41
Open Island	0.379	-0.66 – 2.00	0.433			3/21

	Slope Mean	Slope Range	Slope variance	F-test of variance	Fligner-Killeen	+/- Sl
Shaded Island	0.247	-1.00 – 1.33	0.357			3/20
Speed (cm/s)	0.07	-27.33 – 35.80	145.115	F1,23=0.885, p = 0.772	x2=0.584, p = 0.444	21/26
Open Island	-0.038	-22.58 – 18.27	139.471			12/12
Shaded Island	0.201	-27.33 – 35.80	157.582			9/14
Water loss (mg)	-0.235	-2.24 – 2.90	1.273	F1,17=1.105, p = 0.852	x2=0.055, p = 0.814	3/31
Open Island	-0.233	-2.24 – 2.90	1.375			2/16
Shaded Island	-0.238	-1.96 – 2.27	1.243			1/15

Table 3: Models to test for difference in means of reaction norms. Shaded island is the reference in all models.

not-yet-known not-yet-known not-yet-known unknown

	Estimates	95% C.I.	S.E.	d.f.	F value	P value	R2
Mass (mg)	-9.230	-16.63 – -2.35	2.480	45	7.164	0.0103	0.137
SVL (mm)	-0.132	-0.50 – 0.24	1.837	45	0.5166	0.4760	0.011
Speed (cm/s)	0.240	-6.92 – 7.40	3.553	45	0.0046	0.9463	0.001
Water Loss (ml)	0.004	-0.81 – 0.80	0.393	32	0.0004	0.9902	0.001

Bold text denotes statistical significance at alpha = 0.05